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Drought Resistance in Lentil (*Lens culinaris* Medik.) in Relation to Morphological, Physiological Parameters and Phenological Developments

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A B S T R A C T

In the present investigation lentil genotypes; one macrosperma (IPL-406) and the other microsperma (HUL-57) were subjected to drought stress at mid-vegetative, flower-initiation and pod-formation stages by withholding irrigation. We observed that drought stress of various phenophases significantly decreased shoot length (8% - 22%), leaf area (23% - 52%), CMS (26% - 58%) and phenological developments (0 - 14 DAS) but increased root length (14% - 63%) of plants. Pre anthesis accumulated biomass and nitrogen within the plants parts *viz.* roots, leaves, stem and pod walls and its efficient allocation to growing seeds serves as major sources during active pod filling periods. A significant negative relationship was observed between parameters like leaf area with RWC (R^2 = 0.845*), CMS (R^2 = 0.473*) and GMP (R^2 = 0.876*), shoot length with GMP (R^2 = 0.206*) under drought stress. Meanwhile a significant positively relationship was found between RWC and CMS (R^2 = 0.503*) under drought. Drought indices such as higher DTE and GMP values are proposed to be the selection traits in lentil breeding programme for drought prone environmental conditions. It is suggested that pod formation stage fallowed by flower initiation stage is more sensitive to drought than mid vegetative stage. Genotype HUL-57 was drought resistant and IPL-406 was drought sensitive.

Introduction

Lentil (*Lens culinaris* Medik.) is a prehistoric domesticated crop among food legumes with a vital range of uses as food and feed owing to its protein-rich grains and straw. The cultivated lentils are broadly divided in two types of groups, the small-seeded (microsprma) and the bold seeded (macrosperma) (Erskine *et al.*, 1994, Shrestha *et al.*, 2006a, b, Mishra *et al.*, 2014). Drought stress is an extremely universal and disadvantageous cause for crop yield loss in several areas of the world where lentils are in cultivation by forming community (McWilliams, 1986; Shrestha *et al.*, 2006a, b). Lentil crop when sown during autumn or winter in South Asian countries as well as Mediterranean environments, are to face, occurrence of intermittent drought during the vegetative growth and terminal drought throughout their reproductive period when temperatures are ever-increasing and rainfall is declining (Yusuf *et al.*, 1979; Siddique *et*

al., 1999; Shrestha *et al.,* 2006a, b). Lentil responses to drought are quite disagreeing; some workers reported that lentil is most sensitive to drought at seedling and flowering stages (Yusuf *et al.,* 1979), while others reported that it is sensitive to drought at flowering and pod formation stages (Shrestha *et al.,* 2006a, b; Mishra *et al.,* 2014, 2016). Food legumes including crop plants counteract to drought and adopted themselves to drought through diverse customs *viz.* morphological, phenological and physiological modifications (Vadez *et al.,* 2012; Mishra *et al.,* 2014, 2016). Drought stress is recognized to cause reductions in plant growth, root functioning, leaf area development, cell membrane stability, alterations in biomass and nitrogen allocation in different plant parts, drought tolerance efficiency and geometric mean productivity, similarly, the above parameters and drought indices may gives surety for characterizing drought resistance in many legumes under drought stress (Hamidi and Erskine, 1996; Kurdali *et al.,* 1997; Sio-Se Mardeh *et al.,* 2006; Shrestha *et al.,* 2006a, b; Gunes *et al.,* 2008; Singh *et al.,* 2013; Mishra *et al.,* 2014). Under drought stress the above parameters have been used as source of screening traits in parallel in diverse crop plants, but their relative helpfulness has not been evaluated in lentil groups. Therefore, present investigation was under taken with following objectives: (i) Evaluating response of diverse lentil genotypes (macrosperma and microsperma) to drought stress imposed at specific phenophase on morphological, physiological, phenological parameters. (ii) Variations in apportioning of biomass and nitrogen under drought stress imposed at specific growth stages of crop allocation in different plant parts. (iii) Validation of various drought indices for determining resistant/sensitive genotypes as well as most sensitive stage of crop to drought stress and its relationships with the traits under drought stress.

Materials and Methods

Plant material and growth conditions

The present experiment was conducted in pots at the Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, during *"rabi"* (winter cropping season: November to March) during 2010-11 and 2011-12. Plastic pots (consisted of polyvinyl-chloride) of diameter (30 cm) were taken and filled with well pulverized 15 kg soil taken from farm where lentil crop was grown for many years. Fertilizers were mixed with the soil @ 20, 60 and 40 kg ha^{-1} N, P_2O_5 and K_2O, respectively. Seeds of two genotypes of lentil viz. IPL-406 (macrosperma type) and HUL-57 (microsperma type) were procured from the Department of Genetics and Plant Breeding, Institute of Agricultural Science, B. H. U. Varanasi. Sowing was done on 1st November during experimental year 2010-11 and 2011-12. Each genotype was sown in 150 pots. 20 days after sowing (DAS), five healthy and uniform seedlings were maintained in each pot.

Watering treatments and imposition of drought stress

Twenty five pots of each genotype were kept at normal soil moisture content. Soil moisture content in normal pots was 75% of available moisture. Twenty five pots were exposed to moisture stress at specific phenophase viz. mid-vegetative, flower-initiation and pod-formation. For imposing moisture stress at specific phenophase, irrigation was cheeked in pots well in advance so that plants could experience one cycle of permanent wilting (PWP) at that stage. In stressed pots available soil moisture content was 30% (Mishra et al., 2014). As soon as plants experienced one cycle of permanent wilting; observations were made. Thereafter, these pots were kept at

normal supply of soil moisture till harvest. Control plants were maintained at optimal supply of soil moisture till maturity.

Measurement of shoot length, root length and leaf area

Observations of shoot length, root length and leaf area were taken at mid-vegetative, flower initiation and pod formation stages after imposing drought stress in well watered (WW) plants and drought stressed (DS) plants**.** Roots of plants were removed from pots carefully, cleaned with tape water followed by distilled water and root length was measured. Leaf area of total green leaves (cm^2 $plant^{-1}$) was measured by portable laser leaf area meter (CI-202, CID Bio Science, USA).

