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Heat Tolerance in Lentil under Field Conditions

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Abstract In the present study, 334 lentil accessions were screened for heat tolerance under field conditions in 2011-12 and 160 accessions encounter high temperature (>35 ℃) during the reproductive stage were again screened in 2012-13. Only 37 accessions podded normally and showed pod formation on terminal branch were identified heat tolerant and remaining 59 accessions podded rarely but flowered were identified sensitive to higher temperature. The combined analysis of variance over the years indicated significant genotypic variability for filled and unfilled pods/plant, filled pods on terminal branch and also for 100-seed weight. High heritability was estimated for filled pods/plant (46.3%) and filled pods on terminal branch (58.1%). Based on maximum number of filled pods per plant and on terminal branch along with lower standard error of mean over the years resulted in identification of heat tolerant genotypes (FLIP2009-55L, IG2507 and IG4258). These genotypes also showed higher pollen viability at higher temperature, indicating the usefulness of above trait for identification of heat tolerant donors for lentil breeding program.

Keywords Filled and unfilled pods; 100-Seed weight; Pollen viability; High temperature; Lentil

1 Introduction

Lentil (*Lens culinaris* subsp. *culinaris* Medikus) is an important cool-season legume crop of rainfed agriculture for diversification and intensification of cereal-based cropping systems worldwide. It is grown globally on 3.74 mha area and produces 3.40 mt of grains with an average productivity of 915 kg ha^{-1} (Erskine et al., 2011). India shares about 0.94-1.03 mt (28%) of global lentil production by cultivating it on 1.48-1.59 mha area. It is mostly grown under residual soil moisture conditions during the winter season and hence this crop invariably encounters drought and heat stresses at the time of podding and grain filling period when temperature rises suddenly. As a result it leads to forced maturity and lower yield. In recent years, the global warming has become as a major challenge to rainfed agriculture. It has predicted that heat stress will have more adverse effects on vulnerability of food crops under climate change rather than drought. Therefore, in coming years, high temperature can be an important constraint in lentil production, if night temperature rises by at least 2 °C. Due to this in India, northern part can have higher levels of warming by 2050, while its central and north-eastern parts now have about 11.7 mha as fallow after late harvest of rice and delayed sowing of lentil in these areas encounters force maturity due to high temperature (Subbarao et al., 2001).

In lentil, flowering is known to be very sensitive to changes in external environment especially in temperature and photoperiod. Therefore, heat stress at reproductive stage causes heavy loss in grain yield of lentil (Summerfield et al., 1985). Thus, heat tolerant cultivars can provide not only an opportunity of horizontal expansion of lentil cultivation in rice-fallow lands but also can help to increase lentil productivity by minimizing the yield losses occurring due to forced maturity. It can be visualized that the increases in temperature will have more adverse effects on cool-season crops (e.g. lentil) than the rainy-season crops (Kumar, 2006). Therefore, identification of heat tolerant genotypes in available germplasm and their utilization can help to tackle situation of terminal heat stress through the development of heat tolerant cultivars. Earlier, efforts have been made in other cool-season legume like chickpea to identify the heat tolerant genotypes under field conditions (Dua, 2001; Krishnamurthy et al., 2011).

Yet, it is still not clear how heat affects the growth and development of lentil and whether that can explain part of the differences in seed yield under heat stress. Therefore, it is an urgent need to identify the traits that can be used effectively in field conditions for screening germplasm and breeding materials at reproductive stage. Keeping this in view, the present study aimed (i) to establish an effective screening technique under the field condition by identifying the morphological traits related to heat tolerance and yield and (ii) to validate identified heat tolerant genotypes using laboratory test.

2 Results

2.1 Genetic variability

The procedure used to screen the heat tolerant genotypes is presented in Figure 1. In the present study, out of 334 accessions, 174 accessions flowered early and matured within 80-85 days after sowing. These accessions escaped the heat stress and thus were excluded for further analysis. Another 64 accessions which did not flower or flowered rarely were considered as highly sensitive to heat. The remaining 96 accessions whose flowering and podding stage coincided with high temperature and still flowered were observed for the number of filled pods/plant, number of unfilled pods/plant, and number of filled and unfilled pods on terminal branch of individual plants. These accessions flowered in 56 to 85 days (between March 15 and April 30, 2012) when the maximum day temperature varied between 30.6 and 43 ºC (Figure 2). As a result, development of pods at maximum day temperature (>35 ºC) was used as a criterion to classify accessions as tolerant or sensitive to heat. Thirty seven (37) accessions, which podded normally and showed pod formation on terminal branch, were classified as heat tolerant while remaining 59 accessions that flowered but podded rarely were classified as sensitive to higher temperature. The mean, range and standard error of mean (s.e.m) over two years of these 37 accessions were calculated, which is presented in Table 1. Among 37 accessions, number of filled pods/plant ranged from 3.3 to 51.2 with an average of 23.6 while unfilled pods ranged from 5.5 to 45.0 with an average of 24.2. On terminal branch, filled pods were varied from 1.5 to 10.0 with an average of 3.3 and unfilled pods were varied from 0.0 to 3.8 with an average of 1.3. The 100-seed weight in these 37 accessions ranged from 1.1 to 2.5 g with an average of 1.3 g. The combined analysis of variance over the years indicated significant genotypic variability for filled and unfilled pods/plant, filled pods on terminal branch and also for 100-seed weight (100-SW). Heritability estimates ranged from 8.94 to 58.13% (Table 2). Highest heritability (58.13%) was observed for filled pods/plant on terminal branch and it was lowest (8.94%) for unfilled pods/plant.

