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ISOLATION AND BIOINFORMATICS CHARACTERIZATION OF HORSE GRAM (*Macrotyloma uniflorum* (Lam.) Verdc.) HOMOLOGS (*MuTFL1* and *MuLFY*) OF GENES CONTROLLING GROWTH HABIT

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Growth habit (indeterminate/determinate) is one of the evolutionarily shaped, economically important plant architectural traits in grain legumes, including horse gram. Arabidopsis *AtTFL1* and *AtLFY* genes and their homologs in other grain legume species are known to control growth habit. Taking cues from highly conserved domains in protein encoded by *TFL1* and *LFY* genes, we isolated horse gram homologs (*MuTFL1* and *MuLFY*) genes using reported degenerate primers designed to conserved domains. *MuTFL1* and *MuLFY* homologs were isolated and sequenced using Sanger's sequencing protocol. The nucleotide sequences of *MuTFL1* and *MuLFY* homologs were translated to their corresponding amino acid sequences using "ExPASy" tool. BLASTx analysis of the translated amino acid sequences of *MuTFL1* and *MuLFY* homologs showed high similarity with those of soybean and pigeon pea *TFL1* and *LFY* homologs. Multiple sequence alignment of nucleotide sequences of *MuTFL1* and *MuLFY* with those of related legumes and model species (*Medicago sp.* and *Lotus japonicas*) using "ClustalW" revealed the presence of four synonymous single nucleotide polymorphic (SNPs) sites in *MuTFL1* and three non-synonymous SNP sites in *MuLFY.* These results suggest that not *MuTFL1*, but *MuLFY* could be the main regulator of growth habit in horse gram unlike in other non-model legumes where *TFL1* was reported as the key gene controlling the growth habit. The substitution of glutamine in (determinate genotypes) with histidine

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(indeterminate genotypes) in *LFY*-coded protein appeared to be the cause for switch over from indeterminate to determinate growth habit in horse gram. These results are discussed in relation to strategies for breeding horse gram cultivars with desired growth habits.

Keywords: Determinate, homologs, indeterminate, *LFY*, *TFL1*

INTRODUCTION

Growth habit is one of the evolutionarily shaped, economically important plant architectural traits in grain legumes, including horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) (KRYLOVA *et al.*, 2020). Two types of growth habit, indeterminate and determinate, have evolved during domestication and distribution of legumes from their centers of origin. Horse gram genotypes with indeterminate growth habit are characterized by the presence of several internodes, profuse branching, climbing tendency; non-synchronous flowering, pod and seed maturity. On the contrary, genotypes with determinate growth habit are characterized by fewer branches, compact growth, synchronous flowering and pod and seed maturity (ASHWINI *et al.*, 2021). Cultivars with different types of growth habit are suitable for different purposes and different crop production ecosystems. Cultivars with determinate growth habit are often grown for grains for food use and are suitable for multiple cropping systems and mechanical harvesting. On the other hand, cultivars with indeterminate growth habit are grown both for grains and livestock feed and suitable for mixed cropping systems. However, these cultivars are not suitable for mechanical harvesting. Thus, the cultivars with a desired growth habit should be bred depending on the cropping ecosystems and the desired economic product (grain/fodder).

