PGR NOTE

First Report of a Novel Multi-flowering Germplasm with Fasciated Stem in Lentil (*Lens culinaris* Medik.)

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The mining of entire lentil accessions (2,324) of Indian genebank, ICAR-NBPGR, New Delhi led to the identification of a unique multi-flowering germplasm accession, IC241473, in cultivated lentil (*Lens culinaris* Medik.), forming up to 16 flowers per peduncle at multiple flowering node. Besides, this accession was also having a fasciation of the main stem. The multi-flowering expression was observed under field conditions at ICAR-NBPGR, New Delhi, India, consecutively during the winter seasons of year 2017-18, 2018-19, 2019-20 and was validated at NBPGR, New Delhi and ICARDA, Amlaha (Madhya Pradesh) during 2020–2021. Unlike earlier reported fasciated accessions in legumes, this fasciated accession showed stable multi-flowering expression over the years and locations and was also found fertile with fully developed reproductive organs. This unique and novel germplasm accession can be utilized for genetic studies to identify the locus regulating the multi-flowering trait in lentil and its association with environmental factors.

Key Words: Fasciation, Germplasm, Multi-flowering Lentil, Node

Lentil (Lens culinaris Medik.) is a cool-season selfpollinated pulse crop with genome size of 4.3 Gb (Bett et al., 2016). It is listed as one of the potential future smart food in South and Southeast Asia (Li and Siddique, 2018). It is native to the Near Eastern region and mainly cultivated in South Asia, North America and West Asia (Tripathi et al., 2019). During 2019, the world produced 5.73 million tonnes of lentil from 4.8 million ha of area, led by Canada (2.16 million tonnes) followed by India (1.23 million tonnes), collectively amounting to nearly 60% of the total production (FAOSTAT, 2021). Canada also ranks first (1.48 million ha) in acreage under the lentil, followed by India (1.36 million ha). Lentil is mainly grown in the central and eastern part of India, primarily as a rainfed crop. The average productivity of India is lower (901 kg ha^{-1}) than the world (1,194 kg ha^{-1}) (FAOSTAT, 2021).

Previous researchers studied the inflorescence morphology of diverse legumes such as garden pea, chickpea and lentils (Gaur and Gour, 2002; Sandhu and Singh, 2007; Devi *et al.*, 2018; Mishra *et al.*, 2020). Furthermore, the genetic basis of flowering patterns in any crop is of enormous practical significance for the crop researchers aiming to breed high-yielding cultivars (Sinjushin and Liberzon, 2015). The flowering characters in pulses, such as the number of flowering nodes per plant, the number of flowers per peduncle (FPP), and the number of flowers per plant, seems variable, as these traits directly influence productivity. Multi-flowering (MF) accessions can be identified with an expression of three or more flowers at one or multiple flowering nodes (Gaur and Gour, 2002; Benlloch et al., 2015; Sanwal et al., 2016; Devi et al., 2018). Multi-flowering clusters have been documented for a few flowering nodes in Pisum sativum (up to five FPP) (Devi et al., 2018), chickpea (upto nine FPP) (Gaur and Gour, 2002), lentil (upto seven FPP) (Mishra et al., 2020), having different mechanisms of MF expression (Gaur and Gour, 2002). Generally, lentils have two to four flowers in the axillary racemes on short and very thin peduncles (Muehlbauer et al., 1985; Sandhu and Singh, 2007). At the upper nodes, a more significant number of single and double flower clusters have been reported. Utilization of MF in lentil having fasciated stem for yield improvement

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is not yet explored. To date, we have not found any report highlighting the MF expression to the tune of 16 FPP in any cultivated lentil genotype. Stem fasciation is known to be the heritable trait, and spontaneous fasciation mutants have been reported in crops like soybean (Takagi, 1970), pigeon pea (Bhatnagar *et al.*, 1967) and lupin (Blixt and Gottschalk, 1975).