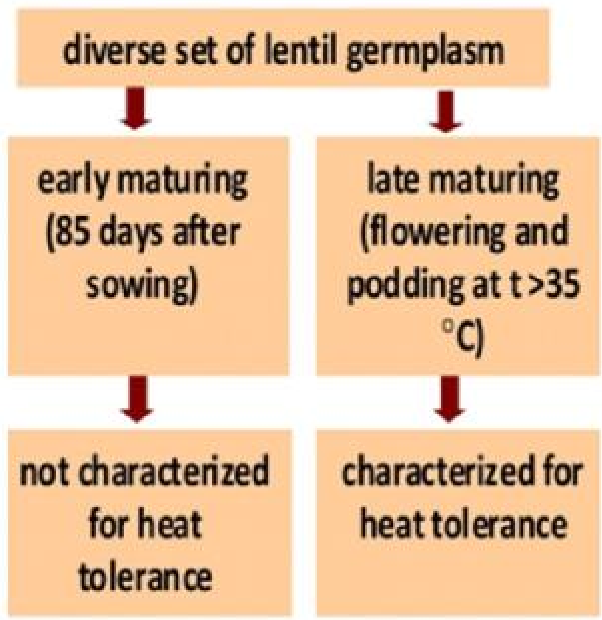

Figure 1 Technique used to screen heat tolerant genotype in lentil under field conditions

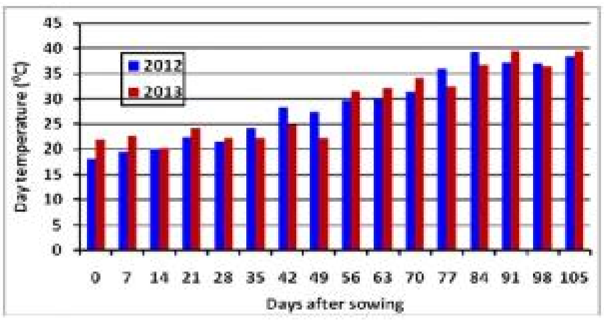

Figure 2 Standard weekly temperature over two years during January to March at experimental farm of IIPR, Kanpur

The results indicated significant year effects on filled and unfilled pods/plant and hence wide variation in s.e.m over years. It was ranged from 0.2 to 37.8 for filled pods/plant and 0.0 to 16.5 for unfilled pods. Therefore, stable genotypes were identified with lower s. e. m. over the years along with highest pod formation per plant and on terminal branch.

Table 1 Mean with standard error of mean over the two years for 37 genotypes of lentil