Classical genetic studies have indicated that growth habit in horse gram is controlled by two genes with determinacy being dominant to indeterminacy (CHANDANA *et al.*, 2021). A precise knowledge on the molecular genetic basis of growth habit polymorphism is expected to enhance the pace and efficiency of breeding horse gram cultivars with desired growth habit. Molecular genetic control of growth habit is well researched in *Arabidopsis thaliana* and *Pisum sativum* (BENLLOCH *et al.*, 2015; KRYLOVA *et al.*, 2020). Experimental evidences have demonstrated that growth habit in *A. thaliana* is controlled by several exogenous factors (temperature and photoperiod) and endogenous factors. Among the endogenous factors, the combination of two key genes namely *AtTFL1* and *AtLFY* are known to determine the fate of the plant with respect to growth habit (determinate/indeterminate) (HANANO and GOTO, 2011). *AtTFL1*, a gene promoting inflorescence meristem identity, represses flowering by repressing expression of *LFY* gene, and hence maintains indeterminate growth of shoot apical meristem (SAM). On the other hand, *LFY*, a floral meristem identity gene, is a key integrator for flowering transition, controlling both floral meristem identity and the activation of floral organ identity genes (MISLATA *et al.*, 2017). *AtTFL1* and *AtLFY* genes in combination maintain the balance between inflorescence and floral meristem identities at the SAM in different phases of plant development (BENLLOCH *et al.*, 2015). A mutation in *AtTFL1* gene is known to increase the expression of *LFY* and hence convert indeterminate to determinate growth habit (WICKLAND and HANZAWA, 2015). In legumes such as pea, *PvTFL1y* restored normal indeterminate growth, similar to that of wild-type *A. thaliana*, when it was introduced into the *tfl1* mutant, which exhibited determinate growth habit (REPINSKI, 2012). *LEAFY* (*LsLFY*) expression increased during the transition from vegetative to reproductive growth in lettuce (FUKUDA, 2017). Thus,

while *TFL1* maintains SAM in indeterminate growth state, the production of determinate floral meristem is accomplished by *LFY* in Arabidopsis. While loss-of-function mutation in *TFL1* result in *LFY* expression in SAM, loss-of-function mutation in *LFY* result in replacement of flowers with shoot or shoot like structures (BENLLOCH *et al.,* 2015; KRYLOVA *et al.,* 2020).

The amino acid sequence of *AtTFL1* is known to be highly conserved among fabaceae family members, as mutations in *AtTFL1* homologs cause similar phenotype in legumes such as pea, soybean, faba bean, common bean, etc. Taking cues from these research leads, the homologs of *AtTFL1* have been identified in pea (*PsTFL1a*) (FOUCHER *et al.,* 2003), soybean (*Dt1*/*GmTFL1*) (LIU *et al.,* 2010), common bean (*PvTFL1y*) (REPINSKI *et al.,* 2014), cowpea (*VuTFL1*) (DHANASHEKAR and REDDY, 2015) and dolichos bean (*LpTFL1*) (KALDATE *et al.,* 2021). Similarly, *AtLFY* homologs have been identified in pea (*PEAFLO*) (HOFER *et al.,* 1997) and soybean (*GmLFY*) (MENG *et al.,* 2007). This research leads in other legumes prompted us to hypothesize that homologs of *AtTFL1* and *AtLFY* genes could also be present in horse gram and that it is possible to isolate and characterize them in horse gram. Our hypothesis is based on the rationale that sequence similarity and highly conserved functional domains and motifs of proteins encoded by *TFL1* [phosphatidyl ethanolamine-binding proteins (PEBP) family] and *LEAFY* (*LFY*-family) genes even among distantly related crop species (PILLITTERI *et al.,* 2004; KALDATE *et al.,* 2021), offer opportunities to isolate and characterize homologs of *TFL1* and *LFY* genes by PCR- amplification by degenerate primers designed to conserved motifs (LEISTER *et al.,* 1996) in horse gram as well. To test the hypothesis, we attempted to isolate and characterize the horse gram homologs (*MuTFL1* and *MuLFY*) of *TFL1* and *LFY* genes using the reported degenerate primers designed for conserved motifs.

MATERIAL AND METHODS

The material for the study consisted of 4 horse gram genotypes differing in growth habit which included two determinate (D) genotypes (IC15735 and HPKM-191) and two indeterminate (ID) genotypes BGM 1 and CRIDA 18-R (Table 1).

