Hyper-variable spontaneous genetic variation for earliness, seed characters and other yield-contributing traits in lentil (*Lens culinaris* Med.)

Sumer Singh Punia[1,*], Baldev Ram[1], Meenakshi Dheer[1], Narendra Kumar Jain[1], Nanag Ram Koli[1] and Om Prakash Khedar[2]

[1]Agricultural Research Station, Ummedganj, Agriculture University, Kota 324 001, India
[2]Agricultural Research Station, Durgapura, Jaipur 302 018, India

The extent of genetic variation obtained naturally is useful in self-pollinated crops like lentil, though the variation is very low in frequency. A single plant was isolated from a commercial population of DPL-62 in 2007–08 assuming that it is a mutant of DPL-62 with earliness and other morphological traits. Interestingly, the progenies of the isolated plant showed segregation up to sixth generation (rabi 2012–13). Wide variation was observed for days to flowering (38–66), days to maturity (95–135), reproductive phase (55–80 days), plant height (35–65 cm), pods per plant (30–185), 100-seed weight (3.10 to 6.80 g), seed coat colour and other traits. Mutation at multiple loci may be the possible reason. In every generation segregation has been seen for major traits, and still the material is segregating which shows a kind of dynamic mutation. Presence of mobile genetic elements in the functional genome may also be a reason. This genetic stock can play a crucial role in understanding the genetics of the target trait and improvement of small-seed Indian lentils.

Keywords: Earliness, genetic variation, *Lens culinaris*, mutation.

LENTIL (*Lens culinaris* Medikus ssp. *culinaris*) is an important food legume of the Indian subcontinent, the Middle East, northern Africa and North America. India produces more than 1 million tonnes (mt) of lentil from an area around 1.6 m ha. The Indian productivity is about 25% lower than the global average (1053 kg/ha). Most of the lentil varieties in India have been developed mainly through pureline selection and intraspecific hybridization. A pedigree analysis of 35 varieties in lentil showed involvement of only 22 ancestors for varietal development, in which the top 10 parents contributed about 30% to the genetic base of released varieties[1]. This has inadvertently led to the narrowing down of genetic base, and has resulted in making lentil vulnerable to biotic or abiotic stresses. Normally in breeding programmes we prefer to select parent with high mean performance for major traits and further in recombination breeding high performing genotypes. The genetic gain by utilizing these good × good crosses appears to be high, and improvements are sufficient to encourage continued breeding within narrow gene pools even though each cycle is expected to lead to reduced genetic variability by performing selection only in one direction[2], i.e. for yield and yield-contributing traits. This has gradually limited the amount of diversity by ignoring introduction of new allelic combinations (because of poor performance for target traits) into the breeding gene pool. In lentil the existing variability among indigenous germplasm has been exploited to reach the present productivity level. It can be exploited further. Therefore, to make a breakthrough in the existing status for yield and for improving stability, new variable source needs to be tapped, developed and incorporated in the Indian germplasm.

Genetic variation can be created both by nature and human intervention. The chance of getting natural variants depends upon the nature of the crop. Lentil is a highly self-pollinated crop with negligible or no cross-pollination. Therefore, the possibility of getting recombinant variables is low; the only option is to get natural mutants. Spontaneous mutants can be useful in lentil if they are variable for major yield-contributing traits. Natural occurrence of mutants is low due to a low mutation rate. Classical and molecular genetic studies have shown various mechanisms which generate natural variation. The possible causes are point mutations, intragenic recombination, unequal crossing over, transposable elements, DNA methylation, paramutation, gene amplification, or by other means[2]. In case of lentil, till date only one case of spontaneous recessive mutation is reported for green cotyledon colour and absence of pigment on the testa[3].

Here we report spontaneous dynamic mutation for multiple economically important traits in lentil. The present study shows generation of incremental genetic variability from a single plant over generations compared to the parental traits. The hyper variable population is yet to be confirmed for genomic reasons for showing this kind of variation, but it has provided a genetic stock with new allelic combination for strengthening the ongoing lentil improvement programmes.

The experiment is being conducted at the Agricultural Research Station, Kota, Rajasthan, starting from rabi 2007–08. This experiment was started with a single

Table 1. Morphological characteristics of lentil variety DPL-62 and selected single plant

Morphological traits	DPL-62	Selected single plant
Maturity	125 days	110 days
Tendrils	Absent	Present
Foliage colour	Dark green	Light green
Plant height	Tall	Medium
Seed coat pattern	Marbled pattern	Absent

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*For correspondence. (e-mail: sumerspunia@hotmail.com)

Box 1. Flow chart: Advancement of progenies of single spontaneous mutant plant

First year (rabi 2007–2008)

(i) Identification of single plant from the commercial population of DPL-62.

