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GENETIC EXPLOITATION OF LENTIL THROUGH INDUCED MUTATIONS

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Abstract

Genetic exploitation through induced mutations has been very instrumental in improvement of crops. Genetic diversity in lentil was created in a local variety Masoor- 85 and exotic cultivar ICARDA- 8 by treating with gamma rays ranging from 100–600 Gy. Desirable segregants were selected in M_2 for high yield, earliness and 100 grain weight. These mutants were confirmed for their yield contributing factors and growth behavior in M_3 generation. True breeding lines were evaluated for yield potential. The promising mutant strains giving better yield potential were tested for yield and other agronomic traits in different station yield trials. Mutant strain AEL 49/20 produced highest grain yield in zonal trials conducted under different agro climatic zones in Sindh province. Observing its better performance, AEL 49/20 was promoted in National uniform yield trials, where it ranked first in the province of Sindh. On the basis of outstanding performance it is approved and released as the first lentil variety “NIA- MASOOR -05” in the province of Sindh for general cultivation. In this study enhancement of genetic potential for improvement of lentil is discussed.

Introduction

Lentil (*Lens culinaris* Medik) 2n =14, is one of the oldest food crops originated in the Fertile Crescent of the Middle East (Barulina, 1930; Renfrew, 1969; 1973; Zohary, 1972; Zohary & Hopt, 1973). Lentil was first domesticated in Southern Turkey. From there it moved to west and east ward (Ladizinsky, 1979, Cubero, 1984). It belongs to genus *Lens* and family Leguminoseae. It is an annually sown, cool season food legume crop. The demand of this protein rich pulse is increasing gradually and will continue to increase to feed the ever-increasing population of the region. As a food, it provides a valuable protein source (about 26%), which coupled with its ability to thrive on relatively poor and marginal lands and even under drought conditions (Jeswani, 1988, Verma *et al*., 1993). Pakistan is one of the major lentil growing countries of south Asia. Lentil is an upcoming crop among the food legumes in Pakistan especially in Sindh. In Pakistan, lentil is grown as a winter crop on an area of about 448,000 hectares (Anon., 2002- 03) with a trial production of 26,200 tons annually. In Sindh, lentil is the second important rabi crop after wheat it is grown on an area of about 7,200 ha (Anon., 2003), with an annual production and grain yield of about 3.9 thousand tons/ha and 533 kg/ha respectively. Success in genetic improvement of lentil has been very limited mainly owing to a narrow genetic base, extreme specificity of adaptation, which leads to the use of ineffective exotic germ plasm. In Sindh, lentil is grown in districts of Hyderabad, Thatta, Dadu, Larkana, Jacobabad, Sukkur, Sanghar, Nawabshah and Mirpur Khas (Anon., 2004).

Low yield potential, susceptibility to diseases (Ascochyta blight, Rust, Wilt, Root rot and Stem rot) and weed infestations are the main production constraints to the lentil crop production in Sindh. Although, lentil is a relatively minor crop on a world scale, in certain regions they assume considerable local importance and can be successfully grown on light to heavy types of soil under both rainfed and irrigated conditions. The prevalent land races have inherent low yield potential in Sindh.

Lentil breeding program is aimed at the improvement of different plant traits and recombining them into one genetic background to enhance the plant productivity. Among other pulses, the lentil has however, high potential and wide adaptability the world over (Singh & Singh, 1997; Tufail, 1989). To generate new useful genetic variability for developing high yielding and widely adopted varieties of lentil induced mutation work was initiated at NIA, Tandojam. Earlier Sharma & Kant (1975); Kharkwal *et al*., (1988); Micke (1988); Tufail *et al*., (1993); Uhlik (1972a, 1972b and 1973) have clearly demonstrated that induced mutation breeding is a useful additional source for creating an innovative genetic variability in lentil. Many workers (Sharma & Chaturvedi, 1981; Thombre *et al.*, 1981; Seth & Chaudhary, 1981; Gottschalk, 1981), reported radio-sensitivity studies and created possible genetic variability through induced mutation in lentil and also in different pulse crops. Mutation breeding acts as a complementary approach and has been resorted to for altering yield potential, flowering habit, crop duration, disease control, improvement in quality and quantity of seed and is more adaptable for inducing recessive genes than dominant ones (Muehlbauer *et al*., 1996). Thus, mutation induction is a means of creating or increasing genetic variability.