Measurement of relative water content (RWC)

RWC of first fully expanded leaf from top was measured adopting standard protocol of Barrs and Weatherly (1962). Leaf samples were collected between 9.00 to 10 hours. RWC was recorded at the end of drought of mid vegetative, flower initiation and pod formation stages. The RWC was calculated as:

RWC (%) = 100 × (Fresh weight–Dry weight) / (Turgid weight–Dry weight).

Determination of cell membrane injury and calculations of cell membrane stability under osmotic stress (PEG 40%) and thermal stress

The cell membrane injures (CMI %) due to osmotic and temperature stress were measured in upper most fully expanded leaf (Blum and Abercorn 1981) at the end of drought stress of mid-vegetative, flower initiation and pod formation stages in well watered (WW) control and drought stressed (DS) plants. Leaves were washed thoroughly, cut into small pieces, and 100 mg leaf pieces were taken. For measuring CMI due to osmotic stress, leaf pieces were placed in test tubes containing 20 mL distilled water or 40% PEG-6000 (Polyethylene glycol - 6000) solution. Samples were incubated at 10 ± 1 °C for 24 h, and then the incubation media was drained out.

Treated leaves were washed several times with distilled water. Both control (distilled water) and PEG-6000 treated samples were incubated in distilled water at 10±1 °C. After 24 h Test tubes were taken out, brought to room temperature and conductivity of the bathing media was measured with direct reading conductivity meter (Systronics model 304). Bathing media was again put in the same test tube containing leaf dishes and autoclaved for 15 minutes at 15 psi. Final conductivity of the media was again measured after autoclaving. Cell membrane stability was calculated on the basis of following equation. Cell membrane injury (CMI %) = $[1-(1-T_1/T_2)/ (1-C_1/C_2)] \times 100$

Where, T and C refers to the conductivities of treatment and control and subscripts 1 and 2 indicates initial (before autoclaving) and final (after autoclaving) conductivities, respectively.

Cell membrane stability (CMS %) was calculated as: (CMS %) = (100 - CMI %)

Cell membrane stability due to temperature stress was measured in the same way with the deviation that leaf samples were taken in 10 mL distilled water and incubated in a water bath for 60 minutes at 50±1 °C. After incubation sample were brought to room temperature. It was then washed thoroughly with distilled water and incubated at 10±1 °C for 24 hours in 15 mL distilled water. Rest of the procedures were same as described above of CMS.

Estimation of dry matter production for determine the dry matter allocation (%) in various plant parts

Plant samples were collected at the end of drought stress imposed at mid vegetative, flower initiation and pod formation stages in well watered (WW) control and drought stressed (DS) plants and separated in individual plant components, oven dried at 65^{o}C till a constant weight was attained. Dry matter allocation (%) was calculated as: [Dry matter of respective plant parts (g) / Total plant dry matter (g)] × 100

Determination of nitrogen (N) for determine the N allocation (%) in different plant parts

Plant samples were collected at specific stages, individual plant components were separated and oven dried at 65 ^{o}C. Dried plant parts were finally ground to fine powder. At maturity study was confined to shoot only as it was not possible to take out intact roots from soil. Total nitrogen content in plant samples was determined by semi-automatic nitrogen analyser (Pelican, Model KEL 20L) adopting Kjeldahl methods.

Nitrogen allocation (%) = [N content (mg) in respective plant parts / Total plant N content (mg)] × 100

Phenological development

Vegetative period was considered as the period (days) from sowing to first flower bud appearance. Reproductive period was taken as the period from first flower bud appearance to the physiological maturity of the crop. Seed filling duration was calculated as the period from first podding (when 50% plants had their yellow coloured visible pods) to physiological maturity of crop.

Physiological maturity was calculated from days after sowing to 90% pods in pot turned brown in colour as mentioned by Shrestha *et al.*, (2006a, b).

Computation of drought indices

Drought indices are mathematical predictions based on the yield data in combined farm obtained from the plants in well watered (WW) and also from drought stressed (DS) conditions. All drought indices were calculated from at least 50 plants seed yield at respective phenophase.

Drought tolerance efficiency (DTE; %) was estimated by formula given by Fischer and Maurer (1978) as: DTE (%) = [Yield under drought stress (DS) condition / Yield under well watered (WW) condition] × 100. Geometric mean productivity (GMP) was calculated according to (Fernandez 1992) as: GMP = √ (Ysi) (Ypi). Genotypes having higher (GMP) exhibited higher yielding capacity.

Statistical analysis

All experimental data recorded are means (n=6) of six replicates (pooled data of two years experiment 2010-11 and 2011-12 for three independent observations with three replicates each year of experimentation).

Data were subjected to ANOVA for factorial completely randomized design by SAS software, fallowing PROC GLM, SAS procedures (SAS 9.2 Foundation for Microsoft® Windows® for x64, SAS Institute Inc., Cary, NC, USA). Least significant differences (LSD $P \leq 0.05$) were considered significant. The relationship between various estimated parameters were subjected to liner regression analysis by fallowing instructions outlined in SAS user's manual for statistical analysis.

Results and Discussion

Shoot length

Plants when exposed to drought stress at various phenophases; shoot length decreased in both genotypes (Fig. 1a). Meanwhile reduction in shoot length was 15 %, 22 %, 16 %, respectively, in IPL-406 as compare to 9, 8, 12%, respectively, in HUL-57 at mid vegetative, flower initiation and pod formation stages. It is well documented that the sequence of events that take place where drought stress develops begins with cellular growth as most sensitive reaction chased by a wide range physiological and biochemical events, but also due to derangement in physiological and biochemical processes.

The events occurring latter on during the drought period or at very negative water potentials are often indirect responses to earlier events rather than direct responses to water stress itself. This picture is further more complicated because of the sensitivity of some response is highly dependent on the plant species. Food legumes; as compared to cereals appear to have more sensitivity towards drought in relation to shoot growth (Shrestha *et al.,* 2006a, b). One of initial effects of drought includes decrease in cell enlargement and cell division resulting in decreased shoot length.