Accessions	DFF	FP/P	UNFP/P	FP/TB	UNFP/TB	100SW
PRECOZ	6.5±0.50	20.0±5.0	9.5±0.5	2.3±0.8	1.0±1.0	2.3±0.1
FLIP2009-55L	68.5±0.50	43.7±3.7*	19.0±4.0	8.3±0.8*	1.5±0.0	2.5±0.2
DPL-58	56±3.01	38.2±1.2	17.5±0.5	3.3±0.3	0.0±0.0	2.5±0.1
DPL-315	68.5±1.50	13.0±7.0	6.0±1.0	4.0±0.5	1.5±0.5	2.1±0.0
DPL-15	78±1.00	13.8±5.9	17.0±7.0	3.0±1.0	1.5±0.5	2.0±0.1
IG-2506	77±6.02	5.7±0.7	12.5±7.5	3.0±0.5	1.5±0.5	1.8±0.1
IG-2507	73.5±5.52	43.7±8.7*	11.5±1.5	7.0±2.0*	2.3±0.8	1.7±0.2
IG-2510	72.5±3.51	8.2±0.2	14.0±4.0	2.3±0.3	1.5±0.5	1.6±0.1
IG-2519	74±5.01	6.2±1.2	23.5±3.5	3.5±0.0	0.5±0.0	2.0±0.0
IG-2525	73±6.02	8.0±2.0	16.0±0.0	2.3±0.8	1.3±1.3	1.4±0.3
IG-2580	73.5±0.50	3.3±0.3	20.0±1.0	4.3±1.8	1.8±0.8	1.6±0.3
IG-2802	74.5±2.51	6.7±2.7	10.0±2.0	1.5±0.5	1.3±0.3	1.4±0.1
IG-2820	76±0.00	8.7±0.7	20.0±7.0	3.8±1.3	2.5±0.5	2.0±0.2
IG-2821	72.5±0.50	11.3±2.7	23.5±16.5	3.8±0.3	1.8±0.8	1.6±0.1
IG-2849	73±3.01	12.5±2.5	26.0±6.0	1.8±0.8	0.8±0.8	1.9±0.1
IG-2878	73.5±3.51	7.7±0.7	22.5±0.5	2.0±1.0	1.5±0.5	1.7±0.1
IG-3263	75±1.00	42.0±36.1	23.0±0.0	3.5±0.5	2.0±1.0	2.0±0.1
IG-3290	75±1.00	7.2±0.8	9.5±2.5	3.3±0.3	0.0±0.0	1.9±0.1
IG-3297	72±4.01	30.5±8.5	15.0±3.0	3.0±0.0	0.0±0.0	1.6±0.2
IG-3312	71±2.01	40.8±5.9	26.0±12.0	2.0±0.0	0.0±0.0	2.3±0.2
IG-3326	75±1.00	15.8±0.8	21.5±3.5	4.3±0.8	0.5±0.5	1.6±0.2
IG-3327	77.5±1.50	43.3±1.3*	16.5±1.5	1.8±0.8	0.0±0.0	1.8±0.2
IG-3330	76.5±2.51	45.5±23.6*	37.0±7.0	4.0±1.0	2.5±0.5	1.7±0.3
IG-3364	80.5±6.52	8.5±3.5	45.0±12.0	4.8±0.8	1.3±0.3	1.8±0.3
IG-3520	72.5±2.51	26.7±6.7	16.5±2.5	3.0±0.5	0.5±0.5	1.9±0.1
IG-3537	79.5±3.51	28.7±6.4	15.5±5.5	2.5±0.5	0.5±0.5	2.1±0.0
IG-3546	74.5±2.51	47.2±2.8*	28.5±0.5	3.3±0.8	3.8±0.3*	1.1±0.7
IG-3568	81.5±6.52	4.8±1.8	43.0±5.0	1.8±0.3	0.3±0.3	1.2±0.0
IG-3641	84.5±4.51	25.8±10.9	15.0±3.0	2.5±0.0	1.5±0.0	1.5±0.1
IG-3745	85.5±10.53	51.2±11.2*	27.0±2.0	2.0±0.0	1.8±0.8	1.9±0.0
IG-3803	83.5±12.54	22.2±7.2	21.0±1.0	2.8±0.3	1.3±0.3	2.3±0.4
IG-3984	84±12.04	28.5±0.5	5.5±2.5	8.0±4.0*	2.3±0.3*	1.7±0.1
IG-4221	68±2.01	8.8±1.2	28.0±4.0	5.3±0.8	0.8±0.8	1.3±0.2
IG-4242	82±7.02	17.3±10.4	29.5±16.5	4.3±0.8	0.8±0.8	1.5±0.1
IG-4258	75.5±0.50	47.7±37.8*	30.5±14.5	10.0±2.0*	3.0±1.0*	2.3±0.2
IG-4318	67.5±0.50	31.2±21.2	16.0±4.0	4.8±0.3	1.5±0.5	1.6±0.2
IG-5146	78±8.02	47.7±2.7*	14.0±4.0	1.8±0.8	1.3±1.3	2.3±0.1
Mean	74.8	23.43	20.32	3.62	1.3	1.82
Range (mean)	56-85	3.3-51.2	5.5-45.0	0.0-3.8	1.5-10.0	1.1-2.5
s.e.m	5.50	9.35	9.05	1.42	0.85	0.28
Range (s.e.m)	0.0-12.5	0.2-37.8	0.0-16.5	0.0-1.3	0.0-2.0	0.0-0.7
CD (at	11.16	18.96	18.40	2.87	1.72	0.56
CV(%)	7.35	39.91	44.54	39.14	66.50	15.19