Materials and Methods

The homogeneous seeds of commercial varieties (Masoor-85 and ICARDA–8), were moisture equilibrated over a 60% glycerol solution in a desiccator for six days prior to exposure to gamma rays having doses ranging from 100, 200, 300, 400, 500 and 600 Gy from a ^{60}Co gamma rays source. The dose rate of the source was 19.8477 Gy/min. The radiated seeds along with control were sown as M_1 in the field in a split plot design with four replications. Each plot consisted of five rows two meters long and planted 30 cm apart. The plant-to-plant distance was kept 10 cm within the row. Seeds collected from M_1 generation were used to raise for further experimentation in M_2 generation. Twenty rows, two meters long from each treatment were grown. One control row was repeated after 10 rows. In M_2 generation quite a few mutants were isolated and confirmed in M_3 generation for their breeding behavior. Different agronomic characters viz., plant height, branches/plant, pod length, seeds/pod, pods/plant, 100 grain weight and grain yield /plant of these mutants were evaluated in $M_{3.}$ Fourteen mutants isolated from radiated population of M-85 and ICARDA–8 were evaluated in micro yield trials I and II. Ten high yielding mutants giving better field performance in micro yield trials were further evaluated in preliminary yield trial. Eight best performing mutants were promoted to zonal trials and evaluated with local check M-85 in different agro-climatic locations in the province of Sindh. Data of each experiment was statistically analyzed. Finally, mutant strain AEL 49/20 performed best and promoted to national trial where it stood first in the province of Sindh.

Results and Discussion

The agronomic data of M_3 generation recorded for confirmation of various characters such as plant height, yield and yield components is shown in Table 1. The mutant lines AEL 15/30, AEL 49/20, AEL 2/20, AEL 12/30, AEL 20/30, AEL 9/20, AEL13/30 and AEL23/40 gave higher grain yield as compared to mother varieties M-85 and ICARDA-8. The number of branches/plant (3.8), 100 grain weight (2.43 g) and grain yield per plant (7.82 g) of mutant line AEL 49/20 is higher than the rest of the mutants and controls while AEL 15/30 produced highest grain yield (10.12 g) per plant. Mutant AEL 49/20 flowered 18 days and matured 5 days earlier than its mother variety M- 85.

Table1. Agronomic evaluations of different mutant lines in M_3 generation during 1995-96.

Genotypes	Doses	Days to flower	Days to maturity	Plant height (cm)	Branches per Plant	Pod length (cm)	Seeds/ pod	Pod/ plant	100 grain weight (gm)	Grain yield/ Plant
AEL 49/20	**200Gy**	**69**	**129**	**42.8**	**3.80**	**1.20**	**2.0**	**159.8**	**2.43**	**7.82**
AEL50/30	300Gy	87	130	37.2	1.20	1.14	1.8	96.4	2.02	2.86
AEL51/30	300Gy	88	131	46.6	2.00	1.14	2.0	76.0	1.36	2.38
AEL52/30	300Gy	87	131	43.4	1.80	1.08	1.4	87.6	1.50	3.28
AEL57/50	500Gy	84	125	37.2	1.40	1.14	2.0	84.0	1.66	3.67
AEL2/20	200Gy	86	136	31.5	2.75	1.12	2.0	215.5	2.41	7.45
AEL9/20	200Gy	72	125	42.8	1.80	1.20	2.0	144.2	2.05	6.01
AEL12/30	300Gy	67	125	42.6	2.20	1.20	2.0	198.8	2.20	6.22
AEL13/30	300Gy	67	126	40.0	3.00	1.18	1.8	209.0	2.05	5.54
AEL23/40	400Gy	75	128	38.5	1.40	1.22	1.8	156.5	1.89	5.17
AEL 28/40	400Gy	91	134	42.0	3.20	1.22	2.0	98.0	2.35	3.67
AEL 45/60	600Gy	85	131	42.2	1.40	1.10	1.8	180.6	1.60	5.83
AEL 15/30	300Gy	75	128	35.0	3.00	1.30	2.0	345.0	2.07	10.12
AEL 20/30	300Gy	87	131	42.6	2.00	1.18	2.0	179.4	1.80	4.95
AEL 41/50	500Gy	68	125	37.8	3.80	1.16	2.0	119.8	2.40	4.61
AEL 54/50	500Gy	88	129	47.4	1.40	1.16	1.8	101.0	1.65	3.00
AEL 55/50	500Gy	88	130	44.4	1.60	1.20	2.0	103.8	1.64	4.11
M-85	Control	86	134	42.2	2.00	1.04	1.4	62.0	1.46	1.85
ICARDA-8	Control	88	135	39.8	2.00	1.06	2.0	188.4	1.73	4.89

Table 2. Evaluation of high yielding mutant lines in preliminary yield trial 1996-97.