Findings of the present experiment suggested that HUL-57 probably maintains it turgor under drought stress and drought effects was less detrimental at various phenophases in this genotypes. By the passion of maintaining cell turgidity, cell division and cell enlargement under drought stress was affected the least in HUL-57; similarly reductions in shoot length of this genotype was of lesser magnitude as compare to genotype IPL-406. Our results are worthy as reported by others (Shrestha *et al.,* 2006a, b).

Root length

In both genotypes root length always more in drought stressed plants than in control plants (Fig. 1b), at all stages studied. Increment was the maximum 63% in HUL-57 when plants were stressed at mid-vegetative stage, while in IPL-406 at this stage increment was (45%). Irrespective of the stage of imposing stress, the increase in root length was always higher in HUL-57 than in IPL-406. Increments in root length under drought stress is a genotypic character associated with water capturing ability from deeper strata of soil to meet the plants water requirement for coping drought stress more effectively, termed as drought avoidance strategy. Longer rooting habit and maintenance of root growth under stress clearly indicated that genotype HUL-57 had some acquired characters to maintain higher root length to absorb available soil moisture more efficiently. Our findings are in close nearness with the result of (Hayatu and Mukhtar, 2010).

Leaf area development

Drought stress, irrespective of stage of imposing, decreased plant leaf area. Reduction in leaf area in stressed plants as compared to control was more in HUL-57 than IPL-406 (Fig. 1c). The reductions, was 43 % upto 52 % in HUL-57 when compared to IPL-406 23 % upto 33% during stress of various phenophases. Drought stress caused the initial effects on decrease in cell enlargement and cell division resulting in decreased leaf area development and expansion. Other possible explanations of reduction in leaf area in stressed plants are due to delay in phylochron index and accelerated leaf senescence and abscission of existing leaves In legumes reduction in leaf area on account of stress is reported to be more deleterious to phylochron index rather than to leaf area. Findings of (Shrestha *et al.,* 2006a, b) support our results.

Vegetative and reproductive periods, pod filling duration and physiological maturity

It was evident that drought stress of respective stage of imposing stress can affect a very marginal reduction in vegetative period in stressed plants of genotype HUL-57 when compared with control plants and genotype IPL-406 (Table 1). But drought stress significantly reduced reproductive period, pod filling duration and physiological maturity of stressed plants. In IPL-406 reproductive period, pod filling duration and physiological maturity shortened by 10-14 DAS, 11-14 DAS and 10-14 DAS, respectively, than the control plants. While in HUL-57 reproductive period, pod filling duration and physiological maturity declined due stress to a lesser magnitude and it was only (0-4 DAS, 1-7 DAS and 2-3 DAS, respectively, irrespective stage of imposing stress than the control plants. Phenological attributes are the well-known potential traits for food legume crops conferring drought resistance and adaptation under drought stress conditions, and variability within genotypes may be used by plant breeders for developing superior lentil cultivars to optimize its production under water scare environments as reports in lentil by (Silim *et al.,* 1993; Thompson *et al.,* 1997; Shrestha *et al.,* 2006a, b). Our observations suggested that genotypic ability of IPL-406 (macrosperma type) for its longer vegetative period when this genotype was re-watered after drought stress of various phenophases positively reduced its reproductive period, pod filling duration and physiological maturity than the control plants, and it was the major reason for reduced seed yield (Hamidi and Erskine 1996). However, shorter vegetative period, longer reproductive period as well as prolonged pod filling duration and longer physiological maturity periods even as under drought stress conditions stabilized more seed yield of genotype HUL-57. Such findings suggested that (microsperma type) lentils are more resistant/adopted well under drought stress conditions and these phenological attributes can be employed in lentil breeding programme for drought stressed conditions (Thompson *et al.,* 1997; Shrestha *et al.,* 2006a, b).

Cell membrane stability (CMS %) on account of osmotic and thermal stresses

As compare to control CMS of leaf tissues of stressed plants at all stages was lower under osmotic (Fig. 2a) as well as under thermal stress (Fig. 2b). It was also evident that decrease in CMS was more in IPL-406 than in HUL-57. Decrease in CMS of leaf tissues under osmotic stress (Fig. 2a) was relatively of lower magnitude in both genotypes than when subjected to thermal stress (Fig. 2b). The maximum reduction in CMS under osmotic or as well as thermal stress was observed when stress was imposed at pod formation stage in both genotypes but the extent of reduction in CMS % was always lower in HUL-57 as 35.88 %, 30.63 % in osmotic and thermal stress respectively, but at same stage CMS % of IPL-406 was 29.60 %, 26.29 %, respectively. The minimum decrease in CMS % was 42.78 %, 37.97 % under osmotic and thermal stress in HUL-57 when stress was imposed at flower initiation stage, whereas, at the same time CMS % in IPL-406 was 33.06 %, 28.50 %, respectively. Drought stress caused cell membrane damage resulting in increased leakage of protoplast solutes, therefore, ion leakage from cells/tissues of leaves could also be used as an index for screening genotypes against various stresses *viz*. heat and drought stresses. Findings of present investigation depicted that decrease in cell membrane stability was more in drought sensitive lentil genotype IPL-406, while in tolerant genotype HUL-57 it was of lesser magnitude consistent findings as we found in our results (Fig. 2a, b) are reported earlier in chickpea (Deshmukh *et al.,* 2002; Gunes *et al.,* 2008). It can also be concluded that a trait

of higher cell membrane stability, (under thermal or osmotic stress) during drought stress conditions is an important physiological selection criteria in lentil. Findings of this investigation are supported as in pigeonpea (Singh *et al.,* 2013).

Dry matter allocation (%) in different plant parts as a proportion of total shoots dry matter

Lentil genotypes apportioned more dry matter to roots shoot under stress as well as control conditions in both genotypes (Table 2a). Pattern of dry matter allocation of stem was generally higher when stress was imposed at flower initiation stage in both genotypes where it was again superior in genotype HUL-57 than IPL-406. But in IPL-406 stress of mid vegetative and pod formation stage decreased stem dry matter allocation in stressed plants as compare to control, meanwhile in HUL-57 reduction in allocation was only under stress at pod formation only (Table 2a).

