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Selection of promising drought tolerant mutant lines in lentil (*Lens culinaris* Medik.)

Ajay Kumar Tomer[1], Kuldeep Tyagi and Jai Prakash Lal

Department of Genetics and Plant Breeding, I. Ag. Sc., B.H.U., Varanasi 221 005
[1]Division of Genetics, Indian Agricultural Research Institute, New Delhi 110 012

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Abstract

Four lentil varieties of small seeded group (PL-639 and PL-406) and bold seeded group (K-75 and L-4076) mutagenised through gamma rays (10, 20 and 30 kR), EMS (0.04 M), sodium azide (0.05 M) and their combinations were evaluated for induced genetic variability and to identify the drought tolerant mutant lines and the trait(s) responsible for enhancing grain yield under rainfed condition. The findings suggest that materials ought to be tested in both moisture stress and moisture non-stress conditions so that the favourable alleles under drought can be maintained as well as the selection response under favorable condition can be maximized. Yield under drought (Y_d), yield potential (Y_p), drought susceptibility index (S) and geometric mean (GM) were considered as the potential indicators for assessing drought resistance of a mutant line. Correlation coefficients between these parameters revealed that GM was positively and significantly correlated with both Y_d and Y_p. There was significant but negative correlation between S and Y_d, while no significant correlation between S and Y_p was observed. It was very clear from the correlation studies that for the enhancement of yield potential under both the conditions selection should be based on GM rather than on S, because S is a better measure of drought tolerance than a measure of performance under stress. Further, selected mutant lines were evaluated for different physiological parameters (nitrate reductase activity, wax content and protein content) in M_4 generation and most of them showed higher values for NR activity and wax content. NR activity and wax content may be used as the more reliable parameters to form the basis of selection under rainfed conditions.

Key words: Induced mutations, drought tolerance, drought susceptibility index, NR activity and lentil

Introduction

Leguminous crops form an important part of the diet of many countries as a major source of protein and play a key role in correcting the incidence of wide spread protein calorie malnutrition. Among legumes, lentils, one of the first agricultural crops grown more than 8,500 years ago are valued for their high nutritive (high protein content) and feed value. This highly valued food legume grown extensively in the Middle East, North Africa, North America, Australia, and South Asia is an excellent supplement to cereal grain diets because of its good protein/carbohydrate content. They are high in fibre, a major source of complex carbohydrates, high in protein, rich in B vitamins and minerals [1].

Grain legumes are important dieting constituents worldwide even though their overall production lags far behind that of the cereals. There are several reasons why grain legume yields, in general, and those of lentil in particular have lagged behind: regulation of these crops to poorer soils, minimal research efforts until very recently, and various biotic and abiotic limitations [2]. As the lentils are predominantly grown in rainfed agriculture, their productivity depends on water conserved in the soil and distribution and frequency of rainfall. Although management practices can contribute to increase yields in water deficit environments, major progress will be realized through genetic improvement [3, 4]. Breeding for drought tolerance involves identification and transfer of morpho-physiological and biochemical traits that may impart drought tolerance to high-yielding cultivars. It is likely that the selection under both optimal and drought conditions represents the ideal environments to select the mutations for high yield under drought as to maintain favourable alleles under drought, at the same time maximising selection response under favourable conditions. Due to established significance of mutation techniques in generating desired, usable variability for breeding or basic research, breeders and plant geneticists have shown great interest in it in the past, which continues to grow. Keeping this in view, the present study was aimed to isolate the mutations for yield related traits and to establish selection criteria on the basis of drought indices and yield means.

Materials and methods

The materials for present study comprised four varieties of lentil, PL-639 (derived from L 9-12 × T-8), PL-406 (selection from P-495), K-75 (selection from Bundelkhand

