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Genetic Variability Analysis for Yield Attributes and Phenotypic Characterization of *TFL1* Homologues for Growth Habit in Pigeonpea [*Cajanus cajan* (L.) Millspaugh]

Isha, K. Mendapara, S.R. Patel, Dinisha Abhishek, D.D. Patel , H.N. Patel, H.H. Mistry **10.18805/LR-4822**

ABSTRACT

Background: Pigeonpea [*Cajanus cajan* (L.) Millspaugh] a versatile food legume, grows mainly in arid and semi-arid regions of the globe. The crop is characterized with distinctive growth habit. An important agronomic trait regulated by mutation in Terminal flower 1 *(TFL1)* locus and decides the onset of reproductive phase in crop plant. The current experiment designed to identify and characterize the pigeonpea genotypes with variation in *TFL1* locus along with other traits, affecting complexity of yield through precise phenotyping, to aid in simplifying the developmental mechanism and puzzle of stagnant productivity in pigeonpea.

Methods: For profiling of *TFL* homologue, phenotyping was done in *kharif,* 2019 at College Farm, N.M. College of Agriculture, Navsari Agricultural University, Gujarat. Sixty four diverse pigeonpea germplasms were screened for growth habit and yield attributing traits. The individuals where shoot apex terminated into reproductive phase was considered as determinate type (DT) and those where main stem continued vegetative growth throughout plant's life was recorded as indeterminate type (IDT).

Result: The experimental findings clearly identify the variants, arises due to mutation in *TFL1* locus. Sufficient variability and flexibility has been observed in pigeonpea to breed short duration, photo-insensitive and determinate plant types with superior seed yield to improve the productivity and area of crop. Genotypes like ICPL 87, GT 100 and BP-16-61 could be incorporated in climate-smart breeding programme to attain substantial and sustainable productivity in pigeonpea.

Key words: Determinate type, Growth habit, Indeterminate type, Pigeonpea, *TFL1* locus, Yield.

INTRODUCTION

India is a major producer and consumer of pigeon pea in the world and presently occupies an area of about 56.02 lakh ha with an annual total production of 40.02 lakh tones and mean productivity of 913.0 kg/ha (Anonymous, 2017). The lifeline to resource-limited farmers, the multi-purpose food legume crop, is majorly limited to lower latitudes, narrowing its efficacy and expansion (Vals *et al.,* 2012). The wider adaptation of crop to different geographical regions depends primarily on the optimal timing of onset of reproduction phase in response to seasonal signals (growth habit), coupled with photo-insensitivity (Blackman, 2017)). Among the two types of growth habit in pigeonpea, the determinate type (DT) genotypes are short in stature and stop vegetative growth after the initiation of flowering, whereas, in indeterminate type (IDT) the genotypes are relatively tall and continue to grow and spread even after flowering (Mir *et al.,* 2012). The IDT growth habit, commonly preferred by pigeonpea growers due to multiple branching, higher yields, feasibility to control insect, lower seed rate and low input among others, is dominant in nature (Gupta and Kapoor, 1991). However, the extended duration and photosensitive nature of IDT cultivars, makes it non-suitable legume crop, in the existing system of cropping pattern (Kumar *et al*, 2016). Consequently, early maturity, initial vigour, ease of mechanical harvesting, tolerance to terminal draught, water logging and ability to fit well in wheat-pulse cropping system illustrated the attention of farmers and breeders towards DT type of growth habit which now has been emerged as a new favourite type in pigeon pea breeding (Shruthi *et al*., 2017).

As most of the cultivars and wild relatives of pigeon pea have indeterminate growth habit, it is supposed that determinate pattern was isolated by the farmers or breeders during the course of domestication. Several studies have been undertaken to dissect the genetic mechanism underlying the growth pattern in different crops, including pigeon pea (Kwak *et al*., 2012, Repinski *et al.,* 2012, Mir *et al*., 2012 and Mir *et al*., 2014). Involvement of a single gene in determinacy control is revealed in some experiments, while other trials showed the control of more than one gene (Tian *et al.,* 2010).Studies have shown that transition from branch to reproductive phase is regulated by members of the

College of Agriculture, Navsari Agricultural University, Campus Bharuch-392 012, Gujarat, India.

Corresponding Author: Dinisha Abhishek, College of Agriculture, Navsari Agricultural University, Campus Bharuch-392 012, Gujarat, India. Email: dinishagpb@nau.in

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phosphatidylethanolamine-binding protein (PEBP) family, which involves *FT* (Flowering Locus T) for determinacy while, *TFL1* (Terminal flower 1) promotes indeterminacy (Prusinkiewicz *et al*, 2007; Lifschitz *et al*., 2014 and Périlleux *et al.,* 2019). A substitution of a single amino acid can transform *FT* into *TFL1* and vice versa and it is supposed that such observable mutations in individual lines have been selected during the process of crop domestication (Ho and Weigel, 2014).