2.2 Identification of heat tolerant genotypes

In the present study, eight genotypes FLIP2009-55L (43.7) IG2507 (43.7), IG3327 (43.3), IG 3330 (45.5), IG 3546 (47.2), IG 3745 (51.2), IG 4258 (47.7) and IG 5146 (47.7) had significantly large number of filled pods per plant at higher temperature (>35 ºC). Also, genotypes, namely, IG2507 (7.0), IG 3984 (8.0), FLIP2009-55L (8.3), and IG4258 (10.0) had significantly more number of filled pods on terminal branch. However, significantly large numbers of unfilled pods were observed in IG3330 (37.0), IG 3364 (45.0), IG3546 (28.5), IG 4242 (29.5) and IG 4258 (30.5). Based on these observations, only three genotypes, FLIP2009-55L, IG2507 and IG4258 were identified as heat tolerance genotypes because these three genotypes had significantly more number of filled pods per plant as well as on terminal branch of each individual plant. These genotypes also had significantly less number of unfilled pods/plant except on IG4258 (Table 3). In order to see the impact of late-sown conditions on seed size, data were recorded on 100-SW under late- and normal-sown conditions. The percent reduction in seed size observed under late-sown condition is presented in Figure 3. It ranged from 2.4 % to 67.2%. This was lowest in IG 4242 (2.4%) and highest in ILL 6002 (67.2%). However, three heat tolerant genotypes FLIP2009-55L, IG2507 and IG4258 had reduction in seed size 5.7%, 26.2% and 28.3%, respectively (Table 2). The pollen viability was used as physiological trait and tested in laboratory by collecting pollens at higher temperature (>35 ºC). The pollen viability of FLIP2009-55L, IG2507 and IG4258 was 63.2%, 72.4% and 60.9%, respectively (Table 2). Pollen viability showed significant positive correlation with filled pods/plant (r=0.79).

Table 2 Mean and critical difference (CD) of heat tolerant lentil genotypes and their pollen viability

Genotype	FP/P	UFP/P	FP on TB	UFP on TB	Reduction in 100-SW (%)	Pollen viability (%)
IG-4258	47.67	30.5	10**	3**	28.3	60.9
IG-2507	43.67	11.5	7**	2	26.2	72.4
FLIP2009-55L	43.67	19	8.25**	2	5.7	63.2
CD (P=0.05)	19.96	18.4	2.8	1.7	-	-

Note- FP/P = filled pods/plant; UFP/P =unfilled pods/plant; FP on TB= filled pods on terminal branch; UFP at TB= unfilled pods on terminal branch

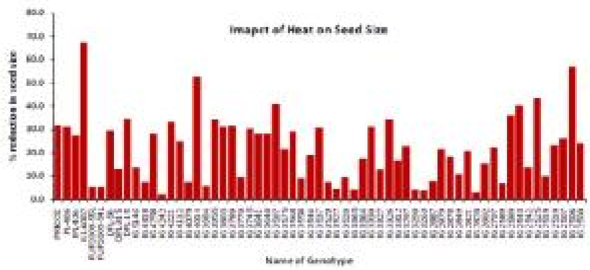

Figure 3 Effect of heat on seed size in 37 lentil accessions

Table 3 Combined ANOVA for different traits under late sown conditions (2011-12 and 2012-13)

Sources of Variation	DF	Mean of Squares					
		DFF	TUFP/P	TFP/P	FP on TB	UFP on TB	100-SW
Year	1	648.12**	39.49	1179.74**	7.15	0.41	0.25
Genotype	36	68.17**	167.23*	555.67**	7.59**	1.56**	0.24**
Error	36	30.29	81.96	87.41	2.01	0.72	0.08
Heritability (%)		38.48	8.94	46.31	58.13	36.84	50

Note: *P<0.05, **P<0.01

3 Discussion

In India, temperature fluctuation during the grain filling period causes drastic yield losses in cool-season legumes. In chickpea, grain yields is estimated to reduce by 53-301 kg/ha if mean temperature rises 1 ºC (Kalra et al., 2008). Photoperiod and temperature are the major factors affecting flowering initiation in crop plants. Pulses are particularly sensitive to heat at flowering and pod development stages. If the crop encounters a few days of exposure to high temperatures (30-35°C) at these stages, heavy yield losses are reported due to flower drop and pod abortion (Summerfield et al., 1985; Sarker et al., 1999; Roberts et al., 1986; Gopesh et al., 2013). However, this sensitivity varies from genotype to genotype. The temperature during the reproductive stage in both years of experimentation was above the threshold level (>30°C; Figure 2), which suggested suitable conditions for identification of heat tolerant genotypes in lentil. Similar environmental conditions have also been used earlier to screen heat tolerant genotypes in chickpea (Krishnamurthy et al., 2011). Based on flowering and podding under higher temperature, in the present study, lentil genotypes were clearly categorized into three main groups, (i) early flowering, (ii) no flowering or rarely flowering and (iii) normal flowering and pod setting. In the present study 174 genotypes flowered early and matured within 80-85 days after sowing. Because these genotypes escaped from high temperature conditions and hence excluded from screening studies conducted further in next year. Earlier studies showed that heat stress delays flowering and accelerates maturity (Krishnamurthy et al., 2011) and hence probably due to this, 64 accessions in the present study did not flower or flowered rarely. The degree of tolerance was studied among 37 genotypes, which had filled and unfilled pods on individual plant as well as on terminal branch. The combined analysis of variance showed significant genetic variability for heat tolerance among these genotypes for filled pods/plant, unfilled pods/plant, filled and unfilled pods on terminal branch, 50% flowering and 100-SW. Similarly, genetic variation for heat tolerance among chickpea genotypes was reported (Krishnamurthy et al., 2011).