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Second year (rabi 2008–2009)

(i) Seeding of three rows of 4 m from selected plant.
(ii) Variation in respect to different morpho-physiological traits were observed.
(iii) Selection of ten single plants on the basis of morpho-physiological traits, viz. maturity, leaflet size, plant height, foliage colour, tendrilled or non-tendrilled, etc.
(iv) Remaining plants harvested in bulk.

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Third year (rabi 2009–2010)

(i) Selected plants were sown in plant progeny rows of 4 m.
(ii) Space seeding of bulk seed in large plot.
(iii) All the single plant progeny rows and space seeding populations were critically evaluated for morphological traits.
(iv) Selections were made in both populations and selected single plants (80 plants) were categorized in seven groups, viz. A–G on the basis of morphological traits (Table 2).

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Fourth year (rabi 2010–2011)

(i) Seeds of all the selected single plants were sown in plant progeny rows according to their categories.
(ii) Critical examination of all the categories between and within for morphological traits.
(iii) Observations were taken on seed traits, viz. size, seed coat colour and pattern, etc. in the laboratory after harvesting.
(iv) Single plants were again selected from those lines which show segregation while few lines were harvested in bulk which show uniformity.

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Fifth year (rabi 2011–2012)

(i) Selected individual plants were again sown in plant to progeny row method.
(ii) Seeds of those lines (113 lines, including parent variety DPL-62) which show uniformity for morphological traits were sown in replicated trials.
(iii) The observations were recorded on five plants from each line in each replication for qualitative and quantitative traits.
(iv) The selection was again made in progenies/lines which show variation resulting in more than 2000 individual plants being selected from different progenies/lines for further evaluations.
(v) Seeds of selected individual plant were evaluated for seed coat colour, pattern and size.

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Sixth year (rabi 2012–2013)

(i) Selected individual plants in previous year were again sown in plant progeny rows.
(ii) Seeds of single plants which showed very high variation for different seed traits, viz. seed size, cotyledon colour, seed coat colour and seed coat pattern were grown by dibbling in single seed descent method for further investigation of trait inheritance.
(iii) The qualitative, quantative and molecular analysis of different traits is ongoing to find out the cause of such huge explosions of genetic variability.

Table 2. Grouping of selected plants on the basis of morphological traits during rabi 2009–2010

Group	Morphological traits
RKL 1003-A	Very early in maturity (90–95 days), tall, erect, dull green foliage, hairy leaves, large seed
RKL 1003-1B	Dwarf, dark green foliage, rudimentary tendrils, early in maturity (90–95 days)
RKL 1003-2B	Dwarf, large seed, dark green foliage, tendrils absent, early in maturity (90–95 days)
RKL 1003-C	Tall, good biomass, large seed (>5.0 g/100) greenish, pinkish and brownish in colour, light green foliage, non-hairy leaves, tendrils present, medium in maturity
RKL 1003-D	Medium height, late in maturity (110–125 days), light green foliage, large seed different in testa colour and pattern
RKL 1003-E	Large seed (>5.0 g/100) brownish in colour, tall, small leaves, prominent tendrils present, early in flowering
RKL 1003-F	Extra large seed (>6.0 g/100) creamish in colour, tall, more branching, late in flowering, light green foliage
RKL 1003-G	Extra large seed, different colour of seed, maturity ranges from 110 to 135 days

