

## Research Paper

## New lentil variety RKL 58F-3715: a case study depicting the emergence of desirable transgressive segregants

Sumer Singh Punia<sup>1</sup>, Khajan Singh<sup>2</sup>, Sarfraz Ahmad<sup>1\*</sup>, Baldev Ram<sup>2</sup>, Meenakshi Dheer<sup>3</sup>, Preeti Verma<sup>2</sup>, Vijay Singh Jat<sup>4</sup>, Ramesh Kumar Solanki<sup>5</sup>, Ashok Kumar Parihar<sup>6</sup>, Shailesh Kumar Jain<sup>1</sup>, Dalip<sup>1</sup>, Rubina Khan<sup>1</sup>, Shiv Kumar Agrawal<sup>7</sup> and Sanjeev Gupta<sup>8</sup>

<sup>1</sup>Sri Karan Narendra Agriculture University, Jobner (Rajasthan), India

<sup>2</sup>Agriculture University, Kota (Rajasthan), India

<sup>3</sup>Vivekanand Global University, Jaipur (Rajasthan), India

<sup>4</sup>SCRS, Government College, Sawai Madhopur (Rajasthan), India

<sup>5</sup>ICAR-Central Arid Zone Research Institute, Jodhpur (Rajasthan), India

<sup>6</sup>ICAR-Indian Institute of Pulses Research, Kanpur (Uttar Pradesh), India

<sup>7</sup>International Center for Agricultural Research in the Dry Areas (ICARDA), Beirut, Lebanon

<sup>8</sup>Assistant Director General, (Oilseeds and pulses), Indian Council of Agricultural Research (ICAR) New Delhi, India

\*Corresponding author e-mail: sarfraz\_firoz@rediffmail.com

Received: 12 January 2024

Accepted: 12 March 2024

### Handling Editor:

Dr. Jitendra Kumar, ICAR-Indian Institute of Pulses Research, Kanpur, India

### ABSTRACT

Lentil is one of the world's most important cool-season grain legumes. The frequency of natural recombinants and segregants under natural conditions is very low in lentil due to their cleistogamous flower. The present investigation depicted the emergence of desirable transgressive segregants obtained from spontaneous mutation. Exploiting these hypervariable mutants, a high-yielding lentil variety RKL 58F-3715 (Kota Masoor 4; KM 4) was developed from the progeny of a natural variant. The identified single deviated plant from the seed production plot of DPL 62 was probably a spontaneous mutation. During generation advancement, the progenies showed hypervariable segregants, especially for seed morphological traits, such as size, shape, colour and texture. The breeding cycles following rigorous selection under modified pedigree and single seed descent delivered the high-yielding stable genotype KM 4. Hypervariable progenies were obtained from natural mutants, distinguished in morphological traits such as earliness, light foliage colour, and prominent tendrils from the parent variety. The national weighted mean over locations over the year of KM 4 for grain yield was 1865 kg/ha, with yield superiority of 22.80% over national check DPL 62. Considerable resistance against major diseases, i.e., wilt and rust, makes it a durable genotype for commercial cultivation in major lentil-growing areas of India.

**Key words:** Kota Masoor 4, Lentil variety, Seed trait, Spontaneous mutation, Transgressive segregants

### INTRODUCTION

Lentil (*Lens culinaris* Medikus ssp. *culinaris*) is one of the most ancient food legumes domesticated and cultivated by humans. It is an important food crop in the Indian subcontinent, Middle East, Northern Africa, and North America. The cultivated lentil species are classified into two groups based on their seed size and other distinct morphological characteristics, viz., small-seeded (*microsperma*) and large-seeded (*macrosperma*) (Ferguson *et al.* 2000). India produces more than 1 million tonnes (mt) of lentil from an area of around 1.4 m ha and shares 18% of production and 29% of the area globally in total pulse production; however, its productivity (752 kg/ha) is about 37% lower than the global average (1197 kg/ha) (Rawal and Navarro 2019). In India, most lentil varieties have been developed mainly through pure line selection, and intra-

specific hybridization showed low yield potential. The pedigree analysis was performed to assess the ancestral contribution, indicating that the extensive and repetitive use of superior genotypes with common ancestors explained the narrow genetic base of released varieties (Tripathi and Katiyar 2018). It inadvertently resulted in lentil lacking seedling vigour, a very high rate of flower drops and low pod setting, a lack of lodging resistance, and vulnerability to biotic or abiotic stresses (Erskine *et al.* 2009). Domestication and breeding have resulted in the loss of adaptive alleles and fixation of deleterious alleles through altered selection, reducing the fitness of modern cultivars across different environments (Warschefskey *et al.* 2014). Further, the repetitive use in cultivating a few genetically uniform varieties makes them vulnerable to pests and diseases, resulting in a considerable yield gap between actual and potential yields.

In lentil, the existing variability among indigenous germplasm has been exploited to reach the present productivity level. Therefore, to make a breakthrough for enhancing genetic gain, increasing the current status for yield and improving stability, a new variable source must be tapped, developed, and incorporated into the Indian germplasm to widen the genetic base of advanced breeding lines. Genetic variability is the clay of evolution, providing the base material on which adaptation and speciation can be created both by nature and human intervention; however, the chances of getting natural variants greatly depend upon the nature of the crop. Lentil is a highly self-pollinated crop, thus having a minimal possibility of new recombinant variants; therefore, natural mutants have the only option to get variability (Punia *et al.* 2014). Spontaneous mutants can play a crucial role in broadening the genetic base in lentil if they are variable for important economic yield-contributing traits. Although the natural occurrence of mutants is low due to a low mutation rate ( $10^{-5}$  to  $10^{-8}$ ) in higher plants, an extensive germplasm collection is still available during long evolutionary history. A spontaneous mutation may result from the activity of mobile genetic elements (transposons) that can move around to different sites within the genome of a cell and affect the action of the gene in which they are inserted (Wessler 2006). Classical and molecular genetic studies have shown various mechanisms which generate a natural variation. The possible causes may be point mutations, intragenic recombination, unequal crossing over, transposable elements, DNA methylation, paramutation, gene amplification and other irregularities (Rasmusson and Phillips 1997).

