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ORIGINAL RESEARCH ARTICLE

Plant Genetic Resources

Identification and characterization of novel penta-podded genotypes in the cultivated lentil

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Abstract

Our study reports the identification of two novel multi-flowering (MF) genotypes, PMF-1 and PMF-2, in cultivated lentil (*Lens culinaris* Medik.), stably forming up to five flowers per peduncle (FPP) on I2 nodes at multiple flowering nodes (beyond the ninth node). These genotypes were identified from the ICARDA (International Centre for Agricultural Research in the Dry Areas) nursery received from Lebanon. Stable MF was observed under open field conditions at IARI (the Indian Agricultural Research Institute), New Delhi, India, during the year 2017–2018 and 2018–2019, and also in the partially controlled glasshouse conditions. In addition, we identified two more stable genotypes, namely, PMF-3 and PMF-4, producing four flowers and a genotype, ILL7663, producing only one to two flowers at multiple flowering nodes under normal growing conditions. It is speculated that the expression of the MF trait is the result of interaction between specific genetic loci with the environmental conditions during the bud formation stage in the identified lines. The possibility of the presence of various genes known in other crops like pea (*Pisum sativum* L.) and chickpea (*Cicer arietinum* L.), along with various environmental factors regulating the MF expression, is also worked out for lentil. Further, these novel resources can be used for genetic studies aimed to identify the locus regulating the MF trait in lentil.

1 | INTRODUCTION

Lentil (*Lens culinaris* Medik. ssp. *culinaris*) is a diploid ($2n = 2x = 14$), cool-season legume, with a genome size of 4,063 Mbp (Arumuganathan & Earle, 1991). Lentil is one of the earliest domesticated plant species consumed since prehistoric times, having its origin from the Near East (Ladizinsky, 1979; Yadav, McNeil, & Stevenson, 2007). It is

Abbreviations: FPP, flowers per peduncle; IARI, Indian Agricultural Research Institute; ICAR, Indian Council of Agricultural Research; ICARDA, International Centre for Agricultural Research in the Dry Areas; MF, multi-flowering; PH, plant height; PL, peduncle length; PPP, pods per peduncle

primarily cultivated in South Asia, North America, West Asia, and North Africa. Lentil grains are known to be rich in proteins, minerals, carbohydrates, and fibers, making it equally useful for both human and animal health (Kumar, Rajendran, Kumar, Hamwieh, & Baum, 2015; Kumar et al., 2019). Lentil is one of the most recommended pulses for people suffering from diabetes, cardiovascular diseases, and obesity (Srivastava & Vasishtha, 2012).

In 2017, the world production of lentil was 7.59 Mg from 6.58 million ha of area, the major producers being Canada (3.73 Mg), India (1.22 Mg), and Turkey (0.43 Mg), accounting for nearly 71% of the total production (FAOSTAT, 2019). In terms of production area, Canada ranks first (2.47 million ha), followed by India (1.66 million ha) and the United States (0.41 million ha). The maximum yield was recorded for New Zealand (2,607 kg ha^{-1}), followed by China (2,314 kg ha^{-1}). In India, lentil is mainly grown in central and eastern regions on the residual moisture as a rainfed crop. Thus, the average yield for India is low (736 kg ha^{-1}), even lower than the world average (1,153 kg ha^{-1}) (FAOSTAT, 2019; Saxena, 2009).

For centuries, inflorescence morphology of various legumes such as garden pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), and lentils have attracted the attention of various researchers (Devi et al., 2018; Gaur & Gour, 2002; Sandhu & Singh, 2007). Furthermore, the genetic regulation of flowering patterns in any crop is of immense practical importance for the breeders aiming to develop high-yielding cultivars (Sinjushin & Liberzon, 2016). The flowering traits in legumes such as the number of flowers per peduncle (FPP), number of flowering nodes per plant, and the number of flowers per plant seems amendable, as these traits directly influence the total number of pods and the yield per plant (Milbourne & Hardwick, 1968).

The number of FPP is a genetically determined trait in several legume crops (Sinjushin & Belyakova, 2015). To date, there is no published report on cultivated lentil genotype stably forming five FPP, but a few wild genotypes—*L. tomentosus* Ladizinsky, *L. ervoides* (Brign) Granade, and *L. orientalis* (Boiss) Ponert (Sharma, 2009)—are known to express MF (multi-flowering) node to the tune of five FPP. Technically, a plant is considered MF when there is expression of three or more flowers at one or multiple flowering nodes (Benlloch et al., 2015; Devi et al., 2018; Gaur & Gour, 2002). Multi-flowering racemes have been reported for a few and not for all the flowering nodes in pulses like *P. sativum* (up to five FPP) (Devi et al., 2018) and chickpea (up to nine FPP) (Gaur & Gour, 2002), where varied mechanisms for flower number regulation operate (Gaur & Gour, 2002; Sinjushin & Liberzon, 2016; Talukdar, 2013).

From the opening of the lowermost bud to the last bud on any single branch, it generally takes two weeks in lentil (Yadav, Phogat, Solanki, & Malik, 2002), whereas pod-setting occurs 3–4 d after the opening of the flowers. In lentils, two to four flowers are generally borne acropetally in the axillary racemes, on short peduncles of 2.5- to 5.0-cm length (Muehlbauer, Cubero, & Summerfield, 1985; Sandhu & Singh, 2007). More single and double flower panicles are reported at the upper nodes. Besides increasing the effective ovules per pod, normally ranging between one to three in lentil (Malhotra, Singh, & Singh, 1974), developing four to five pods per peduncle (PPP) seems promising to significantly increase plant yield (Benlloch et al., 2015; Devi et al., 2018). Moreover, the use of this approach for yield improvement is not yet explored, as there is no published report mentioning the stable expression of five or more FPP in the cultivated lentil. Thus, the present investigation was aimed to identify and characterize the novel MF lentil genotypes bearing five FPP on certain flowering nodes.