When stem dry matter allocation was estimated at maturity in stressed and normal plants it was evident that it increased in control plants of genotype IPL-406 and in stressed in plants at pod formation stage and marginal increase at flower initiation and mid vegetative stage, but in HUL-57 it decreased in control plants with maximum increase in plants stressed at pod formation stage fallowed by flower initiation and mid vegetative stage (Table 2b). Leaf dry matter allocation was generally higher when it was recorded in plants just after the imposing stress (Table 2a) at various phenophases in control and stressed plants as compare to maturity (Table 2b). It was also evident that dry matter allocation of leaves was the maximum in both genotypes and treatments when stress was imposed at mid vegetative stage, but it was always higher IPL-406 as compare to HUL-57 either in control or stressed plants (Table 2b). At maturity stage control and stressed plants generally registered more leaf dry matter allocation in IPL-406 as compare to HUL-57 except at maturity in stressed plants at flower initiation stage (Table 2b). Allocation of pod wall accumulated dry matter during the stress imposed at pod formation stage was the maximum in control and stressed plants of HUL-57 and allocation of pod walls dry matter increased in stressed plants than control as compare to IPL-406 where it decreased on account of stress (Table 2a). But when dry matter allocation at maturity was investigated it decreased in both genotypes but it was generally again higher in HUL-57 than in IPL-406 (Table 2b). When dry matter allocation in seeds at maturity was estimated in HUL-57, it was higher in stressed as well as control when stress was imposed at mid vegetative and pod formation stage as compare to IPL-406, but plants stressed at flower initiation stage it marginally increased in IPL-406 than in HUL-57 (Table 2b).

The findings of present investigation suggested that the pre anthesis stored dry matter in vegetative plant parts (root + stem + leaf + pod walls) serves the major sources of seed filling in lentil. It was also suggested that the major contributors to seed filling at maturity stage were the roots leaves and pod wall than the stem they collective allocated about 60 % – 70 % dry matter allocation to seeds. When dry matter allocation of pod walls at end drought stress imposed at pod formation stage and maturity was investigated it was found that the pod wall development in accomplished in bi–phasic developmental pattern it mean firstly there was an increase in pod walls dry matter allocation and after that the contained seeds grow and allocation of seeds starts after pod walls formation is being completed (Mishra *et al.,* 2014; Shrestha *et al.,* 2006a, b), however, similar conclusions are documented by (Srivastava and Bhardwaj, 1987) in field pea.

Table.1 Phenological developments in two genotypes of lentil under well watered (WW) and drought stress (DS) subjected at specific phenophase of growth. Data are means of six replicates (pooled data of two years experiment)

Phenological development→	Vegetative period (DAS)				Reproductive period (DAS)				Seed filling duration (DAS)				Physiological maturity (DAS)			
Genotypes →	IPL-406		HUL-57		IPL-406		HUL-57		IPL-406		HUL-57		IPL-406		HUL-57	
Treatment →	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS
Mid vegetative ♣	70.00	70.00	63.00	61.00	54.67	44.33	58.33	58.33	48.84	37.67	57.34	56.67	124.34	114.17	121.17	119.67
Flower initiation ♣	70.00	70.00	63.00	63.00	54.67	40.67	58.33	58.67	48.84	35.69	57.34	54.50	124.34	110.67	121.17	121.84
Pod formation ♣	70.00	70.00	63.00	63.00	54.67	40.67	58.33	54.33	48.84	34.67	57.34	50.33	124.34	110.67	121.17	118.40
Treatment →	(NS)				(P ≤ 0.49*)				(P ≤ 0.64*)				(P ≤ 0.54*)			
Genotype →	(P ≤ 1.12*)				(P ≤ 0.51*)				(P ≤ 0.55*)				(P ≤ 0.69*)			
Phenophase →	(NS)				(P ≤ 0.69*)				(P ≤ 0.99*)				(P ≤ 0.77*)			

WW= Well watered control; DS= Drought stressed,
Significant at *P ≤ 0.05; NS=Not significant at P ≤ 0.05
♣ Irrigation was withheld at specific phenophase till plants experienced one cycle of permanent wilting; thereafter, plants were kept at normal supply of water till maturity; observations were made at the specific phenophase and maturity days after sowing (DAS)

Table.2 Dry matter allocation (%) in various plants parts as a proportion of total dry matter (shoot + root) [a] and total dry matter (shoot only) [b] in two genotypes of lentil under well watered (WW) and drought stress (DS) subjected at specific phenophase of growth. Data are means of six replicates (pooled data of two years experiment). Note: At maturity stage it was not possible to dig out intact roots from the pots, so the study was confined to shoot only

(a) Dry matter allocation (%) in various plants parts as a proportion of total (shoot + root) dry matter at the end of stress of specific phenophase

Plant parts →	Root				Stem				Leaf				Pod wall			
Genotype →	IPL-406		HUL-57		IPL-406		HUL-57		IPL-406		HUL-57		IPL-406		HUL-57	
Treatments →	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS
Mid vegetative ♣	12.22	13.72	17.73	20.02	28.31	23.97	35.13	37.68	59.48	62.32	47.20	42.32	-	-	-	-
Flower initiation ♣	12.40	12.29	15.65	13.89	33.16	39.78	38.10	48.92	53.58	46.48	44.77	34.85	-	-	-	-
Pod formation ♣	8.86	10.11	9.45	15.18	30.81	29.76	33.69	25.43	39.80	40.99	37.59	30.42	20.27	18.75	18.99	24.01
Treatment →	(P ≤ 0.58*)				(P ≤ 0.70*)				(P ≤ 0.84*)				(P ≤ 0.40*)			
Genotype →	(P ≤ 0.62*)				(P ≤ 0.75*)				(P ≤ 0.84*)				(P ≤ 0.77*)			
Phenophase →	(P ≤ 0.71*)				(P ≤ 0.92*)				(P ≤ 1.03*)				(NS)			

