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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(7): 750-753

www.thepharmajournal.com
Received: 02-05-2023
Accepted: 04-06-2023

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Optimizing agro-morphological traits of lentil genotypes in response to drought and salt stress

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Abstract
Lentil (*Lens culinaris*) plays a crucial role as a staple food crop in developing countries. However, its production faces significant challenges such as susceptibility to drought and salt stress, along with limited genetic variability. Therefore, it is imperative to develop an efficient prefield drought phenotyping technique and identify diverse drought tolerant and salt tolerant lentil genotypes. In this experiment, the focus was on studying ten different lentil genotypes. Triplicate pots were prepared for each genotype, with approximately five seeds inoculated in each pot. Once the seeds germinated, the 15-day-old seedlings were subjected to drought stress by irrigating them with a solution containing 18% PEG 6000. For inducing salinity stress, the seedlings were watered with a solution containing 200 mM NaCl. This stress treatment was maintained for a period of 30 days. After the stress period, various growth parameters including plant height (PH), seedling vigour index II (SVI II), root length (RL), shoot length (SL), seedling length (SDL), and seedling germination percentage (SGP) were examined. Additionally, other traits such as the number of days required to reach 50% flowering (NFF), 100 seed weight (HSW), number of branches per plant (NBP), and number of days to reach 90% maturity (DM) were also recorded in all three biological replicates of the genotypes. The collected data underwent statistical analysis using ANOVA. Among the ten genotypes studied, PDL 2 and PDL 1 emerged as highly tolerant to drought and salt stress, respectively. These genotypes exhibited maximum seedling survivability and minimal reduction in growth parameters under drought and salt stress conditions. This valuable information can be utilized to further breed lentil varieties with enhanced drought and salt tolerance characteristics.

Keywords: Lentil (*Lens culinaris*), abiotic stress, drought stress, salt stress, growth parameters

Introduction

Lentil (*Lens culinaris*) is a type of legume that is commonly consumed as a food source. It goes by various names such as red dhal, masur, or split peas. Lentils are highly valued in developing countries for their significant contribution to dietary protein. Additionally, they are rich in complex carbohydrates, dietary fibers, vitamins, and minerals, making them a valuable source of essential nutrients (Adsule *et al.* 1989) [1]. Lentil is known for its protein content, which is estimated to be around 24% and can range from 22% to 26%. However, it is deficient in sulfur containing amino acids while containing relatively high levels of lysine (Newman *et al.* 1988) [5]. Therefore, it plays an important role in overcoming the problem of malnutrition and micronutrient deficiencies among the inhabitants of developing countries especially those who cannot afford costly animal protein-based diets (Kumar *et al.* 2016) [6].

In India, lentil cultivation holds a significant position among rabi pulses, following closely behind chickpea. With an extensive cultivation area of 1.56 million hectares (mha) and an annual production of 1.06 million metric tons (mt), lentils play a vital role in the agricultural landscape. The cultivation of lentils in India is primarily concentrated in the states of Uttar Pradesh (UP), Bihar, Madhya Pradesh (MP), and West Bengal. Collectively, these states contribute to over 80% of both the cultivated area and production of lentils in the country.

Lentil, a resilient plant with a relatively high tolerance to drought, is cultivated across the globe. However, the largest contributors to global lentil production, accounting for over three-fourths of the total output, are Canada, India, Australia, Turkey and USA (Alexander, 2015) [2].

Salinity poses a significant challenge for most legumes, as they exhibit limited genetic diversity when it comes to salt tolerance (Farooq *et al.* 2017) [8]. When focusing specifically on lentils, a protein-rich legume that serves as a staple food for vegetarian, vegan, and low meat-consuming communities, it is observed that salinity stress affects all aspects of its germination and early seedling growth (Foti *et al.* 2019) [9]. Lentils are predominantly cultivated in arid and semi-arid regions, and studies have shown that they display moderate sensitivity to salinity, with a 50% reduction in yield observed at a salinity level of 6.0 dS/m (Singh *et al.* 2017) [10].