2 | MATERIALS AND METHODS

2.1 | Plant materials

More than 2,000 genotypes including 500–550 germplasm lines, 25–30 cultivated varieties, 150–200 advanced breeding lines (F_6 to F_7 generations), 125–150 segregating population (F_2 to F_5 generations), and nearly 200 advanced breeding lines (F_7 generation) as nursery from ICARDA (the International Centre for Agricultural Research in the Dry Areas, Lebanon) were grown at the experimental field of Indian Council of Agricultural Research (ICAR)-Indian Agricultural Research Institute (IARI), New Delhi, India, during the winter season (November–April) of 2017–2018 and 2018–2019. These genotypes are part of our ongoing breeding program where plants are continuously bred and/or evaluated for the development of high-yielding varieties. These lines were screened for the number of FPP, and six lentil genotypes—namely, PMF-1 (2011S 56104-5), PMF-2 (2011S 56127-1), PMF-3 (2011S 56104-5-Sel), PMF-4 (ILL10810), IGy50, and ILL7663—were selected to study various inflorescence traits (Table 1) during 2017–2018 and 2018–2019.

The field at IARI, New Delhi, was located at 28°38′39″ N, 77°9′10″ E, at an elevation of 216 m asl. The genotypes were grown under normal field conditions using recommended cultural practices. Plants of six selected genotypes, cultivated varieties, and ICARDA genotypes were raised in three rows, whereas germplasm lines were grown as one row. The plant-to-plant and row-to-row spacing was 5 × 30 cm, and the row length was 5 m, with rows containing ~100 plants per row (Table 1). Depending on the number of plants selected in various generations (F_2 to F_7), the number of plants in different segregating populations

TABLE 1 Details of flowering, maturity, and pedigree of lentil genotypes differing in number of flowers per peduncle

S. No.	Genotype	Pedigree	Maturity	Flowering expression
			d	
1	PMF-1 (2011S 56104-5)	ILL1005 × ILL7012	105–110	Up to 5[a]
2	PMF-2 (2011S 56127-1)	ILL10158 × ILL10074	135–145	Up to 5
3	PMF-3[b] (2011S 56104-5)	ILL1005 × ILL7012	105–110	Up to 4
4	PMF-4 (ILL10810)	ILL7620 × ILL9836	110–115	Up to 4
5	IGy50	–	105–110	Up to 3
6	ILL7663	–	105–110	Up to 2

[a]Six to seven flowers per peduncle in a few plants.
[b]Identified from PMF-1.

and advanced breeding lines varied between 100 and 3,000 individuals.

Of the six selected genotypes, PMF-1 to PMF-4 were obtained from ICARDA, Lebanon, as a nursery for earliness (Lentil International Elite Nursey) and micronutrient (Lentil International Micro-Nutrient Nursey) traits, for further evaluation and selection at IARI, New Delhi (India). Based on the formation of a maximum number of flowers on any peduncle in a genotype, they were classified as up to two- and three-FPP types (most commonly observed), four-FPP types (not very common), and five-FPP types (rare). Until now, no cultivated lentil genotype bearing only one FPP on all the nodes, which is normally known in garden pea, has been identified.

Further, based on mean maturity duration, the lentil genotypes under Indian conditions are broadly classified as extra-early (<100 d), early (100–110 d), normal (110–135 d), and late (>135 d) maturity types. Of the six genotypes studied, two genotypes were of five-FPP type (PMF-1 and PMF-2) and four-FPP type (PMF-3 and PMF-4), whereas one each was of three-FPP type (IGy50) and two-FPP type (ILL7663) (Table 1). During 2018–2019, the genotype PMF-1 was also grown at the ICARDA Station, Amlaha (Sehore, India), which is located at 23°6′36″ N, 76°53′13″ E, and at an elevation of 469 m asl.

2.2 | Data recording

The flowering data recorded include the number of FPP, number of PPP, first and last MF node on a plant, peduncle length (PL, cm) or the length of stalk supporting the inflorescence, plant height (PH, cm) or the maximum height of a mature lentil plant, conversion of flower to pods (%), temperature (minimum, maximum, mean in °C), and bright sunshine hours (h) during which the direct solar irradiance exceeds a threshold value of 120 W m^{-2}. The six selected lentil genotypes were planted in randomized block design in three replications, and the data were recorded for three plants per genotype per replication per year. The nodes were counted considering the presence of scale leaves as Node 1.

2.3 | Weather parameters

The meteorological data, including minimum, maximum, and average temperature (°C) and bright sunshine hours (h), were obtained from the meteorological observatory of the Division of Agricultural Physics, ICAR-IARI, New Delhi, India. The weather data between the first week of February and the last week of March were analyzed for 2018–2019 and 2019–2020, which corresponded with the lentil flowering period under field conditions at IARI, New Delhi.

2.4 | Evaluation under glasshouse conditions

The flowering expression was also studied in the six selected genotypes under the partially controlled glasshouse conditions of the National Phytotron Facility at IARI, New Delhi. The plants were grown in 15.24-cm (6-inch) plastic pots containing growing media that consisted of coco peat/vermiculite/sand (1:2:1), with the temperature ranging from 21 °C (day) to 18 °C (night). Three pots were used to grow each genotype and five plants were grown in a pot. The flowering data were recorded and analyzed in completely randomized design.