Plot size 4.8m²

Genotypes	**Days to flower**	**Days to mature**	**Biological yield (gm)**	**Grain yield/ plot (gm)**	**Grain yield (kg/ha)**
AEL49/20	68.00 c	101.00 cde	1233	443	923
AEL2/20	83.67 a	101.70 cd	1317	453	944
AEL9/20	74.33 bc	99.33 de	1100	402	906
AEL12/30	79.00 ab	99.67 cde	1183	433	902
AEL13/30	68.67 c	101.70 cd	1050	321	669
AEL15/30	69.00 c	103.00 c	1167	333	694
AEL23/40	71.33 c	107.70 d	1350	336	700
AEL28/40	68.67 c	99.33 de	983	298	621
AEL57/50	70.67 c	97.67 e	817	195	406
AEL45/60	80.67 ab	98.33 de	750	194	404
M-85	82.00 a	111.00 a	1067	290	673
ICARDA-8	78.33 ab	112.00 a	1216	253	527
LSD 5%	6.244	3.228	NS	NS	_

Table 3. Evaluation of high yielding mutant lines in micro yield trial 1997- 98.

Plot size 3.6 m²

Genotypes	**Days to flower**	**Days to mature**	**Biological yield (gm)**	**Grain yield / plot (gm)**	**Grain yield (kg/ha)**
AEL49/20	**68 cd**	**113 bcd**	**1933**	**577**	**1602**
AEL28/40	67 d	111 d	1767	503	1397
AEL41/50	67 cd	113 cd	1567	491	1366
AEL54/50	68 cd	116 bc	1617	460	1278
AEL55/50	73 bc	115 bc	1450	397	1102
AEL57/50	68 cd	115 bc	1717	527	1463
AEL45/60	83 a	120 a	1783	436	1213
M-85	81 a	117 ab	1800	473	1315
ICARDA-8	75 b	120 a	1750	568	1579

All values of biological yield and grain yield are Non-significant.

Twelve mutants along with their mother varieties were evaluated in preliminary yield trial during Rabi 1996-97. Data on different morphological characters and yield were taken. Table 2 shows that mutant line AEL 2/20 produced higher grain yield (944kg/ha) followed by mutant strain AEL 49/20 (923 kg/ha) significantly than the mother variety M-85 (673 kg/ha). It is evident from the data that mutant line AEL 49/20 flowered 16 days earlier (68 days) than mutant AEL 2/20 (83.67 days). Seven high yielding mutant lines alongwith their mother were further evaluated in micro yield trial during Rabi, 1997-98. Table 3 shows that mutant strain AEL 49/20 gave higher grain yield (1602 kg/ha) than the mother variety M-85 (I315 kg/ha). The data in Table 3, also shows that mutant line AEL 49/20 flowered significantly earlier (68 days) than the mother (81 days).

Table 4. Performance of lentil mutants in Advance Station Yield Trial at NIA during Rabi 1998-99.

Plot size 7.5 m^2

Genotypes	Days to flower	Days to mature	Biological yield (gm)	Grain yield (gm)	Grain yield (kg/ha)	Rank
AEL 8/92	79.33	110.00	4433c	1613c	2151	6
AEL 9/92	68.00	108.33	4700ab	1777ab	2369	4
AEL 12/92	79.33	111.33	5300 a	1840a	2453	2
AEL 49/20	66.67	107.67	5300a	1852a	2469	1
AEL 15/30	68.67	108.67	4667ab	1823ab	2431	3
M-85 (Check)	80.33	110.33	4167bc	1683bc	2244	5

Table 5. Performance of lentil mutants in zonal yield trial at Tando Jam 1999-00.

Plot size = 4.8 m^2

S. #	Genotypes	Days to flower	Days to mature	Biological yield (g/plot)	Grain yield (kg/ha)	Rank
1.	AEL 8/92	81.00 a	109.00 ab	2117	1340	2
2.	AEL 9/92	74.76 b	109.66 ab	1700	975	3
3.	AEL 10/92	84.64 a	111.67 a	833	362	12
4.	AEL 11/92	82.67 a	110.33 ab	867	408	11
5.	AEL 12/92	85.33 a	112.33 a	1375	640	8
6.	AEL 13/92	84.67 a	111.00 ab	1533	691	6
7.	AEL 14/92	80.33 a	110.00 ab	1575	752	5
8.	AEL 15/92	71.67 b	106.67 b	1083	571	10
9.	AEL 49/20	71.00 b	100.33 c	1883	1467	1
10.	M-85 (Check)	81.33 a	107.67 ab	1200	787	4
11.	M-93 (Check)	73.67 b	107.67 ab	1200	681	7
12.	TC M-85	81.33 a	109.33 ab	1317	577	9
Significance at $p=\leq 0.05$				n.s	n.s	

