Identification of desirable mutants in quantitative traits of lentil at early (M₂) generation

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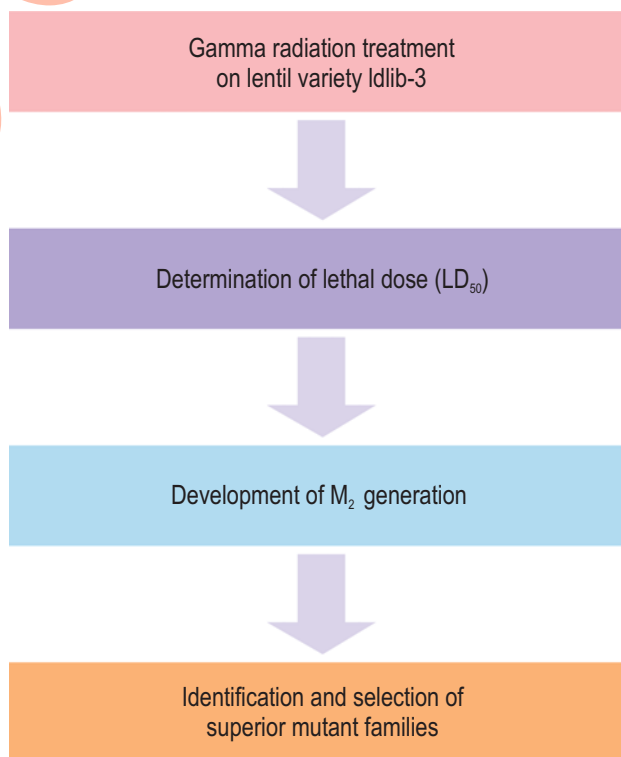
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Abstract

Aim : Narrow genetic base and limited genetic diversity are the major research constraints that affect the efficacy of class breeding methods in lentil. In order to circumvent these conditions, mutation breeding techniques were successfully employed in many studies. The present study was conducted to find the LD_{50} dose of gamma rays in lentil cultivar, Idlib-3 and also to develop and characterize M_2 population for economically important quantitative traits.

Methodology : Initially, seeds of lentil variety Idlib-3 were treated with eight doses (45, 60, 75, 90, 100, 200, 300 and 400 Gy) of gamma rays. Germination percentage was recorded to determine LD_{50} by probit analysis. After determining LD_{50}, M_1 and M_2 generations were raised. At M_2 generation, mutant families were characterized for a set of ten different economically important traits.

Results : The LD_{50} of gamma rays was calculated as 104.34 Gy based on the germination percentage. In M_2 generation many induced mutations such as chlorophyll mutation (2.76%), stunted growth (1.14%) and dwarf mutants (0.35%) were recorded. Dunnett's test revealed a total of 13 superior families over parent for various quantitative traits. The results of Best Linear Unbiased Predictors (BLUPs) confirmed the recurrence and superiority of same seven families, identified in Dunnett's test for high seed yield.

Interpretation : Early selection in M_2 generation was found useful to identify new desirable mutant traits in lentil. The superior families identified for early maturity, high yield and more total number of pods per plants could be either utilized as direct mutants or in the future crossing program.

Gamma radiation treatment
on lentil variety Idlib-3

Determination of lethal dose (LD_{50})

Development of M_2 generation

Identification and selection of
superior mutant families

Introduction

Lentil (*Lens culinaris* Medik.subsp.*culinaris*) is one of the earliest domesticated crops in the Near East (Stefaniak and McPhee, 2015). It is an autogamous, diploid (2n=2x=14) species with a genome size of approximately 4063 Mbp (Arumuganathan and Earle, 1991). It is a drought tolerant legume crop. When compared to other food legumes it can be easily grown in marginal environments (Cubero, 1981). It is predominantly cultivated in the Indian subcontinent, the Middle East, Northern America, Southern Europe, and Eastern and Northern Africa for food (Gupta *et al*., 2011).

Lentil is the fourth most important food legume crop after faba bean, chickpea and peas in Algeria. Even-though, lentil cultivation is promoted by the Algerian Ministry of Agriculture, its production has decreased in the past few years (Riah *et al*., 2014; FAO, 2014) due to lack of adapted varieties to the agro-systems that have legumes production vocation. Moreover, attributes such as limited genetic diversity and narrow genetic base severely affect the crop improvement (Fratini *et al*., 2014). Research efforts are currently underway in many Algerian Agricultural Research Institutes to develop new lentil varieties for Algerian agro-climatic conditions.