Heritability determines the proportion of parental characters that is inherited to their off-springs and hence it is an important parameter to study the inheritance of quantitative characters (Allard, 1960). A trait with high heritability suggests maximum genetic gain in response of selection and can be used reliably for screening the tolerant genotypes under heat stress conditions. In the present investigation, we observed high heritability for filled pods/plant and filled pods on terminal branch. Therefore, these traits could be useful to select tolerant genotypes at higher temperature. Lucas et al. (2012) also used number of pods per peduncle for identification of heat tolerance in recombinant inbred lines population of cowpea. In the present study, three genotypes, IG 3745, IG 4258 and IG 5146 were identified as heat tolerant because these accessions had significantly more pods per plant (>43 pods/plant) at higher temperature. On the basis of the number of pods on the terminal branch at higher temperature, FLIP2009-55L, IG2507 and IG4258 showed more number of pods on terminal branch and thus highly heat tolerant. Though another genotype (IG-3984) also showed more pod formation on terminal branch, it was poor in total number of effective pods per plant. We observed significantly higher unfilled pods for some genotypes, indicating the impacts of high temperature on pod formation. In legumes high temperature during anthesis reduces seed set due to impaired pollen tube growth and fertilization (Gross and Kigel, 1994).

The present study showed instability in performance of filled pods/plant over the years in most of the genotypes. The combined analysis of variation for filled pods/plant reflected that a large proportion of total phenotypic variance was due to environmental factors. In pulses, environment and genotype × environment interactions contribute >70% of total phenotypic variance as reported earlier (Kumar and Ali, 2006). However, two genotypes (i.e. FLIP2009-55L and IG2507) that classified as highly tolerant to heat showed stable performance over the years as reflected by low s.e.m. These genotypes can be used in lentil breeding program for developing improved cultivars having tolerance to terminal heat.

In the present investigation, impacts of high temperature were also observed on seed size, which varied from 2.4% to 67.2% over the normal sown conditions. However heat tolerant genotypes showed 5.7% to 28.3% reduction in seed size which is comparatively lower than other genotypes due to efficient accumulation of photosynthesis in seeds during grain filling at higher temperature. Though a few days of exposure to high temperatures (30–35 °C) during seed filling accelerates senescence, diminish seed set and seed weight, and reduce yield in pluses (Siddique et al., 1999), different genotypes within a species have different capabilities in coping with the heat stress (Wahid et al., 2007).

High temperature leads pollen sterility (Saini et al., 1984) and hence seed yield depends on the temperature during pollen development (Ploeg Van der and Heuvelink, 2005). In the present study, high pollen viability (60-70%) was observed for two heat tolerant genotypes (FLIP2009-55L and IG2507) and showed highly positive correlation with number of pods per plant. Our results suggest that pollen viability test could be used in laboratory for identification of heat tolerant genotypes in lentil. The impact of heat stress on pollen viability has already been demonstrated in several legume crops including chickpea, common bean, groundnut, and soybean (Prasad et al., 1999; Porch and Jahn, 2001; Devasirvatham et al., 2012; Djanaguirama et al., 2013).

4 Conclusion

The present investigation shows that heat stress significantly affects number of flowers, pods, and seeds. Therefore, filled and unfilled pods on a single plant basis and on the terminal branch are important traits for phenotyping heat tolerance under field conditions. Further, the pollen viability is a useful trait for identification of heat tolerant genotype in lentil. Our results clearly demonstrated that significant genetic variability exits for these morphological traits in cultivated gene-pool of lentil. These genotypes can be considered as potential genetic resources to be used in lentil breeding program for the development of heat tolerant cultivars.

5 Materials and Methods

5.1 Plant materials

The present study included 334 lentil genotypes representing local and exotic germplasm originating from drought-prone areas, elite breeding lines from national and international programs and improved cultivars released in India. Breeding lines used in this study were developed at the Indian Institute of Pulses Research (IIPR), Kanpur, India. These lines are derived from crosses involving parents adapted to terminal heat-prone environments. These accessions were evaluated in 2011-12 and 160 accessions (out of above 334 accessions) that faced high temperature (>35 ºC) during reproductive stage were again screened for heat tolerance in 2012-13 (Table 4).

Table 4 Description of pedigree/collection number and collecting/breeding organization of 160 lentil accessions used over two years (2011-12 and 2012-13) in the present study

