

Analysis of gamma rays induced variability in lentil (*Lens culinaris* Medik.)

D. Tabti^{1,*}, M. Laouar^{1,*}, K. Rajendran², S. Kumar² and A. Abdelguerfi³

¹Laboratoire d'Amélioration Intégrative des Productions Végétales (AIPV, C2711100) ENSA El Harrach, DZ16200 Algiers, Algeria

²Biodiversity and Integrated Gene Management Program. The International Centre for Agricultural Research in Dry Areas, Rabat-Institutes, MA10112 Morocco

³Cité des Annassers 4 Bt 68 N°8 Kouba, Algiers, Algeria

*Correspondence: tdahbia1990@gmail.com; m.laouar@ensa.dz

Abstract. In this study, a lentil variety, Idlib-3, was subjected to 100 Gy (LD₅₀) gamma-ray irradiation. At M₂, mutant families were characterized for the most beneficial agronomic traits. High genotypic coefficient of variation, broad sense heritability and genetic advance of the traits such as seed yield per plant and hundred-seed weight indicated expression of additive gene action and confirmed the response at early generation selection. Total number of pods per plant had positive correlation and the highest positive direct effect on seed yield per plant and hence the preference should be given for this trait during selection. The novel mutant families identified with early flowering, early maturity (families 5 and 90) in cluster I, and more first pod height (families 10,70 and 82) in cluster II could be utilized to breed short duration lentil varieties suitable for machine harvest.

Key words: cluster analysis, correlation, genetic advance, mutation breeding, path analysis.

INTRODUCTION

Lentil is an important cool season food legume crop which is cultivated predominantly in the Indian subcontinent, the Middle East, Northern America, Southern Europe, and Eastern and Northern Africa for food (Gupta et al., 2011). Algeria is the second largest lentil producer in North-Africa (FAOstat, 2016). Lentil cultivation has been promoted by the Algerian Ministry of Agriculture since 2007–2008. However, Algerian lentil production fluctuated more in the past four decades; it was around 8,876 tons in 1976, dropped very much to 194 tons in 2000, increased to 8,215 tons in 2011 and more than 10,071 tons in 2016 (FAOstat, 2016). In fact, Algeria is currently importing lentil to meet its domestic demand. In 2015, it imported about 91,136.5 tons (88 million US\$) of lentil from Argentina, Australia, Canada, USA, Turkey and Egypt (Ministry of agriculture 2016, FAOstat, 2016).

The main problem associated with lentil production in Algeria is lack of varieties adapted to drought and heat stress, resistant to diseases and suitable for machine harvesting. The cultivated lentil in Algeria possess narrow genetic base which results low level of genetic variability, poor adaptability and vulnerability to several pests and

diseases. Several authors have also declared that the cultivated lentil has narrow genetic base (Durán et al., 2004; Lombardi et al., 2014; Khazaei et al., 2016). This is an important issue in lentil breeding that needs immediate attention from the breeders.

Broadening the narrow genetic base through intra-varietal, inter-varietal and inter-specific hybridization techniques would bring a possible solution to this issue. But, the tiny size of the lentil flower makes crossing more difficult (Rana & Solanki, 2015). Alternatively, mutation breeding appears to be an efficient method to create new source of genetic variability in lentil. Among various mutagens, gamma rays are highly preferred to induce variability in lentil (Singh et al., 2011). It was successfully employed for the improvement of qualitative and quantitative traits such as high yield, earliness in flowering and maturity and resistance to various diseases (Rajput et al., 2001; Sadiq et al., 2008).

In any mutation breeding programmes, powerful statistical techniques are necessary to analyse the data, ensure its validity and reproducibility. Assessment of the genetic variability is the first important step in mutation breeding. Next, heritability estimates gives information on the magnitude of heritable attributes (Roychowdhury et al., 2012) and the heritability estimates along with genetic advance helps in predicting the gain under selection (Bisne et al., 2009). In addition, understanding the correlations between seed yield and its component traits would facilitate the breeder develop a best selection criterion (Ali et al., 2008). More to this point, it would be useful to understand both the direct and indirect relationships between yield and the other plant characters in order to select the lentil genotypes with high yield potential (Karadavut, 2009). Finally, getting the knowledge of genetic diversity through cluster analysis would facilitate in parent selection (Abna et al., 2012).

With this background, the present investigation was done to (1) characterize M₂ population of lentil for ten morphological traits and (2) evaluate the genetic variability, heritability, character association and diversity through various biometrical analyses for future crop improvement.