However, the economic importance of this trait was recognized only in the recent past, when fasciation was induced in legumes using mutation (Tyagi and Gupta, 1991). A fasciated mutant of soybean and pea was used for producing a variety of valuable recombinants. The increase in yield of fasciated plants is often associated with an increased number of flowers and pods being formed in the upper nodes of plant.

In 2017-2018, this unique accession with MF expression was noticed for the first time during characterization of entire germplasm (2,324 accessions) conserved in NBPGR genebank, which was then re-evaluated in 2018-2019 and 2019-2020. During 2020–2021, this accession, along with checks IPL220, L4727, L4729 and RVL31, was again validated for the descriptor traits and MF associated traits such as number of Flowers Per Peduncle (FPP), Peduncle Length (PL), Pods Per Peduncle (PPP) and Stem Width (SW) at New Area Farm, NBPGR, New Delhi (28 °64'66"N, 77°15'08" E) and ICARDA, FLRP, Amlaha India (23 °12'39"N, 76 °90'29" E). The row-to-row and plant-toplant spacing was 30×5 cm, and the row length was three-meter with each row containing nearly 60 plants. The mean environmental temperature was mostly in the range of 12-18 °C during flowering time.

IC241473 is a vigorously growing genotype having dark green leaves with slight pubescence, days to 50% flowering ranged between 90-95 days, flowers are light purple, plant height ranged between 39.5-45.7 cm, pods per plant ranged between 296-397, the number of secondary branches were recorded from 14-24, peduncle length varied between 4.2-4.5 cm, and the stem width recorded was in the range of 5.08-5.28 mm. The inflorescence in lentil is racemose, with usually one to three flowers per peduncle. MF expression to the tune of 5-FPP has been reported in cultivated lentil (Mishra et al., 2020) and some wild Lens accessions like L. tomentosus, L. orientalis, and L. ervoides (Sharma, 2009). However, in cultivated lentils, MF expression was also reported in some induced mutants. Still, the mutant lines exhibited sterility and did not show stability for subsequent generations' MF trait (Sharma and Kharkwal, 1983). Similarly, occasional expression of a maximum of 7-FPP (Saxena, 2009) and 6-PPP (Malhotra *et al.*, 1974) was also reported in lentils. For the first time, this investigation reports the MF expression to the tune of 16 FPP (on 14th node) in cultivated lentil genotype IC241473 (Fig. 1).

The lentil germplasm IC241473 obtained from the Indian national genebank was uniform for various morphological traits, including MF. We have used single plant selection method (SPS) to maintain the purity of the germplasm. Previous researchers reported an array of genetic architectures underlying the multi flowering or multi-podding characters in *Pisum* (Hole and Hardwick, 1976; Devi *et al.*, 2018). Lamprecht (1947) and Singer *et al.* (1999) reported two polymeric flower number genes (FN and FNA) and Neptune genes (nep-1 and nep-2), respectively.

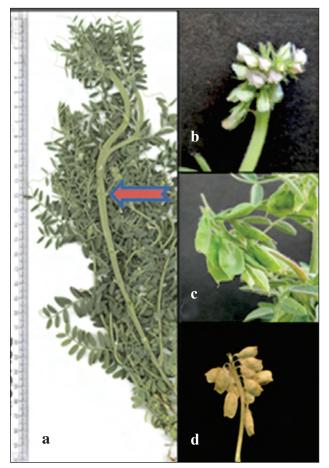


Fig. 1. Multi-flowering (MF) germplasm accession, IC241473 a. fasciated main stem; b. MF accession forming up to 16 FPP; c. MF accession at pod formation stage; d. MF accession at pod maturity stage.

