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GENETIC MANIPULATION OF LENTIL THROUGH INDUCED MUTATIONS

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Abstract

Induced mutation in crops improvement plays an important role in manipulating the genetic structure of plant. Drought is considered to be the major environmental stress that limits lentil yields. Genetic diversity in lentil (*Lens culinaris* Medik) was developed in a local variety Masoor-85 and exotic cultivar ICARDA-8 by treating with gamma rays ranging from 100–600 Gy. Desirable segregants were isolated in M_2 for high yield, earliness and improved 100 grain weight. These mutants were confirmed for their yield, yield components and growth behaviour in M_3 generation. True breeding lines were evaluated for yield potential under residual moisture of rice crop in the field and to screen out suitable drought resistance genotypes which can cope with the prevailing water shortage in Sindh. The promising mutant strains giving better yield potential were tested for yield and other agronomic traits in different station yield trials. Mutant strain AEL23/40 produced highest grain yield in zonal trials conducted under different agro-ecological zones in Sindh province. Observing its better performance AEL 23/40 was promoted to National yield trials, where it ranked first in the province of Sindh and second on Pakistan basis. In this paper performance of lentil mutant strains evaluated under residual moisture of rice crop and enhancement of genetic potential for drought resistance is discussed.

Introduction

Lentil (*Lens culinaris* Medik) 2n =14, of the family Leguminoseae is one of the oldest food crops originated in the Fertile Crescent of the Middle East (Renfrew, 1969). Lentil was first domesticated in Southern Turkey, from there it moved to Europe and Asia (Ladizinsky, 1979; Cubero, 1984). It is an annually sown, cool season food legume crop. As a food, it provides about 26% protein and has the ability to thrive on relatively poor and marginal lands and even under drought conditions (Verma *et al*., 1993).

Pakistan is one of the major lentil growing countries of south Asia, where this crop is grown on an area of 39,000 hectares with a production of 21,100 tones annually (Anon., 2007a). In Sindh, lentil being the second important winter crop occupies an area of about 8200 ha (Anon., 2007a), with an annual production of 5,400 tones/ha and 659 kg/ha grain yield. Being low delta leguminous crop, lentil thrives well on the residual moisture of rice and no additional irrigation is required. However, it is being successfully cultivated in the districts of Hyderabad, Thatta, Dadu, Larkana, Jacobabad, Sukkur, Sanghar, Nawabshah and Mirpur Khas (Anon., 2007b).

Low yield potential of existing varieties, 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 (Fazal Ali & Shaikh, 2007a).In addition, success in genetic improvement of lentil has been very limited mainly owing to a narrow genetic base, extreme specificity of adaptation, and use of ineffective exotic germplasm.

To generate new useful genetic variability for developing high yielding and widely adapted varieties of lentil induced mutation work was initiated at NIA Tandojam in the late nineties. Mutated genes can be influenced in their action to a high degree by environmental factors and / or other genes of the genome. It is possible to manipulate specific genes by cultivating the material under distinct climatic conditions or by transferring the genes into specific genotypic back grounds. The behaviour is of particular interest where genes of economic value are involved. Earlier Kharkwal *et al*., (1988), Micke, (1988) and Uhlik (1973) have clearly demonstrated that induced mutation breeding is a useful additional source for creating an innovative genetic variability in lentil. Gottschalk (1981) conducted radio-sensitivity studies and created genetic variability through induced mutation in lentil and also in other pulse crops. Mutation breeding acts as a complementary approach (Fazal Ali & Shaikh, 2007b) and has been resorted 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 (Fazal Ali & Shaikh, 2007b).

Materials and Methods

The homogeneous seeds of commercial varieties (Masoor-85 and ICARDA–8) of lentil (*Lens culinaris* Medik) , were moisture equilibrated over a 60% glycerol solution in a desiccator for six days prior to exposure to gamma rays having doses 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 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 was evaluated in different yield trials. Eight high yielding mutants giving better field performance in these trials were further evaluated in preliminary yield trial. Ten 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. Mutant strain AEL23/40 produced highest grain yield in zonal trials and was promoted to national yield trials.