2.5 | Statistical analysis

Analysis of variance was performed to determine the genotypic variance for the traits such as PL, FPP, PPP, PH, and number of peduncles (Gomez & Gomez, 1983). Pooled means of all traits for six selected genotypes were calculated, and pairwise comparisons were made among genotypes using Duncan's multiple range test. the frequency

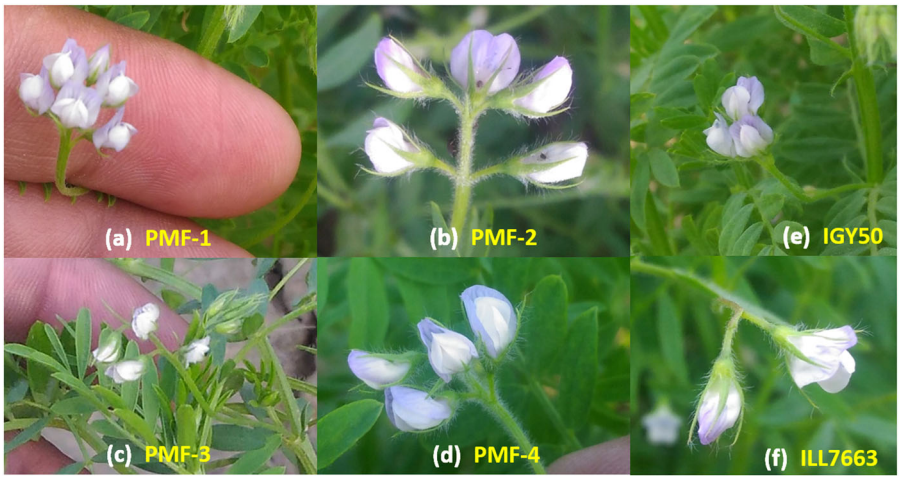

FIGURE 1 Flowering patterns in the cultivated lentil genotypes, expressing (a) six flowers per peduncle, (b) five flowers per peduncle, (c–d) four flowers per peduncle, (e) three flowers per peduncle, and (f) two flowers per peduncle, where PMF-1 to -4, IGY50, and ILL7663 are the lentil genotypes

distribution of different traits, phenotypic correlation coefficients, and the regression coefficient were determined using data from three plants per genotype of six selected genotypes (Al-Jibouri, Millar, & Robinson, 1958). All statistical analyses were conducted using JMP 14 (SAS Institute) and SPSS Statistics 19.0 (IBM Corporation) software.

3 | RESULTS

3.1 | Identification of multi-flowering genotypes

During 2017–2018, we identified two lentil genotypes, namely, PMF-1 and PMF-2, forming up to five FPP at certain nodes in most of the plants at the experimental farm of IARI, New Delhi (Figure 1). Further, a few plants of the genotype PMF-1 (2011S 56104-5) forming only up to four FPP were also used for data recording and were later named as PMF-3. In addition, an early-flowering genotype, PMF-4, was found forming up to four FPP at some nodes. All these genotypes were reevaluated during 2018–2019 for MF expression at IARI, New Delhi, and also under the partially controlled glasshouse at the National Phytotron Facility, India. Two genotypes, PMF-1 and PMF-2, again constantly formed five FPP, whereas the genotypes PMF-3 and PMF-4 formed four FPP at specific nodes, under both open-field as well as controlled growing conditions. The pod formation pattern in various lentil genotypes is illustrated in Figure 2. In a few plants of genotype PMF-1, we could even find six to seven PPP (Figure 3). Besides, the genotype PMF-1 expressed five FPP when grown at Sehore, Madhya Pradesh, India, during 2018–2019. For the

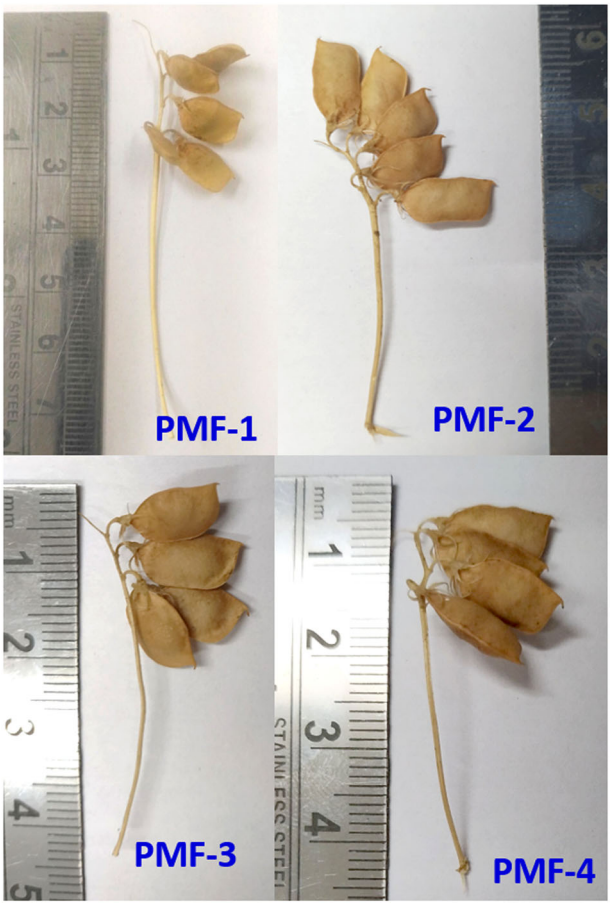

FIGURE 2 Multi-pod formation in various lentil genotypes. The genotypes PMF-1 and PMF-2 are shown expressing the formation of five pods per peduncle, whereas PMF-3 and PMF-4 are shown expressing the formation of four pods per peduncle