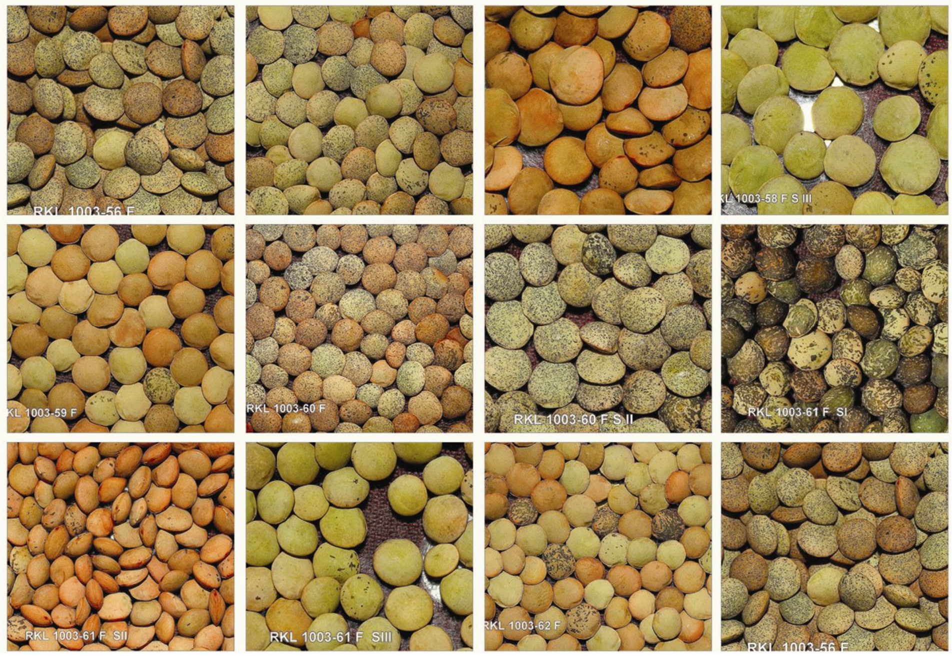


Figure 1. Variation in seed size, colour and pattern of different mutant progenies of group RKL 1003F.

Figure 2. Variation in leaves of different individual mutant progenies in comparison to parent DPL-62.

plant identified from the commercial population of prominent lentil variety DPL-62 (Sheri) during rabi 2007–08. This single plant was selected on the basis of earliness, light foliage colour and prominent tendrils that distinguish the parent variety. The characteristics of the variety and selected single plant are presented in Table 1. The year-wise advancement of single plant progenies and breeding methods are given in flow chart (Box 1).

Progenies of a single plant isolated from a commercial population of DPL-62 were examined during rabi 2008–09 to 2011–12. Interestingly, the progenies of the isolated plant showed segregation in the first generation (rabi 2008–09) for maturity and seed traits. The population size was small, but the extent of variation due to genetic instability was large for traits, viz. seed coat colour, seed coat pattern, seed size and other agro-morphological traits (Figures 1 and 2). Because of the pleiotropic mutations in single gene which determine several distinct and seemingly unrelated traits, we hypothesize that a genetic instability occurred and spontaneously generated diverse progenies for every generation of advancement. Presently (rabi 2012–13) the collection includes more than 2000 variants for all the traits mentioned above. Plants with different kinds of morphological appearance were also observed in different generations. On account of the range of variations observed, these genotypes have been named as ‘hyper variable spontaneously generated plants (HVSGP)’, and they are the source of new alleles for economical traits (Figure 3).

The population has reached the sixth generation (rabi 2012–13) and harvest from single plants is being carried forward by pedigree method; the level of diversity present in the whole set of progenies of the single plant is very high. The frequency distribution for different descriptors in these variant progenies was observed during rabi 2011–12 (Table 3). Most progenies/lines belonged to large (86) and extra-large seeded (19) lentil group with yellow cotyledon colour (85). The range of expression for different descriptors was high and found to be unstable generation after generation in many progenies. The range of variation observed for seed weight was 3.1–6.8 g/100

Figure 3. High-yielding mutant progenies with more number of pods per plant and pods per peduncle.

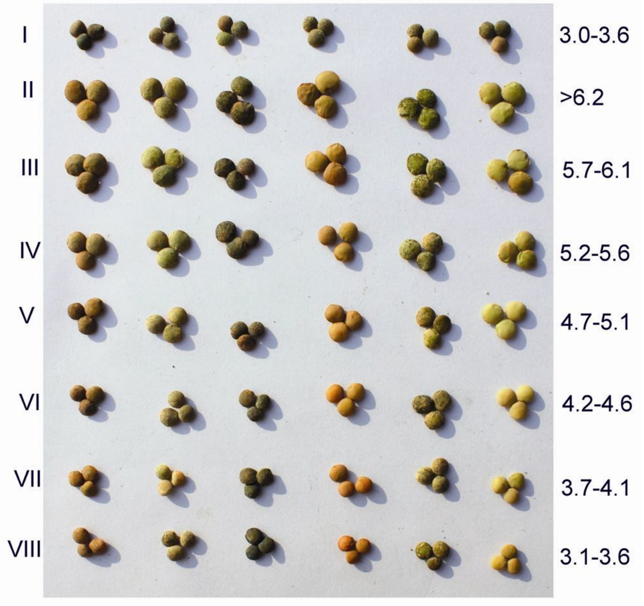


Figure 4. 100-seed weight (g), seed coat colour and pattern among spontaneous mutant progenies. Row I, Seeds of parent variety DPL-62. Rows II–VIII, Range of 100-seed weight in decreasing order. Seed coat colour and pattern left to right from rows II to VIII: Brown with dotted, green with dotted, brown with marbled, tan with absent, grey with marbled and green with absent.

seeds. The seed coat colour and their patterns varied (Figure 4). Interestingly, all the three classes of cotyledon colour, namely olive-green, yellow and orange-red exists in the HVSGP population.

