# Effects of Drought and Growth Media
on Lentil Growth Characteristics

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# Abstract

Lentil (*Lens culinaris* Medik.) is usually grown in regions where lack of moisture limits its production. Drought can be a major limitation to lentil production in the Palliser triangle where annual precipitation is about 300 mm. Root growth and distribution play an important role in crop productivity under dry conditions, enabling plants to access water. The goal of this research was to develop appropriate methods for studying the effects of drought on root and shoot characteristics of a diverse set of lentil genotypes grown in environmentally controlled growth chambers. Two cultivated *L. culinaris* (Eston and CDC Greenstar) and 5 wild lentil genotypes (*L. orientalis* IG 72611, *L. tomentosus* IG 72805, *L. odemensis* IG 72623, *L. lamottei* IG 110813, and *L. ervoides* L01-827A) were grown in Sunshine Mix # 4 (SSM4) and Greens Grade$^{®}$ (GG) media under fully-watered and drought conditions in two growth chambers. SSM4 is a commonly used growth medium at U of S, and GG is known to provide rapid separation of root samples from the growth medium with minimum damage to root systems. Shoot and root characteristics of the genotypes were compared after growing them separately in SSM4 and GG to identify the best growth medium and to compare morphology of different lentil genotypes. The influence of drought on root and shoot characteristics of the lentil genotypes was investigated separately in each growth medium. Shoot traits measured included plant height, number of nodes on the main stem, total number of leaflets per plant, SPAD value, shoot biomass and transpiration rate. Root traits measured were number of nodules, root biomass, root/shoot ratio, total root length, total root surface area, length density, average diameter, volume, and total number of tips and forks. SSM4 was found to be a superior growth medium relative to GG. Most genotypes had significantly higher plant height, SPAD, shoot biomass, transpiration rate and nodule number when grown in SSM4. This was likely associated with higher N concentration in SSM4 compared to GG. It seemed that N mineralization (conversion of organic to inorganic plant available form) in SSM4 was greater relative to GG. *Lens culinaris* Eston had the highest shoot biomass compared to all other lentil genotypes when grown in SSM4 under both fully-watered and drought conditions. However, reduction in root biomass of *L. culinaris* Eston under dry conditions was significantly higher compared to wild lentil genotypes, an indicator that cultivated lentil genotypes experienced drought stress. The lowest reduction in root biomass was observed in *L. odemensis* IG 72623, which makes this genotype a potential candidate for introgression of root characteristics into cultivated lentil genotypes. Drought caused reduction in number of nodes, total number of leaflets and transpiration rate. Drought also reduced root/shoot ratio in cultivated lentil genotypes. No significant difference in root/shoot ratio of wild genotypes was observed between fully-watered and drought conditions, with the exception of *L. tomentosus* IG 72805 and *L. lamottei* IG 110813, which showed significantly greater root/shoot ratio under dry conditions.

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# Dedication

I would like to dedicate this thesis to my lovely mother, Sheedeh Mohsenzadegan, for her unconditional love, continuous support and endless kindness. I could not do this without you mom! I love you so much and I miss you to the moon and back. I would also like to dedicate this thesis to my grandmother, Mahlegh Semsar, who always encouraged me to be happy, optimistic, hopeful and full of life. You will always be present in my life.

## Table of Contents

## List of Tables

## List of Figures

# List of Equations

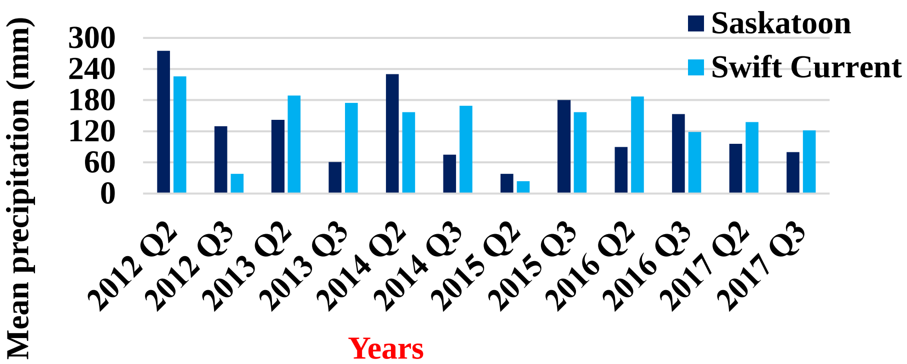
## List of Abbreviations

| | |
|---|---|
| ANOVA | Analysis of Variance |
| BM-3 | Barimasur-3 |
| BM-4 | Barimasur-4 |
| CEC | Cation Exchange Capacity |
| CSFL | Crop Science Field Laboratory |
| DAS | Days after Sowing |
| FC | Field Capacity |
| GG | Greens Grade |
| ICARDA | International Centre for Agricultural Research in Dry Areas |
| NW | North West |
| PH | Plant Height |
| PVC | Polyvinyl Chloride |
| RAD | Root Average Diameter |
| RB | Root Biomass |
| RD | Rooting Depth |
| RDW | Root Dry Weight |
| RL | Root Length |
| RLD | Root Length Density |
| RSA | Root Surface Area |
| RSR | Root/shoot Ratio |
| RV | Root Volume |
| SB | Shoot Biomass |

| | |
|---|---|
| SDW | Shoot Dry Weight |
| SHW | Synthetic Hexaploid Wheat |
| SL | Shoot Length |
| SPAD | Soil-Plant Analysis Development |
| SSM3 | Sunshine Mix # 3 |
| SSM4 | Sunshine Mix # 4 |
| SHW | Synthetic Hexaploid Wheat |
| SYN | Synthetic |
| TE | Transpiration Efficiency |
| TNOL | Total Number of Leaflets |
| TNON | Total Number of Nodules |
| TNORF | Total Number of Root Forks |
| TNORT | Total Number of Root Tips |
| TR | Transpiration Rate |
| TRL | Total Root Length |
| TRSA | Total Root Surface Area |

## 1.0 Introduction

Lentil is an important pulse crop grown in Canada for the last 35 years. Lentil production was 3.2 Mt in Canada in 2016, of which 2.7 Mt came from Saskatchewan (Statistics Canada, 2016). However, limited genetic variation in cultivated lentil germplasm makes it susceptible to biotic and abiotic stress in the prairie environment (Tullu *et al*., 2013). The Prairies are the driest regions in Canada with variable precipitation from year to year and within seasons (Fig. 1.1). This variation will likely continue according to global climate models presented by Nakicenovic and Swart (2000), who predicted the future climate of the Prairies will have shorter and warmer winters. This will cause reduction in snow accumulation, which will subsequently limit available water supplies across the Prairies later in the growing season. It is predicted that the Prairies will experience longer and warmer summers, imposing higher evapotranspiration rates on plants due to higher temperature. To offset the potential effects of drought, identification and development of drought tolerant lentil genotypes is desirable. Investigation of drought tolerance in wild lentil germplasm may result in identification and eventual introgression of desirable genes from wild to cultivated lentil genotypes. Wild lentils originated in the Near East regions with steppe climates (semi-arid climate in mountainous terrain with precipitation below evapotranspiration) (Zohary and Hopf, 1973) and have resources against biotic and abiotic stress, absent in cultivated lentil (Fiala *et al*., 2009). There is little information on lentil root and shoot traits and their contribution to drought tolerance of the crop (Idrissi *et al*., 2015), as screening for root systems under field condition is destructive, costly and time- consuming. The aim of this project was to identify effects of growth media and moisture stress on root and shoot response of both wild and cultivated lentil genotypes in controlled environments.



Q2. Second quarter of the year (Apr-Jun)
Q3. Third quarter of the year (Jul-Sep)

**Fig 1.1.** Mean precipitation at Saskatoon and Swift Current for second and third quarter of years from 2011 to 2017

## 2.0 Literature Review

### 2.1 Lentil: Centre of origin and classification

The centre of origin of cultivated lentil (*Lens culinaris* Medik.) is a mountainous area between the Hindu Kush (Northern Pakistan and Afghanistan) and Himalayas spreading across Bhutan, India, Nepal, China, and Pakistan (Zohary, 1972). Cultivated lentil was introduced to South and Central America from Spain, and it arrived in the United States before 1945 and then into Canada in 1969 (Cubero, 1981). The cultivated lentil genotypes Eston (plant introduction) and CDC Greenstar (locally bred) used in these experiments are commercial cultivars from Canada. *Lens culinaris* subsp. *orientalis* (Boiss.) Ponert, the ancestor of cultivated lentil, originated in the Near East. Distribution of this species is mostly across Turkey, Syria, North Iraq and NW

Iran where it grows in stony habitat with shallow soil under steppe-like climatic conditions (Zohary and Hopf, 1973). The center of origin of *L. ervoides* (Brign.) Grande is mostly the Mediterranean region (Zohary and Hopf, 1973). *Lens ervoides* habitat is usually shady locations under tree canopy or in pine forest (Ladizinsky, 1993). *Lens culinaris* subsp. *odemensis* (Ladiz.) usually grows in open habitat together with legumes like clovers and medics. It was first collected from Israel and Turkey. The species is able to grow in shallow calcareous soil in Syria and Israel at elevation of 700 - 1400 m. It also grows at lower altitudes of 0 to 800 m in shady habitats of Turkey (Ladizinsky, 1993). Three of the wild lentil genotypes used in these experiments originated from Turkey including *L. orientalis* IG 72611, *L. tomentosus* IG 72805 and *L. odemensis* IG 72623, originating from elevations of 2117, 1150 and 1001 m, respectively (http://www.genesis-pgr.org/welcome). *Lens lamottei* IG 110813 originated in Spain at an altitude of 660 m (http://www.genesis-pgr.org/welcome). With respect to relationships among lentil species, the most recent classification, based on genotyping by sequencing, places *L. culinaris*, *L. orientalis* and *L. tomentosus* in the primary gene pool (Wong *et al.*, (2015). *Lens odemensis* and *L. lamottei* formed the secondary gene pool and *Lens ervoides* was placed in the tertiary gene pool. *Lens nigricans* (M. Bieb.) Gordon forms the quaternary gene pool based on phylogenetic tree and structure analysis.

## 2.2 Drought and its effects on plants

Drought is defined as lack of rainfall over a long period of time, causing reduction in soil moisture, damage to plants and decrease in grain yield (Kramer, 1980; Subbarao *et al*., 1995). Drought is one of the most important abiotic stresses (Subbarao *et al*., 1995), imposing large yield limitations for cool season grain legumes (Saxena, 1993; Subbarao *et al*., 1995). Generally, drought stress can be classified into two types described as intermittent or terminal. Intermittent drought happens during the vegetative or reproductive phase (Quisenberry 1982; Subbarao *et al*., 1995), while terminal drought occurs during the pod-filling phase (Rao *et al*., 1985). In response to terminal drought in grain legumes, a reduction in the number of seeds generally occurs rather than a reduction in the size of seeds. This was reported to be due to provision of growing embryos with assimilates without influencing seed growth rate or seed size (Westgate *et al*., 1989; Egli 1998; Munier-Jolain *et al*., 1998). The effect of intermittent drought is mostly observed as variation in seed size (Munier-Jolain *et al*., 1998). However, Samarah *et al.* (2004), reported a reduction in seed size of soybean seeds subjected to water stress at early seed filling stage.

### 2.3 Drought strategies of lentil species

In regions with Mediterranean climates, like Tel Hadya in Syria, total rainfall throughout the growing season accounted for 80% of the variance in seed yield (Erskine and Ashkar, 1993). Lentil crops grown in Mediterranean climate regions usually encounter a rapid rise in maximum temperature during the onset of summer, which occurs from March to May in the northern hemisphere, and September to November in the southern hemisphere. During this period, reduction in rainfall occurs as evaporative demand increases. Lentil reproductive growth coincides with this period and therefore the crop may often experience drought stress during the reproductive stage, resulting in yield loss (Harris *et al.*, 1987). Development of high yielding drought tolerant cultivars in such areas is one of the major objectives of research conducted at the International Centre for Agricultural Research in Dry Areas (ICARDA, 1989).

Lentil crops use specific drought response strategies to survive and produce seed yield. They often exhibit accelerated crop maturity (drought escape) in response to high temperature and low water availability. For example, lentil genotypes grown in South Asia and Ethiopia escape drought through early flowering. In these regions, genotypes originating from northern latitudes cannot adapt to drought conditions due to their late-maturing nature (Hamdi *et al*., 1992). Based on investigation at a mid-hill location near Kathmandu in Nepal, South Asian genotypes produced high yield while West Asian genotypes had poor yield (Shrestha *et al*., 2005). Genotypes from West Asia had a shorter reproductive stage and late flowering due to longer day length photoperiodic requirements and slow dry matter accumulation, which resulted in empty pods and lower yield. Lentil genotypes derived from crosses between South Asian and West Asian parents had the highest yield, due to the combination of early flowering phenology and high seed number from South Asian genotypes and larger seed size from later-maturing West Asian genotypes (Shrestha *et al*., 2005).

A few accessions of cultivated lentil with the ability to escape drought by earlier flowering have been identified (Hamdi *et al*., 1992; Silim *et al*., 1993), for example, BARIMASUR 4, 5, 6, and 7 which have the advantage of showing drought avoidance as rising temperatures and depleting soil moisture encourages early maturity (Erskine *et al*., 1993; Shrestha *et al*., 2006). Alternative drought resistance mechanisms that enable wild lentil species to endure drought have not yet been described in cultivated lentil (Hamdi and Erskine, 1996).

Wild lentil species have genetic potential for transfer of drought tolerance genes to cultivated lentil grown in drought prone areas (Sharma *et al*., 2013). In India, 278 lentil genotypes including cultivated and wild lentil accessions with different origins underwent drought screening at the seedling stage under fully covered polythene tunnels. The results showed that seed yield reduction of wild lentil accessions (ILWL-314 and ILWL-436) was lower than cultivars (PDL-1 and PDL-2) under drought conditions (Singh *et al*., 2016). Based on the morphological and phenological evaluations of *Lens* germplasm collections at ICARDA, *L. odemensis* and *L. ervoides* were recognized as species with drought resistance (Hamdi and Erskine, 1996) based on lower yield reduction in response to drought conditions. *Lens orientalis* was considered the species with the earliest flowering and maturity. One hundred and eleven wild lentil accessions of *L. orientalis*, *L. odemensis, L. ervoides* and *L. nigricans* along with 10 cultivated accessions were grown under dryland conditions and also under supplemental irrigation in Syria. The cultivated lentils had higher seed and straw production compared to wild lentil. Furthermore, only a small percentage of the observed variation in wild lentil yield resulted from time to flowering. The interpretation was that drought escape in wild lentil accessions was not as important as that observed in cultivated lentil genotypes (Hamdi *et al*., 1996).

### 2.4 The role of roots under drought stress

Drying of the soil surface happens very quickly in drought prone areas where most stored water is found in deep soil layers (Mia *et al*., 1996). In such regions, development of crop genotypes with deep root systems is crucial (Gupta, 1992). Rooting depth (RD) is a main contributor to avoidance of drought in many plant species, from woody perennials (Pinheiro *et al*., 2005) to sorghum (*Sorghum bicolor (L) Moench*). Enhanced drought avoidance resulting from deep root systems and early maturity combined with higher transpiration efficiency (TE) were likely the major contributors to the high yield of grain legumes (Soltani *et al*., 2000). In lentil, deep and big root system is found to improve plant water acquisition based on the findings of Kumar *et al.* (2012), who reported relationship between root biomass (RB) and high performance of lentil genotypes when grown in semi-arid to sub-humid regions. Progress has been slow using direct selection for yield in drought prone areas (Richards, 1987). The complex nature of yield formation has caused researchers to suggest other characteristics as selection criteria (Srivastava *et al*., 1988). Selection for deep and extensive root systems with high root length density (RLD) The length of roots in a given soil volume improves plant ability to obtain more water from drying soil in water-limited environments (Subbarao *et al*., 1995; Serraj *et al*., 2004; Sarker *et al*., 2005). Plants with RLD of 0.5 $cm/cm^{-3}$ can absorb water with no difficulty (Passioura, 1983). In lentil, water stress during the reproductive stage was shown to increase the root /shoot ratio by 14-100% (Shreshta, 2005).

Turner *et al.* (2001), reported that RD and RLD are the major drought avoidance traits for legumes exposed to terminal drought stress. Water deficit can cause an increase in RLD in drought tolerant genotypes of soybean (Cortes and Sinclair, 1986), chickpea (*Cicer arietinum* L.) (Kashiwagi *et al*., 2006) and common bean (*Phaseolus vulgaris* L.) (White and Castillo, 1988), an indication that there are examples of legumes with extensive root systems showing tolerance to terminal drought stress.