MATERIALS AND METHODS

All experiments were carried out at the National High School of Agriculture (ENSA), Algiers, Algeria. A microsperma lentil type, Idlib-3 (ILL6994) which is a derivative of a cross between ILL 99, a Moroccan landrace and ILL5588, a Jordanian landrace (El-Ashkar et al., 2004) was used as a parent in this study. ILL6994 is a small red seeded lentil variety (microsperma type). It was originally developed at the International Center for Agricultural Research in the Dry Areas (ICARDA), and was released at Algeria; it was chosen to promote the cultivation of microsperma lentils in Algeria where the cultivation of macrosperma types is predominated. For the development of pure lines of the ILL6994, seeds from the advanced breeding material were raised in long rows, increased at ICARDA. It was collected from the ICARDA and multiplied at the Technical Institute of Field Crops, Algeris, Algeria, where the source seed of ILL6994 was obtained and utilized in this study.

After determining the lethal dose of irradiation (Tabti et al., 2018), a sample of 10,000 homogeneous, air-dried seeds of Idlib-3 were treated with 100 Gy gamma radiation in order to raise M₁ generation, All treated as well as untreated seeds (10,000) were sown during 2013–2014 cropping season. From M₁, a sum of 140 single plants (4,200 seeds), which had at least 30 seeds per plant were selected and sown (40 cm × 30 cm spacing between plants and rows respectively) in a randomized complete block design with three replications during 2014–2015 cropping season to raise M₂ generation. Each row represents one family (10 plants/replication); one row of untreated seeds is sown after each 10 rows of mutants (14 rows in each replication; 10 plants/row) (Tabti et al., 2018).

In M₂ generation, observations were made on ten beneficial agronomical characters including days to first flowering (DF), days to 95% maturity (DM), plant height (PH) (cm), number of primary branches (NPB), number of pods per peduncle (NPP), number of seeds per pod (NSP), total number of pods per plant (TPP), seed yield per plant (SY) (g) and hundred-seed weight (HSW) (g). In order to carry out a selection for machine harvestability, an additional trait, the height of the first pod (HFP) (cm) was added in the study. According to Saxena (2009), the height of first pod was calculated from the base of the plant up to the first node, bearing the first pod.

The ANOVA was done to study the genotypic differences among the families by Genstat software version 18 (VSN International, 2015). Phenotypic and genotypic variances (σ^2_p and σ^2_g) were calculated according to Singh (2000). Phenotypic and genotypic coefficients of variation (PCV and GCV) were calculated to estimate the variability of each trait according to Singh & Chaudhary (1985). The PCV and GCV were classified as low (< 10%), moderate (10–20%) and high (> 20%) (Sivasubramanian & Madhavamenon, 1973). The broad sense heritability (h^2) was estimated by Hanson et al. (1956). The heritability (h^2) percentage was categorized as low (< 30%), moderate (31–60%) and high (> 60%) (Robinson et al., 1949). According to Johnson et al. (1955a) the expected genetic advance as percentage of mean (GA %) was estimated and it was categorized as low (< 10%), moderate (10–20%) and high (> 20%) (Johnson et al., 1955b).

The correlation coefficients (r) among the measured traits were computed using SPSS software, version 20 (IBM Corp. 2011). Path coefficient analysis was carried out using correlation of days to first flowering, days to 95% maturity and yield components on seed yield per plant as illustrated by Dewey & Lu (1959). Standard path coefficients which are the standardized partial regression coefficients were obtained. The indirect effect of the i^{th} variable *via* j^{th} variable on dependent variable 0 (SY) was attained as $P_{0j} \times R_{ij}$; where, P_{0j} is the direct effects of variables j on the dependent variable 0 and R_{ij} is the possible correlation coefficient between independent variables. For cluster analysis, data were analysed to determine the Euclidean distance based on paired group method to determine dissimilar groups of the mutant families using PAST-multivariate software (version 3.13).

RESULTS AND DISCUSSION

Analysis of variance (ANOVA)

Analysis of variance (ANOVA) revealed significant genetic differences across the mutant families for all traits ($p < 0.001$ level) indicating the presence of genetic

variability across the traits. The results shown in Table 1 indicated a wide range of variability which was found for days to first flowering (86–95 days), plant height (22–30 cm), height of the first pod (7–11 cm), total number of pods per plant (15–112 pods), seed yield per plant (0.03–4.83 g) and hundred-seed weight (1.66–3.79 g). Many authors found broad range of variability for total number of pods per plant, hundred-seed weight and seed yield per plant in lentil through induced variability using gamma rays (Tyagi & Khan, 2010; Roy et al., 2013).