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On a similar note, in the lentil also, the 16-FPP expressed on the upper nodes (14th node), suggesting the probable occurrence of an Lf-like locus (late flowering) in this germplasm accession. This necessitates in-depth studies exploring the possibility of linkage between MF genes and late-flowering genes as reported in the case of other pulse crops such as pea and chickpea (Devi et al., 2018). Since lentil is global crop and often exchanged among countries for research purpose, many ecotypes developed due to selection, where trait like flowering is dependent upon the temperature and the photoperiod both (Erskine et al., 1994). In the present study, the lentil genotypes were found initially producing two FPP, followed by the increasing number of FPP upto 16, and again there is a gradual decrease in the number of FPP at the upper nodes. Similarly, Sharma (2009) also reported that MF expression was dependent upon plant age and the environment. MF was one of the most neglected characters to study because of its unstable expression, environmental influence, and poor conversion ratio of flowers to pods (Sharma, 2009). Among various environmental factors, the ambient temperature (11–20°C) during flowering was found to regulate FPP in peas (Hole and Hardwick, 1976; Murfet, 1985; Singer et al., 1999; Devi et al., 2018).

The abortion of flowers is frequent in MF accessions due to numerous factors, viz. genetic architecture, plant nutrition, and other environmental factors (Hole and Hardwick, 1976). In many pulse genera like Cicer and Pisum, the conversion of flower to pods is not always cent percent. Further, pea plants with 3-FPP usually develop 2-PPP (Srinivasan et al., 2006), whereas chickpea produces 9-FPP and not more than 4- or 5-PPP (Gaur and Gour, 2002; Srinivasan et al., 2006). After analyzing seed traits of MF accession, no change in seed size was observed. Moreover, in chickpea and garden pea (Devi et al., 2018), a positive correlation was observed between MF and yield traits in the study of Rubio *et al.* (2004). Globally, the lack of MF genetic resources and meagre studies of this complex trait in lentil limits the utilization of MF traits for yield improvement. Although various lentil genotypes producing 2- to 4-FPP have been reported worldwide (Sandhu and Singh, 2007), this present study is the first global report in cultivated lentil bearing up to 16-FPP as well as 13-PPP. Therefore, the identified MF germplasm accession has a tremendous potential as a promising donor for lentil breeding programs.

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References

- Benlloch R, A Berbel, L Ali, G Gohari, T Millan and F Madueno (2015) Genetic control of inflorescence architecture in legumes. *Front. in Plant Sci.* https://doi.org/10.3389/ fpls.2015. 00543.
- Bett K, C Chan, AG Sharpe, D Cook, R Varma Penmetsa, P Chang, CJ Coyne, R McGee, D Main, J Dolezel, D Edwards, S Kaur, SK Agrawal, SM Udupa and A Vandenberg (2016) The Lentil Genome – from the sequencer to the field. Marrakesh, Morocco. https://hdl.handle.net/20.500.11766/6763.
- Bhatnagar PS, PK Sen Gupta, LC Gangwar and JK Saxena (1967) A fasciated mutant in pigeon pea. *Sci. Cult.* **33**: 120-121.
- Blixt S and W Gottschalk (1975) Mutation in Leguminoseae. Agri. Hort. Genet. 23: 33-85.
- Devi J, GP Mishra, SK Sanwal, RK Dubey, PM Singh and B Singh (2018) Development and characterization of pentaflowering and triple-flowering genotypes in garden pea (*Pisum sativum* L. var. *hortense*). *PLOS ONE* 13(7). https:// doi.org/10.1371/ journal.pone.0201235.
- Erskine W, A Hussain, M Tahir, A Bahks, RH Ellis, RJ Summerfield and EH Roberts (1994) Field evaluation of a model of photothermal flowering responses in a world lentil collection. *Theo. Appl. Genet.* 88: 423-428.
- FAOSTAT (2021) FAOSTAT. Rome: FAO. Retrieved from http:// www.fao.org/faostat/en/#data/QC.
- Gaur PM and VK Gour (2002) A gene producing one to nine flowers per flowering node in chickpea. *Euphytica* 128: 231-235. https://doi.org/10.1023/A:1020845815319.
- Hole CC and R Hardwick (1976) Development and control of flowers per node in *Pisum sativum* L. *Annals of Botany* **0**: 707-722.
- Lamprecht H (1947) The inheritance of the number of flowers per inflorescence and the origin of *Pisum*, illustrated by polymeric genes. *Agri Hort. Genet.* **5**: 16-25.
- Li X and KHM Siddique (2018) Future Smart Food-Rediscovering hidden treasures of neglected and underutilized species for Zero Hunger in Asia, Bangkok, 242 p.
- Malhotra RS, KB Singh and Singh JK (1974) Genetic variability and genotype environmental interaction studies in lentil. *Journal* of Research Punjab Agricultural University **10**: 17-21.
- Mishra GP, HK Dikshit, J Kumari, K Tripathi, J Devi, M Aski, R Mehra, A Sarker and S Kumar (2020) Identification and