The population also varies for earliness and other agro-morphological traits (Table 4). Wide variation was observed for days to flowering (38 to 66 days) and maturity (95 to 135 days) in variant progenies compared to the parent variety DPL-62. Lentil varieties released in India have about 50–60 days of reproductive period (time from flowering to maturity). The parent variety DPL-62 has a reproductive phase of 51 days, but in the HVSGP population a range of about 55–80 days of reproductive phase was observed. The economically important traits like seed size, number of pods per plant and seeds per pod are directly correlated with the reproductive period of the crop. Therefore, seed size and number of pods per plant are more in variant progenies and alleles for this trait can be exploited in future lentil improvement programmes. Spontaneous mutation has been utilized in domestication of narrow-leafed lupin (*Lupinus angustifolius* L.). As a result of domestication, lupin has become a dominant grain legume crop in Western Australia[4]. The other economic important traits, viz. plant height, branches per plant, pods per plant, seeds per pod and number of pods per peduncle also showed a wide range of expression with respect to DPL-62. In brief, it can be said that the small population is representative of the maximum diversity found in cultivated lentils.

This kind of spontaneous occurrence of variation may have happened via many pathways. The variation absent in the parent but present in the progenies can be due to point mutations, intragenic recombination, unequal crossing over, transposable elements, DNA methylation, paramutation, gene amplification and other means[2]. The variation that is still present in long-term selection experiments may not be due to the variation present in the starting materials, but it may be the result of *de novo* variation occurring in generations subsequent to the parent material. A basic cytogenetic comparison (chromosome number and karyotype) of the ten most variable mutant lines with the parent was made by cytological studies of germinated seed root tips. The results indicate no significant difference regarding number and structure of chromosomes in mutant lines and parent. This shows that variation in mutant progenies is not due to change in number and structural changes in chromosomes (Figure 5).

Lentil is a naturally self-pollinated species due to its cleistogamous flowers[5,6]. With less than 0.8% natural cross-pollination[7], the possibility of intragenic natural recombination is negligible. The next possibility may be a point mutation, but this is also not considerable due to multiple segregations for different traits up to the fifth generation. This dynamic, spontaneous genetic variation occurring in every generation forces us to consider the presence of some mobile elements, including the mutator system in the gene blocks which are responsible for the explosion of such HVSGP population. The introduction or activation of transposable elements of various sorts provides the possibility of altering genes to generate phenotypically detectable mutants (forward genetics). The presence of such elements in a gene allows correlating these changes in a given genetic sequence with a specific phenotype (reverse genetics). Fortunately, many genetic stocks have been produced in a variety of organisms to make this a robust technology[8]. For example, in rice there are about 50,000 lines with Tos17 insertions produced at the National Institute of Agrobiological Resources, Japan (http://tos.nias.affrc.go.jp). In addition, about 150,000 lines of rice possess Ac/Ds, enhancer traps, T-DNA, and activation tags which have been produced in Korea, Australia, China, Taiwan, France, Singapore, The Netherlands, and USA. Perhaps a million tagged sites are available in japonica rice[9].

Table 3. Frequency distribution for different descriptors in different spontaneous mutant progenies (mean of five plants) recorded during rabi 2011–2012

Plant descriptors	Range of expression	Number of genotypes (% of total population)
Time of flowering	Early	47 (41.60)
	Medium	29 (25.65)
	Late	37 (32.75)
Intensity of foliage green colour	Light	91 (80.53)
	Medium	05 (04.43)
	Dark	17 (15.04)
Leaflet size	Small	26 (23.00)
	Medium	38 (33.63)
	Large	49 (43.37)
Growth habit	Erect	25 (22.13)
	Semi-erect	88 (77.87)
	Spreading	00 (00.00)
Plant height	Short	04 (03.54)
	Medium	31 (27.43)
	Long	78 (69.03)
Peduncle length	Short	00 (00.00)
	Medium	32 (28.32)
	Large	81 (71.68)
Leaf tendril type	Rudimentary	42 (37.17)
	Prominent	71 (62.83)
Flower colour of standard	White	14 (12.39)
	Blue	99 (87.61)
Seed width	Narrow	00 (00.00)
	Medium	04 (03.54)
	Broad	86 (76.10)
	Very broad	23 (20.36)
Seed profile in longitudinal	Elliptic	06 (05.31)
	Broad elliptic	107 (94.69)
Seed coat colour	Green	39 (34.51)
	Grey	00 (00.00)
	Pink	52 (46.02)
	Brown	17 (15.04)
	Black	05 (04.43)
	Absent	25 (22.13)
Seed coat pattern	Dotted	15 (13.27)
	Spotted	56 (49.56)
	Marbled	17 (15.04)
	Complex	00 (00.00)
Seed: cotyledon colour	Yellow	85 (75.22)
	Orange-red	26 (23.01)
	Olive-green	02 (01.77)
Seed size	Small	00 (00.00)
	Medium	08 (07.08)
	Large	86 (76.11)
	Extra large	19 (16.81)