Table 1. Mean squares (MS), mean and range of different morphological, phenological, and yield attributes in 140 M₂ families of Idlib-3 lentil variety

Traits	MS	Mean ± SE	Range
DF	39.65***	89.08 ± 0.76	86.47–95.76
DM	37.24***	125.40 ± 0.54	122.80–127.40
PH	60.93***	26.57 ± 1.05	22.66–30.69
HFP	17.65***	9.75 ± 0.73	7.59–11.67
NPB	1.73***	2.67 ± 0.21	2–3.19
NPP	0.78***	1.29 ± 0.13	0.88–1.81
NSP	0.05***	1.03 ± 0.02	0.67–1.12
TPP	8350***	39.65 ± 10.86	15.26–112.52
SY	22.1***	1.28 ± 0.46	0.03–4.83
HSW	3.26***	2.81 ± 0.16	1.66–3.79

*** indicates significance at $p < 0.001$ level; SE standard error; DF: Days to first flowering; DM: Days to 95% maturity; PH: Plant height; HFP: Height of first pod; NPB: Number of primary branches; NPP: Number of pods per peduncle; NSP: Number of seeds per pod; TPP: Total number of pods per plant; SY: Seed yield per plant; HSW: Hundred seed weight.

Phenotypic coefficient of variation, genotypic coefficient of variation, broad sense eritability and genetic advance as percentage of mean

The genetic parameters such as phenotypic and genotypic coefficients of variation (PCV and GCV), broad sense heritability (h^2), and genetic advance as percentage of mean (GA %) of the traits are shown in Table 2.

Table 2. Estimates of genetic parameters of 10 different morphological, phenological, yield components, seed yield recorded among 140 M₂ families of Idlib-3 lentil variety

Traits	σ^2_p	σ^2_g	PCV	GCV	h^2	GA %
DF	18.93	10.36	4.89	3.61	54.70	5.51
DM	15.27	10.98	3.12	2.64	71.92	4.62
PH	31.38	14.78	21.08	14.46	47.09	20.45
HFP	11.27	3.19	34.4	18.3	28.31	20.06
NPB	1.02	0.36	37.78	22.38	35.11	27.32
NPP	0.42	0.18	49.69	32.77	43.50	44.52
NSP	0.02	0.02	14.77	12.94	76.78	23.36
TPP	3962.00	2194.00	157.68	117.34	55.38	179.87
SY	9.51	6.29	239.47	194.79	66.17	326.41
HSW	1.35	0.96	41.17	34.71	71.07	60.27

DF: Days to first flowering; DM: Days to 95% maturity; PH: Plant height; HFP: Height of first pod; NPB: Number of primary branches; NPP: Number of pods per peduncle; NSP: Number of seeds per pod; TPP: Total number of pods per plant; SY: Seed yield per plant; HSW: Hundred seed weight.

High and moderate PCV and GCV were observed in plant height and height of the first pod respectively. High PCV and GCV (> 20%) were found for seed yield per plant, total number of pods per plant, number of primary branches, number of pods per peduncle and hundred-seed weight. On the other hand, low PCV and GCV (< 10%) were identified for traits such as days to first flowering and days to 95% maturity, High PCV and GCV were already reported for seed yield per plant (Singh & Srivastava, 2013; Rana & Solanki, 2014) and number of primary branches (Tyagi & Khan, 2010) in lentil.

High difference between PCV and GCV for plant height, height of the first pod, number of primary branches, number of pods per peduncle, total number of pods per plant, seed yield per plant and hundred-seed weight is indicating high influence of environmental factors over the expression of traits. But, the low difference between PCV and GCV for days to first flowering, days to 95% maturity and number of seeds per pod indicates the least influence of environment and a greater contribution of genetic factors on expression of these traits (Gautam et al., 2014).

Nevertheless, GCV is not sufficient enough to determine the heritable variation of the trait. Heritability (h^2) and genetic advance as percentage of mean (GA %) would be useful to study the scope of improvement of the trait through phenotypic selection (Bisne et al., 2009). Further, heritability (h^2) estimates along with GA % and high GCV was helpful in predicting the gain through selection (Chatterjee et al., 2012). Such information is very limited in pulse crops, particularly in lentil. Our results found high h^2 with high GA % with high GCV for seed yield per plant and hundred-seed weight which indicates the expression of additive gene action and confirm the responsiveness of these traits under selection (Chatterjee et al., 2012). But, the high h^2 , high and low GCV of traits such as number of seeds per pod notified that expression of non-additive gene action and phenotypic selection will be less effective for such traits.