Table 1. Horse gram genotypes used to isolate and characterize homologs of AtTFL1 and AtLFY genes controlling growth habit

Genotype	Growth habit	Source	Pedigree
IC 15735	Determinate	NBPGR, Bhowali	Unknown
HPKM-191	Determinate	CSK HPKV, Palampur	Unknown
BGM-1	Indeterminate	Karnataka	Local selection
CRIDA-18-R	Indeterminate	CRIDA, Hyderabad	Mutant of K-42

Isolation of horse gram TFL1 and TFY genes priming-generated amplicons

Genomic DNA from two determinate and two indeterminate genotypes was extracted from young leaves using the Cetyl Trimethyl Ammonium Bromide method (DOYLE and DOYLE,

1987). The extracted DNA quality and quantity was checked on 0.8 per cent agarose gel and normalized to 50 ng/µl for further use at the Marker Assisted Laboratory, Department of Genetics and Plant Breeding (GPB), University of Agricultural Sciences (UAS), Bangalore, India. Reported two degenerate primers one designed to conserved domain of *TFL1*-coded protein [phosphatidyl ethanolamine binding proteins (PEBP) family] (FOUCHER *et al.*, 2005) and other to *LFY*-family genes (FROHLICH and MEYEROWITZ, 1997) (Table 2) were used to isolate and characterize homologs of *TFL1* and *LFY* candidate genes. The *TFL1 and LFY* genes-priming genomic regions of genotypes, IC15735, HPKM-191, BGM-1 and CRIDA-18-R were amplified using PCR with Taq DNA polymerase. PCR mixtures contained approximately 1.5 µl of DNA (50ng per µl), 0.2µl Taq polymerase (5 units per µl), 1.5 µl 10X TE buffer, 1.5 µl DNTPs (2mM) and 0.5 µl each of forward and reverse primer in a total of 15 µl solution. The PCR cycle consisted of 5 min at 95°C (hot start), 0.30 min at 95°C (denaturation), 1 min at 55°C (*TFL1*) and 56°C (*LFY*) (annealing), 1 min at 72°C (extension), 8 min at 72°C (final extension) followed by infinite time at 4°C for holding. The denaturation, annealing and extension step were carried out for 40 cycles. The PCR products was loaded on 3 % agarose gel in 1X TBE buffer stained with ethidium bromide and bromophenol blue as loading dye. The *TFL1* and *LFY* genes priming-generated amplicons from two determinate and two indeterminate horse gram genotypes (hereafter referred to as *MuTFL1* and *MuLFY* amplicons) were separated in an electrophoresis unit at 80 V for three hours using 1X TBE buffer.

Sequencing of amplified products

The *MuTFL1* and *MuLFY* amplicons of determinate and indeterminate genotypes were sequenced (both forward and reverse sequencing approach) at Eurofins Genomics India Pvt. Ltd., Bengaluru, India using Sanger sequencing method. The sequencing was repeated several times to obtain precise and reliable data.

Bioinformatics analysis

To identify corresponding homology of nucleotide sequences of *MuTFL1* and *MuLFY* amplicons, BLASTn (protein database using a translated nucleotide query) analysis was performed by pasting the nucleotide query sequence (both forward and reverse sequence data were used) in the NCBI BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_SPEC=GeoBlast&PAGE_TYPE=BlastSearch) against the genome sequences of model species such as *Arabidopsis*, *Medicago truncatula* and *Lotus japonicus* and other related legume species such as soybean, rice bean, pigeon pea and chickpea (Table 3) available at the National Center for Biotechnology Information (NCBI) database (http://www. ncbi.nlm.nih.gov/). To detect sequencing errors if any, both forward and reverse sequence of *MuTFL1* and *MuLFY* amplicons of both determinate and indeterminate genotypes were aligned using ClustalW2 tool. The non-homologous sequences were trimmed between forward and reverse nucleotide sequences of amplicons using BioEdit software version 7.2.5. Only homologous nucleotide sequences were used for further bioinformatical/statistical analysis. To characterize and identify presence of functional domain, sequence of amplified products was subjected to conserved domain (cd) search tool

(www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and number and type of conserved domain were identified.