Plant breeders and geneticists have a keen interest in understanding the genetic variation present both within and between populations of crop species (Fikiru *et al.* 2007) [12]. It is crucial for breeders to identify and select accessions that are likely to possess the desired traits. This requires knowledge of the trait characteristics of different accessions, enabling more targeted and efficient utilization of germplasm in breeding programs (Fratini *et al.* 2007) [13].

Material and Method

The experiment focused on ten different lentil genotypes. The seeds were subjected to surface sterilization using 1% sodium hypochlorite for 5 minutes. These sterilized seeds were then planted in 12 cm plastic pots containing a mixture of cocopeat, vermiculite, and perlite in a ratio of 3:1:1. Approximately five seeds of each genotype were inoculated in triplicate pots. After germination, the 15-day-old seedlings were exposed to drought stress by watering them with a solution of 18% PEG 6000 (Muscolo *et al.* 2014) [11]. For salinity stress, the seedlings were watered with a 200 mM NaCl solution. The pots were kept in a greenhouse for the next 15 days, with the control counterparts being regularly watered. After a 30-day period of stress treatment, various growth parameters such as plant height (PH), seedling vigour index II (SVI II), root length (RL), shoot length (SL), seedling length (SDL) and seedling germination percentage (SGP) were examined. Three biological replicates were harvested for each genotype in each experiment. Additionally, the number of days to reach 50% flowering (NFF), 100 seed weight (HSW), no of branches per plant (NBP) and Number of days to reach 90% maturity (NM) were observed in all three biological replicates of the genotypes. The data were statistically analysed using ANOVA.

The data were statistically analysed using ANOVA.

S. No	Genotypes	Parentage	Source
1	DPL-15	PL406 × L4076	IIPR, Kanpur
2	DPL-62	JLS1 × LG171	IIPR, Kanpur
3	IPL- 81	K75×PL639	IIPR, Kanpur
4	IPL-406	DPL 35 × EC 157634/382	IIPR, Kanpur
5	IPL-316	Sehore 74-3 × DPL 58	IIPR, Kanpur
6	K-75	local selection from Bundelkhand	CSAUAT, Kanpur
7	JL-3	local selection from Sagar, MP	JNKVV, Jabalpur
8	L-4076	PL 234 X PL 639	IARI, (New Delhi)
9	PDL-1	ILL-7620 x 88522	IARI, (New Delhi)
10	PDL-2	ILL-590´ ILL-7663	IARI, (New Delhi)

Result and Discussion

After subjecting lentil varieties to drought and salt stress for 30 days, the growth parameters were evaluated to assess their performance. Notably, significant differences were observed among the varieties in response to both stress conditions. The plant height of the studied lentil genotypes was significantly influenced by both drought and salt stress. Under control conditions, JL 3 genotype displayed the shortest plant height, measuring 16.8 cm, while PDL 2 genotype exhibited the tallest plant height at 22.5 cm. The imposition of drought stress resulted in a significant decrease in plant height, with JL 3 experiencing the largest reduction (30.43%) and PDL 2 showing the lowest reduction (4.65%). The application of salt stress caused a substantial reduction in plant height, with L 4076 experiencing the highest reduction (41.37%), while IPL 81 showed the lowest reduction (4.67%), as shown in Table 2.

The examined lentil genotypes displayed significant diversity in the number of days to reach 50% flowering (DFF) under both drought and salt stress conditions. Consistent with previous findings (Sinha *et al.* 2018) [14], our study also observed similar results in terms of the reduction in plant height under drought and salt stress. In the absence of stress (Tab.2), PDL 2 genotype had the highest DFF, taking 96 days, while JL 3 genotype had the lowest DFF at 70 days. Drought stress led to a notable decrease in DFF for most genotypes, with JL 3 experiencing the highest reduction (17.98%) and PDL 2 showing the lowest reduction (2.49%). Salt stress caused an enormous decrease in DFF for most genotypes, with DPL 15 experiencing the highest reduction (10.21%) and PDL 1 showing the lowest reduction (3.69%) (Tab. 2).