Correlations and Path analysis

Seed yield is associated with usually many quantitative traits. Information on correlations between seed yield and other quantitative traits would help to establish a suitable selection criterion (Raturi et al., 2015). In this current study, seed yield per plant was positively correlated with plant height, height of the first pod, number of primary branches, number of pods per peduncle, total number of pods per plant, number of seeds per pod and hundred-seed weight, but it showed significant negative correlation with days to first flowering and days to 95% maturity (Table 3). Early studies by Latif et al. (2010) and Tyagi & Khan (2010) showed similar results in lentil.

Table 3 showed correlations between yield components and phenological traits; total number of pods per plant showed positive and significant correlation with number of seeds per peduncle, number of primary branches and number of pods per peduncle; in other hand plant height showed negative and significant correlation with days to first flowering and days to 95% maturity.

More traits in the correlation often cause difficulty to ascertain the characters which are really contributing to the seed yield. While finding the direct contributions of traits as well as the indirect contribution of the traits through other traits, path coefficient analysis is helpful in making right decisions to perform selection. Direct and indirect effects of the observed characters on seed yield per plant are presented in Table 4.

Table 3. Correlation coefficients of 10 different morphological, phenological, yield attributes, seed yield recorded lentil variety

Traits	DF	DM	PH	HFP	NPB	NPP	NSP	TPP
DM	0.251**	1.00						
PH	-0.313***	-0.206*	1.00					
HFP	-0.170*	-0.161	0.595***	1.00				
NPB	-0.083	-0.446***	0.463***	0.312***	1.00			
NPP	-0.151	-0.545***	0.419***	0.449***	0.45***	1.00		
TPP	-0.235**	-0.777***	0.434***	0.253**	0.457***	0.699***	1.00	
NSP	-0.141	-0.366***	0.195*	0.086	0.173*	0.332***	0.489***	1.00
SY	-0.194*	-0.568***	0.365***	0.175*	0.291***	0.574***	0.852***	0.852***
HSW	-0.166*	-0.389***	0.020	0.074	0.236**	0.327***	0.394***	0.394***

*, **, ***: correlation significant at 0.05, 0.01 and 0.001 level of probability respectively; DF: Days to first flowering; PH: Plant height; HFP: Height of first pod; NPB: Number of primary branches; NPP: Number of pods per peduncle; TPP: Total number of pods per plant; SY: Seed yield per plant; HSW: Hundred seed weight.

Table 4. Direct and indirect effects of nine different morphological, phenological, yield attributes, on seed yield recorded Idlib-3 lentil variety

Traits	DF	DM	PH	HFP	NPB	NPP	NSP	TPP
DF	0.01	0.0638	0.0022	0.0017	0.0073	0.0041	-0.0230	-0.2275
DM	0.0025	0.254	0.0014	0.0016	0.0392	0.0147	-0.0597	-0.7521
PH	-0.0031	-0.0523	-0.007	-0.0060	-0.0407	-0.0113	0.0318	0.4201
HFP	-0.0017	-0.0409	-0.0042	-0.01	-0.0275	-0.0121	0.0140	0.2449
NPB	-0.0008	-0.1133	-0.0032	-0.0031	-0.088	-0.0122	0.0282	0.4424
NPP	-0.0015	-0.1384	-0.0029	-0.0045	-0.0396	-0.027	0.0541	0.6766
NSP	-0.0014	-0.0930	-0.0014	-0.0009	-0.0152	-0.0090	0.163	0.4734
TPP	-0.0024	-0.1974	-0.0030	-0.0025	-0.0402	-0.0189	0.0797	0.968
HSW	-0.0017	-0.0988	-0.0014	-0.0007	-0.0208	-0.0088	0.0253	0.3814

In diagonal: direct effect on seed yield; DF: Days to first flowering; DM: Days to 95% maturity; PH: Plant height; HFP: Height of first pod; NPB: Number of primary branches; NPP: Number of pods per peduncle; NSP: Number of seeds per pod; TPP: Total number of pods per plant; SY: Seed yield per plant; HSW: Hundred seed weight.