The available information on lentil root and shoot systems is not as extensive as in cereal crops (Mia *et al*., 1996; Sarker *et al.*, 2005; Gahoonia *et al*. 2005, 2006). Significant differences in root traits (root length, root hair density and length) of the two lentil varieties Barimasur-3 (BM-3) and Barimasur-4 (BM-4) were observed at 20 and 60 days after seeding (DAS). BM-4 had faster root growth as well as longer and thicker root hairs compared with BM-3 (Gahoonia *et al*., 2005). Based on the results of another experiment evaluating root traits of 10 lentil genotypes including BM-3 and BM-4 and the relationship between root traits and nutrient absorption, variation in root length (RL), root hair density and root hair length was reported. BM-3 and BM-4 had the highest RL and the longest and thickest root hairs among the group of lentil genotypes. Significant correlation ($P < 0.05$) between root hair traits (hair length and density) and nutrient uptake of lentil genotypes was observed (Gahoonia *et al*., 2006). Correlation of deep root systems with seed yield, crop growth and water uptake was reported in common bean (Sponchiado *et al*., 1989). In common bean grown under drought conditions, roots influenced the seed yield and shoot dry weight (SDW) more than shoot features (White and Castillo, 1992). In chickpea, higher yield of genotype ICC 4958 compared to the standard cultivar Annigeri under drought conditions was associated with its higher root dry weight (RDW) (Saxena, 1987).

RLD is also associated with drought avoidance and can be used as a factor in developing knowledge on drought avoidance by roots (Kashiwagi *et al*., 2006). Association of RLD in deep soil layers with yield was observed under severe drought stress (Kashiwagi *et al*., 2006). Plant RLD is usually adequate for water uptake in the surface soil layer (Passioura, 1982). However, at soil depth below 0.3 m, RLD might not be sufficient for water absorption. Variation in RLD has been reported in many legumes including faba bean (Looker, 1978), chickpea (Brown *et al*., 1989) and lentil (ICARDA, 1984).

The measurement of RL, RDW, and shoot length (SL) of 43 cultivated lentil genotypes from drought-prone areas with mild winters (during the months of October to March) such as Syria, northern India and Pakistan showed variation in all three traits (Kumar *et al*., 2012). RL ranged from 42 - 83 cm per plant at 65 DAS. RDW varied from 0.18 - 0.76 g plant$^{-1}$ and SL was reported in the range of 14 - 32 cm. Low heritability was estimated for both RL (11.7%) and RDW (12.4%). Genotypes with the greatest RL or RDW originated from drought-prone areas, or they were derived from parents grown under the same conditions. Despite the significant association of RDW with RL and SL, no association between RL, RDW and seed yield was reported in this survey (Kumar *et al*., 2012). At the podding stage, lentil genotypes originating from South Asia had the highest RLD, and those West Asian origin had the lowest RLD at that stage (Shrestha *et al*., 2005).

When RL surpasses a certain size, branching of roots occurs and lateral roots emerge from the root pericycle and epidermis (Morita and Yamazaki, 1993). Lateral roots are important for water absorption under water stress (Banoc *et al*., 2000) and they determine the size and architecture of the lentil root system (Mia *et al*., 1996). RL and root surface area (RSA) contribute to water and nutrient uptake and are mostly influenced by formation of fine roots less than 2 mm in diameter (Zobel *et al*., 2005; 2007). Liu *et al*. (2010), reported that RSA of pulse crops including lentil, is mostly determined by larger roots with diameter of 0.80 to 2.0 mm. Pulse crops, including lentil, influence soil physical properties due to the large diameter of roots (Bengough *et al*., 2006). In oilseeds and wheat, the roots classified in the range of 0 - 0.2 mm diameter accounted for 60% of the total root length (TRL) while roots with diameter of 0.2 - 0.4 mm diameter comprised 30% of the TRL. The remainder of the roots with diameter > 0.4mm formed only a small percentage of TRL. In terms of RSA and total root volume (TRV) of oilseeds [canola (*Brassica napus* L. canola), flax (*Linum usitatissimum* L. flax) and mustard (*Brassica juncea* L. mustard)] the major contribution (>60%) is made by roots in the 0.2-0.4 mm diameter class. In pulse crops, the major contribution to the RL and RSA is made by roots with diameter classes of 0.4-0.6, 0.6-0.8 and 0.8-2.0 mm. The thicker diameter classes for lentil and chickpea caused the increase in RSA (Liu *et al*., 2010). This agrees with findings of Gan *et al*. (2011), who studied and compared RL, root volume (RV) and root average diameter (RAD) of lentil, chickpea, wheat (*Triticum aestivum* L.) and three oilseed crops in tubes (100 cm height) installed at Swift Current, Canada under two moisture levels (low and high-water availability). Results of the experiment suggested that pulse crops had thicker roots with larger diameter and larger root systems with higher RV. Lentil plants had 59% of the TRV at 0-20 cm soil depth when water supply was high, but was reduced to 43%

under conditions of reduced water supply, which also resulted in roots showing a tendency to branch at greater depth.

RV, total number of root tips (TNORT) and total number of root forks (TNORF) are determining factors in root morphology (Raviv *et al.*, 1986). Plants grown in soil containing sufficient nutrient and water supply have better root morphology with higher RV and greater TNORT and TNORF (Lazcano *et al*., 2009). RV represents the size of root systems in plants, and plays significant roles in water and nutrient acquisition (Fageria *et al*., 2004). Increase in RV is observed until late flowering, and afterwards reduction in RV occurs (Gan *et al*., 2011). RV is dependent on RL and RAD (Merrill *et al*., 2002). Approximately 90% of RV is located in the top 60 cm of soil. TNORF is an important root trait enabling plant roots to explore greater soil volume and it shows correlation with other root parameters including RSA, TNORT, RV and RB (Jahuer *et al*., 2008).

Becker *et al.* (2016), reported that increased RB along with the development of fine roots of great length at deeper soil layers can optimize plant water extraction under drought conditions and maintain productivity for wheat. Synthetic hexaploid wheat (SHW) lines, for example, were reported to have variation for this trait.  The genotype SYN-201 had only a small decrease in shoot biomass (SB) when stressed. This line along with 5 more SHW and four U.S. winter wheat cultivars were planted in polyvinyl chloride (PVC) tubes of 99 cm height and 10.2 cm diameter filled with Greens Grade® (GG) medium, which allowed easy separation of roots at harvest. SYN-201 showed plasticity by producing more roots at the bottom of the tubes under drought conditions. SYN-201 and SYN-290 produced fine roots of great length in the diameter classes of 0.00-0.25 mm and 0.25-0.50 mm, contributing to higher water extraction from the GG medium in water-limited treatments.

Based on the review of literature, RD, RB and RLD are reported to be the major contributors to higher water extraction, higher TE and higher yield in lentil genotypes grown in water-limited areas. Despite the importance of root traits for plant survival and growth, there are few experiments on lentil root systems compared to the number of experiments that report shoot characteristics (Sarker *et al.*, 2005). This might be related to difficulty in screening for root trait, and the destructive sampling requirements under field conditions. Use of transparent tubes 5-10 cm in diameter and a minimum of 50 cm in height can provide rapid and convenient phenotyping screening method for root traits in controlled growth rooms (LemnaTech, 2007). Another alternative method is measurement of SPAD, which provides an approximation of leaf chlorophyll content (Uddling *et al.*, 2007). Leaf chlorophyll content is associated with nitrogen content (Filella *et al*., 1995), which shows nutritional status of plants (Menesatti *et al*., 2010). Thus, plants with higher leaf chlorophyll content (higher SPAD) are healthier. Significant positive correlation was reported (r = 0.45**) between SPAD value and root dry weight (RDW) in an experiment involving 43 lentil genotypes (Kumar *et al.,* 2012).

## 2.5 Growth of lentil in indoor growth systems

Phytotron and incubator systems are often used for growing plants in controlled environments to determine response to water limitations. With respect to lentil growth in indoor growth systems, 133 $F_{6-8}$ RILS of lentil together with parents were grown in a greenhouse with temperature set at 14° C day (13h) and 8° C night (11h) under well-watered (75% FC) and water-stress treatments (75-22% FC) (Idrissi *et al*., 2015). Significant reduction was observed for shoot and root biomass of both parents and RILS exposed to drought, and the root/shoot ratio increased under drought conditions.

## 2.6 Growth media used in controlled environments

### 2.6.1 Sunshine Mix growth medium

Sunshine Mix # 4 (SSM4, Sun Gro Horticulture Canada Ltd.) is commonly used at the U of S phytotron facility for a wide range of plant species. It is an organic growth medium with pH range of 5.5- 6.5, electrical conductivity (EC, an indicator of amount of available nutrient in soil) of 0.2 S/m, bulk density (weight of soil in a given volume) of 0.13 Mg/m$^{-3}$ and high nitrogen content (676 mg/kg). SSM4 consists of Canadian sphagnum peat moss, which allows moisture and nutrient retention. The mixture also contains coarse perlite, providing plants with good aeration in the medium. SSM4 also contains dolomitic limestone is another component of SSM4 which is recommended for potted crops. Dolomitic limestone is a combination of calcium and magnesium carbonate, used in the mix to neutralize growth media pH because peat moss has pH 3.5-5.

An example is provided by Zakeri *et al*., (2013) who grew five cultivars of lentil (CDC Greenland, CDC Sedley, CDC Milestone, CDC Blaze and CDC Rouleau) in pots filled with SSM4 at the phytotron of U of S to study the effect of nitrogen application on plant yield. Yuan *et al*., (2017), grew 3 genotypes of *L. culinaris, L. orientalis, L. tomentosus, L. odemensis, L. lamottei* and *L. ervoides* in pots filled with mixture of SSM3 and SSM4 to study the effect of varying light intensity on flowering response of plants.

### 2.6.2 Greens Grade® growth medium

Greens Grade® (GG) (PROFILE Products, LLC, Illinois, USA) is an inorganic growth medium with bulk density of 0.57 Mg/ m$^{-3}$, cation exchange capacity (CEC) (capacity of soil for holding cations) of 33.6 mEq/100g$^{-1}$, low nitrogen content (5.9 mg/kg) and acidic pH of 5.5. known for its contribution to rapid separation of experimental root samples from the medium with minimal damage to the root system. GG contains silica, aluminium oxide, iron (III) oxide and crystalline quartz. Other components of GG included in very small amounts (total <2%) are [CaO, MgO, K2O, Na2O and TiO2 <2%]. GG is manufactured and processed in a rotary kiln at a temperature of about 1200 degrees Fahrenheit and then screened to remove extremely fine particles from the product. This inorganic soil amendment resists compaction and promotes drainage and healthy root growth. An example is provided by Becker *et al*. (2016), who grew synthetic hexaploid wheat (SHW) lines in tubes filled with GG in the greenhouse at Oklahoma State University, USA and reported easy separation of roots from this growth medium. GG is also used for construction of sand based sports fields such as golf course greens where it provides faster drainage compared to sand. The improved drainage of GG makes it ideal for construction of athletic fields because poorly drained soils create problems for growing grass. The equal-sized particles of GG allow this inorganic growth medium to maintain water and nutrient more efficiently compared to sand, contributing to better growth of grass when grown in GG. It can also be used as fertilizer on lawns or as a topdressing treatment for soil.

**Table 2.1.** Comparison of ingredients and their concentration and properties of components of Sun Sunshine Mix # 4 (SSM4) and GreensGrade® (GG) growth media

| Ingredient and concentration of property | Growth media | |
|---|---|---|
| Media components, and their concentration | SSM4 | GG |
| Canadian sphagnum peat moss | Present | NA |
| Coarse perlite | Present | NA |
| Dolomitic limestone | Present | NA |
| Long lasting agent | Present | NA |
| Silicon dioxide (%) | NA | 74 |
| Aluminium oxide (%) | NA | 11 |
| Copper (mg/kg) | 1.3 | < 0.10 |
| Iron (mg/kg) | 155 | 31.1 |
| Manganese (mg/kg) | 11.6 | 18.4 |
| Zinc (mg/kg) | 0.6 | 12.8 |
| Total available N (mg/kg) | 676 | 5.9 |
| Water capacity (%) | 50 | 39 |
| pH | 5.5 - 6.5 | 4.5 - 6.5 |
| Bulk density (Mg/m$^{-3}$) | 0.13 | 0.57 |
| Electrical conductivity (S/m) | 0.2 | NA |
| CEC in (mEq/100g$^{-1}$) | NA | 33.6 |
| Type | Organic | Inorganic |

SSM4: Sunshine Mix # 4; GG: Greens Grade; NA: not available; CEC: cation exchange capacity

## 3.0 Research hypotheses

Despite the importance of the root system in contributing to plant tolerance to drought, few studies have been conducted on root traits of lentil genotypes. Gan *et al*. (2011), studied root traits in cultivated lentil grown in tubes, installed at 100 cm soil profile in Swift Current, Canada. This study was limited to cultivated lentil genotypes. In experiments that have examined wild lentil species, the main focus was on above-ground traits like time to 50% flowering, days to maturity, plant height, and number of pods per plant. An example is a study of 67 wild lentil accessions that included *L. orientalis, L. odemensis*, *L. ervoides* and *L. nigricans* (Gautam *et al*., 2013).

The aim of this project was to study root and shoot traits of both wild and cultivated lentil genotypes using suitable developed methods for conducting research on drought tolerance of lentil in controlled environments. Root biomass is an important trait that is most often measured in drought-related studies (Kumar *et al*., 2012; Sarker *et al*., 2005; Serraj *et al*., 2004). There is a need to assess additional root traits such as root/shoot ratio, root length, root density, root diameter, root volume and total number of root tips and root forks. These can then be compared to shoot traits to gain further insight into root/shoot relationships. Suitable indoor methods for these types of studies are required to gain basic knowledge, and very few are published. As a first step, experiments were planned and designed to identify the effects of growth media and moisture levels on root and shoot response of two cultivated [*L. culinaris* (Eston and CDC Greenstar)] and 5 wild lentil genotypes (*L. tomentosus* IG 72805, *L. ervoides* L01-827A, *L. odemensis* IG 72623, *L. orientalis* IG 72611 and *L. lamottei* IG 110813) grown in two growth media (Sunshine Mix # 4 and Greens Grade®) separately in two growth chambers. Potential drought tolerance of lentil plants can be compared by measuring root and shoot parameters, and their characterization across species and media can provide insight into appropriate indoor methodology and identification of wild lentil species best suited for long term genetic improvement of drought tolerance of cultivated lentil.

Hypothesis 1: Growth media have a differential effect on root and shoot characteristics of the 7 wild and cultivated lentil genotypes regardless of moisture treatments (fully-watered and drought).

Hypothesis 2: Drought has a differential effect on root and shoot characteristics of the 7 wild and cultivated lentil genotypes regardless of the growth media.

Hypothesis 3: Interaction of moisture treatment and growth medium has an effect on growth characteristics of 7 wild and cultivated lentil genotypes.

Hypothesis 4: Cultivated lentil genotypes respond differently to growth media and drought as compared with wild lentil genotypes.

## 4.0 Experiment 1: Effect of water deficit on 7 wild and cultivated lentil genotypes and species grown in polyvinyl chloride (PVC) tubes filled with Greens Grade medium

### 4.1 Objectives

**The objectives of this experiment were to:**

- Measure shoot characteristics including plant height, number of nodes on the main stem, total leaflet per plant, leaf chlorophyll content (SPAD values) and shoot biomass of 7 lentil genotypes grown in Greens Grade®.
- Assess root parameters which included root biomass, total root length, total surface area, length density, volume, and average diameter, total number of root tips and forks, root/shoot ratio and nodules of 7 lentil genotypes grown in Greens Grade®.
- Estimate plant transpiration rate over the experimental period.

### 4.2.Materials and methods

Three seedlings of each of 7 genotypes representing 6 *Lens* species [*L. culinaris* (Eston and CDC Greenstar), *L. orientalis* IG 72611, *L. tomentosus* IG 72805, *L. odemensis* IG 72623, *L. lamottei* IG 110813 and *L. ervoides* L01-827A] were used int the experiment. *Lens orientalis* IG 72611 is considered to be genetically between *L. culinaris* and *L. tomentosus* in the primary gene pool of lentil according to Wong *et al*., (2015), *L. odemensis* and *L. lamottei* are considered the secondary gene pool, and *L. ervoides* constitutes the tertiary gene pool. All plants were grown n polyvinyl chloride (PVC) tubes of 60 cm height and 10 cm diameter and 4710 cm$^3$ volume. The tube bottoms were closed with mesh and filter paper allowing for free drainage of water. The experiment was conducted in a 48 ft$^2$ environmentally controlled growth room (Conviron AGR 48, Winnipeg, MB) in the College of Agriculture and Bioresources phytotron facility at the University of Saskatchewan. At the beginning of the experiment, room temperature/day length was set to 21$^o$C day/16 h/ 15$^o$C night / 8 h and light intensity ranged from 308-392 µmol m$^{-2}$ s$^{-1}$ depending on tube position and plant height. Tubes were randomly moved at each weighing to minimize the effect of light. A change in light intensity and temperature with the aim of accelerating evaporation was made at week 7 after sowing. Temperature was then increased to 26$^o$C day and 16$^o$C night. Light intensity was also raised to the range of 308-530 µmol m$^{-2}$ s$^{-1}$ depending on tube position and plant height. Tubes were placed in plastic tube racks (one rack per replication) to keep them stable.