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 2/20, AEL 12/30, AEL 9/20, AEL 45/60, AEL13/30 and AEL23/40 gave higher grain yield as compared to mother varieties M-85 and ICARDA-8. The number of branches/plant (2.75), 100 grain weight (2.41 g) and grain yield per plant (7.45 g) of mutant strain AEL 2/20 was higher than rest of the mutants and controls. Mutant AEL 12/30 flowered 21 days and matured 10 days earlier than its mother variety ICARDA-8.

Eight mutants along with their mother varieties were evaluated in preliminary yield trial during Rabi 1996-97. Table 2 shows that the mutant line AEL 2/20 produced higher grain yield (944 kg/ha) followed by mutant strain AEL 12/30 (902 kg/ha) than the mother variety ICARDA-8 (527 kg/ha). The data also revealed that mutant line AEL 13/30 flowered 10 days earlier (68 days) than mother ICARDA-8 (78 days).

Table 1. Agronomic evaluations of different mutant strains in M_3 generation during Rabi 1995-96.

Genotypes	Radiation doses (γ rays)	Days to flower	Days to maturity	Plant height (cm)	Branches per plant	Pod length (cm)	Seeds/ pod	Pod/ plant	100 grain weight (g)	Grain yield/ plant (g)
ICARDA-8	Control	88	135	39.8	2.00	1.06	2.0	188.4	1.73	4.89
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
AEL 20/30	300Gy	87	131	42.6	2.00	1.18	2.0	179.4	1.80	4.95
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 41/50	500Gy	68	125	37.8	3.80	1.16	2.0	119.8	2.40	4.61
AEL 45/60	600Gy	85	131	42.2	1.40	1.10	1.8	180.6	1.60	5.83
M-85	Control	86	134	42.2	2.00	1.04	1.4	62.0	1.46	1.85
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
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
AEL57/50	500Gy	84	125	37.2	1.40	1.14	2.0	84.0	1.66	3.67

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

Plot size 4.8m^2

Genotype	Days to flower	Days to mature	Biological yield (g)	Grain yield/ plot (g)	Grain yield (kg/ha)
AEL2/20	84 a	102 cd	1317	453	944
AEL9/20	74 bc	99 de	1100	402	838
AEL12/30	79 ab	99 cde	1183	433	902
AEL13/30	68 c	102 cd	1050	321	669
AEL23/40	71 c	107 d	1350	336	700
AEL28/40	69 c	99 de	983	298	621
AEL57/50	71 c	97 e	817	195	406
AEL45/60	81ab	98. de	750	194	404
M-85	82 a	111 a	1067	290	604
ICARDA-8	78 ab	112 a	1216	253	527
LSD 5%	6.24	3.23	NS	NS	-_

Table 3. Two years overall mean performance of AEL-23/40 (grain yield kg/ha) in ZYT conducted allover Sindh.

Genotypes/ Years	1999-2000	2001-02	Mean
AEL-23/40	767	1648	1208
Masoor-85 (Check)	652	1364	1008

Table 4. Two years yield performance under dubari conditions (rice residual moisture) of candidate variety AEL-23/40.

Genotypes/ Years	2002-03	2003-04	Mean
AEL-23/40	1669	435	1052
Masoor-85 (Check)	1333	303	818

The overall mean performance of mutant line AEL 23/40 in different ecological zones of Sindh for two years 1999-00 and 2001-02 (Table 3) showed that it produced 20% more grain yield than check M-85. Mean performance for two years in rice residual moisture (Table 4) revealed that AEL 23/40 produced 28% more grain yield than check M-85 and has the potential to perform better in acute shortage of water which are prevailing in Sindh.

For extensive evaluation, the best performing ten mutants along with check were further tested over different agro-ecological zones of Sindh province during Rabi 2003-2004. Overall mean performance of five locations in the province of Sindh is depicted in Table 5. The mutant line AEL 23/40 gave the highest grain yield (1648 kg/ha) followed by mutant line AEL 28/40 (1647 kg/ha). Tandojam district Hyderabad was the highest yielding site (2509 kg/ha) as compared to other sites.