The direct effect of total number of pods per plant on seed yield per plant was the highest among others, confirming that yield can be increased both directly and indirectly by increasing the number of pods per plant. Jain et al. (1991) indicated that number of pods per plant is the best selection criterion for yield improvement in lentil. Chakraborty & Haque (2000) and Tyagi & Khan (2010) also reported positive direct effect of total number of pods per plant on seed yield. The lowest significant direct effect on seed yield per plant was observed by number of seeds per pod. The highest indirect effect on seed yield per plant was observed in number of pods per peduncle. The direct effect of number of primary branches on seed yield per plant was negative and significant; whereas its indirect effect via total number of pods per plant was positive. Hundred-seed weight had positive and significant direct effect on seed yield per plant but with low indirect effects via other traits. Though the direct effect of number of pods per peduncle was insignificant, its indirect effect via total number of pods per plant was positive and high. Days to first flowering and days to 95% maturity had positive direct effect on seed yield per plant and negative indirect effect via number of seeds per pod, total number of pods per plant and hundred-seed weight. Plant height and height of the first pod had negative direct effect on seeds per pod whereas they had positive indirect effect via number of seeds per pod, total number of pods per plant and hundred-seed weight. Chakraborty & Haque (2000) reported positive direct effect of number of seeds per pod and negative direct effect of days to flowering and days to maturity. Whereas, Latif et al. (2010) found positive direct effect of days to maturity, plant height, and negative direct effect of days to flowering on seed yield. Reddy (2013) also reported positive direct effect of plant height on seed yield per plant.

Cluster analysis

Cluster analysis using all the ten morphological traits grouped 140 mutant families and control into four major groups at a genetic distance of 48 (Table 5). Cluster IV was the largest one with 72 families (51.06%) followed by cluster II with 35 families (24.82%) including control and cluster III with 31 families (21.99%). The smallest group identified was cluster I with only 3 families (2.13%). The mutant families included in the cluster I were characterized with high mean values for all traits except height of the first pod, days to flowering and days to maturity. The mean value of cluster II was high for the height of the first pod. The mean values of Cluster III were not found noteworthy for all traits. Likewise, cluster IV had lowest mean values for all traits except for days to first flowering and days to maturity. In particular, mutant families 5 and 90 in cluster I would be useful for the development early flowering (86.61 days) and early maturing (123.3 days), short duration lentil varieties. Such varieties would be beneficial for the areas more prone to terminal drought and heat stress. Mutant families such as 5, 42 and 90 in cluster I had high seed yield per plant (4.83 g). Likewise, mutant families such as 10, 70 and 82 in cluster II had more than 11 cm first pod height would be useful to breed lentil varieties suitable for machine harvesting. The clusters might be consisting of diverse mutant families for various qualitative traits. The selected families from various clusters would be useful for the genetic improvement of lentil.

Table 5. Mean values of ten different phenological and morphological characters, yield attributes, and seed yield for four groups revealed by cluster analysis among 140 mutant families and the control

Cluster	(%)	DF	DM	PH	HFP	NPB	NPP	TPP	NSP	SY	HSW
1	2.13	88.13	124.13	28.94	9.88	2.89	1.54	98.22	1.09	3.92	3.03
2*	24.82	88.84	124.26	26.90	9.96	2.78	1.44	58.42	1.05	1.95	2.91
3	21.99	89.16	125.08	26.54	9.66	2.66	1.35	41.18	1.03	1.17	2.79
4	51.06	89.22	126.26	26.11	9.53	2.56	1.21	26.65	1.01	0.55	2.63
mean	-	89.08	125.4	26.57	9.75	2.67	1.29	39.65	1.03	1.28	2.81

* Idlib-3 position; DF: Days to first flowering; DM: Days to 95% maturity; PH: Plant height; HPF: Height of first pod; NPB: Number of primary branches; NPP: Number of pods per peduncle; NSP: Number of seeds per pod; TPP: Total number of pods per plant; SY: Seed yield per plant; HSW: Hundred seed weight.

CONCLUSION

On the whole, mutation induction through gamma rays was found useful to create new source of variability and circumvent the bottleneck in lentil breeding. The induction of mutation by 100 Gy gamma rays on Idlib-3 was effective to generate significant variability for most of the quantitative traits studied. The high genotypic co-efficient of variation, broad sense heritability and genetic advance found for seed yield per plant and hundred-seed weight guaranteed early phenotypic selection and high expected genetic gain. Both correlation and path analysis suggested providing high preference to total number of pods per plant during selection due to its strong relationship and direct effects on seed yield per plant. Cluster analysis results confirm the presence of genetic dissimilarities among the mutant families. The identified mutant families such as 5, 42, 90, 10, 70 and 80 could be used as parents in the future breeding program of Algeria. Moreover, the mutants generated in this study could offer opportunity to perform molecular screening through TILLING (Targeting Induced Local Lesions in Genomes) in the immediate future.

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