The experimental design was factorial. Each genotype was grown under two moisture levels (fully-watered and drought) with 4 replications. There were 8 unplanted tubes for estimation of the background evaporation rate. Four unplanted tubes (equal to replications) were maintained at 80% FC throughout the experiment (Fig 4.1) as a fully watered control. The other 4 unplanted tube was maintained at a water content similar to the drought treatment. To achieve this goal, water was added to the tubes without plants twice a week until they were the same weight as the drought treatment tubes with plants, which had been previously weighed. All the tubes were randomly placed at the growth chamber. After each weighing transpiration rate was calculated by the following equation:

$$Transpiration\ rate\ (\frac{mg}{day}) = \frac{(\mathrm{W2}-\mathrm{W1})-(\mathrm{Wempt2}-\mathrm{Wempt1})}{Diff(Day2-Day1)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.. \quad (4.1)$$

W2 = weight of tube with plant on Day 2

W1 = weight of same tube with plant on Day 1

Wempt2 = weight of empty tube (no soil and plant) on Day 2

Wempt1 = weight of same empty tube on Day 1

Day1 = day on which first measurement was made

Day2 = day on which second measurement was made

To determine weight of water at 100% field capacity (FC) (available moisture found in the soil 2 or 3 days after irrigation or raining when excess moister drains away due to gravity), 7 randomly placed tubes were filled with GG Natural (PROFILE Products, LLC, Illinois, USA). Initial moisture content of 1.6% was measured using a moisture analyzer (MA 30 Sartorius Corp. NY, USA). Tubes filled with GG were saturated with water until visual observation of drainage from the bottom of the tubes. At this point, tops were covered with aluminum to prevent evaporation. Three days later, filled tubes were weighed. Empty tube weight (approximately 1035 g) was subtracted from the weight of filled tubes. This value was then subtracted from weight of dry GG to determine maximum FC of the medium.

$$Weight\ of\ dry\ Greens\ Grade = weight\ of\ wet\ \text{Greens Grade} - amount\ of\ moisture \quad \ldots\ldots\ldots\ldots \quad (4.2)$$

Weight of water at 100% field capacity in Greens Grade $= [(weight\ of\ \ tube\ \ at\ 72\ h -$

$weight\ of\ empty\ tube)] - \text{weight of dry GG}$

…………………………………………………………………….. (4.3)

On December 8th 2015, seedlings that were pre-germinated in a dark incubator and were transplanted into the PVC tubes into which approximately 1.4 g of *Rhizobium leguminosarum* biovar *viceae* strain 1435 (Nodulator XL SCG, Becker Underwood, Canada) had previously been scattered at 2 cm depth of GG. During pre-germination, the seeds were disinfected with buffered bleach containing $KH_2PO_4$, distilled water, tween and bleach (Clorox) and kept in a 150 ml flask with about 5 ml distilled water. On sowing day, 200 ml of modified Hoagland's solution without N containing calcium chloride (60.5 mM), micronutrients (12.1 mM), Fe EDTA (12.1 mM), potassium hydrogen phosphate (12.1 mM) and magnesium sulphate (4.8 mM) was added to all tubes. Seedlings were thinned to one per tube after successful establishment, and then fed with 100 ml of NPK in the amount of 4 g $l^{-1}$ on the 9th and 11th week after sowing when leaf yellowness was observed.

The two moisture treatments in this study were as follows (Fig 4.1.).

1) Fully-watered treatment (control): Plants were grown at 80% field capacity (FC), which provides plants with optimum irrigation (Khadraji and Ghoulam, 2016). This FC was maintained by adding water to each tube and weighing tubes after re-watering throughout the whole experiment (twice a week).

2) Drought treatment: Plants were grown at 80% FC for 6 weeks prior to onset of drought treatment to ensure establishment. At 6 weeks after sowing, plants were established well and FC was reduced from that point until flowering of lentil genotypes (14th weeks after sowing), which was the end of the experiment (Fig 4.1.). Late flowering of plants was related to GG,

where restricted and slowed plant growth and evapotranspiration. *Lens orientalis* IG 72611 and *L. tomentosus* IG 72805 were the only genotypes failed to produce flower at the end of the experiment due to storage problem.

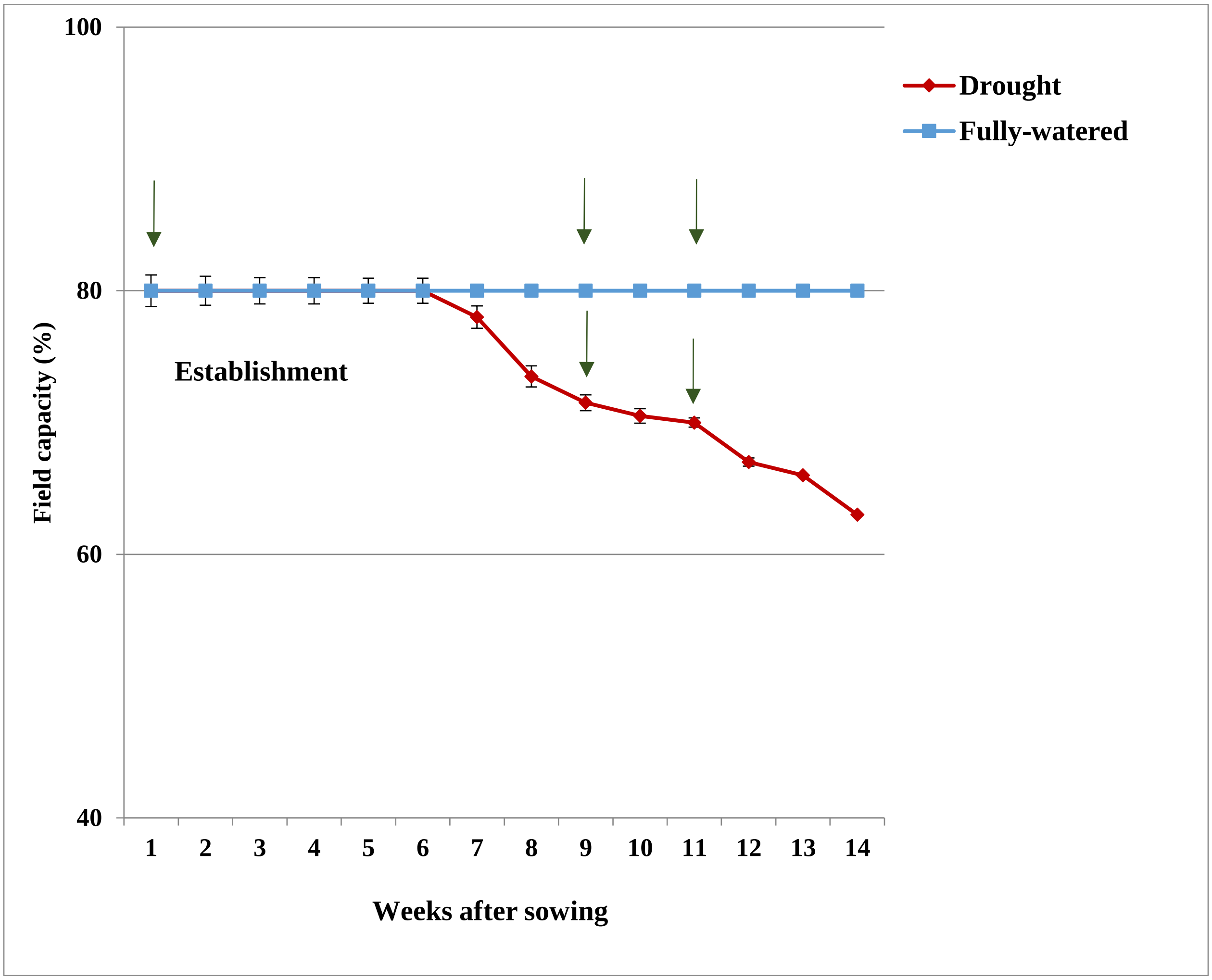


**Fig. 4.1.** Soil moisture profiles of fully-watered and drought treatments applied to lentil plants grown in Greens Grade growth medium for 14 weeks. Bars indicate standard error of mean FC; arrows show time points of fertilizer application.

Nematode sprays of *Steinernema feltiae* (Biobest Canada Ltd., Leamington, ON**)** were performed twice a week as a biological control against thrips (*Frankliniella occidentalis*), which are a major threat to lentil plants grown in controlled environments.

At harvest, lentil roots and shoots produced in each tube were separated. Roots were washed on a sieve to remove the GG medium from plant tissues. Afterwards, they were stored in Ziploc plastic bags containing 45 ml cold tap water and preserved in a refrigerator at about 4 $^{o}$C to prevent disintegration until scanning commenced. Root samples were scanned (Epson Perfection V700 Photo) and the scanned images were analyzed using a WinRhizo scanner image analysis system (Regent Instruments Inc., Ville de Québec, Canada) to determine root parameters, including total TRL, RLD, TRSA, RAD, RV, TNORT and TNORF. The number of nodules was also recorded. Root and shoot samples were oven dried at 72$^{o}$C for 48 h prior to weighing for biomass estimation. An estimation of approximate plant chlorophyll content was measured by taking SPAD readings with the aid of a SPAD-502Plus chlorophyll meter (Konica Minolta, Japan). Plant height, number of nodes on the main stem, total leaflet number per plant and SPAD values were recorded at 50, 80 and 92 DAS. A ruler was used to measure plant height from the base to the topmost growing point of plants.

## 5.0 Experiment 2: Effect of water deficit on 7 wild and cultivated lentil genotypes and species grown in pots filled with Sunshine Mix # 4 medium

### 5.1 Objectives

**The objectives of Experiment 2 were to:**

- Measure shoot characteristic including plant height, number of nodes on the main stem, total leaflet numbers per plant, leaf chlorophyll content (SPAD value) and shoot biomass of 7 wild and cultivated lentil genotypes grown in SSM4.

- Measure root characteristics including root biomass, total root length, total surface area, length density, volume, average diameter, total number of root tips and forks, root/shoot ratio and nodule numbers of 7 lentil genotypes grown in SSM4.
- Estimate plant transpiration rate over the experimental period.

## 5.2. Materials and methods

A set of 7 genotypes comprising of 6 *Lens* species [*L. culinaris* (Eston and CDC Greenstar), *L. orientalis* IG 72611, *L. tomentosus* IG 72805, *L. odemensis* IG 72623, *L. lamottei* IG 110813 and *L. ervoides* L01-827A] were selected for this experiment. *Lens orientalis* IG 72611 falls between *L. culinaris* and *L. tomentosus* according to Wong *et al*., (2015). Three seedlings of each genotype were established in plastic pots 17.5 cm in height by 15.5 cm in diameter and by 3300 $cm^3$ in volume. A filter paper was placed at the bottom to prevent soil loss. Seeds were pre-germinated under two conditions. One group of seedlings was germinated in moist petri dishes kept at room temperature at the Crop Science Field Laboratory (CSFL) for 8 d. The second set of seeds was germinated in petri dishes placed under far red light (650 $\mu mol\ m^{-2}\ s^{-1}$) for 8 d in a MODEL Comco controlled growth room approximately 110 $ft^2$, at Innovation Place, Saskatoon.

The most vigorous seedlings from both germination sets were transplanted into pots filled with SSM4 (Sun Gro Horticulture Canada Ltd., Seba Beach) on March 1, 2016. Granular inoculant was added at the rate of 1.4 g of *Rhizobium leguminosarum* biovar *viceae* strain 1435 (Nodulator XL SCG, Becker Underwood Canada) at 2 cm depth in each pot. Three days after transplanting, modified Hoagland's solution without N containing calcium chloride (60.5 mM), micronutrients (12.1 mM), Fe EDTA (12.1 mM), potassium hydrogen phosphate (12.1 mM) and magnesium sulphate (4.8 mM) was added to all pots. In the second week after transplanting the seedlings were thinned to one per pot.

The pot experiment was conducted in a same experimental design as the tube experiment in a 178 $ft^2$ Conviron AGR 178 environmentally controlled growth room in the phytotron. Temperature settings were also the same as Experiment 1 [at 21°C day (16 h) and 15° night (8 h)]. Light intensity was in the range of 323 to 529 $\mu mol\ m^{-2}\ s^{-1}$. Pots were moved randomly at each weighing to reduce the light effect. Each genotype was grown under fully-watered and drought conditions with 4 replications. Eight unseeded pots were used to estimate the background evaporation rate. All the pots were place in the room randomly.

There were 2 treatments in this study (Fig 5.1.):

1) Fully-watered treatment (control): pre-germinated seeds were grown in pots maintained at 80% field capacity (FC) throughout the whole experiment to provide lentil genotypes with optimum water.

2) Drought treatment: pre-germinated seeds were sown in the pots with 80% FC in the beginning of the experiment to ensure establishment. However, FC rapidly reduced to 57% at 1st week after sowing (Fig. 5.1.), showing rapid evapotranspiration occurred in SSM4. Reduction in FC continued until reaching 40% (water deficit according to Khadraji *et al.*, 2016) at 3rd week after sowing. This FC (40%) was maintained until flowering of plants (6th week after sowing), which was the end of the experiment (Fig. 5.1.).

Eight extra unplanted pots (two per block) were used to estimate the background evaporation rate. Four of these pots had similar moisture content as the control maintained at 80% FC throughout the whole experiment. The other four unplanted pots were maintained with soil water content similar to the drought treatments, receiving water until they were the same weight as drought treatment pots with plants, which had been weighed previously.

To determine 100% field capacity (FC), 7 random pots (93 g) were filled with SSM4. Water was added until it flowed from the bottom of pots. Afterwards, the tops of each pot were covered with aluminum foil to prevent evaporation. Two days later, weight of water at 100% FC was calculated using the following equation.

$$\text{Weight of water at 100\% Field capacity in Sunshine Mix \# 4} = [(weight\ of\ \ pots\ \ at\ 48h - weight\ of\ empty\ pots)] - \text{weight of dry SSM4}$$

……………………………………………………………….. (5.1)

The weight of the dry soil was calculated by subtracting the amount of moisture from Sunshine Mix # 4 measured by using a Sartorius moisture analyzer MA 30 (Sartorius Corp. NY, USA).

$$Weight\ of\ dry\ Sunshine\ Mix\ \#\ 4 = weight\ of\ wet\ \text{SSM4}\ - amount\ of\ moisture$$

…………… (5.2)

Moisture content was measured by placing 3.18 g of SSM4 was in a small plastic tray in the moisture tester and after approximately1 min the reading was recorded.

Plant transpiration rate was estimated by regular measurement of water loss via evapotranspiration obtained by periodic weighing of the both pots with and without plants as shown in equation (1). Insect control methods were the same as Experiment 1. Harvesting of plants was conducted similarly to the first experiment and same measurements were recorded at 2, 16, 29 and 41 d after sowing.

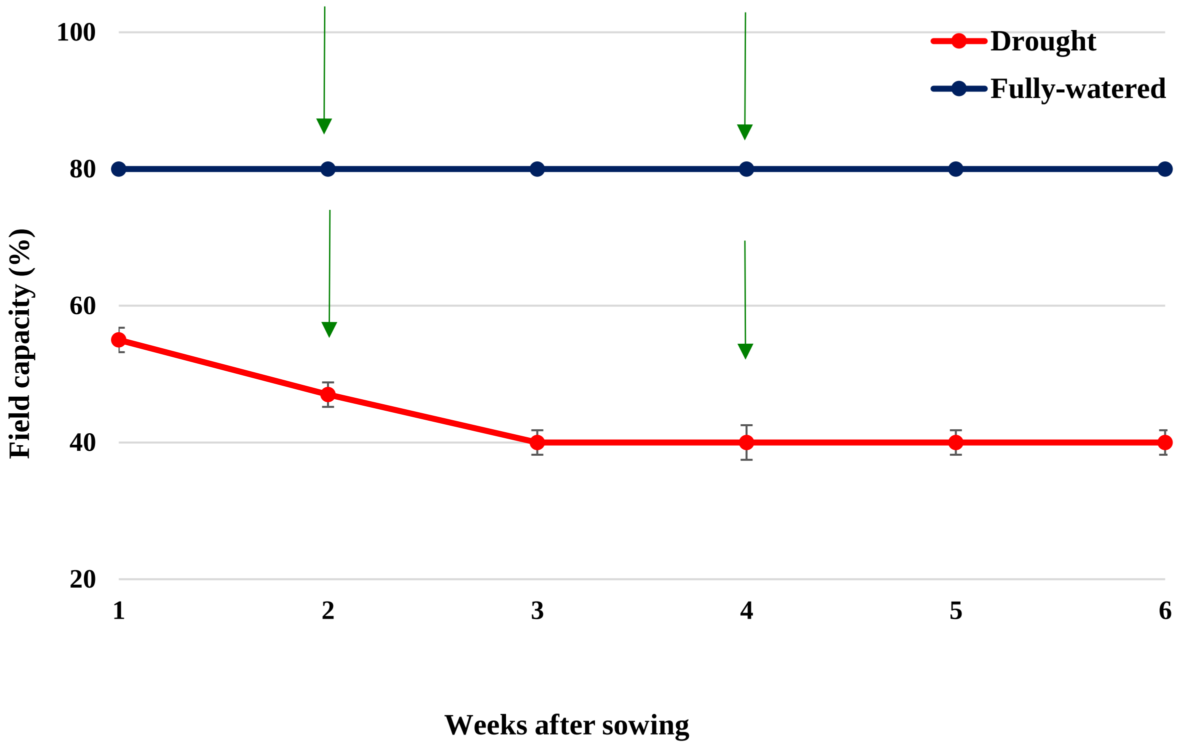


**Fig. 5.1.** Field capacity over 6 weeks for fully watered and drought treatments using Sunshine Mix # 4 growth medium. Bars represent standard error of mean FC and arrows indicate time points of fertilizer application.