Local) and L-4076 (from PL-639 × PL-234) of Indian origin. Two of them (PL-639 and PL-406) are of small seeded group (1.5-2.0g/100 seed weight) and spreading type having light green foliage whereas other two (K-75 and L-4076) are of bold seeded group (2.5-2.8g/100 seed weight) and semi-erect type with dark green foliage. All these four varieties are being used as national checks in All India Coordinated Varietal Trials in their respective groups. But none of them has been bred for drought tolerance or resistance which is the utmost objective of the present study. The experiment was carried out at the Agricultural Farm, Banaras Hindu University, Varanasi (India) situated at 25°18'E longitude and 129.23 meters altitude. To isolate the drought tolerant mutant(s), the pure, healthy and dry seeds of each of the four varieties were subjected to mutagenic treatments (Table 1). For irradiation treatment 900 seeds of each variety were irradiated with 10, 20 and 30 kR doses of gamma rays separately with 60_{Co} source at Gamma Cell in the Division of Genetics, IARI, New Delhi. The individual seed lot (of 900 seeds) for a separate dose was subdivided into three equal splits (300 seeds each). The first part was retained as such for gamma rays treatment alone. The second part was used for the combined treatment with EMS and the third part of 300 seeds was treated in combination with sodium azide (SA). Thus, each treatment consisted of 300 seeds per variety. For chemical treatments, pre-soaked seeds (for 6 hrs) were treated with EMS (0.04M at pH 7.0) and SA (0.05 M at pH 3.0), separately for 6 hrs and then thoroughly washed in running tap water.

Table 1. Plan of treatments

Treatment	Details
T1	Control
T2	Gamma rays (10 kR)
T3	Gamma rays (20 kR)
T4	Gamma rays (30 kR)
T5	EMS (0.04 M)
T6	EMS (0.04 M) + Gamma rays (10 kR)
T7	EMS (0.04 M) + Gamma rays (20 kR)
T8	EMS (0.04 M) +Gamma rays (30 kR)
T9	Sodium azide (0.05 M)
T10	Sodium azide (0.05 M) + Gamma rays (10 kR)
T11	Sodium azide (0.05 M) + Gamma rays (20 kR)
T12	Sodium azide (0.05 M) + Gamma rays (30 kR)

M_1 generation was raised from the treated seeds along with the control of each variety in the field in three replications during the winter season (mid October to March) of 1999-2000. Each treatment was sown in three rows of 4 m spaced at 30 cm apart keeping seed to seed distance 5 cm. On maturity, each M_1 plant was harvested separately and advanced to raise single plant progeny rows in M_2 generation during the winter season of 2000-01. Selection in M_2 was done at inter-family levels following Sarker and Sharma [5]. Five normal looking plants were selected randomly from each progeny row in each treatment of each variety to record the observations. The M_2 mutant lines which showed higher CV and mean than the highest CV and mean of the respective control for any of the character, except for days to flowering and maturity, where lower mean was considered, were selected as promising micromutant lines. Since the minimum number of promising M_2 mutant lines for any one of the treatments was seven, only seven promising mutant lines having higher mean and CV were selected from each treatment for raising the M_3 generation. Each of these selected M_2 mutant lines was bulked and grown separately during the winter season of 2001-02 in split plot design with three replications. Stress treatment i.e. irrigated (non-stress) and non-irrigated (stress) was taken as main factor while varieties and mutagenic treatments were taken as second and third factor, respectively. The stress environment involved naturally occurring intermittent stress, as it was protected from winter rain by covering the plot with polythene cloth. Moisture non-stress treatment received only one supplemental irrigation at the time of flowering (with the start of blooming) i.e. on 68th day of sowing. The soil moisture (30 cm depth) across the treatment ranged from 8.2 to 8.8 percent. Data were recorded on five randomly selected plants in each mutant line under both water stress and non-stress conditions.

Drought susceptibility/resistance of a mutant line in M_3 generation in the field was assessed through the measurement of yield under water stress (Y_d), non-moisture stress, i.e., full genetic yield potential (Y_p), drought susceptibility index (S) and geometric mean (GM). These were considered as the potential indicators for drought tolerance/ resistance of a mutant line. Improved drought resistance is inversely related to S, indicating that a number one ranking would have the lowest S value for a population. In other words, low drought susceptibility index is synonymous with high drought resistance [6].

Data recorded in M_3 generation were computed for analysis of variance for different traits. A drought susceptibility index was used to characterize relative stress tolerance of all the mutant lines. The index was calculated independently using a generalized formula given by Fischer and Maurer [7] and Clarke *et al.* [8], in which

$S = (1-Y_d/Y_p)/DII$

Y_p = Mean yield of a mutant line under non-stress, and

Y_d = Mean yield for the same mutant line under stress

DII (Drought Intensity Index) = 1 - X_d/X_p

X_d = the mean yield averaged across mutant lines in the stress treatment, and

X_p = the mean yield averaged across mutant lines in the non-stress treatment.