Creating genetic variation through hybridization is a cumbersome practice in lentil. The small sized fragile flowers make the emasculation process difficult (Rana and Solanki, 2015). Moreover, self-fertile nature of the crop further imposes limitation to the success of the hybridization program. In view of that, classical plant breeding approaches have become slow and unreliable.

Alternatively, mutation breeding appears to play an important role in the genetic improvement of lentil. Mutants are usually induced by different types of chemical mutagens and ionizing radiations (Amin *et al*., 2015). Among them, gamma rays are commonly used mutagen to create variation in lentil (Singh *et al*., 2011). Gamma rays cause chromosomal rearrangements, deletions, and hence produce new source of genetic variations in plants (Bhat *et al*., 2007). The application of gamma rays is also found easy only on two steps: preparation of seeds and mutagen treatment (Toker *et al*., 2007). Gamma rays were successfully used to develop lentil cultivars such as Elista for resistance to main lentil diseases and Djudje for high protein content and good culinary quality (Tomlekova, 2010).

To avoid high level of mortality rate due to mutation, determination of safe dose at which half of the plant material survives, which is commonly known as Lethal Dose$_{50}$ (LD$_{50}$), should be estimated at initial stage. The LD$_{50}$ helps in determining the sensitivity of a particular variety to the critical dose of mutagen causing 50% mortality rate (Kangarasu *et al*., 2014). It was reported that the factors such as seed size, maturity, hardiness, moisture content and time of the treatment influence variation in LD$_{50}$ dose, even between different varieties of same crop (Karthika and Subba Lakshmi, 2006).

As mentioned in early mutation studies quantitative traits such as yield should undergo selection at early generation. Selections at early generation reduce the loss of desirable combination of favorable alleles (Solanki and Sharma, 2001, 2002; Sneep, 1977). It seems that exercise of selection is independent of the mutagen being used for the development of M$_2$ population (Rana and Solanki, 2015). Moreover, in order to utilize the usefulness of mutations for efficient plant breeding, the study of frequency of chlorophyll mutants and viable mutants in M$_2$ generation is widely recommended (Kharkwal, 2000).

With this background, the following study was conducted to determine the LD$_{50}$ dose of gamma rays in lentil cultivar, Idlib-3 and also to develop and characterize M$_2$ population for economically important quantitative traits.

Materials and Methods

All experiments were carried out at the National High School of Agriculture (ENSA), Algiers, Algeria during 2013-2015 cropping seasons. The experimental plot had sandy loam soil with a pH of 7.6. It had 1.04% nitrogen, 11.69% organic matter and 0.06 ppm available phosphorus. For this study, *a microsperma* type of lentil, Idlib-3 (ILL6994) derived from a cross between ILL99 (Moroccan landrace) and ILL5588 (Jordanian landrace) was employed.

Estimation of germination percentage : A total of 768 well-filled, homogeneous, dry seeds (11% moisture) of ILL 6994 were chosen and divided into eight sets (96 seeds per set). Each of these eight sets were then exposed to 45, 60, 75, 90, 100, 200, 300 and 400 Gy gamma rays treatment using Cobalt 60 (^{60}Co) source in the gamma cell, National Center for Nuclear Research (CRNA), Algeria at the dose rate of 12.11 Gy.min^{-1}. All eight sets of treated seeds along with a one set of untreated control seeds (96 seeds) were sown in pots (one plant per pot). On 25th day after sowing, the total number of germinated seedlings were recorded across all treatments, as well as in the control. The germination percentage and the percentage over control were calculated according to the formula given by Kangarasu *et al*. (2014).

Raising M$_1$ generation : In order to raise M$_1$ generation, samples of 10,000 homogeneous, air-dried seeds of Idlib-3 were treated with 100 Gy gamma radiation. All treated as well as untreated seeds were sown in 600 m^2 areas with 20 cm × 10 cm spacing during 2013-14 cropping season. From this material, a total of 428 single plants were harvested and forwarded to raise M$_2$ generation.