### 5.3. Statistical analysis

Statistical analysis was performed using SAS 9.4 (SAS Institute Inc. 2013 Cary, NC). Analysis of variance (ANOVA) was performed on data obtained from both experiments using PROC GLM to test the effect of growth media, moisture levels, genotypes and the interactive effect of these factors on shoot and root traits [plant height, total leaflet number per plant, SPAD, shoot and root biomass, root/shoot ratio and number of nodules]. Significance was considered at alpha (α) = 0.05, α = 0.001 and α = 0.0001**.** Least significant differences between means of root traits are discussed in the heatmap section of the thesis and were calculated using the PROC MIXED procedure. PROC CORR was used to perform 4 sets of Pearson correlations for plant height, total number of leaves, SPAD value, shoot biomass, root biomass, total root length, root length density, total root surface area, root average diameter and number of nodules in SSM4 and GG under fully-watered and drought conditions. SigmaPlot 13 (Systat Software, Inc, San Jose, CA) was used to draw figures.

## 6.0 Results for Experiments 1 and 2

The results of both experiments will be presented in comparison to each other. The reason for this is: (1) Both experiments were carried out in growth chambers under similar temperature, humidity and lighting conditions. (2) At harvest, all plants had flowered except for *L. orientalis* IG 72611 and *L. tomentosus* IG 72805, implying that they were at same phenological stage. The different size of containers (tubes and pots) might have had effect on plant growth characteristics, specifically root traits. For example, higher height of the tubes (60 cm) compared to pots (17.5 cm) might have influenced root length of lentil genotypes grown in GG compared with SSM4.

However, it seemed that differences in root traits were mostly influenced by growth media rather than container size as explained in the discussion section.

## 6.1 The effect of media on plant height of the lentil genotypes grown under two moisture level

There was a significant interaction between genotypes and media for plant height (P< 0.0001) (Table 6.1). *Lens culinaris* Eston *L. orientalis* IG 72611, *L. tomentosus* IG 72805, *L. odemensis* IG 72623 and *L. ervoides* L01-827A showed significantly higher plant height when grown in SSM4 compared with GG, while there was no significant effect of both media on plant height of *L. culinaris* CDC Greenstar and *L. lamottei* IG 110813.

**Table 6.1.** ANOVA table for plant height of lentil genotypes grown in two growth media at two moisture levels

| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
|---|---|---|---|---|---|
| Geno | 6 | 1558.0 | 259.6 | 4.7 | 0.0004 |
| Media | 1 | 6275.4 | 6275.4 | 113.8 | <.0001 |
| Moist | 1 | 403.6 | 403.6 | 7.3 | 0.0083 |
| Geno*Media | 6 | 6102.3 | 1017.0 | 18.4 | <.0001 |
| Geno*Moist | 6 | 145.9 | 24.3 | 0.4 | 0.8491 |
| Media*Moist | 1 | 77.1 | 77.1 | 1.4 | 0.2403 |
| Geno*Media*Moist | 6 | 461.9 | 76.9 | 1.4 | 0.2262 |

[DF: degree of freedom; Geno: genotypes; Media: was either SSM4 or GG; Moist: fully-watered and drought conditions].

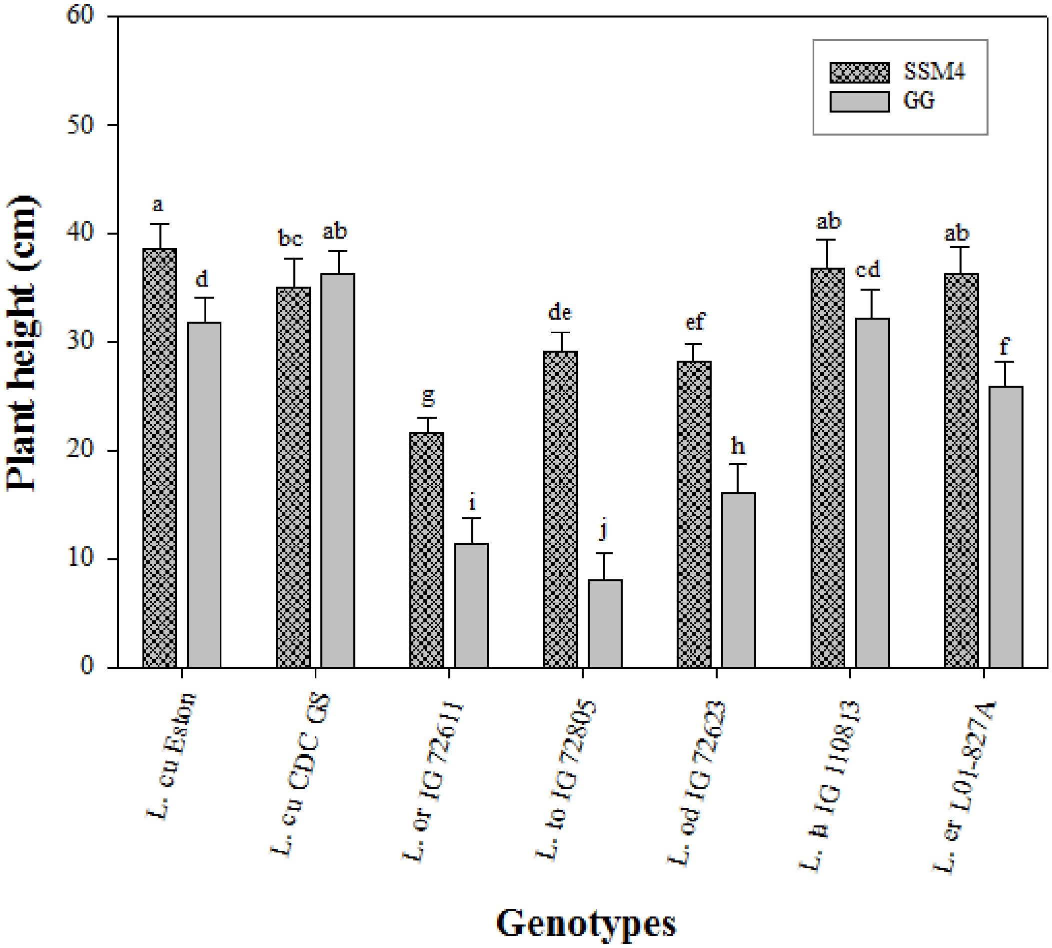


**Fig. 6.1.** Comparison of plant height among 7 lentil genotypes grown on two growth media.
[[Media: SSM4 (Sunshine Mix # 4) and GG (Greens Grade);
*L. cu*: *Lens culinaris*; *L. or*: *Lens orientalis*; *L. to*: *Lens tomentosus*; *L. od*: *Lens odemensis*; *L. la*: *Lens lamottei*; *L. er*: *Lens ervoides*].
Genotypes with same letters are not significantly different. Bars indicate standard error of means]. Subsequent figures have the same order of genotypes.

## 6.2 Effect of media and moisture level on node numbers of the lentil genotypes

There were significant interactions between genotypes, media and moisture levels for the number of nodes (P<0.0001) (Table 6.2). *Lens culinaris* [Eston and CDC Greenstar] and *L. lamottei* IG 110813 had significantly higher number of nodes in GG relative to SSM4 under both fully-watered and drought conditions. *Lens ervoides* L01-827A had significantly higher node number in GG relative to SSM4 under fully-watered conditions, but had no difference in node numbers between SSM4 and GG under drought conditions. *Lens odemensis* IG 72623 had similar number of nodes in both growth media under both fully-watered and drought conditions. *Lens tomentosus* IG 72805 had significantly higher number of nodes in SSM4 compared with GG under both fully-watered and drought conditions. *Lens orientalis* IG 72611 had significantly higher number of nodes when grown in SSM4 relative to GG under drought conditions. However, there was no significant difference for node numbers of this genotype between SSM4 and GG under fully-watered conditions (Fig. 6.2). Drought caused significant reduction in node number of all the genotypes in both media (Fig. 6.2).

**Table 6.2.** ANOVA table for nodes on the main stem of lentil genotypes grown in two growth media at two moisture levels

| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
|---|---|---|---|---|---|
| Geno | 6 | 2084.7 | 347.4 | 215.7 | <.0001 |
| Media | 1 | 98.4 | 98.4 | 61.1 | <.0001 |
| Moist | 1 | 332.5 | 332.5 | 206.5 | <.0001 |
| Geno*Media | 6 | 800.8 | 133.4 | 82.9 | <.0001 |
| Geno*Moisture | 6 | 36.7 | 6.1 | 3.8 | 0.0021 |
| Media*Moisture | 1 | 9.7 | 9.7 | 6.0 | 0.0161 |
| Geno*Media*Moisture | 6 | 38.0 | 6.3 | 3.9 | 0.0016 |

[DF: degree of freedom; Geno: genotypes; Media: was either SSM4 or GG; Moist: fully-watered and drought conditions].

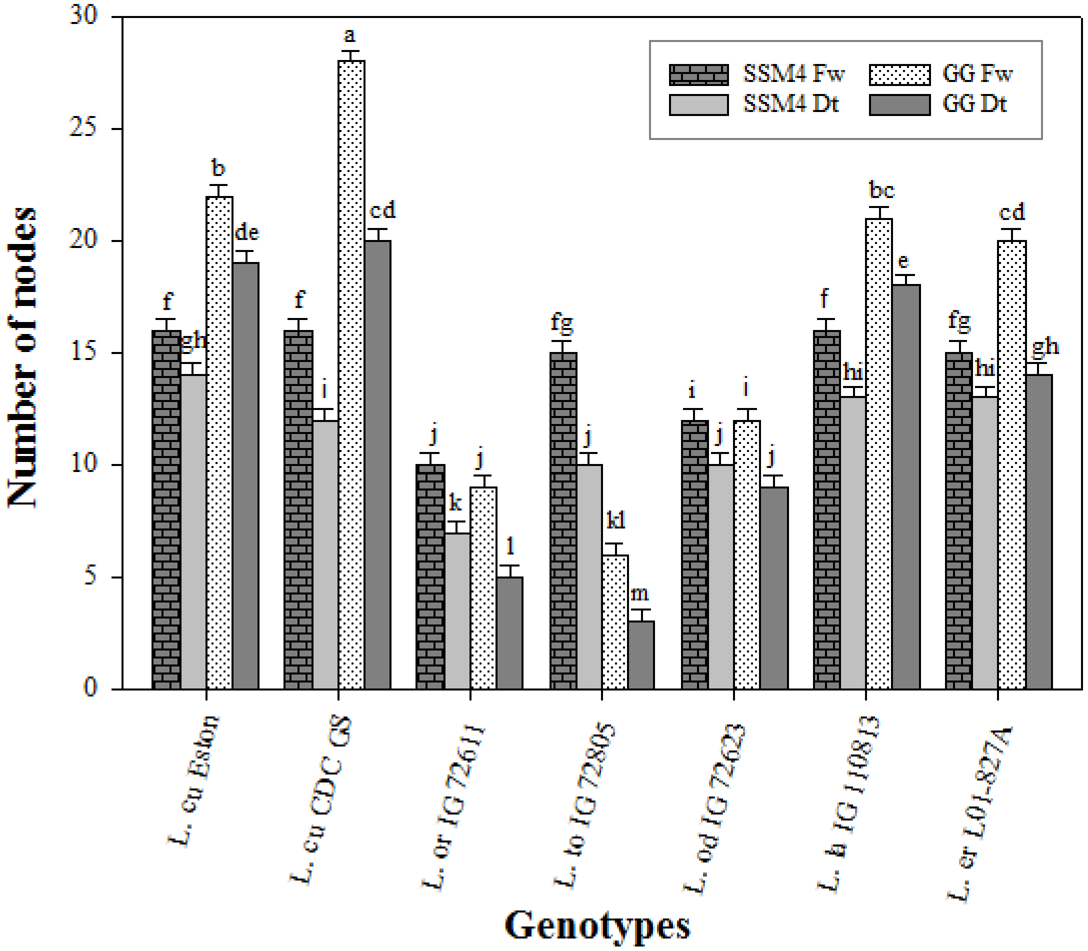


**Fig. 6.2.** Interactions between media and moisture levels for number of nodes of 7 lentil genotypes.
[[Media: SSM4 (Sunshine Mix # 4) and GG (Greens Grade); Moisture levels: Fw (Fully-watered) and Dt (drought)]. Media with same letters are not significantly different. Bars indicate standard.

### 6.3 Effect of media and moisture level on total leaflet numbers of the lentil genotypes

There was a significant interaction between media and moisture for total number of leaflets in all lentil genotypes (P<0.01) (Table 6.3). In both media, total leaflet numbers were significantly higher under fully-watered compared with drought conditions, as expected. Lentil genotypes produced significantly higher (α = 5%) leaflet numbers in GG relative to

SSM4 under fully-watered conditions. However, no significant difference was observed in total leaflet numbers of genotypes between SSM4 and GG under drought conditions (Fig. 6.3).

**Table 6.3.** ANOVA table for total number of leaflets of lentil genotypes grown in two growth media at two moisture levels

| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
|---|---|---|---|---|---|
| Geno | 6 | 304377.2 | 50729.5 | 23.6 | <.0001 |
| Media | 1 | 51679.6 | 51679.6 | 24.1 | <.0001 |
| Moist | 1 | 289821.0 | 289821.0 | 135.1 | <.0001 |
| Geno*Media | 6 | 121772.8 | 20295.4 | 9.4 | <.0001 |
| Geno*Moisture | 6 | 49527.3 | 8254.5 | 3.8 | 0.0023 |
| Media*Moisture | 1 | 14763.0 | 14763.0 | 6.8 | 0.0107 |
| Geno*Media*Moisture | 6 | 8077.6 | 1346.2 | 0.6 | 0.7073 |

[DF: degree of freedom; Geno: genotypes; Media: was either SSM4 or GG; Moist: fully-watered and drought conditions].

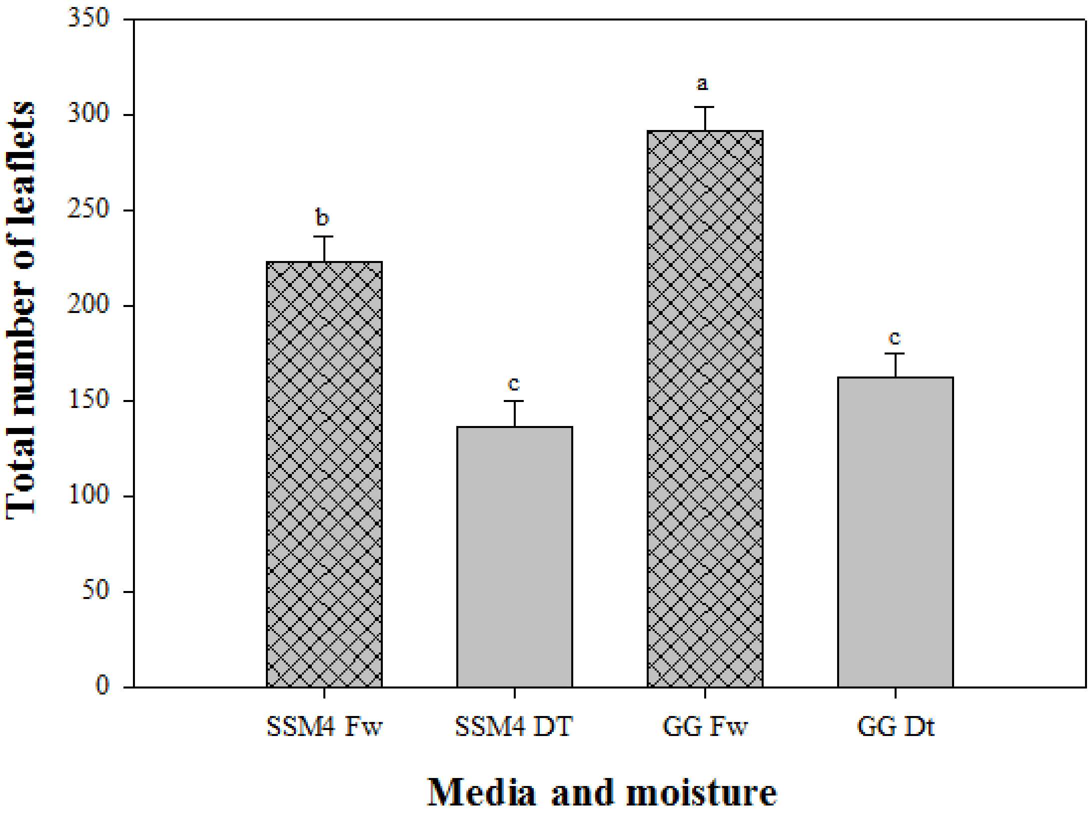


**Fig. 6.3.** Interactions between media and moisture levels for total number of leaflets of 7 lentil genotypes.
[[Media: SSM4 (Sunshine Mix # 4) and GG (Greens Grade); Moisture levels: Fw (Fully-watered) and Dt (drought)].
Media with same letters are not significantly different. Bars indicate standard error of means].