The selection method was followed according to Schneider *et al.* [9], which was based first on the GM, followed by selection based on Y_d.

$$GM = \sqrt{(Y_p \times Y_d)}$$

where, GM = Geometric mean of yield of a mutant line under stress and non-stress.

A number of mutant lines promising for drought tolerance/resistance were selected in M_3 generation and advanced to grow in M_4 generation under moisture-stress condition. Further, a few mutant lines were selected on the basis of higher Y_d than the respective control in each variety and analysed for chemical tests in M_4 generation. The activity of enzyme nitrate reductase [10], total seed protein content by using Micro-Kjeldahl method [11] and wax content of leaves were measured for promising M_4 mutant lines of each variety.

Results and Discussion

Seed yield is the most important economic trait of lentil, and hence, the most practical method to improve its performance is through the direct measurement of yield-related characteristics.

Screening for drought tolerance in M_3 generation: The assessment of mutant lines for drought susceptibility/resistance in M_3 generation in the field was done through the measurement of Y_d, Y_p, S and GM. A proportion of M_3 mutant lines were selected on the basis of high GM. Within this group, a second selection was performed based on higher Y_d to ensure the maintenance of yield performance under stress which resulted in the selection of 38 mutant lines (Table 2) out of 308 total mutant lines (77 in each variety).

Variety PL-639 gave the maximum number of mutant lines (21) followed by PL-406 (7), L-4076 (6) and K-75 (4). The induction of drought tolerant mutant lines was higher in both the small-seeded varieties (PL-639 and PL-406) as compared to bold-seeded varieties (K-75 and L-4076). Among the mutagenic treatments, 20 kR gamma rays was the most efficient treatment accounting for a total of 17 mutant lines either singly or in combination with sodium azide. This was followed by 10 kR gamma rays that gave 8 mutant lines. Here, most of the mutant lines showed different mutagenic response; if one mutant line is better for one parameter then it failed to hold the same position for another parameter. Differing effects on yield and its components under moisture stress have also been observed earlier by Acosta-G and Adams [12], who suggested that yield components are affected differentially because of contrasts in developmental timing and dissimilarities between stress initiation, intensity, and duration. All the selected mutant lines showed higher ranks than their respective controls except T_{6-5} in PL-639 and T_{3-3} in L-4076 which showed higher drought susceptibility index (lower ranks for S). The lowest drought susceptibility index that is desirable (having first rank for drought resistance) was found for the mutant lines T_{5-6} in PL-639, T_{2-3} in PL-406 and T_{11-5} in K-75.

Relationship between drought parameters: All the parameters (Y_p, Y_d, S and GM) studied above helped to select the lines, which may be promising for dry land conditions, but it is difficult to conclude that which parameter(s) is more effective than the other for screening the drought resistant mutant line(s). To solve this problem, correlation studies were made between the drought parameters in each variety and presented in Table 3. Correlations between S and Y_d in all the four varieties were negative but significant. The S was positively associated with Y_p. Thus, selection for low S could improve Y_d and reduce Y_p. Correlation between S and GM were not significant in either variety whereas GM was highly correlated with Y_d and Y_p. Correlations between GM and Y_d and GM and Y_p can, however, be misleading because both Y_d and Y_p are used in the calculation of GM. Since the correlation coefficient between GM and Y_d was high in all the varieties, the response from selection for Y_d would be greater when selection was based on GM than S. In general, the highest correlation coefficients were noticed between GM and Y_d in PL-639 (0.977), PL-406 (0.973) and K-75 (0.961) and between Y_d and Y_p in L-4076 (0.933).