To identify corresponding homology of amino acid sequences of *MuTFL1* and *MuLFY* amplicons, BLASTx (protein database using a translated nucleotide query) analysis was performed by pasting the translated amino-acid query sequence (both forward and reverse sequence data were used) in the NCBI BLASTx tool against the genome sequences of model species such as *Arabidopsis, Medicago truncatula* and *Lotus japonicus* and other related legume species such as soybean, rice bean, pigeon pea and chickpea available at the National Center for Biotechnology Information (NCBI) database (http://www. ncbi.nlm.nih.gov/).

The amino acid sequence homology (~75%) of *MuTFL1* and *MuLFY* amplicons with those of *AtTFL1* and *AtLFY* genes and of other legumes, and presence of conserved domains for PEBP family in *TFL1*-encoded protein and *LFY*-family in *LFY* gene sequences was considered as a criterion to determine if the *MuTFL1* and *MuLFY* amplicons are candidates of *TFL1* and *LFY* genes (KRYLOVA *et al.*, 2020).

Phylogenetic analysis

The translated amino acid sequences of *MuTFL1* and *MuLFY* amplicons were aligned with those of model species and other related legume species to identify legume species closest to those of *MuTFL1* and *MuLFY* by Neighbour joining clustering method with a bootstrap value of 10000 using Phylogeny.fr (http://www. http:// https://ngphylogeny.fr//) tool.

RESULTS AND DISCUSSION

The PCR amplification of *MuTFL1* and *MuLFY* amplicons with expected product size (800bp and 700bp, respectively) was carried out from all the four genotypes (Fig 1; Table 2). BLASTx analysis showed 88.51% and 81.71 % similarity of translated amino acid sequences of *MuTFL1* amplicons with those of soybean (*GmTFL1*) and pigeon pea (*CcTFL1*) homologs of *AtTFL1* respectively (Table 3). Further, conserved amino acid sequence of PEBP family were identified in *MuTFL1* amplicons (Fig 2a). Phylogenetic analysis resulted in grouping of *MuTFL1* and *GmTFL1* into a single clade (Fig 3a). Several researchers have also isolated and reported high amino acid sequence similarity of homologs of *AtTFL1* gene. To quote a few, pigeon pea *CcTFL1* amino acid sequence was reported to be 94% similar to those of *GmTFL1* and *AtTFL1* (Mir et al., 2014) and *Brassica napus* (*BnA10.TFL1*) amino acid sequence was reported to be 85% similar to that of *AtTFL1* (JIA *et al.*, 2019). Our results and those reported by researchers in other grain legumes suggest that *MuTFL1* amplicons could be the putative horse gram homologs of *AtTFL1* gene. Hence, hereafter *MuTFL1* will be referred to as *MuTFL1*. It is not surprising that *MuTFL1* is a homolog of *AtTFL1* and *TFL1* of other legumes as most of dicots have at least one copy of *TFL1* gene in their genomes (KRYLOVA *et al.*, 2020). Evidences indicate that *TFL1* gene has evolved as a result of duplication of one ancestral copy and encode protein containing 177 amino acids. Studies have indicated that even one amino acid (His88Tyr) substitution in protein encoded by *TFL1* can lead to significant change in the protein function (KRYLOVA *et al.*, 2020).