While number of days to reach 90% maturity (DM) under control condition, PDL 2 genotype had the highest DM, taking 136.67 days, while JL 3 genotype had the lowest DM at 113.3 days. Drought stress led to a notable decrease in DM for most genotypes, with IPL 406 experiencing the highest reduction (10.84%) and PDL 1 showing the lowest reduction (1.75%). Salt stress caused a substantial decrease in DM for most genotypes, with JL 3 experiencing the highest reduction (9.32%) and PDL1 showing the lowest reduction (2.01%) (Tab. 2).

Furthermore, the investigated lentil genotypes exhibited notable variation in the hundred seed weight (HSW) in response to drought and salt stress. Under control conditions, PDL 2 genotype had the highest HSW, measuring 3.63 gm, while JL 3 genotype had the lowest number of HSW at 1.80 gm. Drought stress caused a significant decrease in HSW for most genotypes, with DPL 62 experiencing the highest reduction (67.55%) and PDL 1 showing the lowest reduction (3.25%). The imposition of salt stress caused a notable decline in HSW for the majority of genotypes. Particularly, DPL 62 exhibited the highest reduction (59.93%), while PDL 1 demonstrated the lowest reduction (3.25%) (Tab. 2). For the number of branches per plant (NB) under control conditions, PDL 1 genotype had the highest number of branches, measuring 12.9 cm, while K75 genotype had the lowest number of branches at 7.2 cm. Drought stress caused a significant decrease in NB for most genotypes, with IPL 81 experiencing the highest reduction (46.15%) and PDL 2 showing the lowest reduction (10.53%). Salt stress resulted in a substantial decrease in NB for most genotypes, with L 4076 experiencing the highest reduction (34.06%) and PDL 1 showing the lowest reduction (16.21%) (Tab. 2).

Among the tested lentil genotypes, DPL 62 exhibited the highest root length of 9.2 cm under control conditions, while JL 3 had the lowest root length of 5.4 cm. Most of the genotypes experienced a significant decrease in root length due to drought stress, with k 75 experiencing the highest reduction (58.88%) and PDL 2 showing the smallest decline (5.86%). Under salt stress IPL 316 experienced the highest decline (58.88%), while PDL 1 had the smallest reduction (15.49%), as indicated in (Tab 1).

Tabulating the results, it was observed that under control conditions (Tab. 1), IPL 316 genotype exhibited the highest shoot length of 9.4 cm, while DPL 15 genotype displayed the lowest shoot length of 5.9 cm. Drought stress resulted in a significant reduction in shoot length for most genotypes, with DPL 62 experiencing the highest reduction (38.29%) and PDL 2 showing the lowest reduction (8.3%). Salt stress caused a substantial decrease in shoot length for most genotypes, with DPL 62 experiencing the highest reduction (47.73%) and PDL 2 showing the lowest reduction (8.12%) (Tab. 1). It was observed that under control conditions (Tab. 1), PDL 2 genotype exhibited the highest seedling length of 16.23 cm, while L 4076 genotype displayed the lowest seedling length of 13.49 cm. Our study aligns with prior research (Foti *et al.* 2019) [9] by demonstrating comparable results regarding the decrease in shoot and root length when exposed to drought and salt stress. Drought stress resulted in a significant reduction in Seedling length for most genotypes, with IPL 81 experiencing the highest reduction (35.46%) and PDL 2 showing the lowest reduction (7.03%) (Tab. 1). Salt stress caused a substantial decrease in seedling length for most genotypes, with IPL 406 experiencing the highest reduction (58.96%) and PDL 2 showing the lowest reduction (10.43%) (Tab. 1).

Based on the collected data (Tab. 1), PDL 2 genotype displayed the highest seedling vigour index (SVI II) of 1304.12 under control conditions. On the other hand, JL 3 genotype showed the lowest SVI II, measuring 945.57. Drought stress resulted in a significant decrease in SVI II for most genotypes, with IPL-316 experiencing the highest reduction (80.8%) and PDL-2 showing the lowest reduction (7.48%) (Tab. 1). The imposition of salt stress led to a notable decline in SVI II across various genotypes, with DPL 15 exhibiting the most significant reduction (73.32%), while PDL 2 displayed the least reduction (18.29%) (Tab. 1). In accordance with the findings of previous research (Akter *et al.* 2020) [15], our study exhibited concordant outcomes concerning the reduction in growth parameters when subjected to both drought and salt stress.