Seeds of selected individual plants were also examined just after threshing for different seed traits, viz. seed size, seed coat colour, seed coat pattern and cotyledon colour (Figure 6). Here we present only data of ten single plants which were selected from line RKL 1003-61F S2. The yield, 100-seed weight and frequency distribution of different seed traits like cotyledon colour, seed coat colour and pattern of these lines are presented in Table 5. Photographs of seeds of these selected individual plants and their parent line are shown in Figure 7. Seeds of individual plants showed wide expression for different seed traits and seeds from single pod (Figure 6) also showed variation in seed coat colour and colour pattern even after fifth generation of their advancement. The seed size varied from 1 to 9 g/100-seed weight within the seeds of individual plants, indicating that plant bears both

Table 4. Range for different quantitative traits in spontaneous mutant progenies observed during rabi 2011–2012

Traits	Range		Parent (DPL-62) mean
	Minimum	Maximum	
Days to 50% flowering	38	66	72
Days to maturity	95	135	123
Days from flowering to maturity	55	80	51
Plant height (cm)	35	65	38
Primary branches per plant	2	6	2.6
No. of pods per plant	30	185	43
No. of seeds per pod	01	2.5	1–2
No. of pods per peduncle	01	05	1–2
First pod height (cm)	4.8	18.90	17.8
Internode space (cm)	1.78	3.52	2.86
100-seed weight (g)	3.10	6.80	3.34
Seed yield per plant (g)	2.25	8.46	3.18

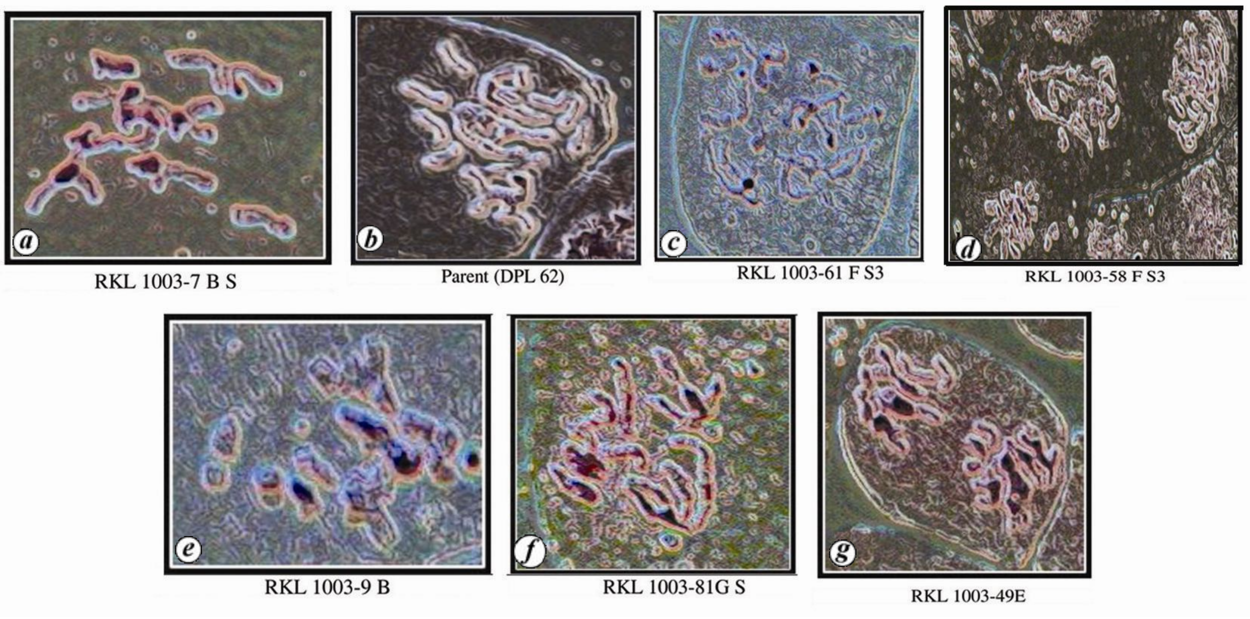


Figure 5. Cytological comparisons of mutant lines and parent. ***a***, Late metaphase of line RKL 1003-7 BS; ***b***, Late metaphase of parent (DPL-62); ***c***, Late metaphase of line RKL 1003-61 F S3; ***d***, Early anaphase of line RKL 1003-58 F S3; ***e***, Late metaphase of line RKL 1003-9 B; ***f***, Early metaphase of line RKL 1003-81G S; ***g***, Late anaphase of line RKL 1003-49E.