Chemical Tests in M_4 Generation: Crop researchers particularly, plant physiologists, are striving to attain enhanced crop production under water limiting conditions. Drought tolerance, involves the development of low osmotic potential (dehydration avoidance) and protoplasmic constitution, which help in maintenance of cellular function at low water potential [13]. Twenty promising drought tolerant mutant lines (6 each from PL-639 and L-4076; 5 from PL-406 and 3 from K-75) selected based on higher Y_d in M_4 generation were further subjected to chemical tests *viz.*, nitrate reductase activity, protein content and wax content. All the mutant lines showed positive relationship of NR activities and wax content with grain yield per plant, while the protein content was negatively associated with grain yield per plant (Table 4). Pandey and Singh [14] observed a positive and significant correlation of nitrate reductase

Table 2. Grain yield under moisture non-stress (Y_p) and moisture-stress (Y_d), their geometric mean (GM) and drought susceptibility index (S) with rank for mutant lines selected first on the basis of geometric mean and then Y_d in M_3 generation

Description	Mutant line	Y_p	Rank	Y_d	Rank	GM	Rank	S	Rank
					PL-639				
Control	T_1	4.80	6	4.16	23	4.47	18	2.14	64
10kR	T_{2-1}	4.74	9	4.32	17	4.53	14	1.00	1.59
	T_{2-3}	4.98	1	4.73	4	4.85	1	0.00	0.90
	T_{2-6}	4.76	8	4.59	10	4.67	9	0.64	25
20kR	T_{3-1}	4.74	9	4.70	6	4.72	6	0.45	16
20kR	T_{3-2}	4.68	13	4.53	11	4.60	11	0.57	19
20kR	T_{3-3}	4.76	8	4.72	5	4.74	4	0.15	8
20kR	T_{3-4}	4.85	3	4.64	8	4.74	4	0.78	32
20kR	T_{3-5}	4.82	5	4.36	15	4.54	13	1.72	57
EMS	T_{5-1}	4.74	9	4.63	9	4.68	8	0.41	15
EMS	T_{5-6}	4.68	13	4.67	7	4.67	9	0.03	1
EMS	T_{5-7}	4.72	11	4.70	6	4.71	7	0.07	4
EMS+10kR	$T_{6.5}$	4.84	4	4.16	23	4.49	17	2.53	68
SA	T_{9-3}	4.69	12	4.38	14	4.53	14	1.19	46
	T_{9-6}	4.86	2	4.63	9	4.74	4	0.85	35
	T_{9-7}	4.72	11	4.52	12	4.62	10	0.76	31
SA+10kR	T_{10-5}	4.74	9	4.39	13	4.56	12	1.33	49
	T_{10-6}	4.80	6	4.28	19	4.53	14	1.95	61
SA+20kR	T_{11-1}	4.80	6	4.74	3	4.77	3	0.22	11
	T_{11-4}	4.78	7	4.26	20	4.51	15	1.96	62
	T_{11-5}	4.73	10	4.35	16	4.54	13	1.45	51
	T_{11-6}	4.80	6	4.78	2	4.79	2	0.07	4
					PL-406				
Control	T_1	5.16	5	4.88	13	5.02	6	2.10	61
10kR	T_{2-3}	5.32	1	5.16	1	5.24	1	–4.93	1
	T_{2-6}	5.18	4	5.10	3	5.14	3	0.39	17
20kR	T_{3-5}	5.10	6	5.02	5	5.06	5	0.39	17
SA	T_{9-3}	5.18	4	4.89	12	5.03	7	1.42	50
SA+10kR	T_{10-3}	5.05	7	5.00	6	5.02	6	0.25	13
SA+20kR	T_{11-6}	5.10	6	5.04	4	5.07	4	0.29	14
	T_{11-7}	5.21	2	5.14	2	5.17	2	0.34	15
					K-75				
Control	T_1	5.34	1	4.97	5	5.15	3	1.40	52
10kR	$T_{2.7}$	5.30	2	5.21	1	5.25	1	0.41	19
SA+10kR	T_{10-4}	5.18	5	5.13	4	5.15	3	0.23	15
SA+20kR	T_{11-5}	5.21	4	5.16	2	5.18	2	–5.00	1
SA+30kR	$T_{12.1}$	5.16	6	5.14	3	5.15	3	0.09	10
					L-4076				
Control	T_1	5.18	3	4.79	13	4.98	7	1.60	43
10kR	T_{2-1}	5.20	2	5.10	1	5.15	1	0.52	21
	T_{2-7}	5.13	5	5.00	2	5.06	2	0.69	27
20kR	T_{3-2}	5.16	34	4.92	5	5.04	4	1.26	37
	T_{3-3}	5.23	1	4.88	7	5.05	3	1.82	49
	T_{3-4}	5.10	6	4.93	4	5.01	5	0.90	34
SA+20kR	T_{11-4}	4.99	10	4.99		4.99	6	0.00	9