Information of genotypes with extreme growth habits and their agronomic characteristics can assist and stimulate the DT/IDT conversion of pigeonpea genotypes through marker assisted backcrossing and genetic manipulation using transgenesis/genome editing, besides being the quick source of experimental material for further decoding the developmental phenomenon controlled by *TFL* locus in pigeon pea improvement programme.

MATERIALS AND METHODS

The present investigation was conducted during *kharif,* 2019 at College Farm, N.M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat which is geographically situated at 20°37′N latitude and 72°54′E longitude as well as at an altitude of 11.98 meters above mean sea level. The location falls under the agro climatic zone-14 with an average rainfall of 1550 mm. This study consisted of 64 different early varieties of pigeonpea. These genotypes were analyzed using randomized block design with three replications and were grown keeping space of 90 cm and 45 cm between rows and plants, respectively. At all stages of the crop growth, the weather condition was favorable for growth and development of the crop. All the recommended cultural practices were followed to raise healthy crop. Observations on days to 50 % flowering and days to maturity were recorded on population basis. All other observations *i.e.* plant height (cm), primary branches per plant, pods per plant, pod length (cm), pod weight (g), seeds per pod, 100 seed weight (g) and seed yield per plant (g) were recorded from five randomly selected plants exclusive of border ones.

For characterization of *TFL* homologue, pigeonpea germplasms were screened for growth habit. The individuals where shoot apex terminated into reproductive phase was considered as determinate type (DT) and those where main stem continued vegetative growth throughout plant's life was recorded as indeterminate type (IDT).

Statistical analysis

The data collected were analyzed using INDOSTAT Statistical software. All the 10 studied characters were subjected to analysis of variance as suggested by Singh and Chaudhary (1977). Phenotypic and genotypic co-efficient of variation (PCV and GCV, respectively) were calculated according to Burton and Devane (1953). Heritability in broad sense was estimated according to Allard (1960) whereas, Genetic advance as per cent of mean for each character was calculated by the formula given by Johnson *et al.* (1955). Path analysis was estimated using Deway and Lu (1959) methods.

RESULTS AND DISCUSSION

Selection has brought remarkable changes in morphology and physiology of crop plants mostly in terms of flowering time and growth habit. These are the key factors in adaptation of any crop to various geographical regions (Pin and Nilson, 2012). Considering this, identification and characterization of genes affecting these traits can help to understand physiology of flowering, growth habit and evolution progression of these traits. To unlock the phenomenon of developmental phase in plants, several studies have been conducted which proposed the involvement of terminal flower 1 locus in regulation of growth habit in pulses and legumes (Campos *et al.,* 2011 Benlloch *et al*., 2015 and Saxena *et al.,* 2017). For the further utilization of such breakthrough in breeding programme, the precise phenotypic characterization of *TFL1* homologue is mandatory. In the present experiment it has been identified that, the extreme phenotypes for growth habit in pigeonpea was regulated by *TFL1* homologue. The phenotypic characterization of 64 genotypes revealed that lines ICPL 87, GT 100 and BP-16-61 could be utilized for the development of short duration, photo-insensitive varieties with improved yield and synchronous maturity (Fig 1a and b). Assessment of growth habit in pigeonpea showed that genotypes GT-100and ICPL 87 produce terminal flower bud at shoot apex describing determinate type whereas, Vaishali did not show terminal flower bud but continued to grow vegetatively at apical bud, while flowering occurred only in lateral buds (Fig 2). Variability for growth habit has also been observed by other researchers in pea (Foucher *et al*., 2003) faba bean (Avila *et al.,* 2007) pigeonpea [Mir *et al.* (2014), Saxena *et al.* (2017)] and Common bean (Campos *et al.,* 2011).