Figure 6. Photographs showing a wide diversity for seed size, cotyledon colour, seed coat colour and seed coat pattern among seeds of individual plants selected from different lines. ***a***, Parent variety (DPL-62) seed. ***b–o***, Individual plant seeds. ***p***, Single pod seed.

microsperma and *macrosperma* group of lentil seeds. The number of *microsperma* lentil seeds is less (1 to 8) compared to *macrosperma*. All the *microsperma* lentil seeds mostly showed dotted coat colour pattern and had orange coloured cotyledons, whereas large seeds (*macrosperma*) had different testa colour pattern with yellow cotyledon colour. Interestingly, the phenotypic expressions of these small seeds are similar to Indian lentils in foliage colour,

Figure 7. Seeds of different single plant progenies of line RKL 1003-61F-S2 showing wide variation for seed size, cotyledon colour, seed coat colour and pattern. ***a***, Seeds of SP_{10}; ***b***, Seeds of SP_{15}; ***c***, Seeds of SP_3; ***d***, Seeds of SP_8; ***e***, Seeds of SP_5; ***f***, Seeds of SP_2; ***g***, Parent line seed; ***h***, Seeds of SP_{18}; ***i***, Seeds of SP_6; ***j***, Seeds of SP_{20}.

Table 5. Yield, 100-seed weight and frequency distribution of different seed traits of individual plants selected from line RKL 1003-61F-S2 during rabi 2011–13

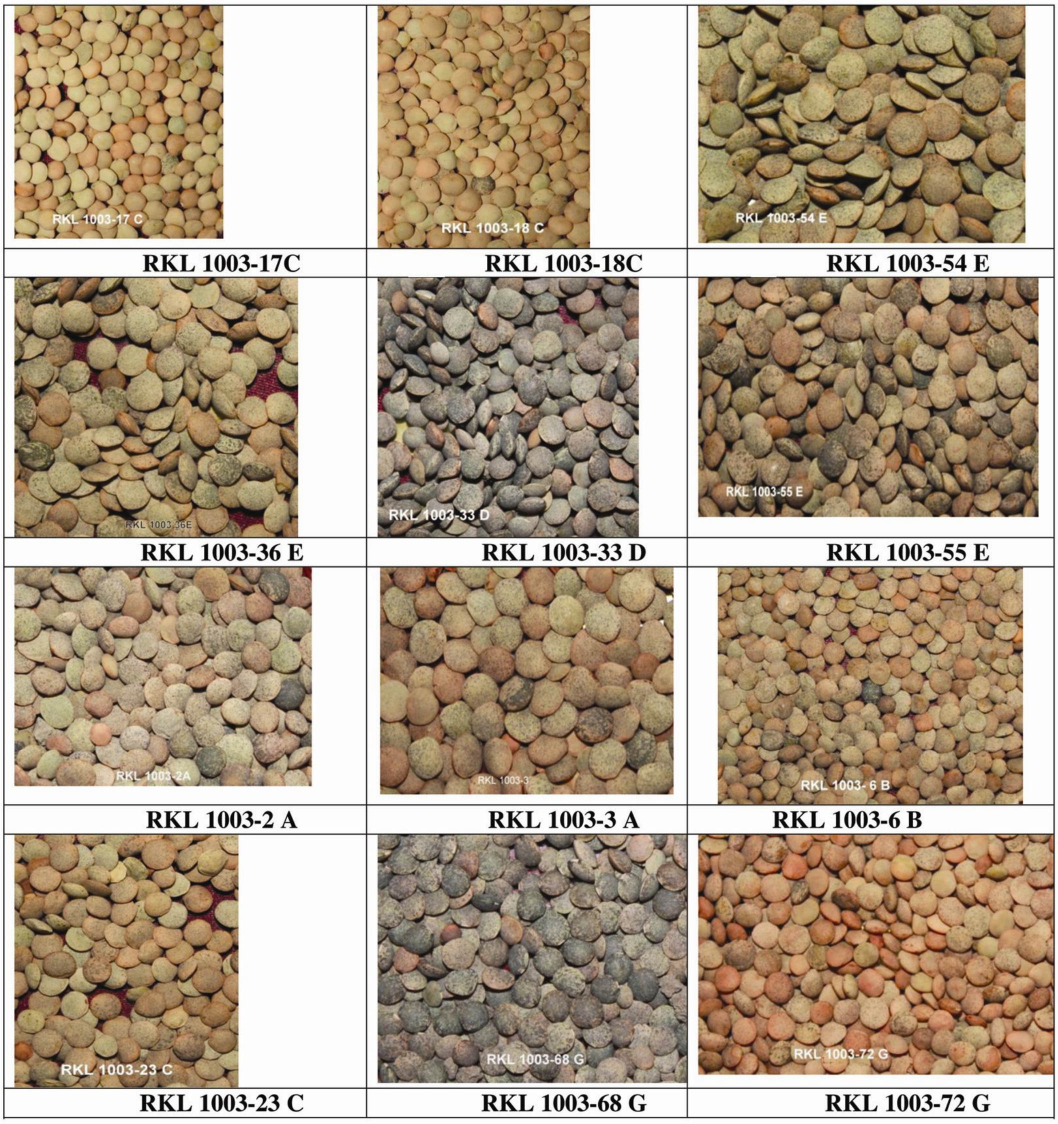
Single plants	Cotyledon colour			Seed coat colour			Seed coat pattern			Total seed	Yield/ plant (g)	100-seed weight (g)
	Y	O	G	Gr	Br	Mar	Spot	Dot	Abs			
SP_2	72 (46%)	80 (52%)	3 (2%)	74 (47%)	81 (53%)	63 (41)	57 (37%)	–	35 (22%)	155	8.08	5.22
SP_3	–	111 (98%)	2 (2%)	2 (2%)	111 (98%)	3 (3%)	2 (2%)	1 (1%)	107 (94%)	113	3.59	3.18
SP_5	–	176 (100%)	–	20 (11%)	156 (89%)	–	110 (63%)	–	66 (37%)	176	5.43	3.09
SP_6	11 (32%)	23 (68%)	–	16 (48%)	18 (52%)	–	5 (15%)	5 (15%)	24 (60%)	34	0.97	2.85
SP_8	–	148 (100%)	–	47 (32%)	101 (68%)	25 (17%)	21 (15%)	1 (1%)	101 (67%)	148	5.37	3.63
SP_{10}	183 (96%)	6 (3%)	1 (1%)	135 (71%)	55 (29%)	15 (8%)	30 (16%)	–	145 (76%)	190	11.59	6.10
SP_{13}	65 (55%)	54 (45%)	–	65 (55%)	54 (45%)	22 (19%)	25 (21%)	–	72 (60%)	119	7.63	6.42
SP_{15}	96 (93%)	7 (7%)	–	66 (64%)	37 (36%)	–	10 (10%)	7 (7%)	86 (83%)	103	4.89	4.75
SP_{18}	–	99 (100%)	–	51 (52%)	48 (48%)	1 (1%)	61 (62%)	1 (1%)	36 (36%)	99	3.87	3.91
SP_{20}	98 (99%)	–	1 (1%)	99 (100%)	–	9 (9%)	26 (26%)	–	64 (65%)	99	6.01	6.08