Values associated with different letters are significantly different from each other

For further evaluation five high yielding mutants were evaluated in advance yield trial during Rabi 1998-99 at NIA, Tando jam (Table 4). The data shows that mutant strain AEL 49/20 gave significantly higher grain yield (2469 kg/ha) followed by AEL 12/92 (2453 kg/ha) and ranked first as compared to all lines tested. For extensive evaluation, the best performing eleven mutants alongwith check were further tested over different agro-climatic zones of Sindh province during Rabi 1999-2000. Performance of lentil mutants in zonal trial conducted at Tando jam is presented in table 5. Data on days to flower, days to maturity, biological yield (g/plot) and grain yield (kg/ha) were recorded. Data on days to flowering showed that mutant line AEL 49/20 flowered significantly 16 days earlier than its mother variety M-85. Grain yield of AEL 49/20 was statistically higher (1274 kg/ha) than its mother variety M-85 (889 kg/ha). Overall mean performance of five locations in the province of Sindh is depicted in Table 6. The mutant line AEL 49/20 gave higher grain yield (953 kg/ha) followed by mutant line AEL 12/30 (862 kg/ha). Manzorabad district Dadu is the highest yielding site as compared to other sites. Five mutant lines along with check M-85 were further evaluated over different agro-climatic zones of Sindh province during Rabi 2000-01.The mutant line AEL 49 /20 gave significantly higher grain yield (1595 kg/ha) followed by AEL 12/30 (1549 kg/ha).

Table 6. Overall mean performance of high yielding mutants in zonal yield trials at different locations in the province of Sindh during Rabi 1999- 2000.

Plot size = 7.5 m^2

Genotypes	Tando jam	Shahdadpur	Manzoorabad	Sindhri	Blochabad	Dokri	Mean of six locations	Grain yield (kg/ha)	Rank
AEL49/20	956a	203a	2167a	517	183	267	715	953	1
AEL8/92	623abc	275a	1066bc	583	317	243	518	690	9
AEL9/92	657abc	226a	1233bc	333	283	247	497	662	11
AEL12/92	520bc	095a	1433ab	583	433	267	515	687	10
AEL2/20	770abc	208a	1750ab	317	450	237	622	829	3
AEL9/20	710abc	317a	1166bc	583	283	265	554	739	8
AEL12/30	933ab	317a	1500ab	403	450	277	647	862	2
AEL15/30	830abc	266a	1400ab	466	317	267	591	788	5
AEL23/40	533bc	117a	1217bc	483	317	268	489	652	12
AEL57/50	923abc	333a	933c	583	383	270	571	761	7
AEL28/40	903abc	250a	1233bc	650	366	260	610	814	4
M-85 (Check)	667abc	200a	1050bc	500	800	230	575	766	6

Table 7. Grain yield (g/plot) of high yielding mutants in zonal trials at different locations in the province of Sindh during Rabi 2000-01.

Plot size 7.5 m^2

Genotypes	Tando Jam	Shahdadpur	Manzoorabad	Sanghar	Blochabad	Mean	Grain yield (kg/ha)	Rank
AEL 8/92	1613c	243a	1163a	1630bc	593a	1048bc	1397	4
AEL 9/92	1777abc	262a	1067a	1633bc	493a	1046bc	1395	5
AEL 12/92	1840abc	258a	1130a	1573c	553a	1071abc	1428	3
AEL 12/30	2117a	285a	983a	1650bc	773a	1162a	1549	2
AEL 49/20	1852abc	298a	1183a	1947a	700a	1196a	1595	1
M-85 (Check)	1683bc	270a	923a	1767ab	470a	1023c	1364	6

Table 8. Performance of lentil candidate variety AEL-49/20 over commercial checks in LNUYT during 2001-02.

S. #	Locations	Grain yield kg/ha AEL-49/20	Yield of checks kg/ha		% Increase over checks
			Name	Yield	
1.	NIA, T. Jam	1162	Masoor-93	477	144
			Markaz	576	102
2.	BARI, Chakwal	437	Markaz	347	26
3.	ARS, Swat	661	Masoor-93	198	234
			Markaz	513	29
4.	ARI, D.I. Khan	1640	Masoor-93	1262	30
			Markaz	1286	28
5.	RRI, Dokri	336	Masoor-93	220	53
			Markaz	290	16
6.	AARI, Faisalabad	2493	Masoor-93	2097	19
			Markaz	2291	09
Increase over the checks		1121		869	29

Table 9. Performance of lentil candidate variety AEL-49/20 over commercial check in LNUYT during 2002-03.