S.No	Accession	Pedigree/Collection Number	Collecting/ Breeding organization
1	IG 2500	PANT-L 538	GBPUAT, Pantnagar
2	IG 2506	PANT-L 643	GBPUAT, Pantnagar
3	IG 2507	LL 3	PAU, Ludhiana, Punjab
4	IG 2508	LL 5	PAU, Ludhiana, Punjab
5	IG 2510	LL 25	PAU, Ludhiana, Punjab
6	IG 2519	PUSA 9	IARI New Delhi
7	IG 2525	T 31	IARI-RS, Kanpur
8	IG 2542	L 543	unknown
9	IG 2543	L 546	unknown
10	IG 2576	L 771	unknown
11	IG 2580	L 1278	unknown
12	IG 2588	LWS 1	JNKVV, Jabalpur, UP
13	IG 2589	LWS 2	JNKVV, Jabalpur, UP
14	IG 2593	LWS 6	JNKVV, Jabalpur, UP
15	IG 2796	P 287	USDA,-RPIP, New Delhi
16	IG 2797	P 290	USDA,-RPIP, New Delhi
17	IG 2802	P 300	USDA,-RPIP, New Delhi
18	IG 2817	P 326	USDA,-RPIP, New Delhi

19	IG 2820	P 332	USDA,-RPIP, New Delhi
20	IG 2821	P 333	USDA,-RPIP, New Delhi
21	IG 2849	P 368	USDA,-RPIP, New Delhi
22	IG 2878	P 405	USDA,-RPIP, New Delhi
23	IG 2879	P 406	USDA,-RPIP, New Delhi
24	IG 2887	P 422	USDA,-RPIP, New Delhi
25	IG 3253	P 912	USDA,-RPIP, New Delhi
26	DPL-58	PL 639 × PRECOZ	IIPR, Kanpur
27	IG 3286	P 949	USDA,-RPIP, New Delhi
28	IG 3290	P 956	USDA,-RPIP, New Delhi
29	IG 3297	P 988	USDA,-RPIP, New Delhi
30	IG 3312	P 1020	USDA,-RPIP, New Delhi
31	IG 3326	P 1046	USDA,-RPIP, New Delhi
32	IG 3327	P 1047	USDA,-RPIP, New Delhi
33	IG 3330	P 1050	USDA,-RPIP, New Delhi
34	IG 3370	P LWS 16	JNKVV, Jabalpur,
35	IG 3365	PI 42	USDA,-RPIP, New Delhi
36	IG 3520	LG 74	PAU, Ludhiana, Punjab
37	IG 3527	LG 112	PAU, Ludhiana, Punjab
38	IG 3529	LG 116	PAU, Ludhiana, Punjab
39	IG 3537	LG 141	PAU, Ludhiana, Punjab
40	IG 3546	LG 150	PAU, Ludhiana, Punjab
41	IG 3558	LG 162	PAU, Ludhiana, Punjab
42	IG 3567	LL 1	PAU, Ludhiana, Punjab
43	IG 3568	LL 3	PAU, Ludhiana, Punjab
44	IG 3575	LL 23	PAU, Ludhiana, Punjab
45	IG 3587	LL 30	PAU, Ludhiana, Punjab
46	IG 3640	NP 22	IARI, New Delhi
47	IG 3641	NP 47	IARI, New Delhi
48	IG 3643	NP 52	IARI, New Delhi
49	IG 3676	P 27	USDA,-RPIP, New Delhi
50	IG 3745	P 175	USDA,-RPIP, New Delhi
51	IG 3770	P 206	USDA,-RPIP, New Delhi
52	IG 3789	P 227	USDA,-RPIP, New Delhi
53	IG 3803	P 241	USDA,-RPIP, New Delhi
54	IG 3955	P 437	USDA,-RPIP, New Delhi
55	IG 3984	P 480	USDA,-RPIP, New Delhi
56	IG 4001	P 505	USDA,-RPIP, New Delhi
57	IG 4014	P 524	USDA,-RPIP, New Delhi
58	IG 4068	P 629	USDA,-RPIP, New Delhi
59	IG 4072	P 633	USDA,-RPIP, New Delhi
60	IG 4079	P 640	USDA,-RPIP, New Delhi
61	IG 4112	P 701	USDA,-RPIP, New Delhi
62	IG 4113	P 702	USDA,-RPIP, New Delhi
63	IG 4147	P 773	USDA,-RPIP, New Delhi
64	IG 4202	P 886	USDA,-RPIP, New Delhi
65	IG 4203	P 887	USDA,-RPIP, New Delhi
66	IG 4206	P 891	USDA,-RPIP, New Delhi
67	IG 4208	P 894	USDA,-RPIP, New Delhi
68	IG 4219	P 916	USDA,-RPIP, New Delhi
69	IG 4221	P 924	USDA,-RPIP, New Delhi
70	IG 4242	P 957	USDA,-RPIP, New Delhi