Cotyledon colour: Y = Yellow; O = Orange; G = Green. Seed coat colour: Gr = Green; Br = Brown. Seed coat pattern: Mar = Marbled; Spot = Spotted; Dot = Dotted; Abs = Absent. SP_{10}: Six small seeds with orange cotyledon; SP_{15}: Seven small seeds with orange cotyledon; SP_{18}: Two small seeds with orange cotyledon.

branching pattern, semi-spreading, late flowering and maturity, glabrous leaves and branches. These results indicate that the *microsperma* type lentils evolved from the *macrosperma* lentil. The progenies of natural variant may also result in phenotypes (Table 5) that may not follow Mendel's rules. This may be due to the presence of wild alleles or occurrence of more than two alleles. Also, the involvement of multiple genes in the expression

Table 6. Mutant lines showing uniform variation for different qualitative and quantitative traits during rabi 2011–12

Mutant lines	Seed traits				Range of maturity (days)	Foliage colour	Growth habit	Mean plant height (cm)	Leaf tendrils (prominent/ rudimentry)
	Cotyledon colour	Ground colour	Seed coat patterns	100-seed weight (g)					
RKL 1003-17C	Yellow	Green	Absent	3.83–8.16	110–120	Light green	Semi-erect	39	Prominent
RKL 1003-18C	Yellow	Green	Absent	2.94–5.62	112–120	Light green	Semi-erect	37	Prominent
RKL 1003-54E	Yellow	Green	Dotted	2.19–4.60	115–120	Green	Semi-erect	44	Rudimentary
RKL 1003-36E	Yellow	Green	Dotted	4.07–6.54	105–110	Light green	Erect	42	Rudimentary
RKL 1003-33D	Orange	Green	Marbled	2.71–4.53	115–120	Dark green	Semi-erect	45	Rudimentary
RKL 1003-55E	Orange	Tan	Dotted	2.94–6.45	116–124	Green	Semi-erect	42	Rudimentary
RKL 1003-2A	Orange	Brown	Dotted	2.15–3.86	115–120	Light green	Semi-erect	46	Prominent
RKL 1003-3A	Yellow	Brown	Dotted	5.23–7.62	103–110	Light green	Erect	38	Prominent
RKL 1003-6B	Orange	Brown	Dotted	2.67–5.56	112–119	Dark green	Semi-erect	43	Rudimentary
RKL 1003-23C	Yellow	Green	Dotted	3.48–6.75	115–124	Light green	Semi-erect	45	Prominent
RKL 1003-68G	Orange	Brown	Marbled	2.15–3.15	118–124	Dark green	Semi-erect	52	Rudimentary
RKL 1003-72G	Orange	Tan	Dotted	1.54–4.74	112–118	Green	Erect	45	Prominent
DPL-62 (Parent)	Orange	Green	Marbled	3.36	120–125	Dark green	Semi-erect	45	Rudimentary