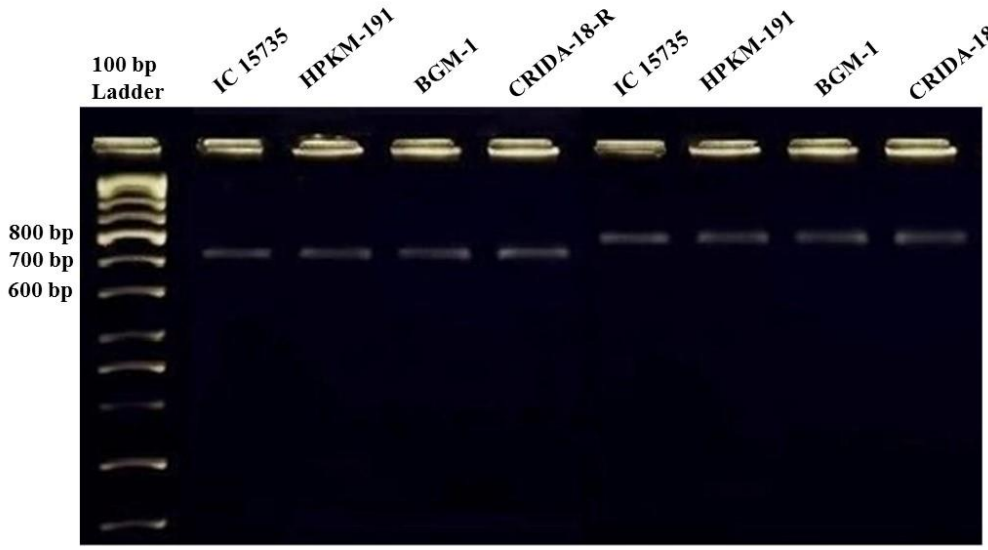

Figure 1. Agarose gel showing PCR amplification of horse gram homologs of *MuLFY* and *MuTFL1*

Table 2. Polymorphisms in horse gram homologs of AtTFL1 and AtLFY genes controlling growth habit

Sl. No.	Primers	Amplicon size	Amplification obtained in				Polymorphism detected in sequence data
			IC 15735	HPKM-191	BGM-1	CRIDA-18-R	
01	TFL3/TFL5 TFL3: GATGTTCCWGGWCCTAGTGAYCC TFL5: CTTGCAGCRGTYYTCYCTYTG	800 bp	+	+	+	+	Single nucleotide polymorphism
02	LFY1a/2a LFY1a: CACCCACGACCITTYATIGTIACIGARCCIGGIG LFY2a: CCTGCCIACRTARTGICKCATYTTIGGYTT	700 bp	+	+	+	+	Single nucleotide polymorphism

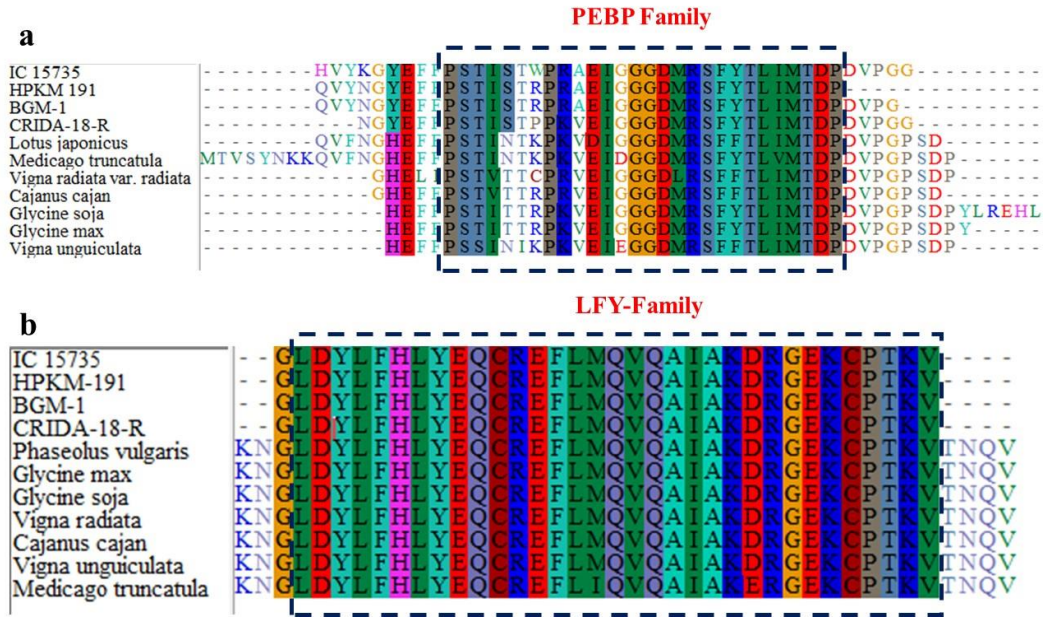

Figure 2. Multiple alignment of the predicted coding domain amino acid sequences of horse gram *MuTFL1* and *MuLFY* homologs corresponding to homologs of soybean, pigeonpea, common bean and *Medicago*. Highly conserved amino acids are in dark color depending on the level of identity (darker = higher level)