Photo-insensitivity in addition to determinate growth habit makes the cultivation of pigeonpea possible throughout the year. Both indeterminate and determinate type of flowering pattern exists in this crop (Mir *et al*., 2012). Wild relatives and most of the cultivars of pigeonpea have indeterminate growth habit and therefore, it is believed that determinate forms of crop were selected by the farmers or breeders during its domestication or breeding process. Flowering pattern or determinacy has been selected long ago by breeders in combination with photoperiod insensitivity to obtain varieties with shorter flowering period, earlier maturation and ease of mechanized harvest (Repinski *et al*., 2012). Determinacy reduces aboveground plant biomass and accelerates synchronizes flowering (Kwak *et al*., 2012). Determinate growth habit has advantage over indeterminacy because of having higher productivity, as photosynthates are transferred to reproductive growth instead of vegetative growth as in indeterminate types. It also confirms early flowering and maturity. It turns out to be more effective when determinacy combines with photo-insensitivity which helps in adaptation to various geographical locations as it can flower throughout the year and because of this ability, breeders does not have to wait for particular season and breeding programme can be run across the year. Thus, *TFL* locus is turned out to be very important for the selective evolution and precisely by utilizing comparative genomics, identify the slight mutation which is useful and through genome editing, mutate or edit the genome and make it favourable for cultivation and consumption.

The experimental materials were also evaluated for growth parameters in order to identify ideal genotypes in terms of growth habit and yield parameters for widespread adoption of pigeon pea. The intra trait variation was found significant for all the traits in 64 different genotypes (Table 1). The mean and range values for all the 10 agronomically important traits showed sufficient variability to be further utilized for their genetic enhancement (Table 2). The GCV and PCV was found high for days to 50% flowering, plant height, pods per plant and seed yield per plant, showcasing existence of vast variation, which can be utilized for the further genetic enhancement of these traits through selection. However, such practises would be ineffective for traits like seeds per pod with lowest extent of both the parameters (Table 3). The values of PCV were greater than GCV, for all the traits but the difference was minor for comprehensive traits. Similar outcomes were also noted by Saroj *et al.,* (2013) and Baldaniya *et. al.,* (2018).

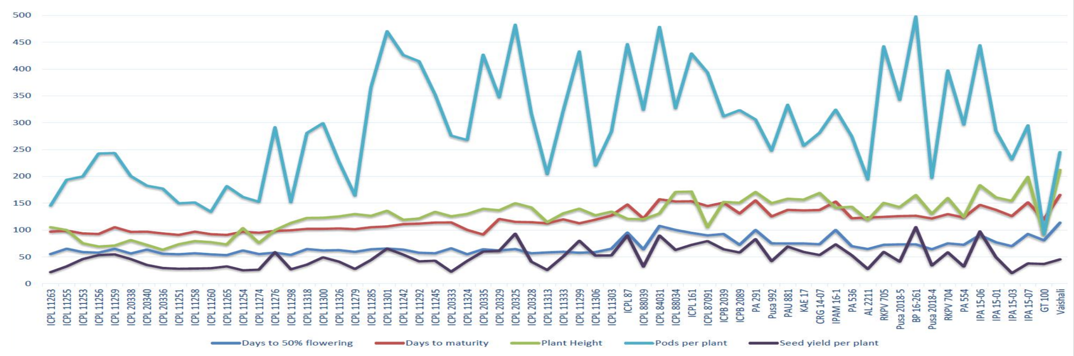

Fig 1(a): Comparative mean performance of 64 pigeonpea genotypes for different traits under study.

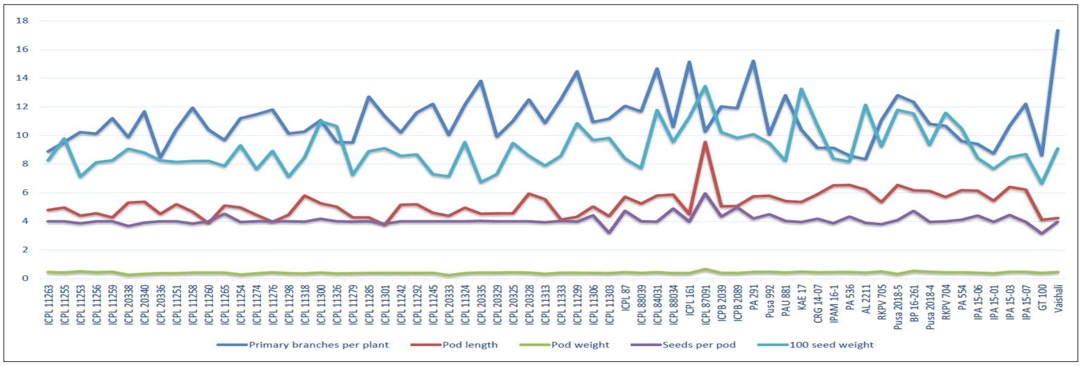

Fig 1(b): Comparative mean performance of 64 pigeonpea genotypes for different traits under study.

Fig 2: Indeterminate and Determinate growth habit in pigeon pea.