S. #	Locations	Grain yield kg/ha		% Increase over checks
		AEL-49/20	Masoor-93 Check	
1.	NIA, T. Jam	2956	2375	24
2.	NARC Islamabad	2418	2075	17
3.	BARI, Chakwal	2167	1484	46
4.	BARS, Kohat	1292	510	153
5.	RARI, Bahawalpur	1875	1563	20
Increase over check		2142	1601	34

Keeping in view the performance of mutant line AEL 49/20 in the province of Sindh, it was promoted as a candidate variety in Lentil National Uniform Yield Trial. The high yielding mutant AEL 49/20, derived from the irradiated population of M-85 was evaluated for its yield performance in National Uniform Yield Trial for consecutive three years from 2001 to 2004. During Rabi 2001-02 the candidate line AEL 49/20 gave more than 29% grain yield (Table 8) over national checks (Masoor-93 and Markaz). In LNUYT 2002-03 (Table 9) shows that candidate variety AEL 49/20 produced 34% more grain yield than commercial check Masoor -93. In the year 2003-04, the LNUYT data showed (Table 10) that the candidate variety AEL 49/20, gave 21% more grain yield over commercial checks (Masoor -93 and Markaz). Table 11 shows the three years (2001-04) performance of candidate variety at Sindh level gave 68% higher grain yield over national checks. Three years over all performance of candidate variety AEL 49/20 (Table 12) gave 18% more grain yield than the national checks.

Table 10. Performance of lentil candidate variety AEL-49/20 over commercial checks in LNUYT during 2003-04.

S. #	Locations	Grain yield kg/ha AEL-49/20	Yield of checks kg/ha		% Increase over checks
			Name	Yield	
1.	NIA, T. Jam	2028	Masoor-93	1390	46
			Markaz	854	137
2.	NARC, Islamabad	1736	Masoor-93	1592	9
			Markaz	1597	9
3.	NIAB, Faisalabad	549	Masoor-93	458	20
			Markaz	521	5
4.	BARI, Chakwal	281	Markaz	256	8
5.	PRS, Sohawali	430	Masoor-93	226	90
			Markaz	371	16
6.	AZRI, Bhakkar	1232	Markaz	744	66
7.	BARS, Kohat	1250	Markaz	778	61
8.	ARI, D.I. Khan	2032	Masoor-93	1985	2
			Markaz	1528	33
9.	ARI, Tandojam	1056	Markaz	750	41
10.	AZRI, Quetta	124	Markaz	106	17
11.	ARI, Sariab	1428	Masoor-93	1012	41
			Markaz	1377	8
Increase over checks		1104		914	21

Table 11. Three years (2001-04) yield performance of candidate variety AEL-49/20 in LNUYT in the province of Sindh.

Locations	Grain yield kg/ha AEL-49/20	Yield of checks		% Increase over checks
		Name	Yield	
NIA, T. Jam	2031	Masoor-93	1414	44
		Markaz	715	184
ARI, T. Jam	1056	Markaz	750	41
RRI, Dokri	336	Masoor-93	220	53
		Markaz	290	16
Increase over the checks	1141		678	68

Note: At Dokri 336 kg/ha is misleading for calculation of potential yield of AEL-49/20

Table 12. Three years performance of candidate variety (AEL-49/20) in LNUYT on Pakistan basis.

Year	Locations	AEL-49/20 yield kg/ha	Masoor-93 yield kg/ha	Markaz-2001 yield kg/ha	Mean of checks
2001-02	19	1016	941	1050	996
2002-03	23	1679	1582	-	1582
2003-04	18	1218	1161	949	1055
Mean		1304	1228	1000	1114

- Three years performance (pooled)
- 6% increase over Masoor-93
- 30% increase over Markaz-2001 and
- 18% increase over both checks

Keeping in view the over all better performance of AEL 49/20 (NIA – MASOOR – 05) from M_3 population to advance and national yield trials, a proposal for its approval and release as a new first high yielding variety of lentil in Sindh province was submitted to Technical Sub-committee for Approval of new crops Varieties and Techniques during year 2005. This committee has recommended for submission to the Provincial Seed Council Government of Sindh for its approval and release for general cultivation in the province of Sindh as the first ever lentil variety (NIA – MASOOR – 05) of Sindh on the basis of short duration, better quality, high yielding and disease resistant .The Provincial Seed Council Government of Sindh in its 27th meeting held on 19th January 2006 at Karachi has approved the release of NIA – MASOOR – 05 for general cultivation in the province of Sindh as the first ever lentil variety endowed with high yielding, disease resistant, high protein and hydration capacity.

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