## 6.4 The effect of media on SPAD values of the lentil genotypes grown under two moisture levels

There were significant differences (P< 0.0001) in SPAD value measurements among lentil genotypes grown in SSM4 and GG (Table 6.4). Wild lentil genotypes had significantly higher SPAD values when grown in SSM4 relative to GG. *Lens culinaris* (Eston and CDC Greenstar) genotypes did not differ in SPAD value when grown in either media (Fig. 6.4).

**Table 6.4.** ANOVA table for SPAD values of lentil genotypes grown in two growth media at two moisture levels

| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
|---|---|---|---|---|---|
| Geno | 6 | 3485.6 | 580.9 | 8.82 | <.0001 |
| Media | 1 | 13003.2 | 13003.2 | 197.4 | <.0001 |
| Moist | 1 | 6.0 | 6.0 | 0.09 | 0.7620 |
| Geno*Media | 6 | 6207.7 | 1034.6 | 15.7 | <.0001 |
| Geno*Moisture | 6 | 216.9 | 36.1 | 0.55 | 0.7688 |
| Media*Moisture | 1 | 1.7 | 1.7 | 0.03 | 0.8704 |
| Geno*Media*Moisture | 6 | 520.8 | 86.8 | 1.3 | 0.2 |

[DF: degree of freedom; Geno: genotypes; Media: was either SSM4 or GG; Moist: fully-watered and drought conditions].

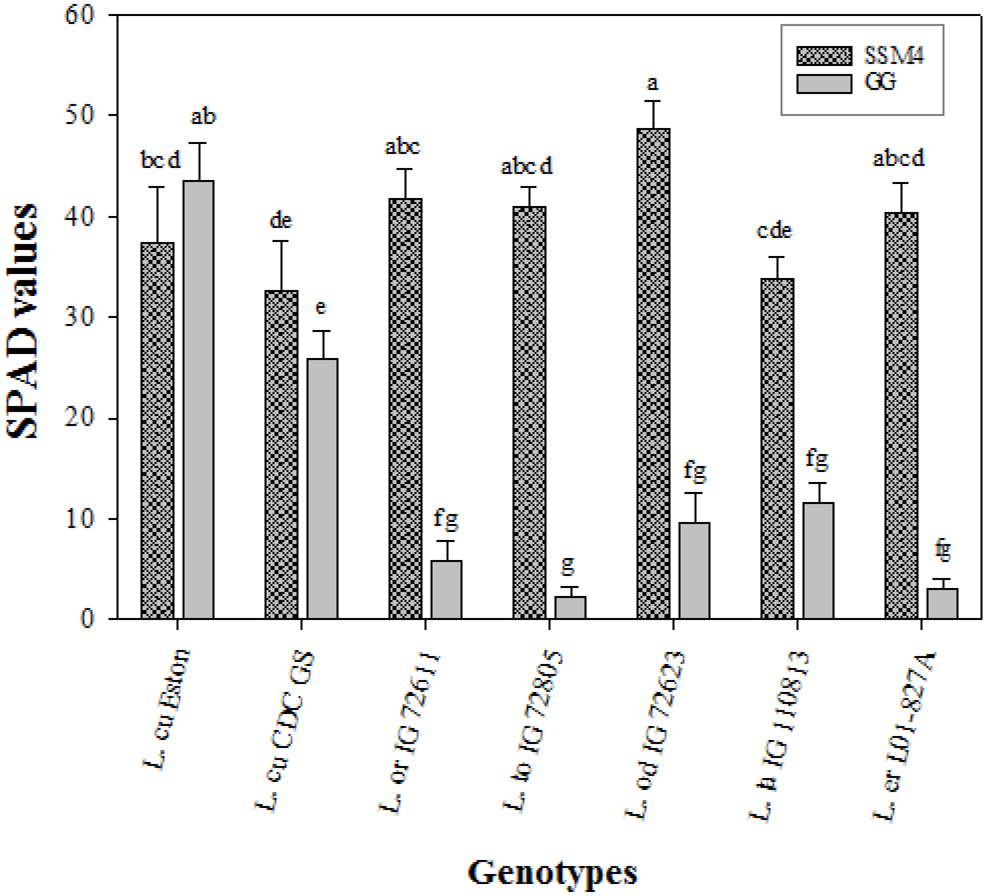


**Fig. 6.4.** Comparison of SPAD values among 7 lentil genotypes grown on two growth media.
[[Media: SSM4 (Sunshine Mix # 4) and GG (Greens Grade)].
Genotypes with same letters are not significantly different. Bars indicate standard error of means].

### 6.5 Effect of media and moisture level on shoot biomass of the lentil genotypes

There were significant interactions among genotypes, media and moisture levels for shoot biomass ($P<0.001$) (Table 6.5). Most genotypes, including *L. culinaris* Eston, *L. orientalis* IG 72611, *L. tomentosus* IG 72805, *L. odemensis* IG 72623 and *L. ervoides* L01-827A had significantly higher shoot biomass in SSM4 compared with GG under both fully-watered and drought conditions. *Lens culinaris* CDC Greenstar had no significant difference for shoot biomass between SSM4 and GG under fully-watered conditions. However, it had significantly higher shoot biomass in SSM4 relative to GG under drought conditions. *Lens lamottei* IG 110813 produced similar shoot biomass under fully-watered and drought conditions in SSM4 and in GG media. (Fig. 6.5).

**Table 6.5.** ANOVA table for shoot biomass of lentil genotypes grown in two growth media at two moisture levels

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Geno | 6 | 85.5 | 14.2 | 61.8 | <.0001 |
| Media | 1 | 72.1 | 72.1 | 312.8 | <.0001 |
| Moist | 1 | 36.3 | 36.3 | 157.5 | <.0001 |
| Geno*Media | 6 | 23.7 | 3.9 | 17.1 | <.0001 |
| Geno*Moisture | 6 | 4.9 | 0.8 | 3.5 | 0.0041 |
| Media*Moisture | 1 | 0.7 | 0.7 | 3.0 | 0.0857 |
| Geno*Media*Moisture | 6 | 6.2 | 1.0 | 4.5 | 0.0006 |

[DF: degree of freedom; Geno: genotypes; Media: was either SSM4 or GG; Moist: fully-watered and drought conditions].

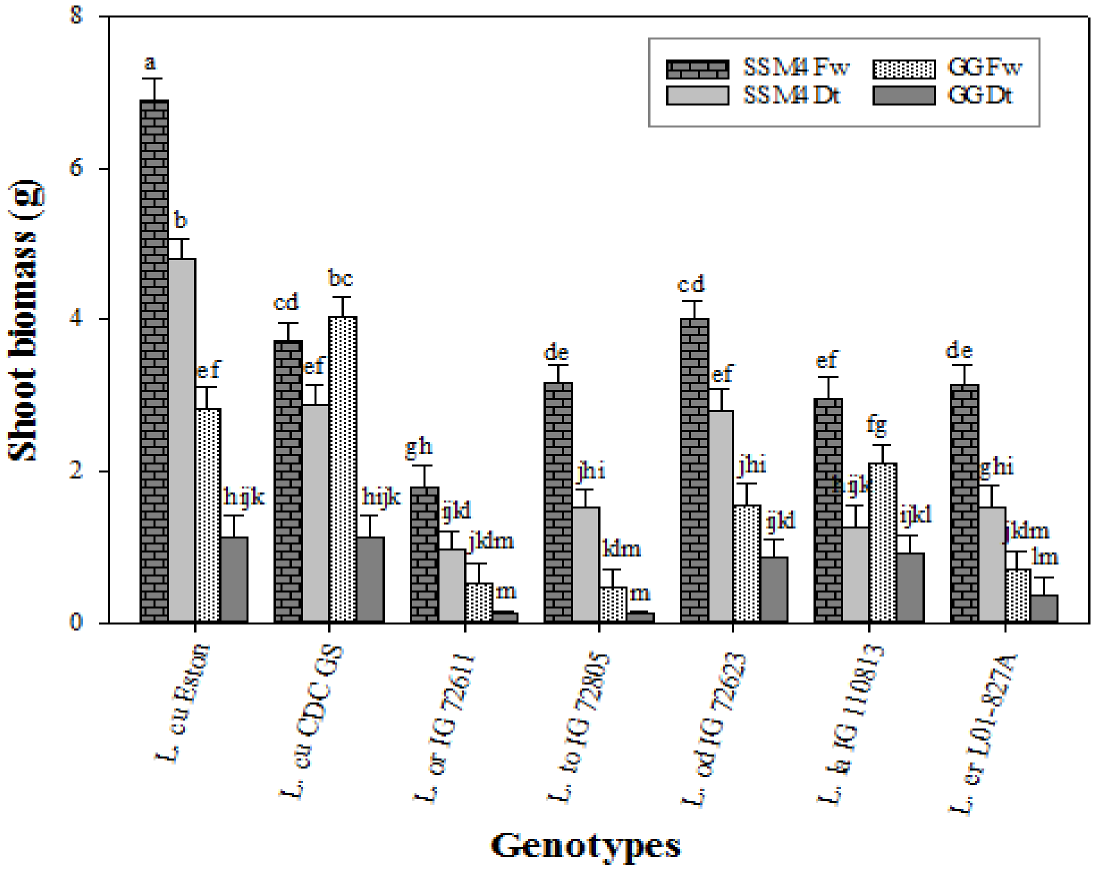


**Fig. 6.5.** Interactions between media and moisture levels for shoot biomass of 7 lentil Genotypes.
[[Media: SSM4 (Sunshine Mix # 4) and GG (Greens Grade); Moisture levels: Fw (Fully-watered) and Dt (Drought)].
Genotypes with same letters are not significantly different. Bars indicate standard error of means].

## 6.6 Effect of media and moisture level on root biomass of the lentil genotypes

Significant interactions were also observed among genotypes, media and moisture levels for root biomass (P<0.0001) (Table 6.6). *Lens lamottei* IG 110813 had significantly higher root biomass in GG compared with SSM4 under both fully-watered and drought conditions. *Lens culinaris* [Eston and CDC Greenstar] along with *L. odemensis* IG 72623 had significantly higher root biomass in GG relative to SSM4 under fully-watered conditions. However, there was no significant difference in root biomass of these genotypes between SSM4 and GG under drought conditions. *Lens orientalis* IG 72611, *L. tomentosus* IG 72805 and *L. ervoides* L01-827A produced statistically similar root biomass in SSM4 and GG under both fully-watered and drought treatments (Fig. 6.6). Drought had no statistically significant effect on root biomass for most genotypes grown in SSM4 except for *L. culinaris* Eston. However, most genotypes grown in GG had significantly reduced root biomass under drought conditions including *L. culinaris* [Eston and CDC Greenstar], *L. odemensis* IG 72623 and *L. lamottei* IG 110813 (Fig 6.6).

**Table 6.6.** ANOVA table for root biomass of lentil genotypes grown in two growth media at two moisture levels

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Geno | 6 | 39.5 | 6.5 | 50.8 | <.0001 |
| Media | 1 | 8.8 | 8.8 | 68.2 | <.0001 |
| Moist | 1 | 11.6 | 11.6 | 89.9 | <.0001 |
| Geno*Media | 6 | 11.3 | 1.8 | 14.6 | <.0001 |
| Geno*Moisture | 6 | 7.6 | 1.2 | 9.7 | <.0001 |
| Media*Moisture | 1 | 6.4 | 6.4 | 50.1 | <.0001 |
| Geno*Media*Moisture | 6 | 7.9 | 1.3 | 10.1 | <.0001 |

[DF: degree of freedom; Geno: genotypes; Media: was either SSM4 or GG; Moist: fully-watered and drought conditions].

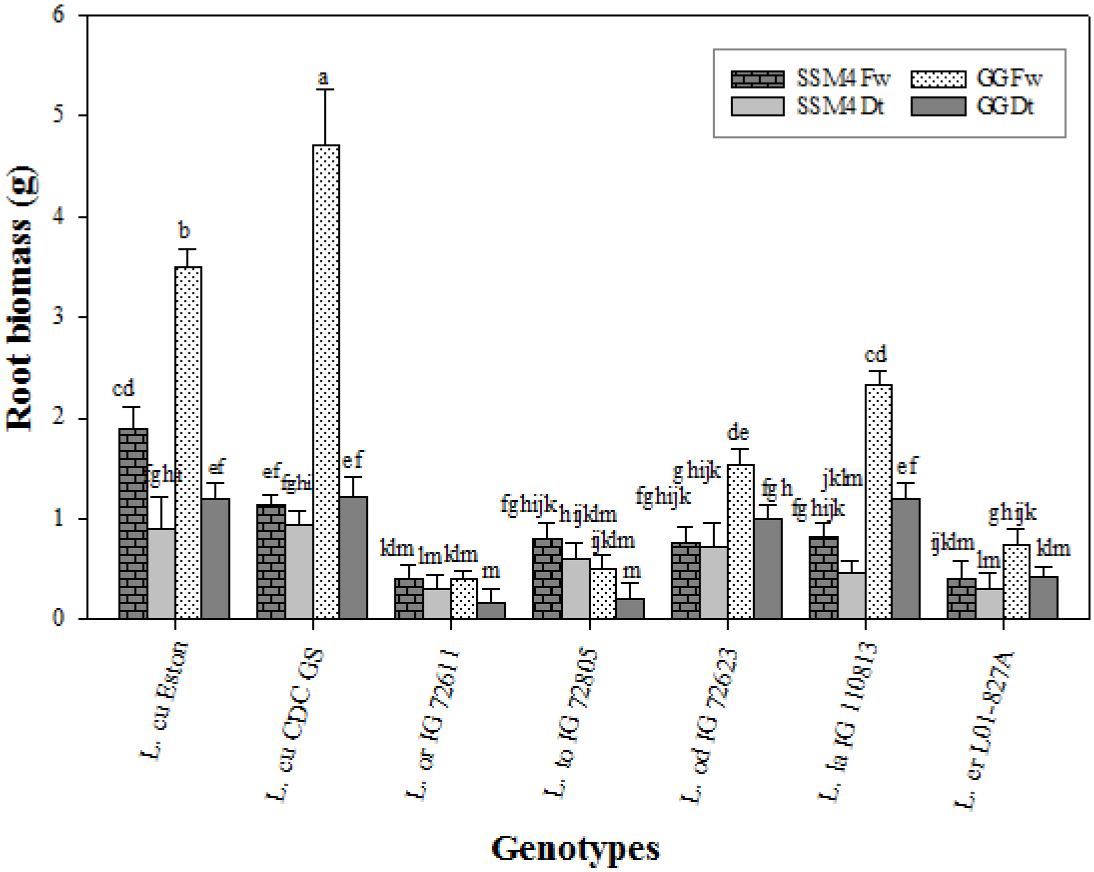


**Fig. 6.6.** Interactions between media and moisture levels for root biomass of 7 lentil Genotypes.
[[Media: SSM4 (Sunshine Mix # 4) and GG (Greens Grade); Moisture levels: Fw (Fully-watered) and Dt (Drought)].
Genotypes with same letters are not significantly different. Bars indicate standard error of means].

## 6.7 Effect of moisture level on root/shoot ratio of lentil genotypes grown in two growth media

*Lens culinaris* [Eston and CDC Greenstar] had significantly higher root/shoot ratio (RSR) under fully-watered compared with drought treatment, while *L. tomentosus* IG 72805 and *L. lamottei* IG 110813 produced significantly higher RSR under drought relative to fully-watered conditions. The other wild lentil genotypes like *L. orientalis* IG 72611, *L. odemensis* IG 72623 and *L. ervoides* L01-827A produced statistically similar RSR under both fully-watered and drought conditions (Fig. 6.7).