(b) Dry matter allocation (%) as a proportion of total dry matter (shoot only) at maturity

Plant parts →	Stem				Leaf				Pod wall				Seed			
Genotype →	IPL-406		HUL-57		IPL-406		HUL-57		IPL-406		HUL-57		IPL-406		HUL-57	
Treatments →	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS
Mid vegetative♣	32.51	32.76	25.92	26.34	8.93	12.89	6.14	8.06	11.74	12.49	12.39	12.99	46.82	41.88	54.73	52.61
Flower initiation♣	32.51	33.92	25.92	33.21	8.93	6.96	6.14	14.62	11.74	11.89	12.39	11.49	46.82	47.18	54.73	40.72
Pod formation♣	32.51	46.82	25.92	41.93	8.93	8.90	6.14	5.67	11.74	11.40	12.39	12.39	46.82	34.12	54.73	39.82
Treatment →	(P ≤ 0.50*)				(P ≤ 0.35*)				(P ≤ 0.28*)				(P ≤ 0.60*)			
Genotype →	(P ≤ 0.55*)				(P ≤ 0.38*)				(P ≤ 0.33*)				(P ≤ 0.68*)			
Phenophase →	(P ≤ 0.67*)				(P ≤ 0.47*)				(NS)				(P ≤ 0.83*)			

WW= Well watered control; DS= Drought stressed

Significant at (*P ≤ 0.05; NS=Not Significant at P ≤ 0.05; respectively)

♣ Irrigation was withheld at specific phenophase till plants experienced one cycle of permanent wilting; thereafter, plants were kept at normal supply of water till maturity; observations were made at the end of stress cycle of specific phenophase (a) and at maturity stage (b)

Note: At maturity stage it was not possible to dig out root from the pots, so the study was confined to shoot only

Table.3 Nitrogen allocation (%) in various plants parts as a proportion of total [shoot + root] nitrogen (a) and total nitrogen [only shoot] (b) in two genotypes of lentil under well watered (WW) and drought stress (DS) subjected at specific phenophase of growth. Data are means of six replicates (pooled data of two years experiment). Note: At maturity stage it was not possible to dig out intact roots from the pots, so the study was confined to shoot only

(a) Nitrogen allocation (%) in various plants parts as a proportion [a] total nitrogen (shoot + root) at the end of stress of specific phenophase

Plant parts →	Root				Stem				Leaf				Pod wall			
Genotypes →	IPL-406		HUL-57		IPL-406		HUL-57		IPL-406		HUL-57		IPL-406		HUL-57	
Treatments →	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS
Mid vegetative ♣	8.97	8.14	14.02	15.74	21.57	18.94	27.57	29.63	69.46	75.22	58.50	54.63	-	-	-	-
Flower initiation♣	9.39	9.10	13.53	11.72	24.99	27.51	31.84	37.34	65.67	63.49	54.74	50.93	-	-	-	-
Pod formation ♣	7.02	8.41	9.42	10.73	20.45	14.29	24.39	14.12	49.56	55.95	47.46	43.71	21.33	20.84	16.12	31.33
Treatment →	(P ≤ 0.30*)				(P ≤ 0.33*)				(P ≤ 0.83*)				(P ≤ 0.29*)			
Genotype →	(P ≤ 0.34*)				(NS)				(P ≤ 0.86*)				(P ≤ 0.33*)			
Phenophase →	(P ≤ 0.42*)				(P ≤ 0.41*)				(P ≤ 1.05*)				(NS)			

(b) Nitrogen allocation (%) in various plants parts as a proportion of total nitrogen (shoot only) at maturity

Plant parts →	Stem				Leaf				Pod wall				Seed			
Genotypes →	IPL-406		HUL-57		IPL-406		HUL-57		IPL-406		HUL-57		IPL-406		HUL-57	
Treatments →	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS
Mid vegetative ♣	10.72	10.68	6.58	7.91	6.32	13.22	4.09	5.01	1.36	2.54	2.52	2.62	78.56	71.84	86.81	84.49
Flower initiation ♣	10.72	9.85	6.58	7.74	6.32	4.81	4.09	6.82	1.36	1.24	2.52	1.80	78.56	84.17	86.81	83.69
Pod formation ♣	10.72	19.29	6.58	12.20	6.32	5.71	4.09	3.36	1.36	1.24	2.52	0.68	78.56	73.84	86.81	83.81
Treatment →	(P ≤ 0.70*)				(P ≤ 0.36*)				(P ≤ 0.27*)				(P ≤ 0.39*)			
Genotype →	(P ≤ 0.76*)				(P ≤ 0.37*)				(P ≤ 0.22*)				(P ≤ 0.45*)			
Phenophase →	(P ≤ 0.93*)				(P ≤ 0.46*)				(P ≤ 0.34*)				(P ≤ 0.55*)			

WW= Well watered control; DS= Drought stressed

Significant at (*P ≤ 0.05; NS=Not Significant at P ≤ 0.05; respectively); actual (%) values presented in table was reanalysed after arc sign transformation to compared validation of statistical analysis test

♣ Irrigation was withheld at specific phenophase till plants experienced one cycle of permanent wilting; thereafter, plants were kept at normal supply of water till maturity; observations were made at the end of stress cycle of specific phenophase (a) and at maturity stage (b)

Note: At maturity stage it was not possible to dig out root from the pots, so the study was confined to shoot only

Table.4 Drought resistance indices in two genotypes of lentil when subjected to drought stress at specific phenophase of growth. Data are means of six replicates (pooled data of two years experiment)

	Drought tolerance efficiency DTE (%)		Geometric mean productivity (GMP)	
Genotype →	IPL-406	HUL-57	IPL-406	HUL-57
Mid vegetative ♣	54.69	79.07	4.77	6.17
Flower initiation ♣	40.46	60.83	4.10	5.41
Pod formation ♣	30.41	40.55	3.56	4.42
Genotype →	(P ≤ 2.55**)		(P ≤ 0.14*)	
Phenophase →	(P ≤ 3.12**)		(P ≤ 0.17*)	
Genotype × Phenophase →	(P ≤ 4.41**)		(P ≤ 0.25*)	

Significant at **P ≤ 0.01; *P ≤ 0.05; respectively)

♣ Irrigation was withheld at specific phenophase till plants experienced one cycle of permanent wilting; thereafter, plants were kept at normal supply of water till maturity; observations partitioning various drought sensitivity indices were calculated on the basis of seed yield obtained from the plants grown under control treatments and drought stressed at specific phenophases. Seed yield was recorded in plants after harvesting at the maturity.