(NR) with protein accumulation and seed yield in different lentil cultivars. It was found that NR activity and wax content of most of the mutant lines were higher than their respective controls. All the mutant lines showed lower protein content than their respective control, exception being T_{3-4} in PL-639 which gave higher protein content along with higher wax content and NR activity over control. Out of 20 mutant lines, there was only one mutant line (T_{3-4} in L-4076), which showed the lower values for all three chemical tests as compared to respective control. Mutant line T_{2-3} in PL-639, T_{3-5} in PL-406, T_{10-4} in K-75 and T_{2-1} in L-4076 showed the highest NR activity and wax content than the other mutant lines.

Overall the highest NR activity was found for T_{2-1} mutant line in L-4076 variety. A stable cell membrane that remains functional during water stress appears central to adaptation to high temperatures and found related to heat and drought tolerance [15]. The parameter wax content was found positively associated with the parameters grain yield and the NR activity in all the mutant lines. Overall, NR activity and wax content may be used as the reliable physiological parameters to form the basis of selection under rainfed conditions. Breeding for yield performance in areas where drought conditions are inconsistent often involves costly experiments including tests in both stress and non-stress conditions. A control or non-stress treatment

Table 3. Correlation coefficients between drought parameters in MS generation

Traits	Correlation coefficients			
	PL-639	PL-406	K-75	L-4076
Y_d & Y_p	0.682*	0.972*	0.855*	0.933*
S & Y_d	–0.219*	–0.243*	–0.329*	–0.224*
S & Y_p	0.167	–0.087	0.071	0.183
S & GM	–0.032	–0.123	–0.099	–0.008
GM & Y_d	0.977*	0.973*	0.961*	0.902*
GM & Y_p	0.756*	0.967*	0.928*	0.887*

*Significant at the 0.05 probability level

Table 4. Mean of protein content, NR activity, wax content and grain yield/ plant of some selected promising mutant lines in M_4 generation

Mutant line	Protein content (%)	NRA	Wax content (mu/cm^2)	Yield/plant (g)
		PL-639		
Control (T_1)	25.45	1.62	107.23	4.36
$T_{2\text{-}3}$	24.12	2.21	155.87	4.85
$T_{11\text{-}6}$	24.36	2.08	142.36	4.63
$T_{11\text{-}1}$	24.36	2.02	141.89	4.61
$T_{0\text{-}6}$	23.87	2.16	153.64	4.80
$T_{3\text{-}4}$	25.52	1.87	128.42	4.51
$T_{3\text{-}1}$	24.16	1.98	136.36	4.62
		PL-406		
Control (T_1)	24.16	1.73	114.65	4.76
$T_{2\text{-}6}$	23.89	2.34	162.51	5.19
$T_{11\text{-}6}$	24.12	1.73	110.32	4.85
$T_{3\text{-}5}$	23.47	2.46	171.84	5.45
$T_{9\text{-}3}$	23.59	2.19	159.62	5.12
$T_{10\text{-}3}$	24.06	2.06	146.89	5.05
		K-75		
Control (T_1)	25.88	1.98	121.42	4.24
$T_{10\text{-}4}$	23.65	2.29	187.56	4.98
$T_{2\text{-}7}$	23.81	2.02	172.36	4.76
$T_{12\text{-}1}$	24.76	1.83	146.28	4.72
		L-4076		
Control (T_1)	23.97	1.78	119.35	4.42
$T_{2\text{-}1}$	22.76	2.53	148.42	4.96
$T_{2\text{-}7}$	22.93	2.26	136.08	4.83
$T_{3\text{-}2}$	23.32	1.98	130.42	4.73
$T_{3\text{-}3}$	23.16	1.94	129.36	4.78
$T_{3\text{-}4}$	22.45	1.69	108.48	4.62
$T_{11\text{-}4}$	23.05	2.11	131.39	4.81

is important to measure the true yield potential of genotypes and to determine the degree of drought to which the genotypes were subjected [9]. We propose a simple, conventional breeding strategy that involves selecting a proportion of genotypes (here, mutant lines) based on a high GM. Within this group, a second selection would be performed based on high Y_d to ensure the maintenance of yield performance under stress.

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