Several spontaneous mutations have been found significant for developing erect-growing type field pea varieties in some countries (Redden *et al.* 2005). Similarly, Ladizinsky (1997) isolated two spontaneous dwarf mutations (genes *dfl* and *df2*) from a segregating population of lentil. Early maturing mutants have the potential to generate varieties or hybrids with better adaptability (Sharma and Sharma 1978, Sharma and Sharma 1980). Therefore, identifying natural variants in crops like lentils can be rewarding in addressing breeding objectives. Lentil variety RKL 58F-3715, also known as Kota Masoor 4 (KM 4), has been developed from hypervariable lines generated from the progenies of a single plant natural variant isolated from the DPL 62 cultivar. During the generation advancement of the natural mutant, a wide range of variation was

seen for seed morphological traits. The progressive generations of the natural mutant were advanced following selection and advancement as per the pedigree method and modern single-seed descent methods. Rigorous screening for grain yield and adaptability for prevailing biotic or abiotic challenges narrowed down the genetic stock for developing a set of diverse lines, of which genotype RKL 58F-3715 was promoted for cultivar identification. The present case shows the potential of a chance variant to become a variety for cultivation in the conserved moisture conditions of central India.

## MATERIALS AND METHODS

### *Identification of natural mutants and handling of their segregating generations*

The breeding program was started with a single plant identified from the commercial population of prominent lentil variety DPL 62 (Sheri) during *rabi* 2007–08 at the Agricultural Research Station, Agriculture University, Kota, Rajasthan. The handling of natural mutant progenies and the breeding strategy used for year-wise advancement for improvement and development of variety KM 4 are given in Figure 1. The hypervariable progenies were initially advanced (*rabi* 2008–09 to 2011–12) by single plant progeny rows (pedigree) and used pedigree and SSD methods simultaneously in later stages. The extensive extent of genetic variation showed in each generation of advancement for different qualitative and quantitative traits (Figures 2 and 3).

Different progenies of a single plant isolated from a commercial population of DPL 62 were examined in the field and the lab for various agromorphological and seed traits at every season of their advancement. In *rabi* 2009–10, ten single plant seeds were sown as a plant to progeny rows, and space seeded the remaining bulked seed for critical evaluation. Based on evaluating different agromorphological traits under open field and seed characteristics in lab conditions, progenies were categorized into seven groups named A to G (Figure 2). After evaluation for different seed traits, *viz.*, cotyledon colour ground colour, seed coat pattern, seed size, and seed weight, all the single plants of other groups were sown in single plant progenies and critically evaluated in the field. The progenies selected from group F were advanced by adopting pedigree and single seed descent (SSD) methods. The remaining groups (A, B, C, D, E, and G) were advanced using the pedigree method. The SSD