Fig.1 (a) Shoot length (cm), (b) Root length (cm), (c) Leaf area (cm^2 $plant^{-1}$) in two genotypes of lentil under well watered (WW) and drought stress (DS) subjected at specific phenophase of growth. Values of each Colum represents the data of means ± SE of six replicates; (pooled data of two years experiment; whereas; P1= mid vegetative stage; P2 = flower initiation stage; P3 = pod formation stage; at which drought stress was imposed)

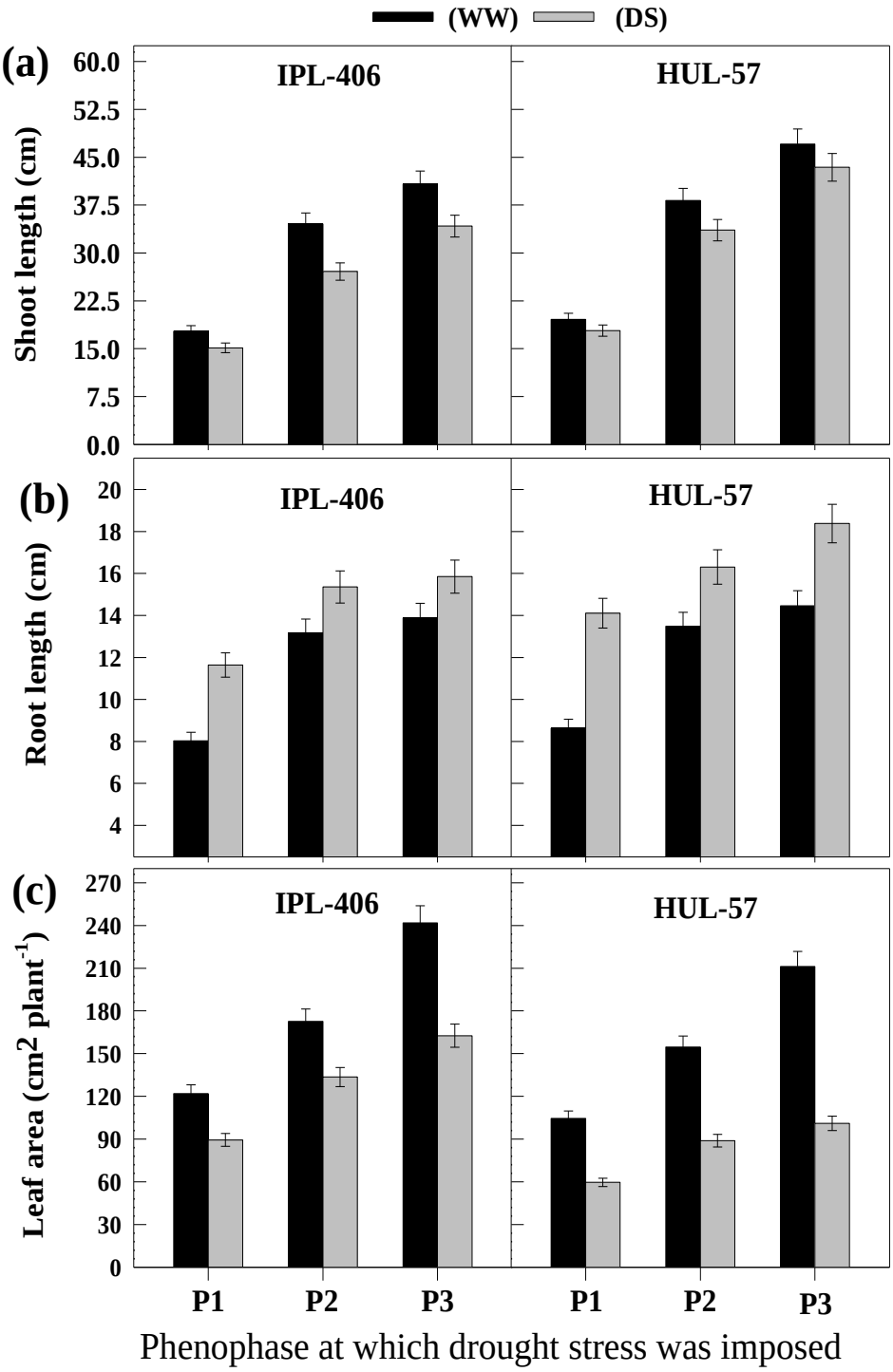


Fig.2 (a) Cell membrane stability (CMS %) under osmotic stress PEG (40 %); (b) Cell membrane stability (CMS %) under thermal stress (±50 ^{0}C), in two genotypes of lentil under well watered (WW) and drought stress (DS) subjected at specific phenophase of growth. Values of each Colum represents the data of means ± SE of six replicates; (pooled data of two years experiment; whereas, P1= mid vegetative stage; P2 = flower initiation stage; P3 = pod formation stage; at which drought stress was imposed)