**Table 6.7.** ANOVA table for root/ shoot ratio of lentil genotypes grown in two growth media at two moisture levels

| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
|---|---|---|---|---|---|
| Geno | 6 | 0.3 | 0.06 | 5.29 | 0.0001 |
| Media | 1 | 18.9 | 18.9 | 1630.03 | <.0001 |
| Moist | 1 | 0.0 | 0.0 | 3.64 | 0.0602 |
| Geno*Media | 6 | 0.1 | 0.0 | 2.63 | 0.0226 |
| Geno*Moisture | 6 | 0.5 | 0.0 | 7.91 | <.0001 |
| Media*Moisture | 1 | 0.0 | 0.0 | 0.24 | 0.6229 |
| Geno*Media*Moisture | 6 | 0.1 | 0.0 | 2.17 | 0.0547 |

[DF: degree of freedom; Geno: genotypes; Media: was either SSM4 or GG; Moist: fully-watered and drought conditions]

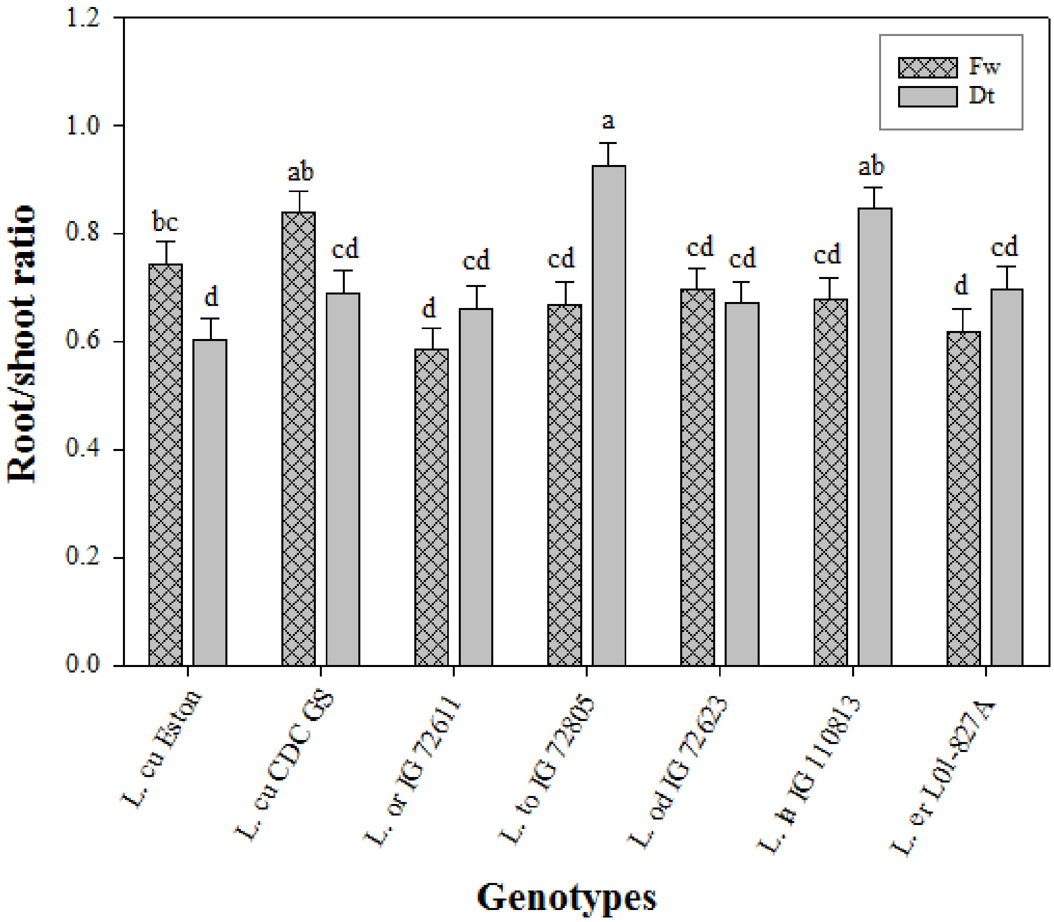


**Fig. 6.7.** Comparisons of root/shoot ratio among 7 lentil genotypes grown on two growth media.
[[Moisture levels: Fw (Fully-watered) and Dt (Drought)].
Genotypes with same letters are not significantly different. Bars indicate standard error of means].

## 6.8 Effect of growth media on total root length (TRL), total root surface area (TRSA) and root length density (RLD) of the lentil genotypes grown under fully-watered and drought conditions

Growth media did not have a significant effect on total root length (TRL) of lentil genotypes, while significant effect of media on total root surface area (TRSA) and root length density (RLD) was observed under both fully-watered and drought conditions ($P \leq 0.05$) (Figs. 6.8a, 6.8b). Lentil genotypes had significantly greaer TRSA in GG compared to SSM4 under both fully-watered and drought conditions ($P \leq 0.05$). Lentil genotypes had significantly greater RLD in SSM4 under both fully-watered and drought conditions ($P \leq 0.05$) (Figs. 6.8a, 6.8b) .

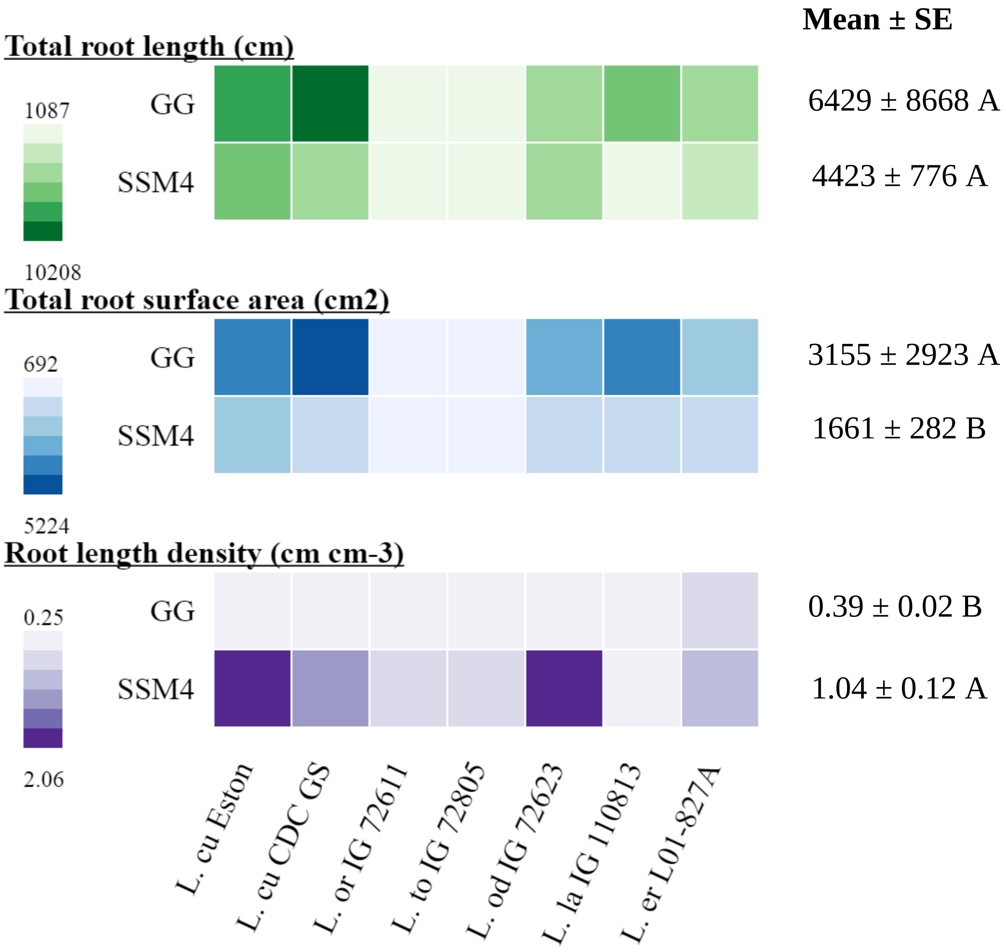


**Fig. 6.8a.** Heatmap showing the effects of two growth media on total root length, total root surface area and root length density of 7 lentil genotypes grown under fully-watered conditions
[[GG: Greens Grade; SSM4: Sunshine Mix # 4].
*L. cu*: *Lens culinaris*; *L. or*: *Lens orientalis*; *L. to*: *Lens tomentosus*; *L. od*: *Lens odemensis*; *L. la*: *Lens lamottei*; *L. er: Lens ervoides*]. Subsequent figures have the same order of genotypes

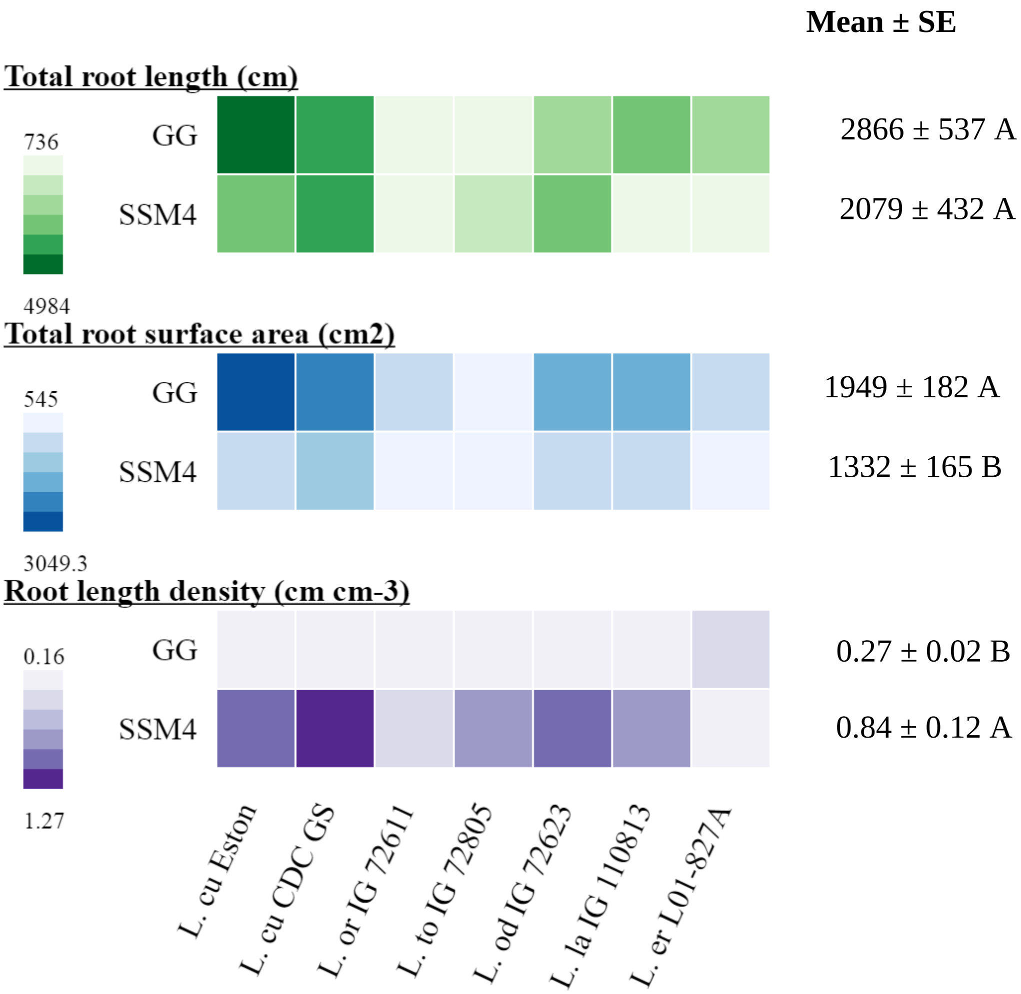


**Fig. 6.8b.** Heatmap showing the effects of two growth media on total root Length, total root surface area and root length density of 7 lentil genotypes grown under drought conditions [GG: Greens Grade; SSM4: Sunshine Mix # 4].

## 6.9 Effect of moisture level on total root length (TRL), total root surface area (TRSA) and root length density (RLD) of the lentil genotypes grown in either SSM4 or GG

Moisture level (Fully-watered and drought) had significant effect on TRL of lentil genotypes grown in both GG and SSM4 ($P \leq 0.05$). Moisture level had no significant effect on TRSA of lentil genotypes grown in SSM4. However, significant effect of moisture level was observed on TRSA of genotypes grown in GG (P ≤ 0.05). Similar to TRSA, moisture level had no significant effect on RLD of genotypes grown in SSM4, while significant effect of moisture level was observed on RLD of genotypes grown in GG (P ≤ 0.05) (Figs. 6.9a, 6.9b). Lentil genotypes had significantly reduced RLD under drought relative to fully-watered conditions in GG (Fig. 6.9b).

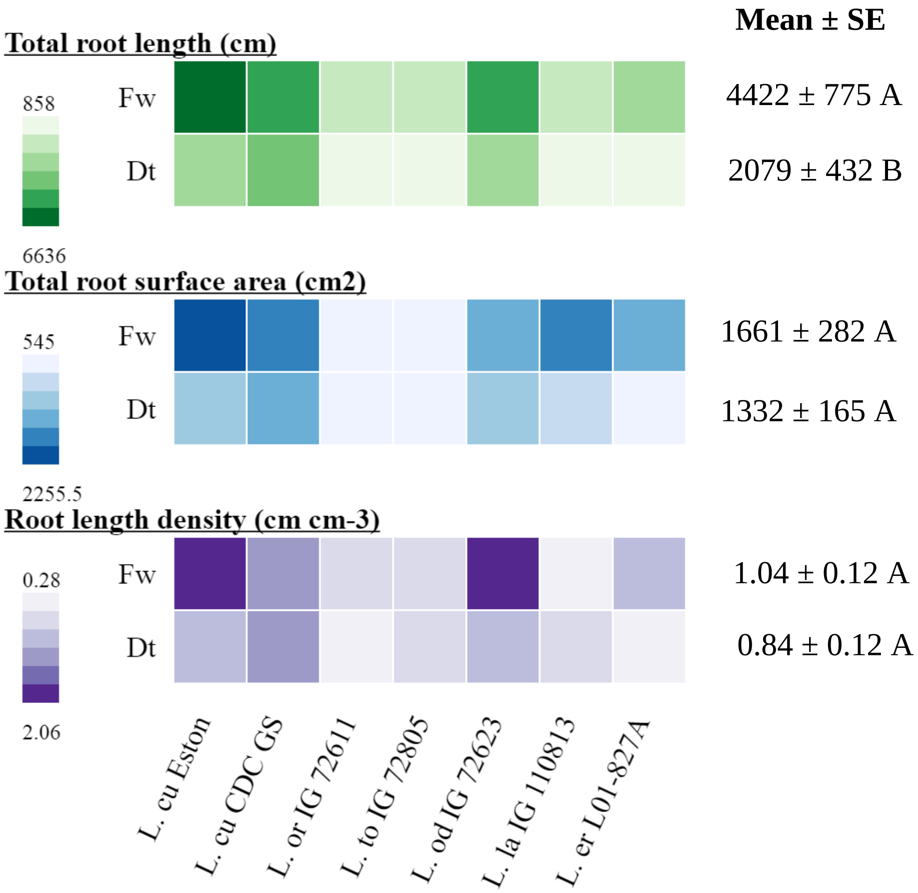


**Fig. 6.9a.** Heatmap showing the effects of two moisture levels on total root length, total root surface area and root length density of 7 lentil genotypes grown in Sunshine Mix # 4 [Fw and Dt represent fully-watered and drought conditions, respectively].

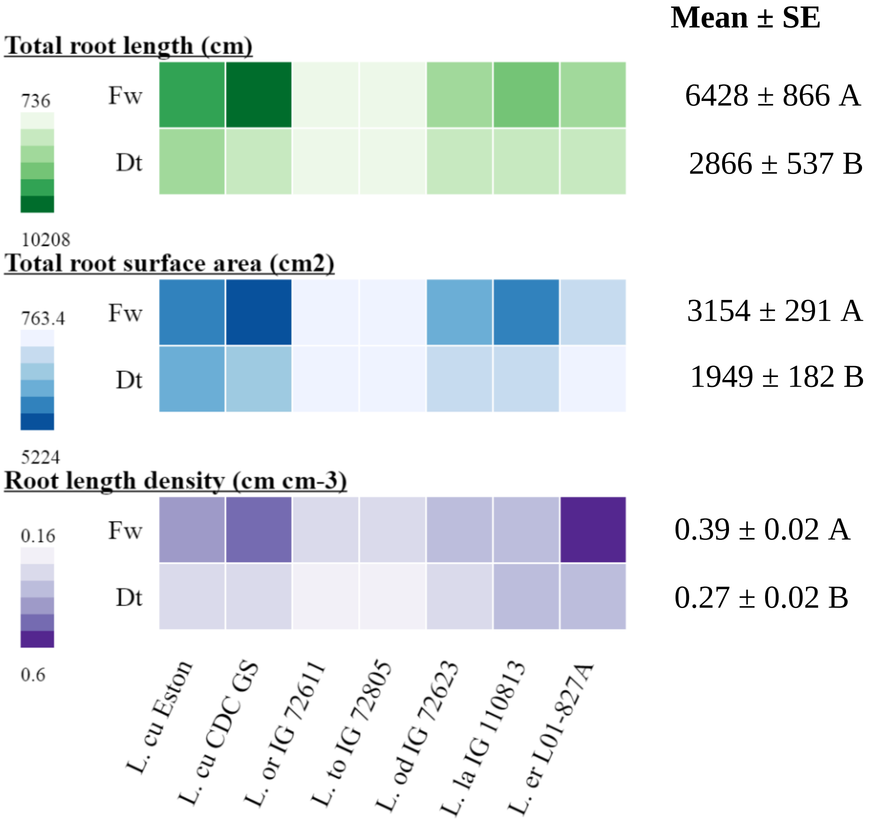


**Fig. 6.9b.** Heatmap showing the effects of two moisture levels on total root length, total root surface area and root length density of 7 lentil genotypes grown in Greens Grade [Fw and Dt represent fully-watered and drought conditions, respectively].