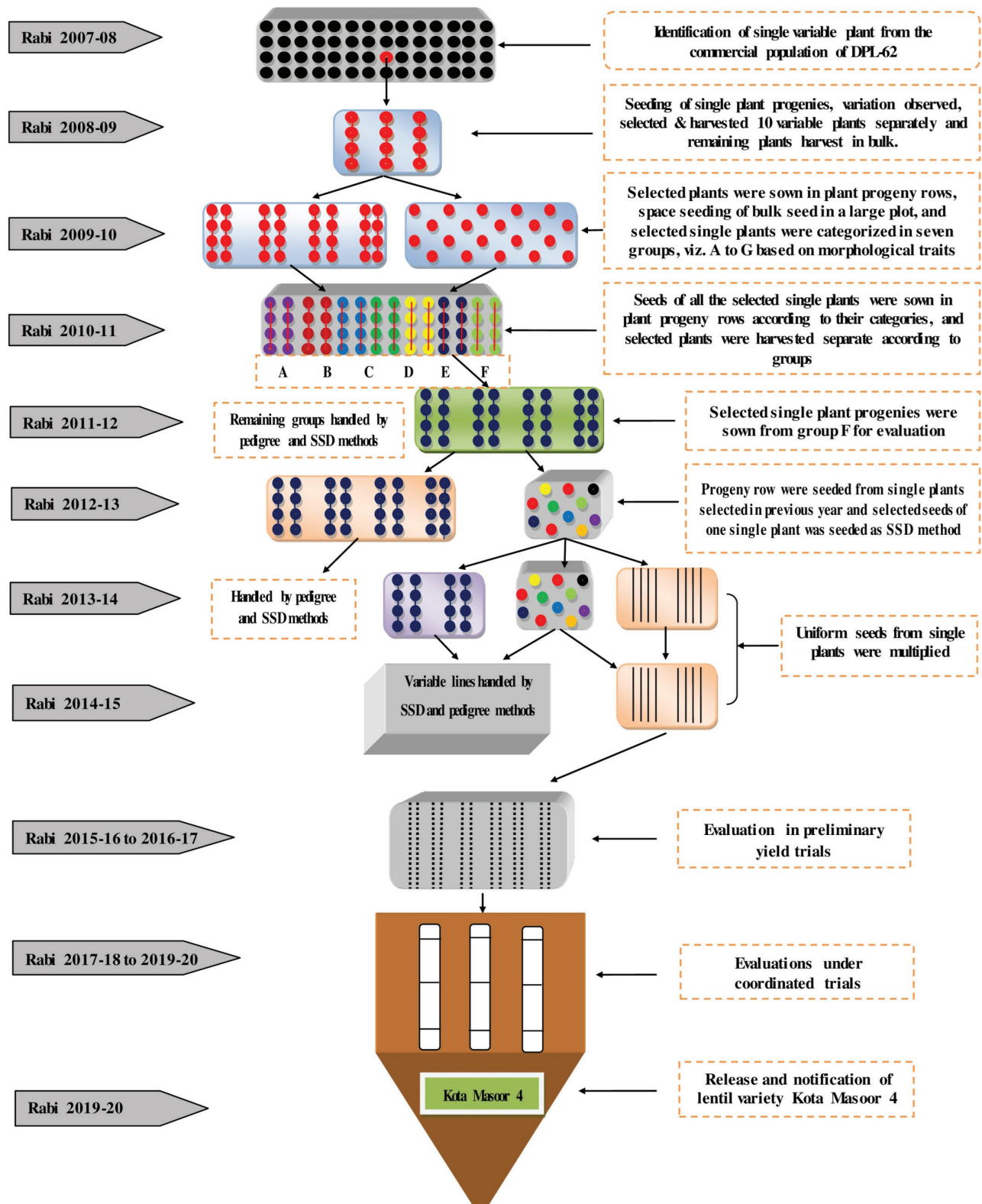


Fig. 1. The breeding strategy used for year-wise advancement of plant progenies to develop a variety RKL 58F-3715 (KM 4).



Fig. 2. Agro-morphological and seed traits of different groups (A to G) categories during rabi 2009-10.

method was applied to those progenies where the extent of genetic variation was enormous for seed traits and other agro-morphological traits (Rabi 2012-13).

Single plant seeds of progeny (RKL 58F-S3-SP7) selected from progenies of a single plant (RKL 58F-S3) showed variations for different seed traits, viz., seed size, seed coat pattern, ground colour,

and cotyledon colour (Figure 3) and advanced by SSD method, where medium seed size was used for planting with orange cotyledon color and spotted seed coat pattern during *rabi* 2012-13. The progeny of these seeds showed uniformity for the traits mentioned above and for agro-morphological characteristics. Hence, seeds were multiplied in separate blocks for the next two years (*rabi* 2013-14 to 2014-15) to get sufficient seeds for further experiments.

#### ***Stability and performance test at multi-locations***

Based on the yield test, reaction to diseases and other critical agro-morphological traits in Preliminary Yield Trials (PYT) for two conjugative years (*Rabi* 2015-16 to 2016-17), the most promising homozygous uniform lines, including KM 4, were entered into different varietal yield trials for first-year (*Rabi* 2017-18) for testing yield potential under ICAR-All India Coordinated Research Project on MULLaRP (Mungbean, Urdbean, Lentil, Lathyrus, Rajmash and Fieldpea) at different locations. Test variety (KM 4) was found promising with more than 10% increased yield under Initial Varietal Trial (IVT) and so promoted for rigorous testing for yield stability, resistance for major disease and insect pests and other agro-morphological traits under Advance Varietal Trials (AVT 1 and AVT 2) for two years at different locations of the central zone of India.

In IVT and AVTs, a randomised block design was used for varietal testing at all the locations. The mean averaged over locations for different traits has been given only for KM 4 and check varieties to interpret the results in this article. However, several other varieties were also tested in the coordinated trials.

#### ***Screening against major diseases and insect pests***

Resistance against different diseases (rust and wilt) and insect pests (pod borer and aphid) was tested at several locations in the country under coordinated trials (Tables 3 and 4). Damage caused by pod borer was recorded as a percentage of pod damage, and aphid infestation was recorded by counting the number of aphids present on the 10 cm top shoot. The standard inoculation and post-inoculation procedures and practices were followed to generate comparable information (Nagarajan *et al.* 1986).

#### ***Statistical analysis***

The data obtained from various locations were

tabulated and statistically analysed using the RBD (Randomized Block Design) method. The software SPSS was used to perform the one-way ANOVA (analysis of variance). Tukey's range test was followed to compare the mean values of different treatments. The tables present the new variety of KM 4's mean values and checks. Values followed by letters in a row are significantly different ( $p < 0.05$ ) using Tukey's range test.

## **RESULTS AND DISCUSSION**

### ***Hypervariable progenies obtained from natural mutant***

The identified variable single plant was distinguished in morphological traits such as earliness, light foliage colour and prominent tendrils from the parent variety DPL 62. Interestingly and fortunately, the progenies of the single isolated plant showed segregation in the first generation (*rabi* 2008-09) for different agro-morphological and seed traits, *viz.*, foliage colour, maturity, tendrils, leaflet shape, plant height, seed size, seed coat pattern, cotyledon colour, seed ground colour etc. Progenies of this single plant showed continuous variation in each generation of their advancement for different agro-morphological and seed traits up to *Rabi* 2020-21 (Figures 2 and 3). Over 80% of progenies reach uniformity for different characteristics, and the remaining progenies still show segregation for different traits.

The harvested seeds of individual progenies of different groups showed wide variation for different seed traits, *viz.*, seed size, seed coat colour, seed coat pattern, cotyledon colour and 100-seed weight (Figure 2). Groups A and B comprised early maturing lines (90-95 days), and other traits like foliage colour and seed traits showed variation. On the other hand, late-maturing progenies were grouped under D, F and G with variable seed traits. Extra-large seed (>5.0 g/100 seed) progenies are categorized into groups C, D, E, F, and G, with late and medium maturity. Based on evaluations for two years, 2010-11 and 2011-12, group F was the most variable group for different seed traits and yield components.