Coefficient of variation only describes variation present in the genotypes, it does not partition variation into heritable and non-heritable variation whereas, heritability shows heritable variation. High magnitude of heritable variation was observed for all the traits except, primary branches per plant, unfolding less environmental influence (Table 3). Equivalent results were attained by Reddy *et al.* (2013), Saroj *et al.* (2013), Singh *et al.* (2013), Kesha *et al.* (2016), Meena *et al.* (2017) and Kumar *et al.* (2018). Selection for the improvement of such traits may or may not be useful as it includes both fixable and non-fixable variance.

Genetic advance complements heritability for comprehending improvement in mean genotypic value of selected plants over parental population. In this experiment, high extent of genetic advance was observed for comprehensive traits except seeds per pod which describe the ruling effect of additive genes for all these traits. Thus selection for these traits would be rewarding. Whereas, for seeds per pod showing less magnitude of genetic advance because of the involvement of non-additive genes, heterosis breeding strategy can be effective to improve the trait. These findings are in agreement with Reddy *et al.* (2013), Saroj *et al.* (2013), Kumar *et al.* (2018), Rajwade *et al.* (2018) and Satyanarayana *et al.* (2018). High heritability coupled with high genetic advance was perceived for days to 50% flowering, days to maturity, plant height, pods per plant, pod length, pod weight, 100 seed weight and seed yield per plant showing role of additive gene effects and less influence of environment. Seeds per pod showed high heritability coupled with moderate genetic advance, indicates role of non-additive gene action and in this case, high heritability observed might be due to environmental influence. Moderate

Table 1: Analysis of variance for yield attributing traits in pigeon pea.

Character		Df	Days to 50 % flowering	Days to maturity	Plant height	Primary branches per plant	Pods per plant	Pod length	Pod weight	Seeds per pod	100 seed weight	Seed yield per plant
MSS	Replication	2	84.74**	69.91**	932.12**	30.36**	5990.20**	0.26**	0.00	0.02	0.03	45.26
	Genotypes	63	663.71**	1250.18**	3338.06**	9.42**	31515.30**	2.56**	0.01**	0.44**	7.05**	1261.67**
	Error	126	8.06	6.9	172.77	2.14	167.69	0.01	0	0.01	0.05	15.52

** indicate significance at 1 % level of probability.

Table 2: Range, mean and components of variance for yield attributing traits in pigeon pea.

Characters	Range	Mean	Component of variance		
			Genotypic	Phenotypic	Environmental
Days to 50% flowering	53.33-113.67	69.71	218.55	226.61	8.06
Days to maturity	91-165.33	118.65	414.43	421.33	6.91
Plant height (cm)	63.24-212	127.35	1055.10	1227.86	172.77
Primary branches per plant	8.33-17.33	11.11	2.42	4.57	2.14
Pods per plant	91.93-497.87	290.02	10449.20	10616.89	167.69
Pod length (cm)	3.75-9.53	5.20	0.85	0.86	0.01
Pod weight (g)	0.24-0.66	0.41	0.004	0.004	0.001
Seeds per pod	3.14-5.95	4.09	0.14	0.15	0.01
100 seed weight (g)	6.64-13.44	9.12	2.33	2.38	0.05
Seed yield per plant (g)	19.78-105.47	49.78	415.38	430.90	15.52

Table 3: GCV, PCV, h2 (bs), GA and GAM for yield attributing traits in pigeon pea.

Characters	GCV (%)	PCV (%)	h2 (bs) (%)	GA	GAM (%)
Days to 50% flowering	21.21	21.50	96.40	29.91	42.90
Days to maturity	17.16	17.30	98.40	41.59	35.05
Plant height (cm)	25.51	27.52	85.90	62.03	48.71
Primary branches per plant	14.02	19.24	53.10	2.34	21.04
Pods per plant	35.25	35.53	98.40	208.91	72.03
Pod length (cm)	17.72	17.82	98.80	1.89	36.27
Pod weight (g)	14.92	16.04	86.50	0.12	28.60
Seeds per pod	9.24	9.59	92.90	0.75	18.34
100 seed weight (g)	16.74	16.92	97.90	3.11	34.12
Seed yield per plant (g)	40.95	41.70	96.40	41.22	82.82

GCV-Genotypic coefficient of variation; PCV-Phenotypic coefficient of variation, h2 (bs)-Heratibility (Broad sense), GA-Genetic advance, GAM-.

Table 4: Genotypic path coefficient analysis indicating direct and indirect effects of casual variables on seed yield per plant.