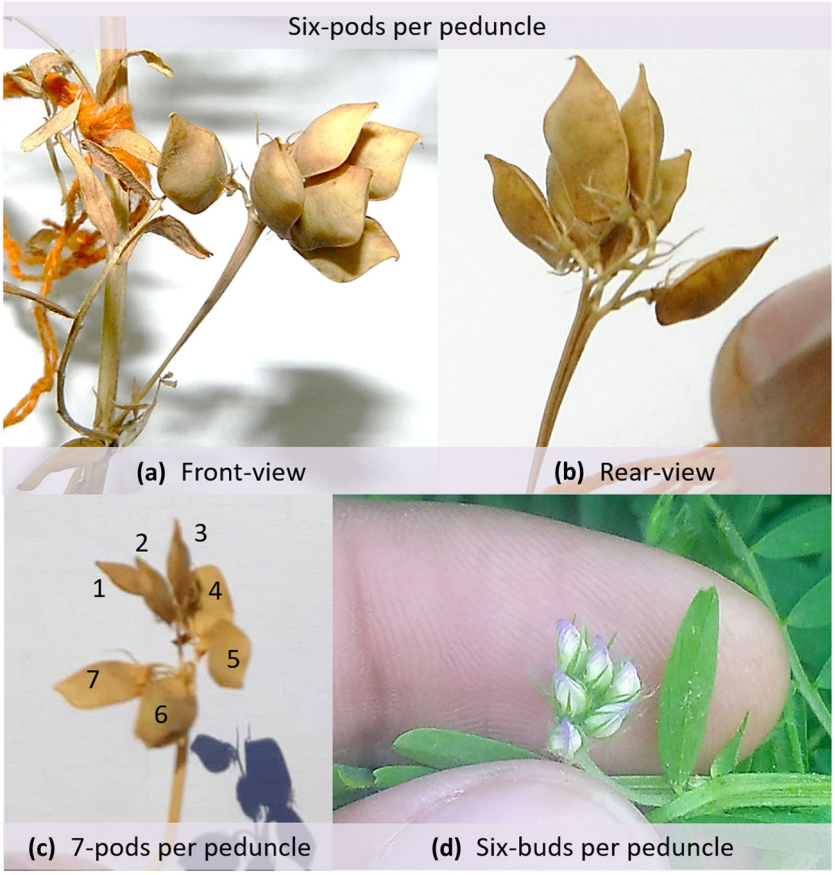

FIGURE 3 The multi-flowering genotype PMF-1, sometimes forming even six to seven pods per peduncle

comparison of flowering details, we used two more genotypes, IGy50 and ILL7663, stably forming three FPP and two FPP at certain nodes, respectively.

3.2 | Node-by-node analysis of the genotypes bearing four and five flowers per peduncle

Pooled node-by-node analysis of four and five FPP expression for 2017–2018 and 2018–2019 showed that the ninth node (from the base) was the first node expressing the four FPP phenotype in the genotype PMF-1, whereas the 20th node was the last in the MF genotype PMF-2. Further, the expression of four FPP was recorded in the genotypes PMF-1, PMF-2, PMF-3, and PMF-4 on the 9th to 18th, 10th to 20th, 10th to 17th, and 10th to 18th nodes, respectively. In the case of early-flowering genotype like PMF-1, the expression of five FPP was observed on the 12th to 17th nodes, whereas in the late-flowering genotype, PMF-2 was recorded on the 10th to 12th nodes under open field conditions during both years (Figure 4). Further, an overlap of the actual period for the expression of five FPP was observed in both PMF-1 and PMF-2 genotypes. Among the genotypes bearing four and five FPP, the node number 13 was found to bear the maximum four and five FPP, which was followed by node number 14. Interestingly, the pooled MF data gave a nearly perfectly bell shape curve starting at the ninth node and ending at the 20th node, suggesting that the MF expression in lentil is influenced by both genotype and environment.

3.3 | Temperature influencing the pattern of multi-flowering expression

Multi-flowering expression was also analyzed with specific weather data such as temperature (daily maximum, minimum, and mean) and bright sunshine hours (h), which was recorded in the experimental field at IARI, New Delhi. In 2018, the expression of five and six FPP was recorded from 13 to 23 February (Figure 5), whereas in 2019, this was observed from 25 February to 5 March (Figure 6) when the mean environmental temperature was mostly in the range of 12–18 °C. An entire shift in the flowering pattern from two to five FPP (sometimes six to seven FPP on a few nodes of a few plants) and again back to one or two FPP was recorded in the identified MF genotypes. It seems that besides genetic regulation, temperature has also played some role in the expression of MF phenotype. Moreover, bright sunshine hours had no direct role in the expression of the MF trait during both the years of the study period.

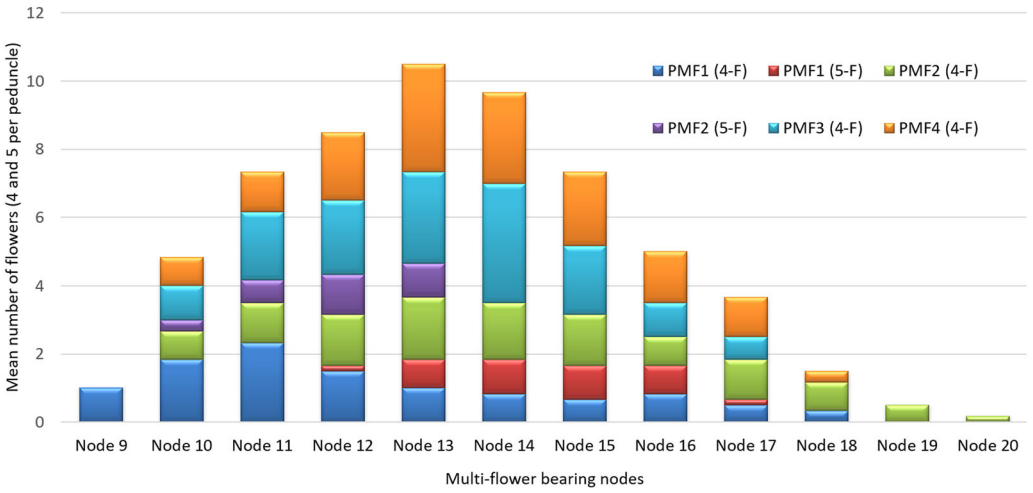

FIGURE 4 Relationship between multi-flower-bearing nodes and the number of multi-flowering peduncles in multi-flowering lentil genotypes, where PMF-1 to PMF-4 are the multi-flowering lentil genotypes, and the number in the parentheses shows the number of flowers per peduncle. The data are for 2017–2018 and 2018–2019 from New Delhi, India