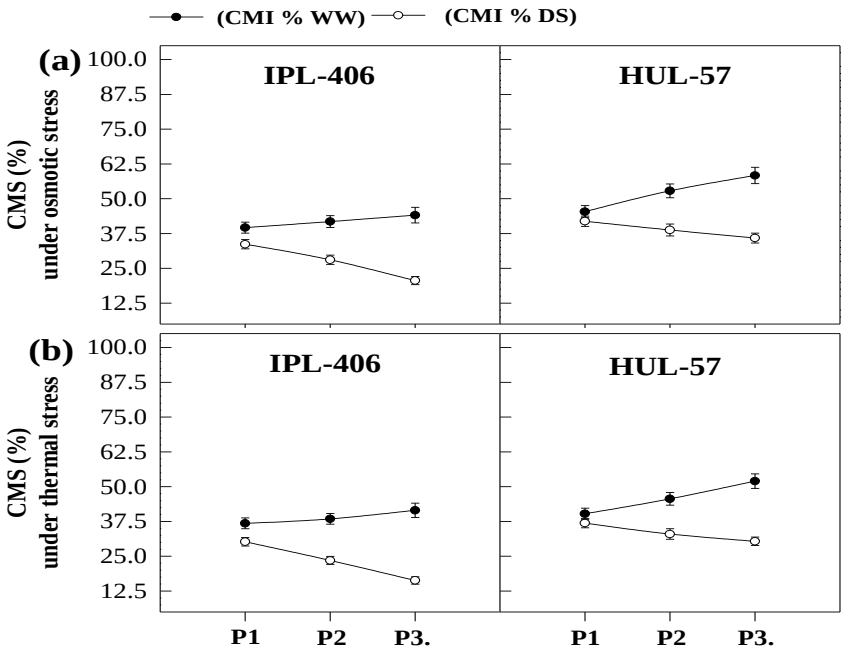


Fig.3 The relationship between (a) leaf area and relative water content of leaves; (b) relative water content of leaves and cell membrane stability; (c) leaf area and cell membrane stability. Solid lines represent the least square regressions for twelve replicates of both genotypes under drought stress conditions and the broken lines represent the 95% confidence intervals for the regressions. (Where, RWC% = relative water content of leaves expressed in per cent, CMS% = cell membrane stability expressed in percent)

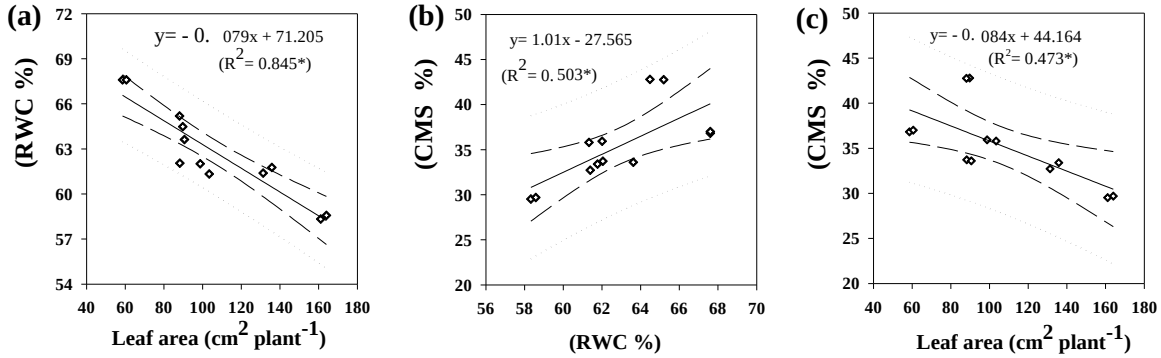
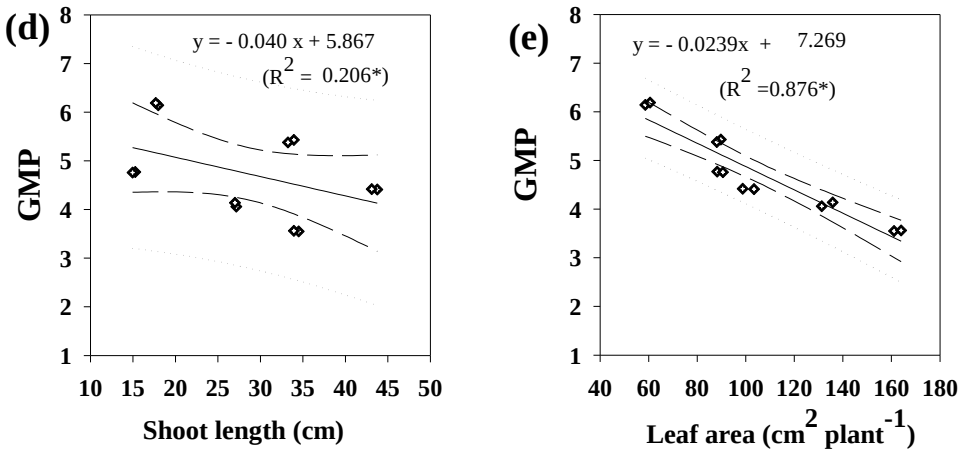


Fig.3 The relationship between (d) shoot length and GMP; (e) leaf area and GMP. Solid lines represent the least square regressions for twelve replicates of both genotypes under drought stress conditions and the broken lines represent the 95% confidence intervals for the regressions. (Where, GMP = geometric mean productivity)



Though, in some cases where dry matter allocation under drought stress is exceed as compare to control plants such situations might be due to dearrangments in metabolic activities which is responsible for dry matter allocation in plant organs or due to imbalance between source and sink strength. Such findings are in agreements to the previous results (Silim *et al.,* 1993; Hamidi and Erskine 1996; Thompson *et al.,* 1997; Davis *et al.,* 2000) and (Shrestha *et al.,* 2006a, b) documented that under drought stress sometimes allocation of dry matter is being enhanced.