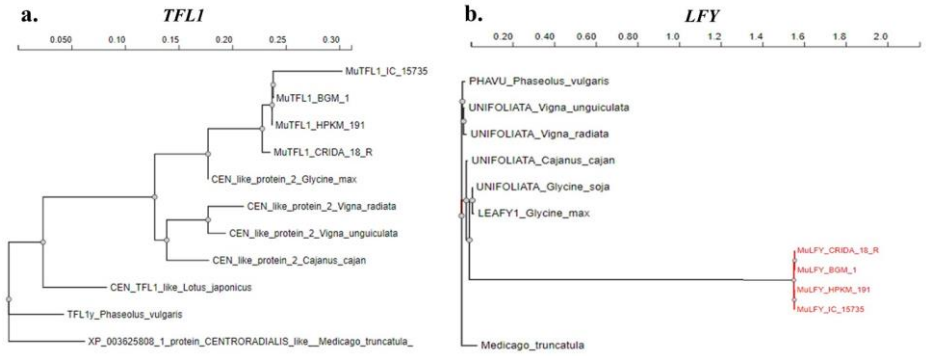

Figure 3. Phylogenetic tree depicting clustering of horse gram *MuTFL1* and *MuLFY* homologs with those of different model crops and legume crops.

BLASTx analysis also showed high similarity (85.37%) of translated amino acid sequences of *MuLFY* amplicons with those of soybean (*GmLFY*), *UNIFOLIATA* of green gram, chickpea, pigeon pea, and cowpea, and *Medicago sativa* (80.61%) (Table 3). Phylogenetic analysis showed that *MuLFY* formed a separate clade with soybean, whereas other related legumes formed different clade (Fig 3b). A fairly high amino acid sequence similarity of *MuLFY* amplicons with those of other legumes indicates that *MuLFY* could be a homolog of *AtTFL1* and other legumes. Hence, hereafter *MuLFY* amplicons will be referred as *MuLFY*. Homologs of *AtLFY* have also been reported in other legumes. To name a few, *M. truncatula* and soybean are reported to harbor one and two *AtLFY* homologs, respectively (KIM *et al.*, 2013). Based on our results and those reported by other researchers, we argue that *MuLFY* could have the same internal structure and regulating mechanism as that of soybean and cowpea. Presence of conserved amino acid sequence of *LFY*-family protein in the *MuLFY* amplicons provide further evidence that *MuLFY* is the homolog of *AtLFY* and of other legumes (Fig 2b).

Table 3. Amino acid sequence similarity of horse gram homologs (MuTFL1 and MuLFY) with those of related legumes, Lotus japonicus and Medicago

Horse gram homologs	Nucleotide Sequence similar to query sequence	GenBank accession number	Crop	E value	Maximum identity
MuTFL1	SELF-PRUNING	NP_001240029.1	*Glycine max*	9e-108	88.51
	CEN-like protein 2	XP_020201996.1	*Cajanus cajan*	2e-100	81.71
	TFL1y/*TFLa*	XP_014512639.1	*Vigna radiate*	3e-95	78.16
	CEN-like protein 2	XP_027938387.1	*Vigna unguiculata*	5e-98	73.56
	CEN-like protein	AAQ93599.1	*Lotus japonicas*	1e-89	73.10
	TFL1y	ABR53775.2	*Phaseolus vulgaris*	3e-51	72.41
	CEN like protein	XP_003625808.1	*Medicago sp.*	2e-88	71.84
MuLFY	*LEAFY1*	ABP94176.1	*Glycine max*	3e-13	85.37
	UNIFOLIATA	KHN09705.1	*Glycine soja*	7e-13	85.37
	UNIFOLIATA	KYP59722.1	*Cajanus cajan*	8e-13	85.37
	UNIFOLIATA	XP_027905350.1	*Vigna unguiculata*	8e-13	85.37
		XP_022633651.1	*Vigna radiate*	8e-13	85.37
	_	XP_007137848.1	*Phaseolus vulgaris*	7e-13	85.37
		AY770393.1	*Medicago sativa*	9e-15	80.61