71	IG 4243	P 959	USDA,-RPIP, New Delhi
72	IG 4246	P 967	USDA,-RPIP, New Delhi
73	IG 4247	P 971	USDA,-RPIP, New Delhi
74	IG 4258	P 985	USDA,-RPIP, New Delhi
75	IG 4278	P 1036	USDA,-RPIP, New Delhi
76	IG 4284	P 1047	USDA,-RPIP, New Delhi
77	IG 4318	P 1132	USDA,-RPIP, New Delhi
78	IG5146	LC 33	POSRS, Berhampore,WB
79	ILL 10965	ILL 8090 X ILL 7980	ICARDA
80	ILL 10969	ILL 7723 X ILL 8090	ICARDA
81	ILL 10712	ILL6783 X ILL 98	ICARDA
82	ILL 10711	ILL 7012 X ILL 4404	ICARDA
83	L-9-12	Selection from local variety	
84	T-36	Local selection from Badaun, UP	CSAUAT, Kanpur
85	PL-406	Selection of P45	GBPUAT, Pantnagar
86	JL-1	Local selection from Madhya Pradesh	JNKVP, Jabalpur
87	IPL-307	L 4076 × DPL 44	IIPR, Kanpur
88	IPL 98/193	(Sehore 74-3 × DPL44) × DPL35	IIPR, Kanpur
89	SEHORE 74-3	Local selection from Sehore	JNKVP, Jabalpur
90	ILL-6002	ILL 4349 × ILL 4605	ICADA, Syria
91	PRECOZ	Argentina cultivar	ICARDA
92	L-4603	Precoz × L 3991	IARI, New Delhi
93	DPL-15	PL406 × L 4076	IIPR, Kanpur
94	DPL-62	JLS 1 x LG 171	IIPR, Kanpur
95	IPL-81	K 75 × PL 639	IIPR, Kanpur
96	IPL-406	DPL 35 x EC 157634/382	IIPR, Kanpur
97	IPL-315	PL 4 × DPL 62	IIPR, Kanpur
98	IG 2639	P 41	USDA,-RPIP, New Delhi
99	IG 2649	P 55	USDA,-RPIP, New Delhi
100	IG 2794	P 285	USDA,-RPIP, New Delhi
101	IG 2836	P 353	USDA,-RPIP, New Delhi
102	IG 3072	P 720	USDA,-RPIP, New Delhi
103	IG 3563	LG 167	PAU, Ludhiana, Punjab
104	IG 3589	LL 31	PAU, Ludhiana, Punjab
105	IG 3662	P 10	USDA,-RPIP, New Delhi
106	IG 3667	P 15	USDA,-RPIP, New Delhi
107	IG 3673	P 23	USDA,-RPIP, New Delhi
108	IG 3750	P 183	USDA,-RPIP, New Delhi
109	IG 3794	P 232	USDA,-RPIP, New Delhi
110	IG 3801	P 239	USDA,-RPIP, New Delhi
111	IG 3802	P 240	USDA,-RPIP, New Delhi
112	IG 3905	P 366	USDA,-RPIP, New Delhi
113	IG 3964	P 450	USDA,-RPIP, New Delhi
114	IG 3973	P 467	USDA,-RPIP, New Delhi
115	IG 3982	P 477	USDA,-RPIP, New Delhi
116	IG 4000	P 504	USDA,-RPIP, New Delhi
117	IG 4013	P 523	USDA,-RPIP, New Delhi
118	IG 4059	P 613	USDA,-RPIP, New Delhi
119	IG 4060	P 617	USDA,-RPIP, New Delhi
120	IG 4071	P 632	USDA,-RPIP, New Delhi
121	IG 4073	P 634	USDA,-RPIP, New Delhi

122	IG 4074	P 635	USDA,-RPIP, New Delhi
123	IG 4076	P 637	USDA,-RPIP, New Delhi
124	IG 4078	P 639	USDA,-RPIP, New Delhi
125	IG 4098	P 667	USDA,-RPIP, New Delhi
126	IG 4149	P 779	USDA,-RPIP, New Delhi
127	IG 4195	P 873	USDA,-RPIP, New Delhi
128	IG 4197	P 876	USDA,-RPIP, New Delhi
129	IG 4216	P 910	USDA,-RPIP, New Delhi
130	IG 4228	P 934	USDA,-RPIP, New Delhi
131	IG 4244	P 961	USDA,-RPIP, New Delhi
132	IG 4253	P 978	USDA,-RPIP, New Delhi
133	IG 4281	P 1044	USDA,-RPIP, New Delhi
134	IG 4286	P 1057	USDA,-RPIP, New Delhi
135	IG 4303	P 1095	USDA,-RPIP, New Delhi
136	IG 4312	P 1109	USDA,-RPIP, New Delhi
137	IG3371	P LWS 19	JNKVV, Jabalpur,
138	ILL 10824	ILL 7620 × ILL 91517	ICARDA, Syria
139	ILL 10835	ILL 7723 × IL× 87062	ICARDA, Syria
140	ILL 10972	ILL 8199 × ILL 7979	ICARDA, Syria
141	ILL 10963	ILL 8090 × ILL 7980	ICARDA, Syria
142	ILL 10970	ILL 7537 × ILL 590	ICARDA, Syria
143	ILL 10833	ILL 358 × IL× 87062	ICARDA, Syria
144	IC 15110	UNKNOWN	NBPGR, NEW DELHI
145	IC 15112	UNKNOWN	NBPGR, NEW DELHI
146	IC 15113	UNKNOWN	NBPGR, NEW DELHI
147	ILL 832	ILL 4402 × ILL 2501	ICARDA, Syria
148	ILL 10831	ILL 2501 × ILL 7537	ICARDA, Syria
149	ILL 10707	ILL 8090 × ILL 7685	ICARDA, Syria
150	ILL 10829	ILL7620 × ILL 91517	ICARDA, Syria
151	ILL 10968	ILL 8090 × ILL 7980	ICARDA, Syria
152	ILL 10708	ILL 7617 × ILL 4404	ICARDA, Syria
153	ILL 10713	ILL 8077 × ILL 6994	ICARDA, Syria
154	ILL 10725	ILL 7713 × ILL 7201	ICARDA, Syria
155	ILL 10825	ILL 7620 × ILL 91517	ICARDA, Syria
156	ILL 10827	ILL 7620 × ILL 91517	ICARDA, Syria
157	ILL 10826	ILL 7620 × ILL 91517	ICARDA, Syria
158	ILL 10973	ILL 6024 × ILL 6829	ICARDA, Syria
159	ILL-7663	Cross between two locals	ICARDA, Syria
160	EC-542161	Unknown	NBPGR, New Delhi