Table 1: Comparison of Traits under Drought and Salt Stress in 10 Lentil (*Lens culinaris*) genotypes.

Genotype	Plant Height (Cm)			Days to 50% Flowering			No of Branches per plant			Days to 90% Maturity			100 Seed Weight (gm)		
	C	T1	T2	C	T1	T2	C	T1	T2	C	T1	T2	C	T1	T2
DPL-15	18.60	15.50	16.34	86.33	82.33	78.33	9.10	6.90	6.80	125.00	120.00	120.00	2.50	1.98	2.02
DPL-62	18.64	16.98	17.22	74.33	70.00	71.00	10.40	7.20	8.70	117.00	110.67	108.33	3.17	1.89	1.98
IPL- 81	21.48	19.56	20.52	85.33	81.67	80.33	11.40	7.80	8.60	120.67	116.67	118.00	2.70	2.25	2.43
IPL-406	18.76	17.02	15.72	86.33	80.33	82.33	9.27	7.01	7.60	122.67	110.67	113.67	2.30	2.01	2.01
IPL-316	21.52	19.60	18.70	87.33	81.33	80.67	8.37	6.90	6.10	121.00	117.33	113.33	1.90	1.76	1.72
K-75	21.26	18.50	20.20	80.00	74.33	72.67	7.20	5.30	4.80	128.67	124.00	121.33	3.07	2.85	2.43
JL-3	16.80	12.88	14.60	70.00	59.33	64.00	5.90	5.10	5.40	113.33	108.33	103.67	1.80	1.42	1.32
L-4076	18.52	14.98	13.10	86.67	76.67	80.33	12.30	9.10	9.10	128.67	124.00	121.33	2.30	2.07	2.01
PDL-1	22.60	20.14	20.82	93.67	85.67	90.33	12.90	9.10	11.10	135.33	133.00	132.67	3.07	2.97	2.97
PDL-2	22.50	21.50	20.20	96.00	93.67	88.33	12.60	11.40	10.70	136.37	132.00	131.33	3.63	3.42	3.23
GM	20.06	17.66	17.74	84.6	78.53	78.83	9.94	7.58	7.89	124.9	119.6	118.36	2.64	2.20	2.21
CD 5%	1.07	0.95	0.95	4.4	4.23	4.3	0.59	0.41	0.43	3.4	4.67	2.85	0.06	0.08	0.12
SED	0.51	0.45	0.46	2.12	2.04	2.07	0.28	0.20	0.21	1.64	2.25	1.37	0.03	0.04	0.05
SEM	0.36	0.32	0.32	1.5	1.44	1.46	0.20	0.14	0.14	1.16	1.59	0.97	0.02	0.02	0.04
CV	3.15	3.18	3.18	3.07	3.18	3.22	3.51	3.24	3.26	1.61	2.30	1.42	1.43	2.31	3.25
MAX	22.60	20.14	20.82	96.00	93.67	90.33	12.90	9.10	11.10	136.37	133.00	132.67	3.63	3.42	3.23
MIN	16.80	12.88	13.10	70.00	59.33	64.00	5.90	5.10	4.80	113.33	108.33	103.67	1.80	1.42	1.32

Where, PH= plant height, NFF= Number of days to 59% flowering, DN= number of days to 90% maturity, NB= Number of branches per plant, HSW= Hundred Seed weight

Table 2: Comparison of Traits under Drought and Salt Stress in 10 Lentil (*Lens culinaris*) genotypes.