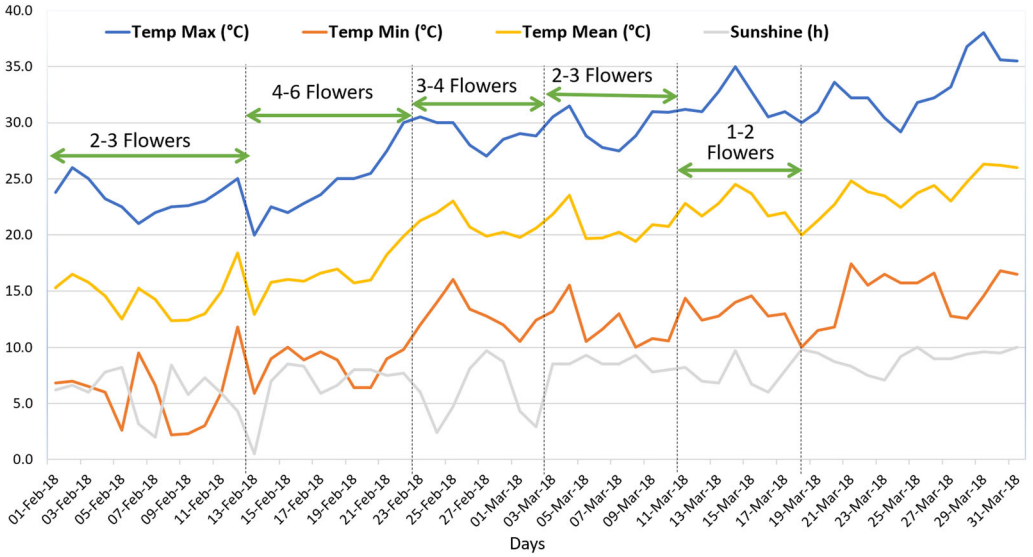

FIGURE 5 Relationship between various weather parameters and the induction of multi-flowering expression in the multi-flowering genotypes in cultivated lentil in 2018

3.4 | Correlation studies between inflorescence traits and conversion of flowers to pods

Separate correlation analysis was performed for the inflorescence traits for the genotypes forming two to three and four to five FPP (Table 2). In the case of both MF (forming four to five FPP) and other genotypes (forming two to three FPP), a highly significant positive correlation (r = .9134 and .8737, respectively) was observed between the number of peduncles and PPP per plant. A significant correlation (r = .8213) has been recorded between PL and the number of PPP in the two to three FPP forming genotypes and for the genotypes forming four to five PPP (Table 2).

An increasing trend was observed between the number of FPP and PL in all the genotypes studied (Supplemental Figure S1a). On a similar note, the correlation study was highly significant, and a positive correlation between the number of FPP and PL, for both MF (r = .9367) and other (r = .7968) genotypes, was observed. Since all the peduncles (1–3 FPP) do not give rise to an exact number of PPP, there is an increase in the number of one PPP and two PPP in the two- to three-FPP-forming genotypes (Table 2).

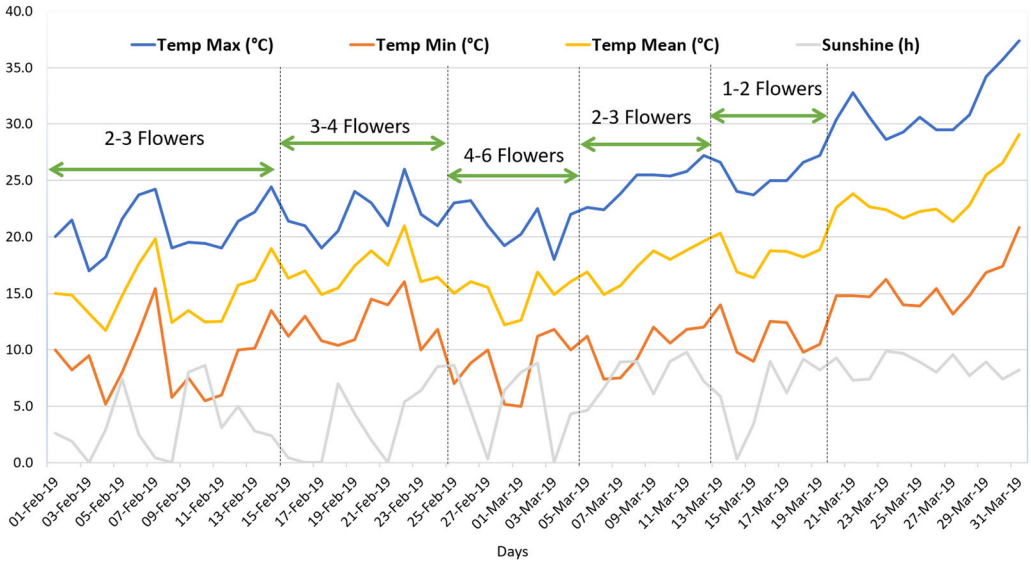

FIGURE 6 Relationship between various weather parameters and the induction of multi-flowering expression in the multi-flowering genotypes in cultivated lentil in 2019

TABLE 2 Correlation coefficient between various inflorescence traits in the lentil genotypes

Traits	Peduncle length	Flowers per peduncle	No. of peduncles	Pods per peduncle	Plant height
Peduncle length		.9367**	.3616	.1225	.0293
Flowers per peduncle	.7968**		.2503	.0043	.0000
No. of peduncles	.8593**	−.7213**		.9134**	.0093
Pods per peduncle	.8213**	−.8479**	.8737**		−.0615
Plant height	−.0275	.0000	.0115	−.0290	

Note. Upper diagonal represents the genotypes forming four to five flowers per peduncle (FPP), whereas the lower diagonal represents genotypes forming two to three FPP.