To perceive the genetics of different seed traits in progenies of group F in further generations, progenies were advanced using the pedigree and single seed descent methods. Here, we dispense the results of different progenies of single plant progeny RKL 58F-S3-SP7 advanced with the SSD

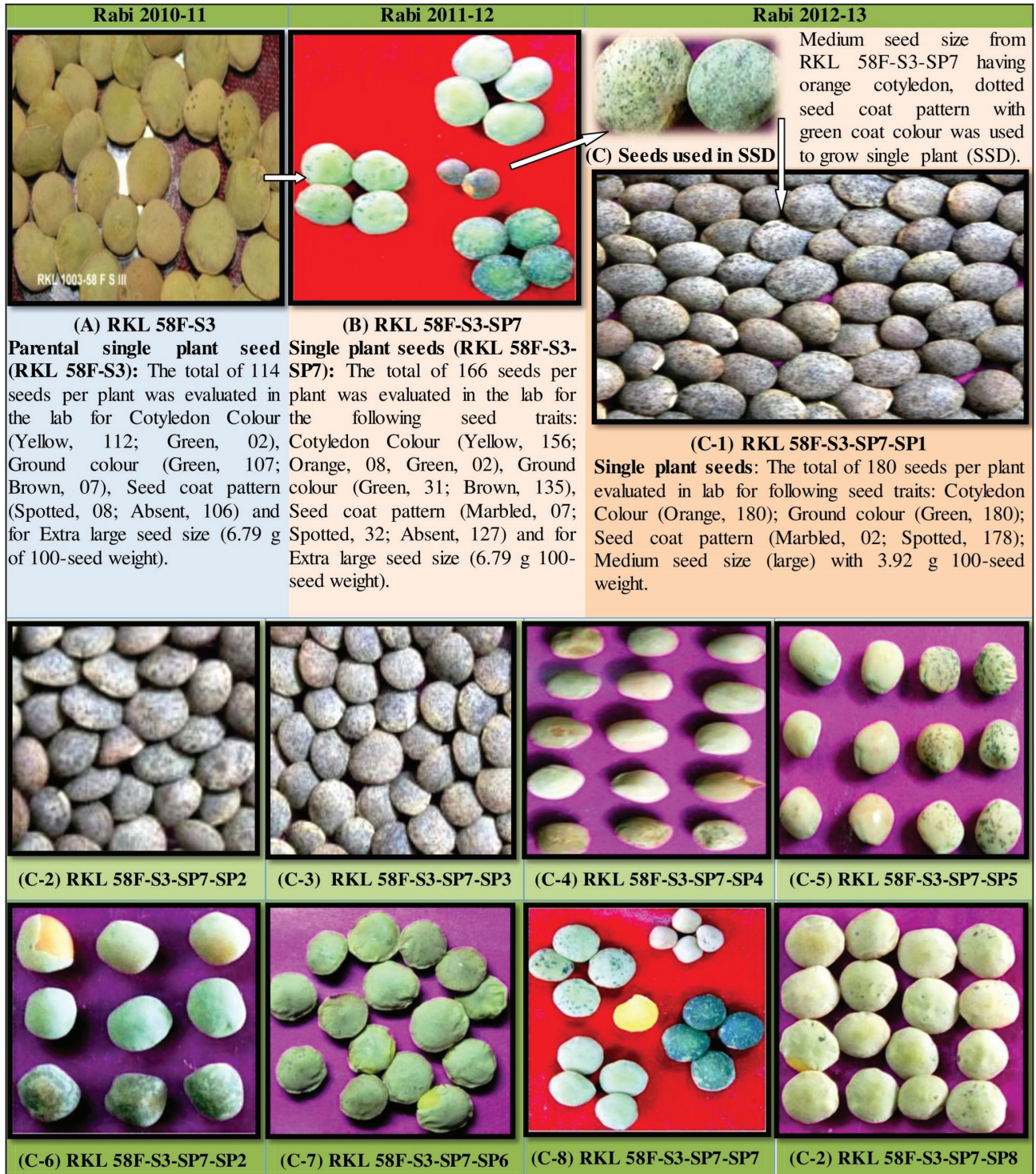


Fig. 3. (A to C) Handling of single plant progenies of selections during rabi 2010-11 to 2012-13, (C-1) identified prominent selection which further turns to KM4, (C-2 to C-9) depicting seed variation shown by other selections of 58F-S3-SP7.

method. The different seed traits of progeny RKL 58F-S3-SP7 are presented in Figure 3. This progeny has an extra-large seed (6.43 g/100 seed). Out of 166, the majority of seeds (127 seeds) showed an

absence of seed coat pattern, yellow cotyledon (156 seeds), and brown seed ground colour (135 seeds), except only eight seeds having orange cotyledon, dotted seed coat pattern and medium seed size. All

the categories above said single plant seeds were advanced with the SSD method during *Rabi* 2012-13. The categories' progeny showed uniformity for different agro-morphological and seed traits, as shown in Figure 3.

The progeny of RKL 58F-S3-SP7-SP1 have uniform seeds with orange cotyledon, green ground colour, and spotted seed coat pattern with medium seed size, whereas other categories of seeds communicated genetic instability and produced variable progenies for different traits. The other progenies of group RKL 58F and their sub-groups of RKL 58F-S1 and RKL 58F-S2 were advanced with pedigree. The SSD method also showed wide variability for different morphological and seed traits, as shown in Figure 3. The invariable progenies of single plant RKL 58F-S3-SP7-SP1 (KM 4) were multiplied in separate blocks to achieve sufficient seed for further evaluations during *rabi* 2013-14 to 2014-15.

The harvested seeds of individual progenies of different groups showed wide variation for various seed traits, viz., seed size, seed coat colour, seed coat pattern, cotyledon colour and 100-seed weight.

In the case of lentil, very few cases of spontaneous mutations were reported. A wide range of variations was observed in progenies of single plant selection. These variable genotypes were named Hyper Variable Spontaneously Generated Plants (HVSGP) and are the source of new alleles for economically important traits (Punia *et al.* 2014). In another case, a recessive mutation was identified for green cotyledon colour and the absence of pigment on the testa in lentils (Milczak 1990).

#### *Yield superiority and adaptability and agro-morphological characteristics*

Based on the yield superiority over checks and stable resistance reaction to prevalent diseases and insect pests in preliminary yield trial and station trial at ARS, Kota (Rajasthan), the genotype was proposed for Initial Varietal Trial- Large Seed (IVT-LS) and AVTs under AICRP on MULLaRP. The variety KM 4 was tested for three years (*Rabi* 2017-18 to 2019-20) across different locations in the Central Zone (CZ) of India for yield stability to explain that the weighted mean for seed yield of KM 4 was 1870 kg/ha with maturity duration of 108-115 days.