Nucleotide and amino acid differences between determinate and indeterminate genotypes

An examination of nucleotide sequences of *MuTFL1* amplicons revealed differentiation of determinate and indeterminate genotypes at four nucleotide base pairs. Bioinformatic analysis of translated amino acid sequences of *MuTFL1* amplicons suggested that none of these contributed to amino acid substitution. Thus, the four single nucleotide polymorphic (SNP) sites were synonymous. On the contrary, one of the three detected SNP sites in *MuLFY* amplicons was found associated with amino acid substitution of glutamine (in indeterminate genotype) to histidine (in determinate genotype) (Table 4). We believe that this amino acid substitution in *LFY*-coded protein might be the cause for the switch over of indeterminacy to determinacy in horse gram. Our unpublished data indicate that determinate growth habit is a result of non-synonymous (sn) SNP in dolichos bean (*Lablab purpureus*) homolog (*LpTFL1*) of *AtTFL1*. In a latest study, KALDATE *et al.* (2021) have reported that the splice site SNP present at the end of the third exon of *LpTFL1* locus is responsible for transformation of SAM into inflorescence in determinate genotype in dolichos bean. The splice site SNP leads to mutant *LpTFL1* locus that code for a non-functional protein due to the absence of 14 amino-acids in determinate genotypes (KALDATE *et al.*, 2021).

Table 4. Nucleotide and amino acid sequence differences in MuTFL1 and MuLFY homologs of AtTFL1 and AtLFY genes in horse gram determinate and indeterminate genotypes

Sl. No.	Homolog	Nucleotide in determinate genotypes	Nucleotide in indeterminate genotypes	Amino acid substitution from determinacy to indeterminacy
01	*MuTFL1*	A	T	Synonymous
02		T	G	
03		T	A	
04		G	T	
01	*MuLFY*	T	A	Synonymous
02		T	A	Glutamine to histidine
03		T	A	Synonymous

High degree of amino acid sequence homology and presence of conserved domains of PEBP family and *LFY* family provide adequate evidence that homologs of *AtTFL1* and *AtLFY* are present in horse gram as well. However, considering presence of synonymous SNPs in *MuTFL1* while presence of sn-SNPs in *MuLFY*, we opine that not *MuTFL1* but *MuLFY* could be the master regulator of growth habit in horse gram unlike in other non-model legumes where *TFL1* reported as is the key gene controlling the growth habit. We argue that difference in plant architecture in determinate genotypes in horse gram compared to their counterpart genotypes in other legumes could be cause for key role of *LFY* gene controlling growth habit in horse gram. Unlike in other legumes, where SAM terminates in inflorescence, it only stops growth in horse gram (CHANDANA *et al.*, 2021).