**Significant at the .01 probability level.

This could be the reason for the highly negative correlation between the number of FPP and PPP ($r = -.8479$) in the genotypes forming two to three FPP. However, no correlations have been recorded in MF genotypes for these traits.

The range for the number of peduncles bearing two FPP was the maximum recorded, followed by one FPP, whereas the least was observed for five FPP and six FPP (Supplemental Figure S1b). Furthermore, independent correlation analysis for inflorescence traits found that PL does play an essential role in the expression of the MF trait. No significant correlation has been recorded between PH and the flowering traits such as PL, FPP, number of peduncles, and PPP with any of the studied lentil genotype (Table 2). However, a very high correlation ($r = .756$) was observed among all the genotypes studied between the total number of flowers produced and the height of the plant (in cm) at maturity (Supplemental Figure S2).

3.5 | Comparison of inflorescence traits among various lentil genotypes

The comparison analysis among various lentil genotypes differing in their flowering traits revealed MF genotypes PMF-1 and PMF-2 as statistically similar in their expression of five FPP or PPP. A significantly higher number of four and three FPP or PPP was recorded in the genotypes PMF-3/PMF-4 and PMF-2, respectively. However, a maximum of one to two FPP or PPP were recorded in the genotypes IGy50 and ILL7663. Significantly longer PL was recorded in the genotypes PMF-1 and PMF-2 for one to five pods bearing peduncles compared with other studied genotypes. Also, the shortest PL was recorded for the genotypes PMF-3 and PMF-4 for two and one pod bearing peduncles. Overall, a trend was recorded between the increasing number of pods bearing peduncles and increasing PL (Table 3).

TABLE 3 Mean performance and their comparison for various inflorescence traits of lentil genotypes differing for the number of flowers per peduncle (FPP)

Trait	Genotype						
	PMF-1	PMF-2	PMF-3	PMF-4	IGy50	ILL7663	Overall mean
6 FPP	0.67 ± 0.33a**	–	–	–	–	–	0.11 ± 0.06
5 FPP	3.33 ± 0.42a**	3.17 ± 0	–	–	–	–	1.08 ± 0.27
4 FPP	10.83 ± 0.60b**	12.00 ± 0.89b**	15.00 ± 0.68a**	15.67 ± 0.66a**	–	–	8.92 ± 1.12
3 FPP	20.83 ± 0.47a**	21.83 ± 0.87a**	20.50 ± 0.71a**	19.00 ± 0.68b**	3.33 ± 0.33c**	–	14.25 ± 1.53
2 FPP	2.50 ± 0.22e**	3.50 ± 0.50d**	8.17 ± 0.74c**	9.17 ± 0.70c**	46.00 ± 1.36b**	48.83 ± 1.27a**	19.69 ± 3.35
1 FPP	1.17 ± 0.30c**	1.17 ± 0.16c**	2.67 ± 0.21b**	2.00 ± 0.51b**	25.17 ± 1.19a**	24.17 ± 0.98a**	9.22 ± 1.86
6 PPP	0.33 ± 0.33a**	–	–	–	–	–	0.06 ± 0.05
5 PPP	1.67 ± 0.021a**	1.00 ± 0.00b**	–	–	–	–	0.44 ± 0.11
4 PPP	9.67 ± 0.42b**	5.67 ± 0.66c	12.50 ± 0.76a**	11.83 ± 0.30a**	–	–	6.61 ± 0.88
3 PPP	14.33 ± 1.22bc**	18.00 ± 0.77a**	13.33 ± 0.95c**	15.83 ± 0.70b**	3.00 ± 0.25d**	–	10.75 ± 1.17
2 PPP	3.67 ± 0.21f**	5.83 ± 0.70e**	9.17 ± 0.60d**	12.33 ± 0.88c**	34.17 ± 1.37a**	26.33 ± 1.45b**	15.25 ± 1.92
1 PPP	2.33 ± 0.33d**	3.17 ± 0.74cd**	3.17 ± 0.30c**	3.83 ± 0.40c**	37.50 ± 0.50a**	33.67 ± 0.66b**	13.94 ± 2.60
PL (6 PPP)		–	–	–	–	–	–
PL (5 PPP)	6.80 ± 0.10a**	6.35 ± 0.16b**	–	–	–	–	2.19 ± 0.52
PL (4 PPP)	6.70 ± 0.09a**	5.92 ± 0.15b**	4.17 ± 0.10c**	3.28 ± 0.07d	–	–	3.34 ± 0.44
PL (3 PPP)	6.07 ± 0.09a**	5.27 ± 0.15b**	3.15 ± 0.05c	2.95 ± 0.07c**	1.92 ± 0.85d**	–	3.22 ± 0.36
PL (2 PPP)	4.98 ± 0.05a**	4.58 ± 0.06b**	2.07 ± 0.03d**	1.95 ± 0.04e**	3.62 ± 0.10c	3.67 ± 0.04c	3.48 ± 0.19
PL (1 PPP)	4.07 ± 0.049a**	4.03 ± 0.042a**	1.28 ± 0.070c**	1.20 ± 0.026c**	2.60 ± 0.026b	2.68 ± 0.017b	2.64 ± 0.19

Note. Values represent mean values ($n = 3$) ± SE at $P = .05$. Means followed by the a common lower-case letter within a row are not significantly different. Duncan's multiple range test was used for the comparison. FPP, flowers per peduncle; PPP, pods per peduncle; PL, peduncle length; (6PPP) to (1PPP), respective number of pods per peduncle per plant.

[a]Data not taken.

**Significant at the .01 probability level.

Yield is the outcome of several traits, including the number of flowers produced on each peduncle and their percentage conversion into pods. We studied flowering behavior in a small population of nearly 250–300 plants per genotype and never aimed to investigate the yield per se in such a small population. However, the maximum number of filled pods per plant was recorded in the genotype PMF-4, whereas PMF-3 produced the maximum number of flowers per plant. Also, the maximum flower-to-pod conversion was observed in the MF genotype PMF-4 (88.1%), followed by PMF-3 (79.36%) (Supplemental Figure S3). The conversion of flowers to pods among all the studied genotypes was the lowest in PMF-2. The flowering in PMF-2 was very late and coincided with a sudden rise in temperature during the last week of March, ultimately leading to severe flower abortion in this genotype. The total number of flowers and pods was significantly higher in the genotype PMF-4, whereas percentage pod conversion was maximum in the genotype IGy50 and PMF-4 (Supplemental Figure S4).