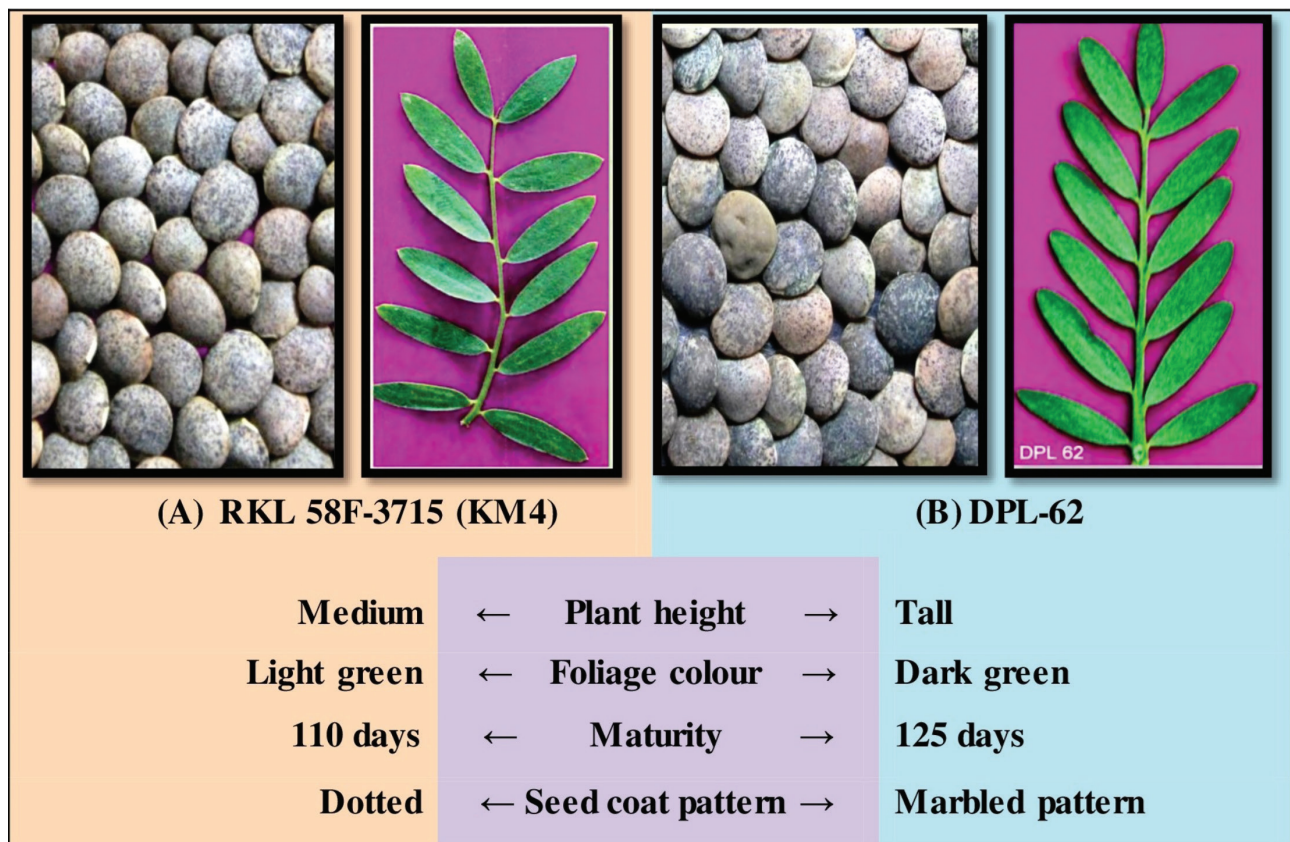


Fig. 4. Morphological characteristics of new lentil variety RKL 58F-3715 (KM4) and parent DPL-62.

**Table 1.** Weighted mean grain yield, percent superiority, and frequency data of KM 4 in coordinated varietal trials (2017-18 to 2019-20)

Particulars	Year	No. of trials/ Locations	New variety	Check 1	Check 2	Check 3	Check 4
			KM 4	L 4076	DPL 62	JL 3	IPL 316
Mean yield (q/ ha)(Zonal)	IVT (2017-18)	7	1962	1718	1595	1685	1570
	AVT 1 (2018-19)	6	1785	1593	1496	1560	1598
	AVT 2 (2019-20)	8	1847	1679	1465	1593	1607
Weighted Mean yield (kg/ha)		21	1870	1666	1523	1616	1591
Percentage increase or decrease over the checks	IVT (2017-18)	7	-	(+)14.20	(+)23.00	(+)16.44	(+)24.97
	AVT 1 (2018-19)	6	-	(+)12.06	(+)19.32	(+)14.42	(+)11.71
	AVT 2 (2019-20)	8	-	(+)10.01	(+)26.08	(+)15.95	(+)14.94
Weighted mean percent superiority		21		(+)12.09	(+)22.80	(+)15.61	(+)17.21

**Table 2.** Location-wise grain yield (kg/ha) of new lentil variety RKL 58F-3715 (KM 4) along with checks in Coordinated Varietal Trials (2017-18 to 2019-20)