## 6.10 Effect of media on root average diameter (RAD) and root volume (RV) of lentil genotypes grown under fully-watered and drought conditions

Growth media had no significant effect on RAD of lentil genotypes grown under fully-watered conditions. However, significant effect of growth media on RAD of genotypes grown under drought condition was observed ($P \leq 0.05$). Significantly greater RAD of lentil genotypes was observed in GG compared to SSM4 under drought conditions (Figs. 6.10a, 6.10b). Growth media also had significant effect ($P \leq 0.05$) on RV of lentil genotypes under both fully-watered and drought conditions and plants with significantly greater RV were observed in GG (Figs. 6.10a, 6.10b).

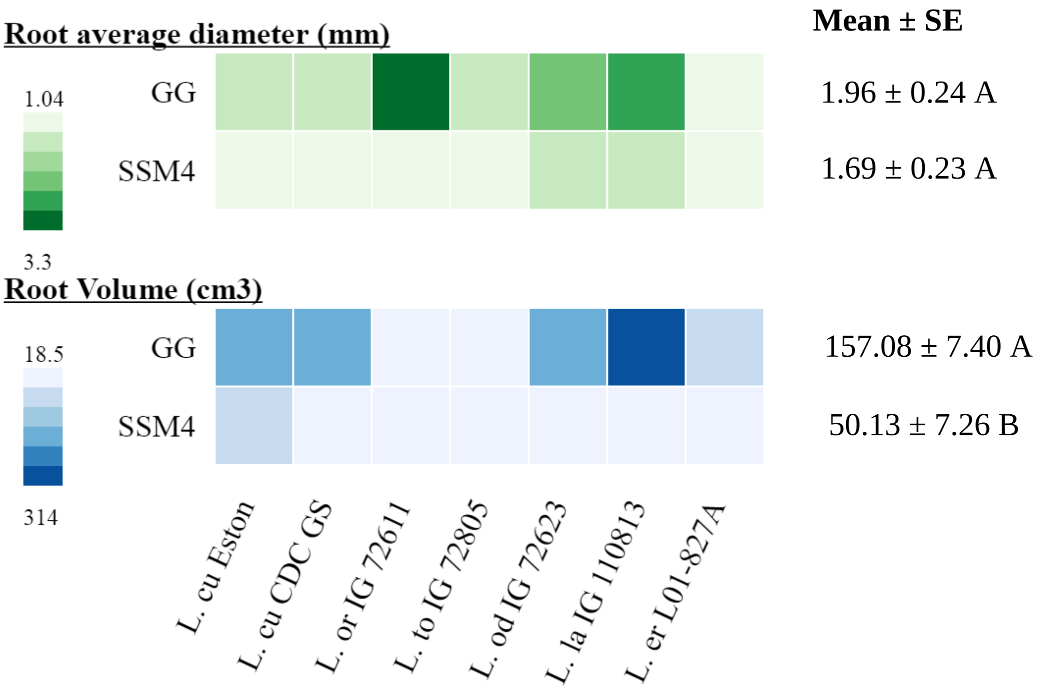


**Fig. 6.10a**. Heatmap showing the effects of two growth media on root average diameter and root volume of 7 lentil genotypes grown under fully-watered conditions [GG: Greens Grade; SSM4: Sunshine Mix # 4].

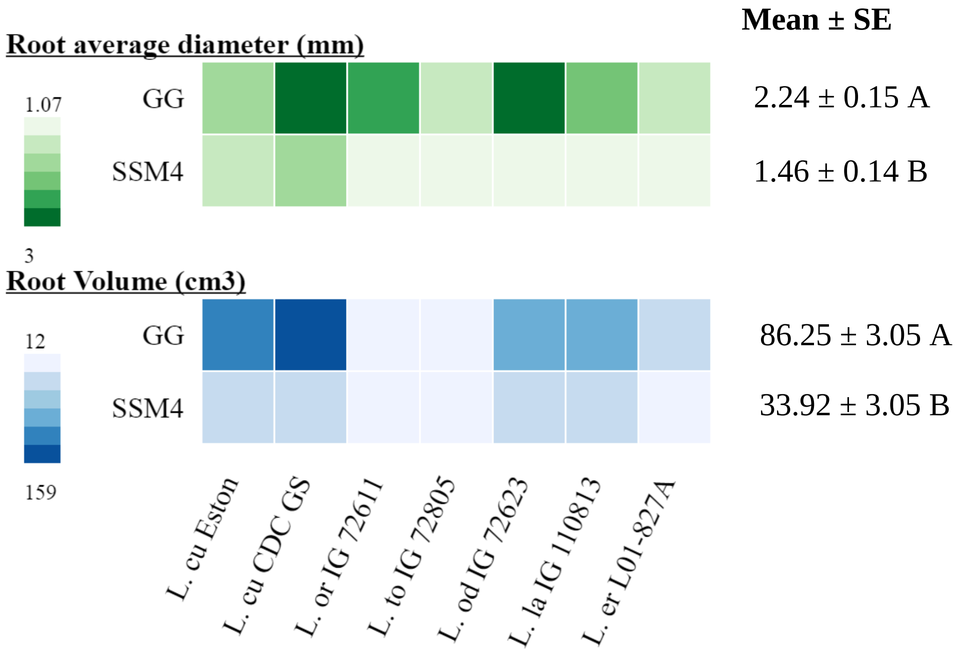


**Fig. 6.10b.** Heatmap showing the effects of two growth media on root average diameter and root volume of 7 lentil genotypes grown under drought conditions [GG: Greens Grade; SSM4: Sunshine Mix # 4].

### 6.11 Effect of moisture level on root average diameter (RAD) and root volume (RV) of the lentil genotypes grown in either SSM4 or GG

Growth media had no significant effect on RAD of lentil genotypes grown under fully-watered and drought conditions. However, growth media had significant effect ($P \leq 0.05$) on RV of lentil genotypes grown in GG (Figs. 6.11a, 6.11b).

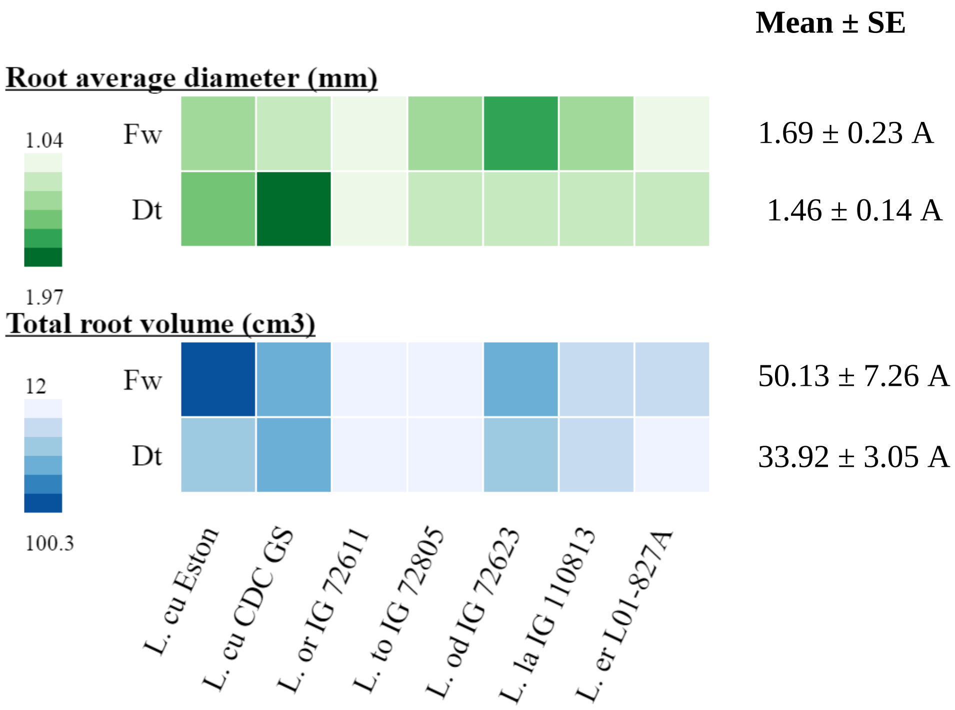


**Fig. 6.11a** Heatmap showing the effects of two moisture levels on root average diameter and root volume of 7 lentil genotypes grown in Sunshine Mix # 4 [Fw and Dt represent fully-watered and drought conditions, respectively].

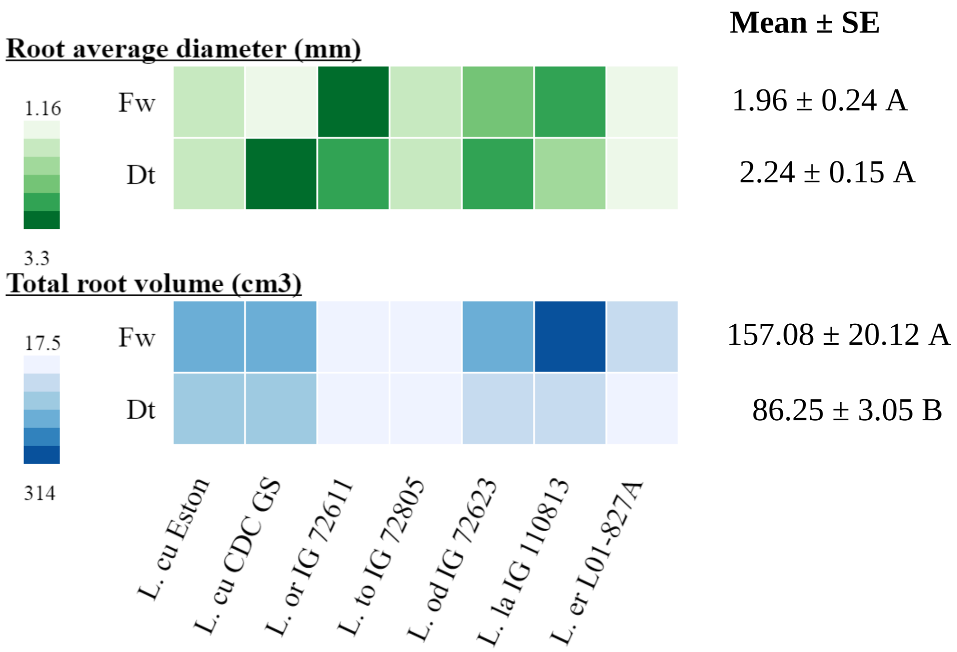


**Fig. 6.11b** Heatmap showing the effects of two moisture levels on root average diameter and root volume of 7 lentil genotypes grown in Greens Grade [Fw and Dt represent fully-watered and drought conditions, respectively].

## 6.12 Effect of media on total number of root tips (TNORT) and root forks (TNORF) of lentil genotypes grown under fully-watered and drought conditions

Growth media had a significant effect on total number of root tips of lentil genotypes grown under both fully-watered and drought conditions ($P \leq 0.05$) (Figs. 6.12a, 6.12b). Significant effect of growth media on total number of root forks of lentil genotypes was also observed under both fully-watered and drought conditions ($P \leq 0.05$) (Figs. 6.12a, 6.12b).

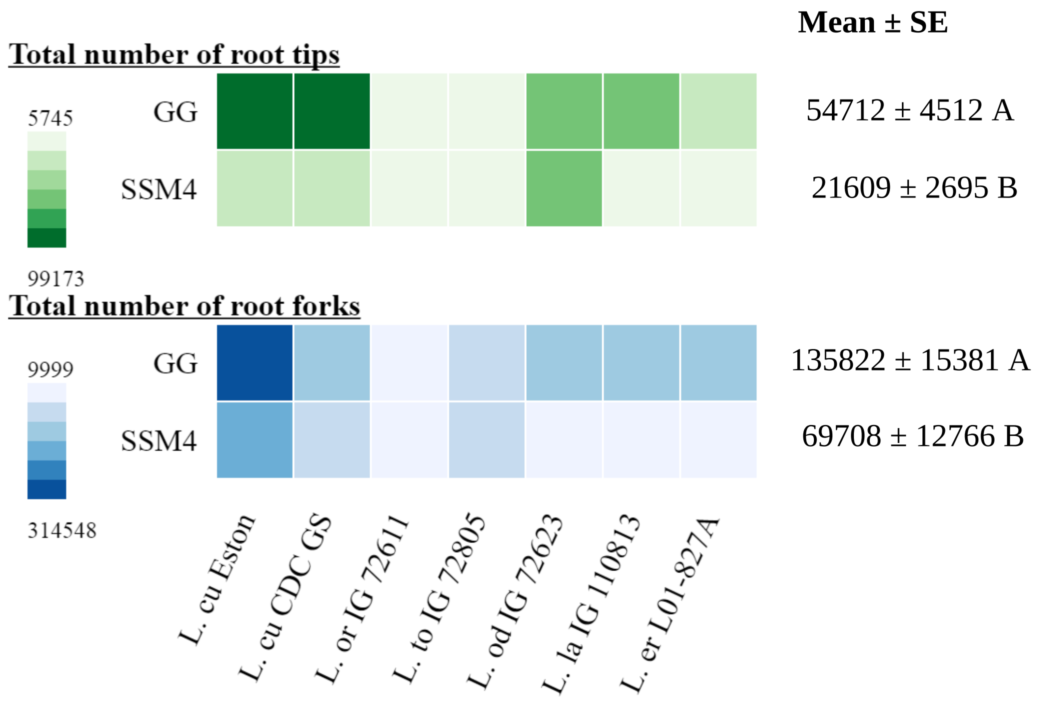


**Fig. 6.12a.** Heatmap showing the effects of two growth media on total number of root tips and forks of 7 lentil genotypes grown under fully-watered conditions [GG: Greens Grade; SSM4: Sunshine Mix # 4].

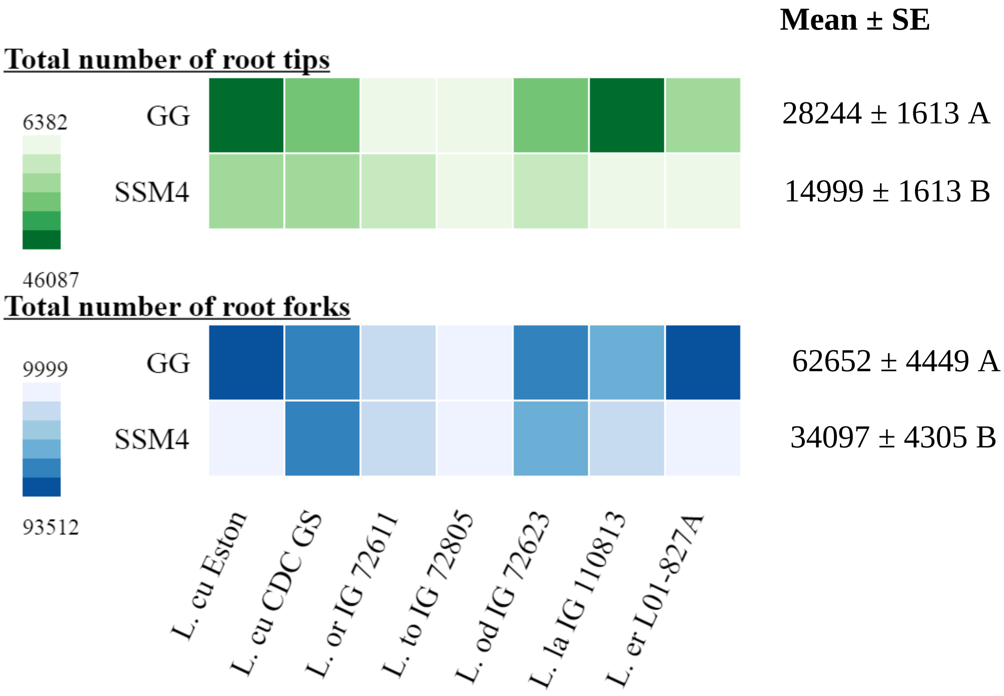


**Fig. 6. 12b** Heatmap showing the effects of two growth media on total number of root tips and forks of 7 lentil genotypes grown under drought conditions [GG: Greens Grade; SSM4: Sunshine Mix # 4].