Characters	DTFF	DM	PH	PB	PPP	PL	PW	SPP	SW	Genotypic correlation with SY/P
DTFF	0.26	0.19	-0.16	-0.04	0.28	-0.11	0.06	0.03	0.04	0.55**
DM	0.24	0.21	-0.20	-0.04	0.33	-0.13	0.06	0.03	0.05	0.55**
PH	0.17	0.16	-0.25	-0.05	0.40	-0.07	0.04	0.02	0.04	0.46**
PB	0.11	0.08	-0.12	-0.09	0.34	0.05	0.01	0.002	0.02	0.41**
PPP	0.10	0.10	-0.14	-0.04	0.72	-0.06	0.05	0.03	0.04	0.79**
PL	0.10	0.10	-0.07	0.02	0.15	-0.28	0.08	0.06	0.06	0.22^{NS}
PW	0.09	0.07	-0.06	-0.006	0.21	-0.13	0.18	0.05	0.04	0.44**
SPP	0.07	0.06	-0.04	0.002	0.20	-0.17	0.08	0.10	0.04	0.34**
SW	0.09	0.09	-0.08	-0.02	0.24	-0.14	0.06	0.03	0.11	0.39**

** -Significant at 1.0 % level of probability, Residual = 0.4775.

DTFF-Days to 50% flowering, DM-Days to maturity, PH- Plant height (cm), PB-Primary branches per plant, PPP-Pods per plant, PL-Pod length (cm), PW-Pod weight (g), SPP-Seeds per pod, SW-100 seed weight (g), SY/P-Seed yield per plant (g).

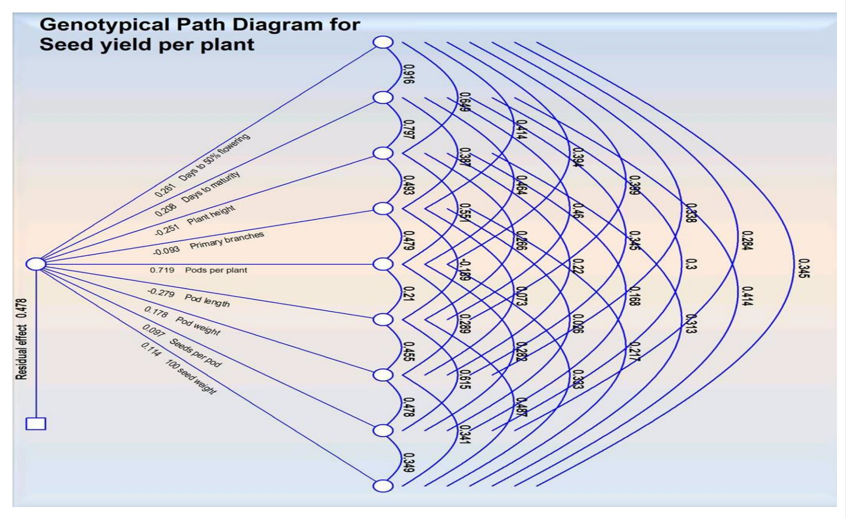

Fig 3: Genotypic path diagram for seed yield per plant.

heritability coupled with high genetic advance was attained for primary branches per plant indicating effect of additive genes because of high genetic advance (Table 3). The data were also subjected to path analysis to identify the component traits influencing seed yield either directly or indirectly through other traits, high positive direct effects on seed yield per plant were observed by pods per plant followed by days to 50 % flowering, days to maturity, pod weight, 100 seed weight and seeds per pod (Table 4; Fig 3). Such positive direct effects were also perceived by Kesha *et al. (*2016), Baldaniya *et al. (*2018), Satyanarayana *et al. (*2018) and Kandarkar *et al.* (2020). Negative direct effects on seed yield were unveiled by plant height, primary branches per plant and pod length. Comparable outcomes were revealed by Pandey *et al*. (2016) and Kesha *et al. (*2016). Pods per plant showed highly significant correlation with seed yield per plant. The reason behind this can be due to high direct effects and high indirect effects via other casual traits. Plant height, primary branches per plant and pod length showed negative direct effects might be due to negative indirect effects through other component traits. Present investigation showed 0.48 residual effects, meaning more traits are there which are not under study might show accountable variation. The traits under study only accounted 52.2% of the total variation thus, there is a scope in future study to include more traits.

Analysis of agronomic data revealed that for improving seed yield per plant in pigeonpea, ideal traits are days to 50% flowering, days to maturity, plant height, primary branches pre plant, pod weight, seed per pod and 100 seed weight.

CONCLUSION

It can be concluded that precise profiling of germplasm for extreme growth habits and agronomic features can assist and stimulate the DT/IDT conversion of pigeonpea genotypes. The identified lines could be incorporated in climate-smart pigeonpea breeding programme, to develop short duration, photo-insensitive varieties with improved yield and ability to thrive in inter cropping system to attain substantial and sustainable productivity.

Conflict of interest: None.

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