Raising M$_2$ generation and data collection : From M$_1$ (428 single plants), a sum of 140 single plants (4200 seeds), which had at least 30 seeds per plant were selected and sown (40cm × 30cm spacing) in a randomized complete block design with three replications during 2014-15 cropping season. All mutant progenies were characterized for viable mutants and chlorophyll mutants at different growth stages. The frequency of chlorophyll and viable mutations was calculated following the method of Khan and Tyagi

(2009). Observations were recorded for a set of ten economically important traits namely, days to first flowering (DF), days to 95% maturity (DM), plant height (PH), height of first pod (HFP), 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 (SY) and hundred seed weight (HSW).

Statistical analysis : The LD_{50} value was calculated by probit analysis using SPSS software version 20 (IBM Corp., 2011). The probit vs log of the doses were plotted in a graph to fit a line of regression. From the plot of probit, the average dose response was calculated and the LD_{50} value was obtained (Heinrichs *et al.*, 1981).

In order to identify mutant families which were significantly superior over the parent (untreated control) in M_2, Dunnett's test was employed using Genstat software, version.18 (VSN International, 2015). The Best Linear Unbiased Prediction (BLUP) values were calculated (genotypes modeled as a random effect) for all quantitative characters and the twenty four superior mutant families over control for seed yield (SY) were selected. The BLUPs were calculated using IBP Breeding Management System Version 3.0.8 (2015).

Results and Discussion

Germination under different doses of gamma rays were recorded, and the percentage over control was calculated (Table 1). Germination was found to decrease progressively with the increasing dose of treatment. The highest germination percentage (96.55%) was obtained at 45 Gy treatment and the lowest germination percentage was obtained at 400 Gy treatment (4.6%). Similar results were observed by Solanki and Sharma (1994), Singh *et al.* (2007) in lentil and by Girjia and Dhanavel

Table 1 : Number of seeds germinated, germination percentage and percentage over control calculated for different doses of gamma ray treatment in lentil variety, Idlib-3

Dose (Gy)	Number of seeds germinated	Germination percentage	Percentage over control
0	87	90.62	100[d]
45	84	87.5	96.55[d]
60	75	78.12	86.21[c]
75	73	76.04	83.91[c]
90	71	73.96	81.61[c]
100	30	31.25	34.4[b]
200	9	9.37	10.34[a]
300	8	8.33	9.20[a]
400	4	4.16	4.60[a]
	CV (%): 10.4		

Means followed by same letter are not significantly different at 5% (Duncan Multiple range test)

(2009) in cowpea. Usually, the altered physiological balance caused by mutagenic treatments causes reduction in seed germination (Singh *et al.*, 2007).

Based on the germination percentage and probits, LD_{50} of Idlib-3 was estimated. The LD_{50} value was obtained at 104.34 Gy gamma ray treatment (R^2=0.92). Sharma and Sharma (1986) reported that the LD_{50} of *microsperma* lentil types usually vary from 135 to 186 Gy. In M_2 population, a significant reduction in survival of plants was observed at each growth stage. Even though only 95.88% seeds were germinated, 90.40% and 83.12% of seedlings survived at the stage of flowering and maturity respectively. It was further decreased to 37.29% and only a total of 1302 single plants (with seeds) were harvested at the end. These results indicate the

Table 2 : Mean performances of thirteen significantly superior mutants' families over parent, Idlib-3 identified by Dunett's test for ten traits