A very high correlation ($r = .801$) was recorded between the mean of the total number of peduncles per plant and the total number of pods per plant (Figure 7a) when all the genotypes were considered together. Further, a still higher correlation ($r = .889$) was recorded when the same

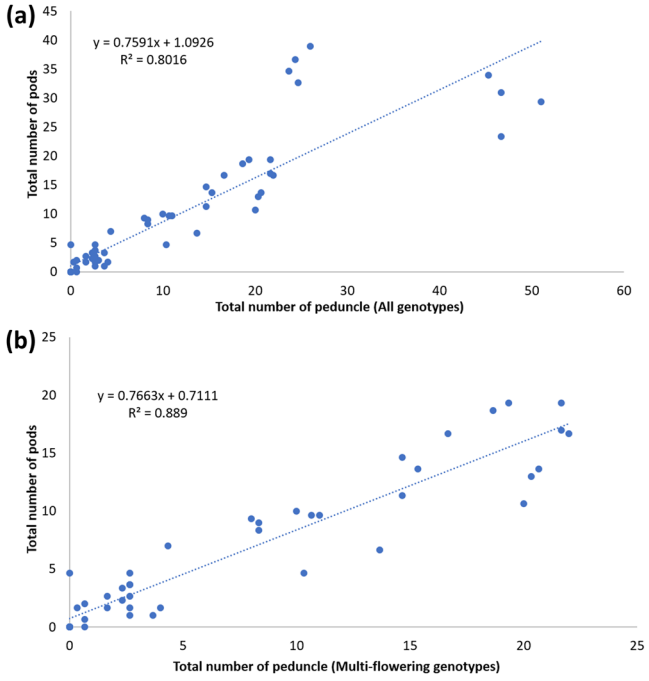

FIGURE 7 Correlation between the means of the total number of peduncles and total number of pods per plant in (a) all the lentil genotypes and (b) multi-flowering lentil genotypes

was calculated for the MF genotypes (Figure 7b), which indicates the potential of MF as an essential trait for yield improvement.

3.6 | Pedigree analysis

Incidentally, all genotypes under New Delhi conditions that expressed MF at multiple flowering nodes are of ICARDA origin. We further checked the pedigree details of these MF lines to find common parent(s) imparting the MF expression. However, all the parents of the MF lines were different (Table 1).

3.7 | Evaluation of genotypes for multi-flowering expression under glasshouse conditions

The MF genotypes PMF-1 and PMF-2 expressed five FPP, whereas PMF-3 and PMF-4 formed four FPP (Supplemental Figure S5). The number of flowers and pods under the glasshouse condition was significantly lower than under open-field conditions. This could be because the pots used for growing the plants were very small (15.24-cm [6-inch] diameter). However, the results do confirm the expression of the five FPP in the identified genotypes, even under partially controlled conditions.

4 | DISCUSSION

In cultivated lentil, the inflorescence is racemose with normally one to three flowers at the axis. Multi-flowering expression to the tune of five FPP has been reported in some wild *Lens* genotypes like *L. tomentosus*, *L. orientalis,* and *L. ervoides*, (Sharma, 2009). However, in cultivated lentils, expression of five FPP was reported in the induced mutants generated in the L-235 genotype through *N*-nitroso-N-methylurea (NMU, 0.005%) treatment (Sharma & Kharkwal, 1983), but the mutant lines exhibited sterility and did not show stability for the MF trait in subsequent generations. Similarly, unstable expression of a maximum of seven FPP (Saxena, 2009) and six PPP (Malhotra et al., 1974) was also observed in lentils under field conditions.

Stable expression of five FPP was recorded for the first time in two cultivated lentil genotypes, PMF-1 (seldom six to seven FPP) and PMF-2, across different years. On a similar note, in garden pea, genotypes were reported expressing MF to the tune of three to five FPP at multiple flowering nodes (Devi et al., 2018; Sanwal, Kumar, & Singh, 2016). This indicates the possible presence of some allelic combinations in these lentil genotypes that stably expressed five FPP under the Indian climatic conditions. A few studies, mostly in pea and chickpea, describe the genetic control of the number of FPP. In lentil, Gill and Malhotra (1980) reported the number of FPP as a monogenic trait, with the two-FPP phenotype as dominant over the three-FPP phenotype.

Although the lentil genotypes obtained from ICARDA as nursery are generally in the fixed or homozygous condition (F_7 generation), we observed some heterogeneity for the expression of MF trait in the genotype 2011S 56104-5. Thus, the plants differing for flowering behavior in the genotype 2011S 56104-5 are selected using a single-plant selection method and are renamed as PMF-1 (Pusa Multi-flowering) and PMF-3 for five- and four-FPP types, respectively. Thus, it appears that the lentil nursery received from ICARDA was homozygous for various traits, but heterogeneous for the MF trait. The genotypes PMF-1 and PMF-2 stably expressed the formation of up to five FPP in different years (2017–2018 and 2018–2019) and locations (open-field conditions and glasshouse conditions) at multiple flowering nodes. Furthermore, the genotype PMF-1 has also expressed MF even at the Sehore location (Madhya Pradesh, India) during 2018–2019. Similar results were reported in other pulses like garden pea (Devi et al., 2018; Sanwal et al., 2016) and chickpea (Gaur & Gour, 2002).