Year	Trial	Name of locations	New Variety	Check 1	Check 2	Check 3	Check 4	General mean of locations	CV%
			KM 4	L 4076	DPL 62	JL 3	IPL 316		
2017-18	IVT (Large seed)	Raipur	1293	1199	990	1347	1072	1180	14.1
		Sehore	1486	1257	1160	1220	995	1224	8.7
		Sagar	2777	2292	2125	1993	1896	2217	8.5
		Kota	1714	1653	1420	1018	1565	1474	10.7
		Chitrakoot	2885	2555	2411	3154	2176	2636	3.9
		Banda	1961	1971	1764	2033	1620	1870	11.8
		Bhatapara	1615	1102	1297	1028	1669	1342	5.5
		Zonal mean	1962	1718	1595	1685	1570	1706	
% Increase or decrease			-	(+)14.20	(+)23.00	(+)16.44	(+)24.97	(+)15.00	
2018-19	AVT 1 (Large seed)	Raipur	2233	2078	1499	2257	2058	2025	7.4
		Sehore	1414	1138	1095	1416	1160	1245	8.8
		Sagar	1722	1396	1580	1198	1563	1492	12.0
		Kota	2235	2008	1720	1268	1948	1836	11.2
		Chitrakoot	1571	1552	1297	1662	1272	1471	5.1
		Banda	1533	1389	1786	1562	1587	1571	12.2
		Zonal mean	1785	1593	1496	1560	1598	1606	
		% Increase or decrease			-	(+)12.06	(+)19.32	(+)14.42	(+)11.71
2019-20	AVT 2 (Large seed)	Raipur	1476	1237	805	1090	914	1104	8.3
		Sehore	1815	1672	1389	2198	1612	1737	5.1
		Sagar	2429	2098	1973	1834	2170	2101	10.8
		Kota	1968	1758	1554	1191	1739	1642	7.5
		Chitrakoot	1798	1642	2023	2183	1854	1900	5.8
		Banda	1594	1666	1045	1060	1355	1344	13.6
		Jabalpur	690	637	799	650	787	713	19.4
		Indore	526	336	341	638	323	433	3.7
Zonal mean	1847	1679	1465	1593	1607	1638			
% Increase or decrease			-	(+)10.01	(+)26.08	(+)15.95	(+)14.94	(+)12.76	

Three years of screening in coordinated varietal trials revealed that the maximum yield superiority (22.80%) of KM-4 was obtained over its parent variety DPL 62 used as one of the national checks, followed by 17.21%, 15.61% and 12.09% over IPL 316, JL 3 and L 4076 checks, respectively (Table 1). The location-wise yield data analysis showed that in the IVT trial testing centre, Chitrakoot recorded the highest seed yield of 2885 kg/ha, followed by 2777 kg/ha at Sagar (Table 2). In AVT-1, maximum

seed yield (2235 kg/ha) was observed at the Kota location, followed by 2233 kg/ha at Raipur. Similarly, location Sagar yielded the highest seed weight (2429 kg/h), followed by Kota (1968 kg/h) in AVT-2. The variety had shown superior yield across the testing locations, indicating its greater stable performance for the Central Zone of India.

Comparative morphological features of the new mutant variety KM 4 with its parent variety

DPL 62 are present in Figure 4. It is semi-erect in growth habit with a green stem, pubescence on leaves, ovate leaflet, and rudimentary tendrils. Its higher yield is likely due to higher biomass, more primary branches (6-10 numbers), and a more significant number of pods per plant (60-150). It grows up to 40-45 cm in height, bears 50% flowering in the 65-70 days range, and requires 108-115 days to attain maturity. The flower color of this variety is purple. The 100-seed weight is about 3.28-3.56 grams, and the seeds produce orange cotyledon, dotted seed coat pattern, and brown ground color with a flat seed shape. Thus, these traits will help maintain its genetic purity and admixture from other varieties during large-scale seed production.

The role of spontaneous mutations in changing the flower morphology, specially opened types of flowers, was also reported in the lentil (Dheer *et al.* 2014) and chickpea (Srinivasan and Gaur 2012), which might be utilized in shifting the crop pollination behavior from self to cross-pollination. However, such flower openness and other major morphological variants of the flower were not observed in all our segregating progenies. The progenies of selected plant RKL 58F-S3-SP7 showed uniformity for different agro-morphological and seed traits after six to seven generations of segregations. At the same time, other categories of seeds continued to show genetic instability and produced variable progenies for different qualitative and quantitative traits. In contrast to our study, Mishra *et al.* (2022) reported that inconsistent expression of seed-coat colour was not restricted to a single plant and has persisted in the population since they studied this anomalous expression of seed-coat colour on a single plant via pod-to-pod basis. In addition, they showed anomalies in seed coat colour due to an altered anthocyanin pathway in the lentil genotype. The seed coat pigmentation in legumes occurs by depositing various flavonoids chiefly derived from anthocyanin (Yang *et al.* 2010). In addition, the presence of several mutants regulates the expression of numerous anthocyanin biosynthetic pathway genes, changing seed colour development in various crops (Quattrocchio *et al.* 2006). Unfortunately, such comprehensive studies on the seed coat colour and responsible genes for seed pigmentation must be included in lentils. Thus, the identified variety and segregants may provide evidence for discovering the key switches that efficiently regulate the enzymes, resulting in such a hypervariable expression of seed coat pigmentation.

The spontaneous mutation may induce either

or a combination of abnormal phenomena such as chromosomal aberration, point mutation, intragenic recombination, unequal crossing over, transposable elements, DNA methylation, paramutation and gene amplification (Rasmusson and Phillips 1997). However, the segregants still present even after long-term selection may not be due to the variation in the starting materials. It may result from de novo variation occurring in generations after the parent material. Lentil is a natural self-pollinated species due to its cleistogamous flowers (Wilson 1972, Kumar and Singh 1998). With less than 0.8% natural cross-pollination (Phillips and Howard, 2009), the possibility of intragenic natural recombination is negligible. Although lines may be unpromising phenotypically in the legume crop, they may contain untapped alleles or allelic combinations that could be utilized for plant breeding with adequate investment in conventional and marker-assisted selection (Ahmad and Belwal 2020). The seed coat is derived from the tissue of maternal origin, ultimately determined by the genotype of the seed-producing plant. Thus, the seed coat in any lentil plant should display only one colour. Unfortunately, the seed-coat colour was not stable and incredibly varied with segregants, which presumes the probable existence of some hyperactive transposable element regulating seed-coat colour in the lentil (Punia *et al.* 2014). The seed variations in soybean were controlled via the methylation of a large CACTA transposon from an MYB transcription factor, affecting the transposition to regulate anthocyanin synthase genes responsible for seed coat colour variation (Mishra *et al.* 2022, Zabala and Vodkin 2014). We may expect such genetic elements driven spontaneously to regulate the seed coat colour in the identified KM 4 variety, which can be tested in the future by using these natural segregants.