Families	DF	DM	PH (cm)	HFP (cm)	NPB	NPP	TPP	NSP	SY (g)	HSW (g)
68	89.7	**122.8****	27.59	9.79	3.19	1.34	66.17	1.06	2.24	2.76
70	87.97	123.5	**30.5****	11.12	2.98	1.41	75.01	1.06	2.18	3.23
5	86.61	124.5	**30.69****	10.14	2.70	1.24	97.72	1.10	**4.83****	3.20
11	89.17	127	25.54	9.18	2.48	1.27	31.37	**1.11****	2.14	2.6
84	89.92	124.5	26.68	10.03	2.52	1.45	50.46	**1.11****	1.64	2.53
118	90.38	125.6	26.49	9.73	2.52	1.54	37.71	**1.12****	1.196	2.29
90	87.09	123.3	29.34	9.83	3.10	1.72	**112.52****	1.08	**3.07****	2.83
33	88.74	126.5	24.01	9.28	2.25	0.97	18.53	1.007	0.62	**3.78****
59	86.6	123	27.67	10.32	2.62	1.57	71.71	1.07	**2.90****	3.02
75	89.66	126.7	27.79	10.29	2.25	1.35	40.44	1.06	**2.91****	2.91
134	89.39	125.2	26.99	10.43	2.65	1.48	62.98	1.05	**2.99****	2.89
133	90.01	125.6	26.9	10.26	2.82	1.52	58.27	1.07	**3.84****	3.14
42	90.69	124.6	26.79	9.64	2.86	1.65	84.42	1.09	**3.84****	3.04
Idlib-3	**86.47**	**124.4**	**26.73**	**10.4**	**2.633**	**1.807**	**68.8**	**1.049**	**1.668**	**3.209**
SD	1.505	1.368	1.797	0.505	0.283	0.215	25.85	0.0309	1.137	0.366
SE (±)	0.402	0.366	0.480	0.135	0.0757	0.057	6.908	0.0082	0.304	0.0979

**: significantly superior

Table 3 : Identification of twenty four "best" mutant families, based on seed yield by BLUPs

Families	DF	DM	PH (cm)	HFP (cm)	NPB	NPP	TPP	NSP	SY (g)	HSW (g)
5*	87.17	124.63	**29.45**	9.91	2.68	1.26	**83.34**	**1.07**	**3.56**	2.99
42*	90.29	124.74	26.68	9.67	2.76	1.52	**72.62**	**1.06**	**2.73**	2.89
90*	87.57	**123.56**	**28.43**	9.75	**2.89**	1.57	**94.46**	**1.07**	**2.57**	2.81
133*	89.77	125.54	26.76	9.94	2.73	1.43	53.20	**1.05**	**2.47**	2.90
59*	87.22	**123.41**	**27.25**	9.95	2.63	1.46	62.69	**1.06**	**2.25**	2.89
134*	89.31	125.23	26.82	**10.02**	2.65	1.42	57.01	1.04	**2.15**	2.82
80	89.32	123.55	26.73	**10.14**	2.67	1.52	62.29	1.03	**2.03**	2.98
92	88.44	124.26	**27.66**	**10.03**	2.66	1.49	55.61	1.03	**2.00**	2.87
82	88.09	**123.99**	26.60	**10.53**	**2.77**	1.33	53.06	1.04	**1.92**	2.91
68*	89.56	**123.15**	**27.25**	9.74	**2.94**	1.32	59.96	**1.05**	**1.91**	2.77
70*	88.21	**123.75**	**29.32**	**10.39**	**2.83**	1.38	**66.48**	**1.08**	**1.90**	**3.04**
123	88.52	124.58	**28.50**	9.85	2.69	1.39	58.20	**1.06**	**1.84**	2.86
75*	89.52	126.52	**27.37**	9.97	2.43	1.33	40.23	1.04	**1.80**	2.81
72	89.40	124.53	26.11	9.39	2.65	1.33	52.15	**1.07**	**1.78**	2.87
137	87.93	124.89	**27.91**	9.79	2.65	1.32	52.19	**1.08**	**1.76**	2.70
112	88.69	**123.29**	25.55	9.37	**2.91**	1.34	60.19	1.04	**1.75**	2.90
10	87.75	124.76	**28.75**	**10.63**	2.82	1.40	52.07	**1.05**	**1.75**	2.94
1	89.66	124.82	**27.61**	9.76	**2.90**	1.50	55.04	**1.07**	**1.70**	2.72
129	88.26	**123.26**	**27.51**	**10.13**	2.62	1.45	61.22	1.03	**1.68**	2.98
88	88.70	124.58	25.47	9.52	2.64	1.33	48.13	1.03	**1.67**	2.90
30	89.39	124.94	25.80	9.61	**2.71**	1.42	44.77	**1.05**	**1.66**	2.83
60	89.22	125.11	25.68	9.61	**2.80**	1.37	50.90	1.02	**1.65**	2.75
50	88.14	124.82	**27.32**	**10.28**	2.63	1.42	50.42	1.02	**1.63**	2.80
61	88.42	124.83	**27.92**	9.68	**2.86**	1.35	54.59	**1.05**	**1.61**	2.88
Idlib-3	**87.05**	**124.52**	**26.66**	10.04	**2.64**	**1.64**	**62.31**	**1.04**	**1.57**	**3.07**

*: kept the same ranking as superior families; In bold are higher values of predicted mean compared to the control

deleterious effect of mutations on survival of plants over different growth stages. Arisha *et al*. (2015) noticed similar reduction in survival of M₂ mutant plants at maturity stage in pepper.