Breeding implications

Understanding the molecular genetic basis of growth habit, one of the domesticated syndrome traits provide useful clues to breed horse gram cultivars with a range of flowering time in desired growth habit. The availability of many such cultivars with desired growth habit offer greater cultivar choice to farmers/growers that helps broaden the area planted to horse gram depending on the desired economic product needs and cropping ecosystem, as the demand for the grain legumes as sources of food (protein) and fodder is increasing worldwide (KRYLOVA *et al.*, 2020). Growth habit is also directly connected to flowering time, duration, pod-maturity and suitability to different cropping ecosystems and mechanized harvesting. Considering the putative role of *MuLFY* in controlling growth habit in horse gram, it is possible to design gene-specific markers for use as powerful surrogates for identification and selection of genotypes with desired growth habit in germplasm and breeding populations in seedling stage itself. Selection of plants at seedling stage assumes importance given that plants in germplasm/breeding populations could be differentiated with respect to their growth habit only at reproductive phase of plant development which generally require from 30 to 60 days depending on their genetic backgrounds and climatic conditions. Identification and selection of genotypes with desired growth habit in seedling stage is also highly relevant and desirable considering that it is most often difficult to discriminate determinate and indeterminate genotypes on phenotype basis under short days and extreme climatic conditions. The use of gene-specific markers helps shorten the breeding cycles and efficiency (MIR *et al.*, 2014). Further, with the use of CRISPR-Cas9 technology, it is possible to create and identify new alleles in *MuLFY* controlling a range of flowering time in desired growth habit that are not naturally available (BENLLOCH *et al.*, 2015).

CONCLUSION

The strategy to identify homologs of known genes using degenerate primers designed from validated sequence information from databases of model species and extensively researched related species was effective in horse gram. Further, it could be hypothesized that basic growth habit controlling genes are likely to be relatively well conserved between horse gram and other legumes such as soybean, green gram and pigeon pea. Higher amino acid sequence homology and clustering of horse gram homologs, *MuTFL1* and *MuLFY* with those of soybean, green gram and pigeon pea provided preliminary evidence for their hypothetical role in the control of growth habit in horse gram. However, confirmatory analysis such as gene expression and transformation studies are required to validate their candidacy of growth habit. The validated *MuLFY* gene opens new avenues for further studies on molecular genetic control of growth habit in horse gram.

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IZOLACIJA I BIOINFORMATIČKA KARAKTERIZACIJA HOMOLOGA (*MuTFL1* i *MuLFI*) GENA KOJI KONTROLISU RAST VRSTE *Macrotiloma uniflorum* (Lam.) Verdc.

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Izvod

Tip rasta (nedetrminisan/determinisan) je jedna od evolucijski oblikovanih, ekonomski važnih biljnih arhitektonskih osobina zrnastih mahunarki, uključujući *Macrotiloma uniflorum* (Lam.). Poznato je da geni Arabidopsisa *AtTFL1* i *AtLFI* i njihovi homolozi u drugim vrstama mahunarki kontrolišu način rasta. Uzimajući znake iz visoko konzerviranih domena u proteinima kodiranih *TFL1* i *LFI* genima, izolovali smo homologe *Macrotiloma uniflorum* (Lam.) (*MuTFL1* i *MuLFI*) koristeći prajmere dizajnirane za očuvane domene. *MuTFL1* i *MuLFI* homolozi su izolovani i sekvencionirani korišćenjem Sangerovog protokola sekvenciranja. Nukleotidne sekvence *MuTFL1* i *MuLFI* homologa su prevedene u njihove odgovarajuće sekvence aminokiselina korišćenjem „EkPASi“ alata. BLAST analiza prevedenih aminokiselinskih sekvenci *MuTFL1* i *MuLFI* homologa pokazala je veliku sličnost sa onima *TFL1* i *LFI* homologa soje i graška. Višestruko poravnanje nukleotidnih sekvenci *MuTFL1* i *MuLFI* sa srodnim mahunarkama i model vrsta (*Medicago sp*. i *Lotus japonicas*) korišćenjem „ClustalV“ otkrilo je prisustvo četiri SNP mesta u *MuTFL1* i tri nesinonimna SNP u *MuLFI*. Ovi rezultati sugerišu da *MuLFI* može biti glavni regulator načina rasta kod ove vrste za razliku od drugih mahunarki gde je *TFL1* prijavljen kao ključni gen koji kontroliše vrsturasta. Zamena glutamina u (determinisani genotipovi) sa histidinom (nedeterminisani genotipovi) u *LFI* kodiranom proteinu je izgleda bila uzrok prelaska sa nedetrminisanog na determinisani tip rasta. Ovi rezultati se razmatraju u vezi sa strategijama oplemenjivanja sorti sa željenim tipom rasta.

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