#### ***Resistance to major diseases and insect pest***

Data on major diseases like rust, wilt, Ascochyta blight, and stem Phyllium blight at different central zone locations were assessed under coordinated trials for three years from 2017-18 to 2019-20 (Table 3). The candidate variety KM 4 displayed an enhanced level of resistance to wilt (23.04%) compared to susceptible local check, which had 63.57% population mortality. Similarly, a mean rust score of 2.88 was observed, showing less rust incidence than the susceptible check (8.50). The average incidence of Ascochyta blight score of 5.0 was recorded in KM 4 and DPL 62. In contrast, JL 3 and IPL 316 observed the least incidence score of 3.0

**Table 3.** Reactions to major diseases of new lentil variety KM 4 at different locations during *rabi*, 2017-18 to 2019-20

Disease name	Trial (Year)	New Variety	Check 1	Check 2	Check 3	Check 4	Local check
		KM 4	L 4076	DPL 62	JL 3	IPL 316	
Rust (1 -9)	IVT (2017-18)	2.40	NA	2.60	4.20	3.80	8.00
	AVT 1 (2018-19)	4.00	NA	2.00	6.00	4.00	9.00
	AVT 2 (2019-20)	2.25	3.50	3.50	2.75	3.00	
	Mean	2.88	3.50	2.70	4.32	3.60	8.50
Wilt (%)	IVT (2017-18)	21.17	NA	28.27	30.28	24.49	62.17
	AVT 1 (2018-19)	22.60	NA	26.68	31.84	35.37	64.96
	AVT 2 (2019-20)	25.35	26.73	19.58	24.86	21.48	
	Mean	23.04	26.73	24.84	28.99	27.11	63.57
Ascochyta Blight (1-9)	IVT (2017-18)	5.00	NA	5.00	3.00	3.00	
Stem Phyllium Blight (1-9)	AVT 2 (2019-20)	1.00	4.00	1.00	3.00	4.00	

\*Data presented in the table are mean over locations in each year

**Table 4.** Reactions of new lentil variety KM 4 to insect pests' incidence

Insect Name	Trial (Year)	KM 4	Check 1	Check 2	Check 3	Check 4	Local /Susceptible check
			L 4076	DPL 62	JL 3	IPL 316	
Pod borer (% pod damage)	IVT (2017-18)	6.33	NA	6.16	8.34	6.64	6.94
	AVT1 (2018-19)	2.13	NA	1.72	1.09	1.44	2.97
	AVT2 (2019-20)	2.51	3.56	3.96	2.77	3.78	3.35
	Mean	3.66	3.56	3.95	4.07	3.95	4.42
No. of aphids/ 10 cm top shoot	IVT (2017-18)	0.86	NA	6.38	1.24	4.41	5.55
	AVT1 (2018-19)	13.94	NA	21.99	1.70	27.30	14.24
	AVT2 (2019-20)	2.39	3.12	4.78	5.09	1.70	3.62
	Mean	5.73	3.12	11.05	2.68	11.14	7.80

and showed medium tolerance against *Ascochyta* blight. In contrast, a minor incidence of stem *Phyllium* blight (score 1.0) was observed in KM 4, and DPL 62 showed higher resistance than the rest of the checks.

As inferred from Table 4, the pod borer and aphid incidence are other major lentil reproductive constraints. The new variety KM 4 showed the most minor pod damage (3.66%) of all the checks except L 4076 (3.56%). Similarly, in candidate variety, aphid incidence (5.73) was also observed to be lower than checks DPL 62 (11.05), IPL 316 (11.14) and local susceptible but was higher for the checks L 4076 (3.12) and JL 3 (2.68). In addition, the moderate to high resistance to disease and insect pests offered more durability, ensured optimum crop stand in the field, and was expected to give a higher yield per unit area.

It is evident that the lentil crop is prone to soil disease wilt and aerial incidence of rust, *Ascochyta* blight, and stem *Phyllium* blight due to the seasonal variations in climate leading to the intensity of the major diseases. Hence, evaluation of candidate genotype KM 4 with recommended central zone released varieties, i.e., DPL 62, JL 3, IPL 316, L 4076, screened-out good material

showed resistance to the biotic stresses. These biotic stresses have reduced lentil productivity by 20-25% (Maheshwari *et al.* 2008). In addition, sincere efforts were made to assess the promising lentil genotypes with suitable agronomic interventions like timely sowing, matching nutrients application and biotic and abiotic tolerance/resistance, etc.; technological backups help scale lentil productivity (Singh 2018).

Considering all these agronomic, disease resistance reaction parameters and based on the unbeatable performance of our candidate genotype (KM 4) in ICAR-AICRP trials, it was identified and further recommended for release by CVRC (Central Variety Release Committee). Finally, the variety with the name KM 4 was released on 29th January 2021 in the Gazette Notification S.O. No. 500(E) for rainfed conditions of the Central Zone of India, which comprises Uttar Pradesh (Bundelkhand region), Madhya Pradesh, Chhattisgarh, Gujarat, South-East Rajasthan and Maharashtra.

## CONCLUSION

The spontaneous mutation creates random genetic variations with a low frequency of desirable alleles and thus has little possibility of identification for generating favorable natural recombinants. The

lentil variety KM 4 is one of the kinds identified from the natural variation, which showed a series of hypervariable segregants during generation advancement. The HVSGP population showed unpredictable segregating patterns and variations for economically important traits like the number of pods per plant, seeds per pod, and seed size. The possible mechanism for wide variation assumed mutation at multiple alleles and the presence of transposable elements; however, the curiosity to know the exact possible reason requires a detailed molecular study. Moreover, the favorable alleles of yield contributing traits and other important characteristics are utilized and can be exploited in future lentil improvement programs. The KM 4 is one of the esteemed examples of tapping these variations, resulting from a rigorous selection. Under rainfed conditions, the recorded grain yield was 1870 kg/ha with enhanced resistance against wilt and rust diseases, ensuring economic gain under the well-managed field. Therefore, being superior in several aspects, this new variety would be popular among farmers and consumers.