The effectiveness of a given mutagen can be fully explicated through the frequency of induced mutations. Chlorophyll mutation frequency is a dependable index to evaluate the efficiency of mutagenic treatments, destined to generate a wide array of variability (Usharani and Kumar, 2015). In M_2 generation, different types of chlorophyll mutants such as xantha, viridis and chlorina were observed in the present study. The frequency of chlorophyll mutation was found to be 2.76% (116 mutants). Sharma and Sharma (1981) recorded 2.55% and 1.26% chlorophyll mutants in *macrosperma* and *microsperma* lentils at 100 Gy. Similarly, Paul and Singh (2002) reported 0.8% of chlorophyll mutants in *microsperma* lentils using 100 and 150 Gy. The frequency of chlorophyll mutation was observed as 1.21% and 2.42% in two varieties of soybean at 150 Gy (Khan and Tyagi, 2009). In cluster bean, Bhosle and Kothekar (2010) recorded 1.66%, 2.21% and 2.61% of chlorophyll mutations at 50, 100 and 150 Gy, respectively.

The viable mutants observed in the present study consisted of forty eight stunted growth mutants (1.14%), fifteen

dwarf mutants (0.35%), three mutants with four pods per peduncle (0.07%) and three mutants with abnormal leaves (0.07%). In previous studies, similar spectra of viable mutants were reported in M_2 population of chickpea (Kharkwal, 2000), cowpea (Girija and Dhanavel, 2009) and grasspea (Talukdar, 2009). Viable mutations are the result of gross chromosomal rearrangements caused by ionizing radiations and the application of different mutagens could alter the spectra of viable mutations (Ehrenberg *et al*., 1961).

In the present study, the superiority of mutant families over parent was studied through "comparison with control" (Dunnett's test). The total number of mutant families showing significant superiority over the parent varied from 1 to7 for all traits. Only one family was found superior over the parent for days to maturity, total number of pods per plant and hundred seed weight; two families for plant height; three families for number of seeds per pod and seven families for seed yield. For other traits, no family was found superior over the parent. It appears that among the 140 evaluated mutant families; only 13 families (0.09%) demonstrated superiority over the parent for yield and yield components, phenological and morphological characters (Table 2). Ali *et al*. (2010) found mutant lines which had superiority over parent for flowering, yield, number of branches and hundred seed weight in lentil.

The Best Linear Unbiased Predictors (BLUPs) were more commonly utilized to increase the sugarcane breeding efficiency (Resende and Barbosa, 2006). It was found more effective method than routine phenotypic selection in walnut breeding (Aleta *et al*., 2004). However, there were no similar evidences found for legumes and other crops. In most of the fruit breeding programs (Imai *et al*., 2016), mutagenesis often accumulates highly unbalanced phenotypic records, which create serious obstacles for comparison and phenotypic selection. To overcome this obstacle, the best linear unbiased prediction (BLUP) method is widely recommended. In this study, the BLUP values were calculated for the set of ten economically important traits. A total of twenty four high yielding mutants were sorted out from the BLUP values. The range of BLUPs for seed yield varied from 1.61 to 3.56 g. The three best mutants families identified for seed yield also recorded high values for total number of pods per plant. Similarly, these high yielding families had demonstrated other desirable characters such as more plant height and early maturity. Nine families classified as best by Dunnett's test were also classified as the best ones by BLUP. It confirms again the superiority of mutants identified in the present study (Table 3).

The findings of the present study revealed some putative mutants of Idlib-3 at 100 Gy treatment in M_2 generation which can be used as either direct mutants or in the future crossing program. However, it is recommended to have more number of individuals in M_2 population to facilitate selection for seed yield, especially when LD_{50} is effective.

Acknowledgments

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