Nitrogen allocation (%) in different plant parts as a proportion of total shoots nitrogen

It was evident from the findings that root nitrogen allocation when recorded at just after drought stress imposed at various phenophase root nitrogen allocation was always higher in HUL-57 than in IPL-406 under control as well as stressed treatment (Table 3a). Stem nitrogen allocation at end of stress imposed at mid vegetative and flower initiation stage was again higher in HUL-57 as compare to IPL-406, however, it decreased in both genotypes due to stress imposed at pod formation stage in both genotypes (Table 3a). Nevertheless, stem nitrogen allocation when determined in stressed plants at mid vegetative and flower initiation stage either in control plants or drought stressed it was observed that it generally decreased in both genotypes (Table 3b), but decrease was more in HUL-57 than in IPL-406 (Table 3b). But in IPL-406 stem nitrogen allocation marginally increased in stressed plants at pod formation stage (Table 3b). It was observed that nitrogen allocation of leaves at the end of drought stress of various phenophases was generally more in IPL-406 in stressed as well as control plants than in HUL-57 (Table 3a). When leaves nitrogen allocation was determined at maturity (Table 3b) in stressed plants at various phenophases and control it was recorded that it decreased about 10 times in both genotypes as compare to the previous allocation when it was measured at just after end of stress of various phenophases (Table 3a), but the decline was always more in HUL-57 than in IPL-406 (Table 3b). Nitrogen

allocation of pod walls during stress imposed at pod formation stage (Table 3a) was higher in both genotypes as compared to maturity (Table 3b) in stressed plants at different phenophase and control plants also. But in stressed plants of HUL-57 pod walls nitrogen allocation was two times higher than control plant (Table 3a). At maturity pod walls nitrogen allocation drastically reduced and it was 1.36 % in control and 1.24 % upto 2.54 % in stressed plants of IPL-406 (Table 3b), while in HUL-57 it was 2.52 % in control and 0.68 % upto 2.62 % in stressed plants (Table 3b). Seeds nitrogen allocation at maturity (Table 3b) revelled that HUL-57 was most of the stage were superior in allocation in control and stressed plants as compare to IPL-406 (Table 3b). Nitrogen allocation under drought stress and control treatments at various phenophase of lentil genotypes in different plant parts of this experiment depicted that there was a liner relationship between pre anthesis accumulated nitrogen in various plant organs (Table 3a) as roots, leafs, stem and pod wall to support seed filling at later stage of active seed development (Table 3b). It was also evident that the pod walls and seed accumulated nitrogen fallowed a bi-phasic developmental pattern because the pod walls develops first and until pod walls formation is not completed seeds will not formed and not getting nitrogen allocation. Results of nitrogen allocation suggested that as compare to dry matter allocation the remobilization of nitrogenous substances were more efficiently moves to seeds from all plant parts and this gives higher nitrogen allocation to seeds (Whitehead *et al.,* 2000). Similar results are also reported in lentil (van Kassel, 1994; Kurdali *et al.,* 1997; Mishra *et al.,* 2014) and chickpea (Davies *et al.,* 2000) under drought stress.

Drought tolerance efficiency (DTE %)

The minimum (DTE %) values was noticed when stress was imposed at pod formation stage and fallowed by flower initiation stage, but DTE was the maximum under the stress of mid vegetative stage. Genotype HUL-57 always had a higher DTE than IPL-406 (Table 4). Higher DTE values of HUL-57 exhibited its superior performance under the drought stress conditions at various phenophases than IPL-406. Ranking on the basis of DTE it can be concluded that drought stress of pod formation stage fallowed by flower initiation stage was found to be most damaging in both lentil genotypes than the drought of mid vegetative stage. However, HUL-57 always registered better for coping drought stress than IPL-406 (Table 4). It indicates that DTE can be used in lentil breeding programme for identifying drought resistant genotypes and such estimates are used by others in other crops by (Fischer and Wood, 1978; Somarian and Mahmoodabad, 2011).

Geometric mean productivity (GMP)

Drought stress significantly decreased geometric mean productivity (GMP). Drought stress of pod formation stage affects the maximum reduction in GMP while, stress of mid vegetative stage caused the minimum, but decline was always more in genotype IPL-406 in comparison to HUL-57 (Table 4). Results suggested that GMP was found an efficient tool in identifying high yielding genotypes (Fernandez, 1992; Somarian and Mahmoodabad, 2011). HUL-57 genotype always registered a higher GMP it means this genotype exhibited higher yielding capacity than IPL-406 (Table 4). Pod formation stage fallowed by flower initiation stage was found the most susceptible stage to drought stress at which genotypes possessed the lower GMP than the stress of mid vegetative stage. Findings of present investigation suggested that GMP can be used as selection traits in lentil breeding programme and microsperma types lentils performed better than macrosperma types.

Relationship between estimated parameters

Drought affects morphological and physiological parameters in plants. These parameters are interlinked with each other and responsible for various developmental process, modifications and alterations in plants under stress conditions. It was observed that when relationship of leaf area was estimated with RWC (Fig. 3a), RWC with CMS (Fig. 3b) and leaf area with CMS (Fig. 3c) it revealed a significant negative relationship between leaf area and RWC (R^2 = 0.845*), leaf area with CMS (R^2 = 0.473*) and a positive relationship between RWC and CMS (R^2 = 0.503*). Such findings suggested that under drought stress a higher leaf area caused the more transpiration by this reason RWC of leaves decreased and decreased in RWC content decreased CMS. Findings of this investigation suggested that in lentil lower leaf area is proposed to be desirable trait under drought stress. When relationship between shoot length (Fig. 3d) and leaf area (Fig. 3e) was determined with GMP the relationship was found negatively (R^2 = 0.206*) and (R^2 = 0.876*) with both measured traits. This is possibly might be due to shoot length and leaf area were not going to increase in GMP. The stabilised relationships between estimated parameters are in coordination with earlier evidences documented (Sio-Se Mardeh *et al.*, 2006; Singh *et al.*, 2013).

Now it can be concluded that under drought stress functioning of shoots and root growth significantly affected, under drought stress lower leaf area in desirable character in lentil. A trait of higher CMS is linked with drought resistance in lentil. Drought of various phenophases had long lasting significant effects on various phenological developments and physiological maturity. Pre anthesis accumulated biomass and nitrogen within the plants parts *viz.* roots, leaves, stem and pod walls and its efficient allocation to growing seeds serves as major sources during active pod filling periods. As compare to dry matter, contribution of nitrogen from different plant parts is more to seed filling in lentil. DTE and GMP indicated that microsperma types to more resistant to drought than macro sperma types. Pod formation stage fallowed by flower initiation stage was most sensitive to drought than the mid vegetative stage. Stabilized relationship with various measured traits can be taken in consideration in lentil breeding programme for drought stress conditions.

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