#### ACKNOWLEDGEMENT

The author acknowledges the support from the technical staff of the Agriculture Research Station, Agriculture University, Kota, during the research. The author also thanks the ICAR-IIPR Kanpur for providing financial assistance over time.

#### REFERENCES

- Ahmad S and Belwal V. 2020. Morphological Characterization and Evaluation of Mungbean [*Vigna radiata* (L.) Wilczek] Germplasm for Various Yield Attributing Traits. *International Journal of Current Microbiology and Applied Science* **9**(1): 780-790.
- Dheer M, Punia SS, Ram B, Jain NK, Koli NR, Bhatnagar V and Khedar OP. 2014. Morphological features of an open flower mutant plant and characterisation of their progenies in lentil (*Lens culinaris* Medik.). *Genetic Resources and Crop Evolution* **61**: 879-886.
- Erskine W, Muehlbauer FJ, Sarker A and Sharma B. 2009. The lentil, botany, production and uses. CAB International, Pp.1-3.
- Ferguson ME, Maxted N, Slageren MV and Robertson LD. 2000. A re-assessment of the taxonomy of lens. *Botanical Journal of Linnean Society* **133**(1): 41-59.
- Kumar A and Singh DP. 1998. Hybridization technique in lentil under field conditions. *Lentil Experimental News Service* **25**: 1-3.
- Ladizinsky G. 1997. Dwarfing genes in the genus *Lens* Mill. *Theoretical and Applied Genetics* **93**: 1270-1273.
- Maheshwari SK, Bhat NA, Masoodi SD and Beig MA. 2008. Chemical control of lentil wilt caused by *Fusarium oxysporum* f. sp. lentis. *Annals of Plant Protection Sciences* **16**(2): 419-421.
- Milczak M. 1990. An interesting case of spontaneous mutation in lentil (*Lens culinaris* Medik.). *Acta Geologica Polonica* **31**: 3-4.
- Mishra GP, Aski MS, Tontang MT, Choudhary P, Tripathi K and Singh A. 2022. Morphological, molecular, and biochemical characterization of a unique lentil (*Lens culinaris* Medik.) genotype showing seed-coat color anomalies due to altered anthocyanin pathway. *Plants* **11**: 1815.
- Nagarajan S, Nayar SK, Bahadur P and Kumar J. 1986. Wheat pathology and wheat improvement. Indian Agricultural Research Institute, Regional Station, Flowerdale, Shimla-171002, 12 pp
- Phillips RL and Rines HW. 2009. Genetic analyses with oat-maize addition and radiation hybrid lines. In *Handbook of maize*. Springer, New York, NY: 523-538.
- Punia SS, Ram B, Dheer M, Jain NK, Koli NR and Khedar OP. 2014. Hyper-variable spontaneous genetic variation for earliness, seed characters and other yield-contributing traits in lentil (*Lens culinaris* Med.). *Current Science* **106**: 75-83.
- Quattrocchio F, Baudry A, Lepiniec L and Grotewold E. 2006. The regulation of flavonoid biosynthesis. In *The Science of Flavonoids*, Grotewold, E.; Springer: New York, NY, USA Pp 97-122.
- Rasmusson DC and Phillips RL. 1997. Plant breeding progress and genetic diversity from *de novo* variation and elevated epistasis. *Crop Science* **37**: 303-310.
- Rawal V and Navarro DK. 2019. The global economy of pulses. FAO Rome, Italy.
- Redden B, Lenoforte A, Ford R, Croser J and Slattery J. 2005. Pea (*Pisum sativum* L.). In *Genetic Resources, Chromosome Engineering and Crop Improvement: Grain Legumes*, CRC Press, Boca Raton **1**: 49-83.
- Sharma SK and Sharma B. 1980. Induced fasciation in lentil (*Lens culinaris* Medic.). *Genetica Agraria* **37**: 319-326.
- Sharma SK and Sharma B. 1978. Induction of male sterility in lentil. *Legume Research* **2**: 45-48.
- Singh NP. 2018. Pulses as a candidate crop for doubling farmers' income. *Indian Farming* **68**: 36-43.
- Srinivasan S and Gaur PM. 2012. Genetics and characterization of an open flower mutant in chickpea. *Journal Heredity* **103**: 297-302.
- Tripathi A and Katiyar P. 2018. Determination of ancestral relationship towards the development of advanced breeding lines of lentil (*Lens culinaris* Medic) varieties in India. *Bangladesh Journal of Botany* **47**: 165-172.

- Warschewsky E, Penmetsa RV, Cook DR and Von Wettberg EJ. 2014. Back to the wilds: tapping evolutionary adaptations for resilient crops through systematic hybridization with crop wild relatives. *American Journal of Botany* **101**: 1791-1800.
- Wessler SR. 2006. Transposable elements and the evolution of eukaryotic genomes. *The Proceedings of the National Academy of Sciences* **103**: 17600-17601.
- Wilson VE. 1972. Morphology and technique for crossing *Lens esculenta* Moench. *Crop Science* **12**: 231-232.
- Yang K, Jeong N, Moon JK, Lee YH, Lee SH, Kim HM, Hwang CH, Back K, Palmer RG and Jeong SC. 2010. Genetic analysis of genes controlling natural variation of seed coat and flower colors in soybean. *Journal of Heredity* **101**: 757-768.
- Zabala G and Vodkin LO. 2014. Methylation affects transposition and splicing of a large CACTA transposon from a MYB transcription factor regulating anthocyanin synthase genes in soybean seed coats. *PLoS One* **9**: e111959.