Genotype	Root Length (Cm)			Shoot Length (Cm)			Seedling Length (Cm)			Germination Percentage (%)			Seedling Vigour Index II		
	C	T1	T2	C	T1	T2	C	T1	T2	C	T1	T2	C	T1	T2
DPL-15	8.20	6.47	5.60	5.90	4.40	5.02	14.10	10.87	10.62	78.33	67.00	60.00	1105.13	728.38	637.62
DPL-62	9.20	7.27	6.20	6.50	4.70	4.40	15.70	11.97	10.60	72.00	55.00	67.00	1130.53	658.49	710.62
IPL- 81	9.10	6.53	7.20	7.20	5.50	5.20	16.30	12.03	12.40	84.33	72.00	72.00	1375.09	866.75	893.30
IPL-406	6.21	4.23	3.40	8.10	7.20	5.60	14.31	11.43	9.00	84.33	58.33	71.00	1206.53	667.22	639.36
IPL-316	6.37	3.77	3.60	9.40	8.60	7.90	15.77	12.37	11.50	80.67	51.67	65.00	1271.87	639.26	747.96
K-75	5.67	3.57	3.40	9.20	8.40	8.20	14.87	11.97	11.60	72.67	63.00	55.00	1080.76	754.30	638.46
JL-3	5.47	4.10	5.40	9.30	8.20	7.80	14.77	12.30	13.20	64.00	60.00	58.33	945.57	738.00	769.34
L-4076	6.42	4.77	4.40	7.07	6.20	6.10	13.49	10.97	10.50	80.33	69.00	63.00	1084.00	757.01	661.92
PDL-1	8.20	6.80	6.20	6.40	5.70	6.20	14.60	12.50	12.40	87.33	81.67	85.00	1275.22	1021.45	1054.74
PDL-2	8.43	7.97	7.80	7.80	7.20	6.90	16.23	15.17	14.70	80.33	80.00	75.00	1304.12	1213.33	1102.50
GM	7.32	5.54	5.32	7.68	6.61	6.33	15	12.15	11.65	78.43	65.7	67.13	1177.88	804.4	785.58
CD 5%	0.33	0.19	0.29	0.41	0.35	0.33	0.41	0.52	0.62	4.3	3.03	3.56	88	68	76
SED	0.16	0.09	0.14	0.19	0.17	0.16	0.20	0.25	0.30	2.07	1.46	1.71	42.81	33.14	36.84
SEM	0.11	0.06	0.1	0.14	0.12	0.11	0.14	0.17	0.21	1.46	1.03	1.21	30.27	23.43	26.05
CV	2.67	2.04	3.28	3.15	3.18	3.16	1.64	2.53	3.16	3.23	2.72	3.13	4.4	5.04	5.74
MAX	8.43	7.97	7.80	9.40	8.60	8.20	16.23	15.17	14.70	87.33	81.67	85.00	1304.12	1213.33	1102.50
MIN	5.47	3.57	4.40	5.90	4.40	4.40	13.49	10.87	9.00	64.00	55.00	55.00	954.57	639.26	637.62

Where, RL= root Length, SL= shoot length, SDL= seedling length, SGP= Seedling germination percentage and SVI II= seedling vigour index II.

Conclusion

Maintaining genetic variability in germplasm resources is crucial for ensuring ongoing genetic advancements in cultivars. Understanding the extent of genetic variation and relationships between populations is vital in comprehending the existing genetic diversity and its potential application in lentil breeding programs. The current study indicates the existence of noteworthy genetic variability among the evaluated genotypes. This presents a promising opportunity for improvement through direct selection and hybridization, which entails crossing of different genotypes. By utilizing these approaches, significant enhancements and advancements can be achieved in lentil crop breeding. The degree of reduction in growth parameters under drought and salt stress was more significant in drought-susceptible (DS) and salt-susceptible (SS) genotypes compared to drought-tolerant (DT) and salt-tolerant (ST) genotypes, in relation to their respective control conditions. These findings imply that DS and SS genotypes have a lesser capacity to endure and adapt to stress compared to DT and ST genotypes. The observed variations in the reduction of growth parameters indicate that DS and SS genotypes possess a limited ability to cope with the applied stresses, highlighting their diminished capability to confront and tolerate unfavourable environmental conditions when compared to DT ad ST genotypes. The current study revealed a substantial genetic variability across the 10 traits examined. However, it is recommended to expand the investigation by including a larger number of genotypes across different locations and over multiple years. This expanded research would help validate the significance of these traits as direct contributors to grain yield. By incorporating more genotypes and considering multiple environmental factors, a more comprehensive understanding of the traits' impact on grain yield can be obtained, providing valuable insights for future breeding and improvement efforts.

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