5.2 Field experiments

Field experiments involving a set of 334 and 160 accessions were conducted on 15 January 2011-12 and 2012-13, respectively at Main Research Farm of Indian Institute of Pulses Research, Kanpur (268270N, 808140E; 152.4 m above sea level). These experimental materials were evaluated both years in augmented design. Planting of these accessions was delayed 60-70 days from normal planting of lentil during the winter season in order to synchronize high temperature at reproductive stage. Planting was done on ridges and the plot size was a single row of 3 m length with 30 cm spacing between rows and 5 cm between plants within the rows. To avoid possible drought effects, sufficient moisture in soil was maintained by applying regular irrigation. Other standard agronomic practices were also followed in order to raise a good crop.

5.3 Meteorological data

Data was collected weekly on temperatures [(ºC), maximum and minimum] and precipitation (mm) during the growth period of crop from the agrometeorological observatory of the Indian Institute Pulses, Research, Kanpur.

5.4 Data recording

Delayed sowing exposed plants to higher temperature at reproductive phase under irrigated conditions in field. Ten plants were selected randomly from each single row plot in order to take observation on individual plant. Observations were recorded visually on formation of flowers and quantitatively on filled and unfilled pods per plant. Moreover the top 7-8 cm terminal branch of each individual plant was quantitatively recorded for number of filled and unfilled pods under high temperature (>35^{o}C). The post-harvest data was recorded on 100-seed weight (g) for each genotype. Based on these traits, present germplasm accessions were characterized into (i) sensitive (i.e. plants with flowers but no or rare pods), (ii) highly sensitive (i.e. plants with no or rare flowers and pods), (iii) tolerant (i.e. plants with filled pods but rare/no pods on terminal branch) and (iv) highly tolerant (i.e. individual plans and terminal branch with normal podding) categories (Figure 4).

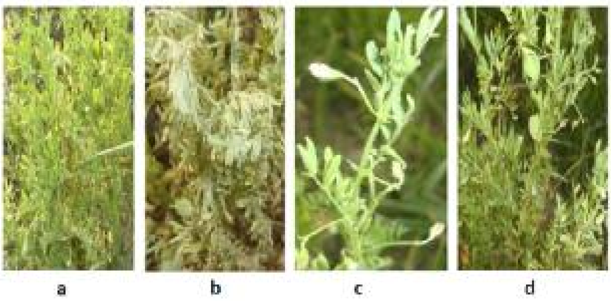

Figure 4 Sensitivity of lentil plants during reproductive period under the high temperature (a) sensitive, (b) highly sensitive, (c) tolerant and (d) highly tolerant

5.5 Determination of pollen viability

Pollen viability of the fresh pollens was studied in those accessions which showed tolerance on the basis of morphological characters. It was determined by acetocarmine technique (Robert, 1977) and those pollen grains stained deeply and looking normal were counted as viable and weakly stained were counted as non-viable (Pearsonand Harney, 1984). For each genotype, 2000 pollen grains were recorded by counting 200 pollen grains per slide. The pollen viability (%) was calculated using the following formula.

Pollen viability (%) = (Number of stained pollen / Total number pollen counted) × 100

5.6 Statistical analysis

Analyses of variance (ANOVA) were carried out for year-wise data on various traits using the statistical analysis tool of GENSTAT 14th edition (Payne et al., 2011). A combined analysis of variation over years was carried out for partitioning the phenotypic variance into year, genotype, and error variances. Phenotypic correlations were calculated using the mean values over years (SAS Institute, 2007). The critical difference (CD) at 5% significance level was calculated by using following formula.

CD= √2 MSe/no. of years × $t_{5\%}$ for error degree of freedom

Where, MSe is the error means of square.

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