A variety of genetic architectures have been reported to underly the MF trait in pea (Devi et al., 2018; Hole & Hardwick, 1976; Sinjushin & Liberzon, 2016). Furthermore, two polymeric flower number genes (*FN* and *FNA*) (Lamprecht, 1947), and Neptune genes (*nep-1* and *nep-2*) (Singer et al., 1999) were found to govern the MF in peas, whereas three flowering loci—sterile nodes (*Sn*), day neutral (*Dne*), and high response (*HR*)—along with the late flowering (*Lf*) gene determines the node on which MF appears (Devi et al., 2018; Murfet, 1973; Reid, 1979). In lentil also, the nodes forming five FPP expressed between the 10th and 17th node, suggesting the possible presence of an *Lf*-like locus in these lentil genotypes. This needs further detailed genetic studies.

The MF genes are reported to be linked with late flowering genes in other pulses (Devi et al., 2018; Gritton, 1986). However, in lentil, a very tight association of MF with late-flowering phenotype under New Delhi conditions could not be found (Table 1). Various genotypes differing for the flowering expression (two to five FPP) have recently been used for crossing in different combinations, which could help in deciphering the genetics of MF trait in the cultivated lentil.

In lentil, several reports state a positive correlation between the yield and number of flowers per plant (Singh & Singh, 1976; Wilson, 1977), number of pods per node (Kumar, Sharma, Malik, Dahiya, & Sharma, 2002, Vir & Gupta, 2002), and number of pods per plant (Yadav,

Phogat, Solanki, & Tomer, 2005). However, there is no published report stating any lentil genotype stably expressing five FPP. Further, in the three PPP lentil genotype, the frequency of different types of PPP showed one PPP as the most common, followed by two PPP, whereas three PPP was the least common (Muehlbauer, 1974). However, in the genotypes PMF-1, PMF-2, PMF-3, and PMF-4, maximum flowers and pods frequency were recorded for three PPP, whereas one PPP was reported as the maximum for the genotypes ILL7663 and IGy50.

Erskine et al. (1994) observed that the movement of lentil across the globe has resulted in the selection of various region-specific genotypes, where flowering is controlled by a balance between the photoperiod and the temperature. Further, large genotype × environment interactions have been testified to contribute to the seed yield for several traits (Kumar, Sharma, Luthra, & Sharma, 2005; Yadav et al., 2002). The lentil genotypes were found initially producing two FPP, followed by three, four, and five FPP in the middle, and again there is a gradual decrease in the number of FPP at the upper nodes. On the similar note, MF expression was found to decline with plant age and was also highly influenced by the environment (Sharma, 2009). Differential expression of MF on certain nodes, as observed in the lentil, was also reported in garden pea (Devi et al., 2018; Hardwick, Andrews, Holeand, & Salter, 1979). Likewise, Emami (1996) and Kumar (2002) also classified a lentil plant to be of three- or four-FPP type, if it produced that many flowers even on a single peduncle.

Ironically, MF in lentil is mentioned indifferently in the literature, mostly concerning four FPP, and in one case, even seven FPP has been reported (Sharma, 2009). Moreover, MF was never considered a trait to study, due to unstable expression through generations, environmental influence, and poor conversion to multi-pods (Sharma, 2009). Among various environmental factors, the ambient temperature (11–20 °C) during flowering was found to regulate the number of FPP in peas (Devi et al., 2018; Hole & Hardwick, 1976; Murfet, 1985; Singer et al., 1999). In this study, the expression of five and six FPP showed some association with the mean ambient temperature in the genotypes PMF-1 and PMF-2. Similarly, MF expression was found to be influenced by the ambient growing conditions, along with the mutations in the flowering time genes, in peas (Hole & Hardwick, 1976; Murfet, 1985; Singer et al., 1999) and chickpea (Gaur & Gour, 2002; Sheldrake, Saxena, & Krishnamurthy, 1978). Also, the number of peduncles per plant was found to be dependent on both the genotype and its interaction with the environment (Saxena 2009).

In MF or multi-podded genotypes, flower abortion is common, which is due to several factors such as genetic makeup, nutrition, and other environmental factors (Gritton, 1986; Hole & Hardwick, 1976; Meadley & Milbourn, 1970). In crops like pea and chickpea, higher number of FPP does not result in increased PPP. Further, pea plants with three FPP usually develop two PPP (Srinivasan, Gaur, Chaturvedi, & Rao, 2006), whereas chickpea produces up to nine FPP and not more than four or five PPP (Gaur & Gour, 2002; Srinivasan et al., 2006). Similarly, in lentil, an inevitable reduction in the total number of five PPP compared with the total number of five FPP was observed. Further, no change in seed size or maturity duration was found in the MF genotypes. Moreover, in chickpea (Kumar, Srivastava, & Ganesh, 2000) and garden pea (Devi et al., 2018), the MF trait has been reported to have a positive impact on the overall yield and yield stability (Rubio, Flores, Moreno, Cubero, & Gil, 2004). Thus, there is a need to perform large-scale multilocation replicated yield trials on a plot basis before conclusively commenting on the actual yield potential of MF trait in lentil.

In pulses, the peduncle traits play diverse roles in improving the productivity by regulating the assimilate transportation from the leaves (source) to the growing pods (sink) (Devi et al., 2018; Marshall, 1995). Therefore, in the near future, the possibility of increasing the yield of any grain legume by increasing the total number of pods per plant seems an outstanding choice. Thus, in addition to the identification of lentil natural mutants having MF expression, we should also aim to identify and manipulate the gene(s) controlling the I2 meristem activity leading to the desired MF expression in lentil using the latest biotechnological tools.

5 | CONCLUSION

Despite the positive effect of MF on the overall pod and seed yield, no attention has been paid to a detailed understanding of this complex trait in lentil. Although various lentil genotypes producing two to four FPP have been reported worldwide (Sandhu & Singh, 2007), this study for the first time reports the stable expression of five PPP. Hence, the identified MF genotypes appear to be potential parents for lentil breeding programs aiming to incorporate the MF trait.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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