Keeping in view the superior performance of mutant strain AEL 23/40 in the province of Sindh, it was promoted as a candidate variety in Lentil National Uniform Yield Trial during year 2002.The performance of mutant was very encouraging. The performance of AEL23/40 was reconfirmed in national trial during 2004 and 2007.Three years (LNUYT 2002 to 2004 and 2007-08) performance data on Pakistan basis (Table 6) showed that candidate variety AEL 23/40 produced 6% more grain yield than commercial check Masoor-93 and 21% more grain yield on Sindh basis. In LNUYT 2007-08, the genotypes, locations and their interaction were highly significant ($p<0.01$). The candidate variety AEL 23/40 produced mean seed yield of 1179 kg/ha while rest of the genotypes, including check varieties Masoor-93 and Masoor -06 were inferior in yield producing 819 and 859 kg/ha (44% and 37%) respectively (Table 7).

Table 5. Grain yield (g/plot) of high yielding mutants in zonal trials at different locations in the province of Sindh during Rabi 2003-04.

Plot size 7.5 m^2

Genotypes	Tando Jam	Shahdad Pur	Manzoorabad	Sanghar	Blochabad	Mean of five locations	Grain yield (kg/ha)	Rank
AEL 8/92	1613c	243a	1163a	1630c	593a	1048bc	1397	9
AEL9/92	1777abc	262a	1067a	1633c	493a	1046bc	1395	10
AEL12/92	1840abc	258a	1130a	1573c	553a	1071abc	1428	8
AEL2/20	1883abc	297a	1247a	1917abc	807a	1230a	1640	3
AEL9/30	1932abc	352a	1257a	1733bc	793a	1213ab	1617	4
AEL12/30	2117a	285a	983a	1650c	773a	1162abc	1549	7
AEL23/40	2080ab	273a	1087a	1907abc	833a	1236a	1648	1
AEL57/50	1935abc	287a	1107a	1800bc	840a	1194abc	1592	5
AEL28/40	2010abc	352a	1240a	1992ab	580a	1235a	1647	2
AEL49/20	1852abc	298a	1183a	1947abc	700a	1196abc	1595	6
M-85	1683bc	270a	923a	1767bc	470a	1023a	1364	11
Mean over genotypes	1882	289	1126	1777	676	1150		

Table 6. Performance of AEL-23/40 (Grain yield kg/ha) in (LNUYT) during three years (2002-2004, 2007-08).

Year	Pakistan (Mean of locations)			Sindh (Mean of locations)		
	AEL23/40		Masoor-93	AEL23/40		Masoor-93
2002-03	1576	(12)	1582	2903	(1)	2375
2003-04	1185	(12)	1161	1493	(2)	1289
2007-08	927	(14)	724	1478	(2)	1174
Mean of three years	1229		1156	1958		1613

Values given in parentheses indicates number of locations

Table 7. Performance of lentil candidate variety AEL-23/40 over commercial checks in LNUYT during 2007-08.

Location	Grain yield kg/ha			% Increase over checks	
	AEL 23/40	Masoor -93 (Check)	Masoor-06 (Check)	Masoor -93	Masoor-06
NIA Tando.Jam	1304	903	818	44	59
NARC, Islamabad	1014	917	871	11	16
NIAB, Faisalabad	1746	594	1035	194	69
AARI, Faisalabad	1540	1375	1368	12	13
BARS, Kohat	861	542	743	59	16
ARI, Dera Ismail Khan	717	553	628	30	14
AZRI, Dera Ismail Khan	600	226	239	165	151
QAARI, Larkana	1653	1444	1166	14	42
Mean over locations	1179	819	859		

Conclusion

Mutant AEL23/40 selected in M_2 and confirmed in M_3 for superior yield components produced higher grain yield than the check varieties in station, zonal and national yield trials over the years. It performed exclusively well under water stress conditions. AEL23/40 has therefore the potential for release as a candidate lentil variety for the Sindh province particularly in areas where water is a limiting factor in winter season.

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