characterization of novel penta-podded genotypes in the cultivated lentil. *Crop Sci.* **60(4)**: 1974-1985.

- Muehlbauer FJ, JI Cubero and RJ Summerfield (1985) Lentil (*Lens culinaris* Medik.). In: Summerfield RJ and EII Roberts (eds) *Grain legume crops* Collins, London, pp. 266-311.
- Murfet IC (1985) *Pisum sativum*. In: AH Halevy (ed.), Handbook of flowering Vol IV: Boca Raton, FL: CRC Press pp. 97-126.
- Rubio J, F Flores, MT Moreno, JI Cubero and J Gil (2004) Effects of the erect/bushy habit, single/double pod and late/early flowering genes on yield and seed size and their stability in chickpea. *Field Crops Res.* **90**: 255-262.
- Sandhu JS and S Singh (2007) History and origin. In: Yadav SS, DL McNeil and PC Stevenson (eds) *Lentil: An ancient crop for modern times* Springer, Dordrecht, the Netherlands, pp. 1-9.
- Sanwal SK, R Kumar and B Singh (2016) VRP-500 (IC610501; INGR15009), a garden pea (*Pisum sativum*) germplasm with triple pods at every node. *Indian J. Plant Genet. Resour.* 29(1): 90.
- Saxena MC (2009) Plant morphology, anatomy and growth habit. In: Erskine W, F Muehlbauer, A Sarker and B Sharma (eds) *The lentil: Botany, production and uses.* CAB International, Wallingford, UK, pp. 3446.
- Sharma B (2009) Genetics of economic traits. In: Erskine W, F Muehlbauer, A Sarker and B Sharma (eds) *The lentil:*

Botany, production and uses. CAB International, Wallingford, UK, pp. 34-46.

- Sharma B and Kharkwal MC (1983) Mutation breeding of lentil, cowpea and chickpea. *Mutation Breeding Newsletter* 21: 5-6.
- Singer S, J Sollinger, S Maki, J Fishbach, B Short and C Reinke (1999) Inflorescence architecture: A developmental genetics approach. *Botanical Review* 5: 385-410.
- Sinjushin AA and AS Belyakova (2015) Ontogeny, variation and evolution of inflorescence in tribe Fabeae (Fabaceae) with special reference to genera *Lathyrus*, *Pisum* and *Vavilovia*. *Flora* 211: 11-17.
- Srinivasan S, PM Gaur, SK Chaturvedi and BV Rao (2006) Allelic relationships of genes controlling number of flowers per axis in chickpea. *Euphytica* 152: 331-337.
- Takagi Y (1970) Monogenic recessive male sterility in oil rape (*Brassica napus* L.) induced by gamma irradiation. *Z. Pflanzenzuchtg* 64: 242-247.
- Tripathi K, PG Gore, A Pandey, R Bhardwaj, N Singh, G Chawla and A Kumar (2019) Seed morphology, quality traits and imbibition behaviour study of atypical lentil (*Lens culinaris* Medik.) from Rajasthan, India. *Genet. Resour. Crop Evol.* 66(3): 697-706.
- Tyagi BS and PK Gupta (1991) Induced mutations for fasciation in lentil (*Lens culinaris* Medik.). *Indian J Genet Plant Breed.* 51(3): 326-331.