RKL 1003-17C **RKL 1003-18C** **RKL 1003-54 E**

RKL 1003-36 E **RKL 1003-33 D** **RKL 1003-55 E**

RKL 1003-2 A **RKL 1003-3 A** **RKL 1003-6 B**

RKL 1003-23 C **RKL 1003-68 G** **RKL 1003-72 G**

Figure 8. Seed size, seed ground colour and seed coat patterns of stable mutant lines.

of a trait with high epistatic effect can result in hyper variable expression. A possibility of unequal crossing over is not negligible, but is difficult to explain due to the extent of variability for many qualitative and quantitative traits after the fifth generation. Ultimately, a reliable hypothesis that fits into the structure of the HYSGP population occurrence is the presence of mobile genetic elements in the genome of lentil, leaving footprints after every generation of advancement.

Heritable genotypic variation is a major contributor to phenotypic diversification, and thus a fundamental driver of evolution. Naturally occurring alleles can be used as tools to study plant gene function and to develop crops with agronomically important traits. The extent of genetic variation obtained in the present study is useful for developing bold seeded lines having high seed yield along with early in maturity. After fifth generation of evaluation and selection, 12 lines showed stability for different qualitative and quantitative traits (Table 6). Out of these, six lines have yellow cotyledon with green ground colour of seed, except line RKL 1003-3A which shows brown ground colour. While the remaining six lines have orange cotyledon colour with green, orange and tan ground colour. The seed coat pattern of stable mutant lines was absent or dotted type (Figure 8). The 100-seed weight (g) ranged from 1.54 to 8.16 g/100 seeds, indicating that mutant lines were still segregates for seed size. These lines were bold-seeded and high-yielding, early in maturity and had long reproductive period. Presently, the bold-seeded lines used in genetic improvement are a *macrosperma* type of Mediterrean region having low adaptability in the Indian subcontinent, particularly in central India. This genetic stock can play a crucial role in understanding the genetics of the target trait and a useful genetic stock for improvement of Indian lentils. Here, we have put forward the hypothesis that natural generated variation in lentil makes an important contribution for lentil improvement programme. Further studies are needed to analyse the reasons behind the expression of variations for many traits in a wide range generation after generation.

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Nanocrystalline silica from termite mounds

Ashok K. Ganguli[1,2,*], Sandeep Kumar[2], Arabinda Baruah[2] and Sonalika Vaidya[3]

[1]Institute of Nano Science and Technology, Habitat Centre,
Phase-X, Sector–64, Mohali 160 062, India
[2]Department of Chemistry, Indian Institute of Technology, Delhi,
Hauz Khas, New Delhi 110 016, India
[3]Department of Chemistry, Hindu College, University of Delhi,
Delhi 110 007, India

To develop a better understanding of the physical properties and microstructures of the termite hill soils, we have carried out studies on the soil samples collected from two locations: near Dehradun, Uttarakhand and near Hauz Khas, New Delhi. Their elemental composition and microstructure were studied using different instrumentation techniques, such as powder X-ray diffraction, field emission scanning electron microscopy, transmission electron microscope (TEM) and energy-dispersive analysis of X-ray (EDAX). α-Quartz (SiO_2) was present in both the samples, while the sample collected from Hauz Khas, Delhi also contained β-cristobalite phase of SiO_2. TEM–EDAX showed that termite hill soil consisted of silica (quartz), aluminium oxide, manganese oxide and iron oxide along with small percentage of potassium and calcium. Our studies highlight the potential to generate α-quartz from all termite hills. It is also possible to obtain the less common β-cristobalite form of silica in certain termite mounds.

Keywords: β-Cristobalite, α-quartz, termite mound, silica.

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*For correspondence. (e-mail: ashokganguliiitd@gmail.com)