## 6.13 Effect of moisture level on total number of root tips (TNORT) and root forks (TNORF) of lentil genotypes grown in either SSM4 or GG

Moisture level had significant effect on TNORF of lentil genotypes grown in SSM4 ($P \leq 0.05$) (Figs. 6.13a, 6.13b). Moisture level also had significant effect on TNORT and TNORF of lentil genotypes grown in GG ($P \leq 0.05$). In both growth media (SSM4 and GG), drought significantly reduced TNORF of genotypes (Figs. 6.13a, 6.13b). However, Drought caused significant reduction only in TNORT of lentil genotypes grown in GG.

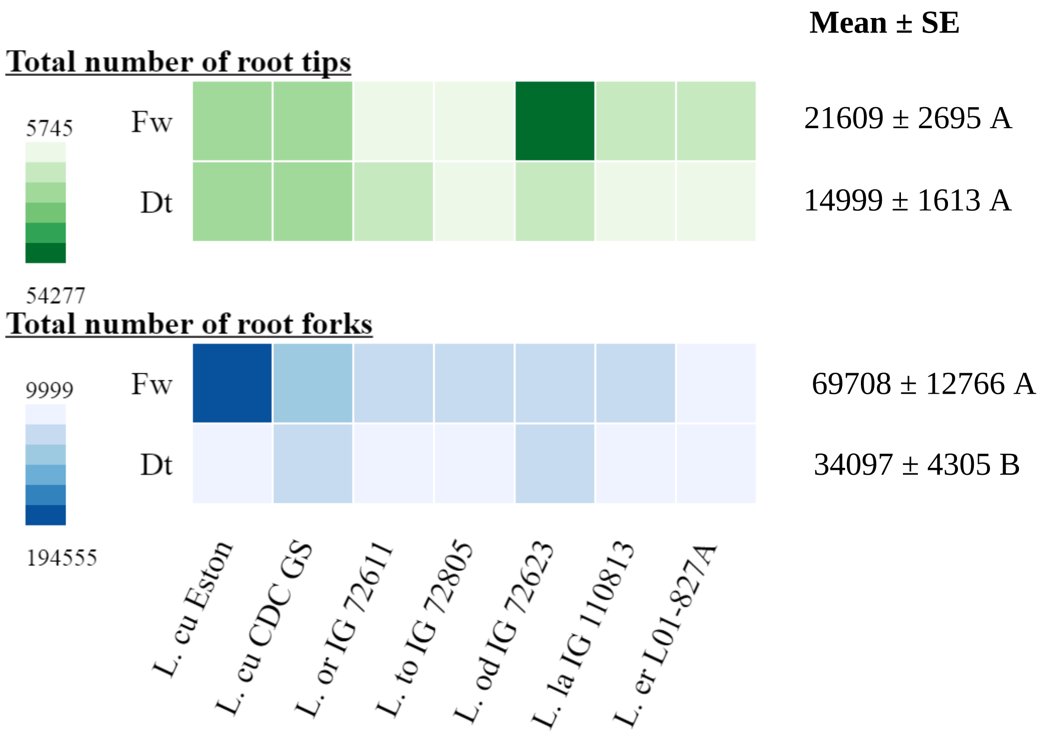


**Fig. 6.13a.** Heatmap showing the effects of two moisture levels on total number of root tips and forks of 7 lentil genotypes grown in Sunshine Mix # 4 [Fw and Dt represent fully-watered and drought conditions, respectively].

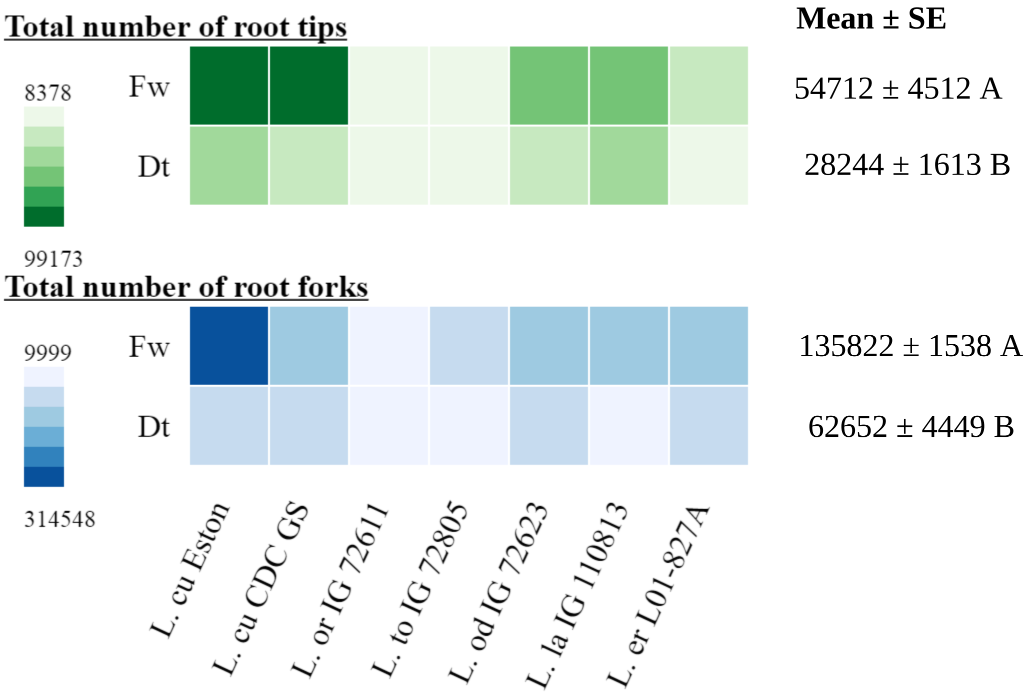


**Fig. 6.13b** Heatmap showing the effects of two moisture levels on total number of root tips and forks of 7 lentil genotypes grown in Greens Grade [Fw and Dt represent fully-watered and drought conditions, respectively].

### 6.14 The effect of media on nodule count of lentil genotypes grown under two moisture levels

Significant effects of media were observed on number of nodules in lentil genotypes (P<0.0001) (Table 6.14). *Lens culinaris* [Eston and CDC Greenstar], *L. orientalis* IG 72611 and *L. odemensis* IG 72623 had significantly higher number of nodules in SSM4 compared with GG. However, *L. tomentosus* IG 72805, *L. lamottei* IG 110813 and *L. ervoides* L01-827A showed statistically similar number of nodules in SSM4 and GG (Fig. 6.14).

**Table 6.14.** ANOVA table for nodule counts of lentil genotypes grown in two growth media at two moisture levels

| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
|---|---|---|---|---|---|
| Geno | 6 | 2582028.7 | 430338.1 | 65.4 | <.0001 |
| Media | 1 | 759978.6 | 759978.6 | 115.5 | <.0001 |
| Moist | 1 | 46296.0 | 46296.0 | 7.0 | 0.0102 |
| Geno*Media | 6 | 719419.4 | 119903.2 | 18.2 | <.0001 |
| Geno*Moisture | 6 | 28019.4 | 4669.9 | 0.7 | 0.6430 |
| Media*Moisture | 1 | 747.9 | 747.9 | 0.1 | 0.7371 |
| Geno*Media*Moisture | 6 | 46173.1 | 7695.5 | 1.1 | 0.3344 |

[DF: degree of freedom; Geno: genotypes; Media: was either SSM4 or GG; Moist: fully-watered and drought conditions]

*Lens culinaris* Eston grown in SSM4 had the highest number of nodules among all other genotypes, followed by *L. culinaris* CDC Greenstar grown in the same growth medium (SSM4). *Lens odemensis* IG 72623 grown in SSM4 was the next genotype with the highest number of nodules, followed by *L. culinaris* [Eston and CDC Greenstar] grown in GG as well as *L. orientalis* IG 72611 grown in SSM4. The other genotypes such as *L. tomentosus* IG 72805, *L. lamottei* IG 110813 and *L. ervoides* L01-827A grown in both media together with *L. orientalis* IG 72611 grown in GG were genotypes with the lowest number of nodules (Fig. 6.14).

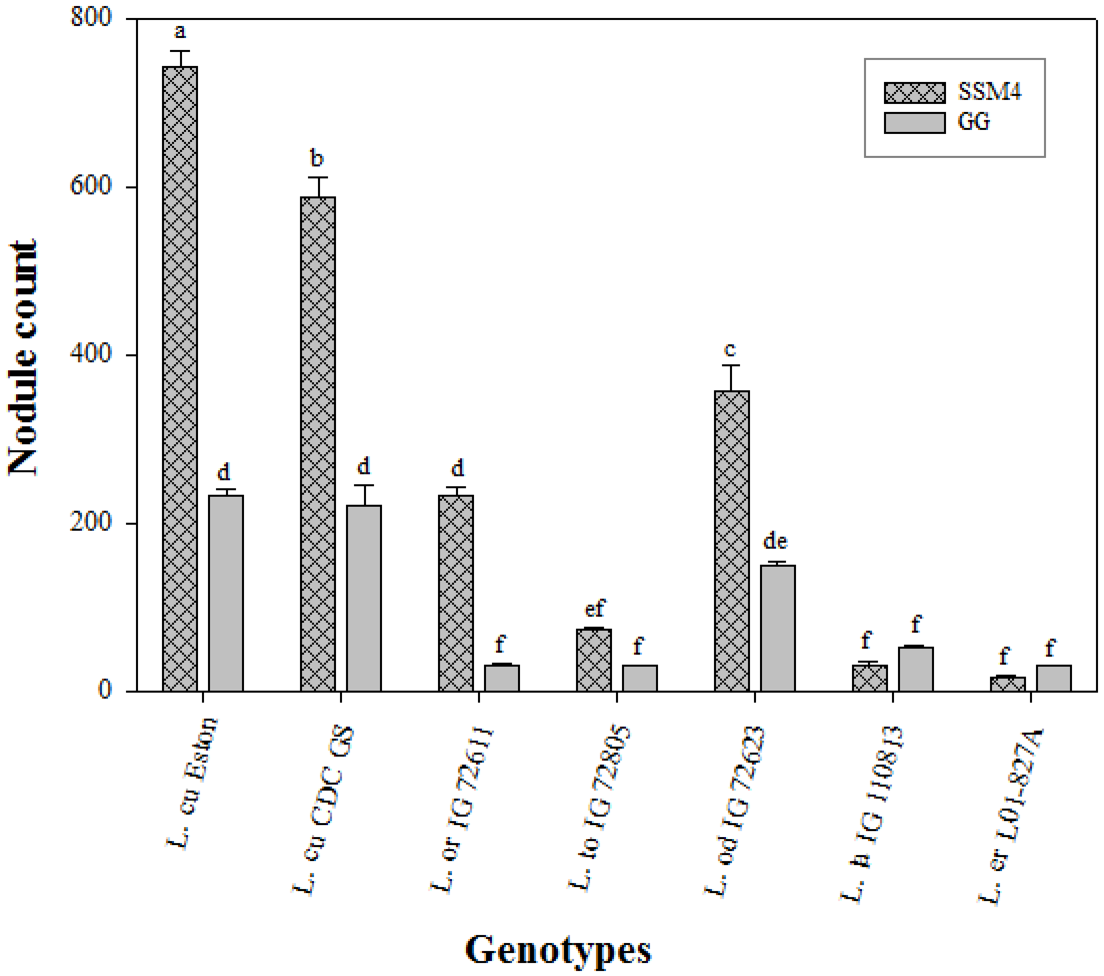


**Fig. 6.14.** Comparison of nodule counts among 7 lentil genotypes grown on two growth media.
[[Media: SSM4 (Sunshine Mix # 4) and GG (Greens Grade)].
Genotypes with same letters are not significantly different. Bars indicate standard error of means].

### 6.15 Effects of media and moisture levels on transpiration rate (TR) of lentil genotypes

Significant interactions were observed among media and moisture level for TR of lentil genotypes (Table 6.15). Lentil genotypes had significantly higher TR in SSM4 compared with GG under both fully-watered and drought conditions (Fig. 6.15).

**Table 6.15.** ANOVA table for mean transpiration rate of lentil genotypes grown in two growth media at two moisture levels.

| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
|---|---|---|---|---|---|
| Geno | 6 | 36618.7 | 6103.1 | 20.5 | <.0001 |
| Media | 1 | 36141.9 | 36141.9 | 121.6 | <.0001 |
| Moist | 1 | 17913.3 | 17913.3 | 60.2 | <.0001 |
| Geno*Media | 6 | 10235.3 | 1705.8 | 5.7 | <.0001 |
| Geno*Moist | 6 | 7460.4 | 1243.4 | 4.1 | 0.0011 |
| Media*Moist | 1 | 1372.6 | 1372.6 | 4.6 | 0.0347 |
| Geno*Media*Moist | 6 | 1724.7 | 287.4 | 0.9 | 0.4530 |

[DF: degree of freedom; Geno: genotypes; Media: was either SSM4 or GG; Moist: fully-watered and drought conditions].

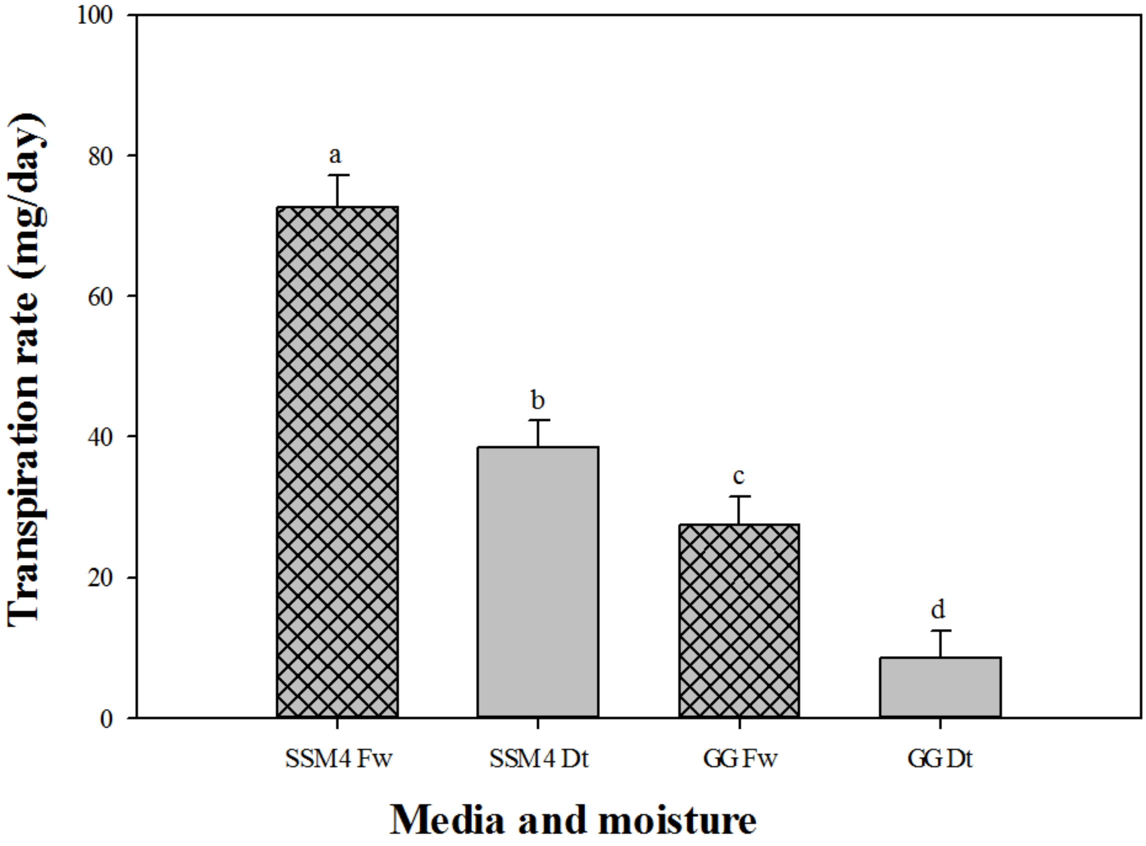


**Fig. 6.15.** Interactions between media and moisture levels for mean transpiration rate of 7 lentil genotypes.
[[Media: SSM4 (Sunshine Mix # 4) and GG (Greens Grade); Moisture levels: Fw: (Fully-watered) and Dt (Drought)].
Growth media with same letters are not significantly different. Bars indicate standard error of means].

### 6.16 Correlation between shoot and root traits of lentil genotypes grown in SSM4 under fully-watered conditions

The correlation matrix is discussed in the numerical order presented in the first column of Table 6.16. The first four characteristics are above ground measurements, and the remainder are below ground measurements. Significant and positive correlation existed among all above ground traits except for SPAD that showed no correlation with PH and SB. SB and TNOL had the strongest positive and significant correlation among above ground traits (r= 0.54, $P \leq 0.05$) and the weakest correlation existed between SB and PH (r= 0.42, $P \leq 0.05$). Most of below ground traits were significantly and positively correlated with each other. For example, TNORT, TNORF and TRL had significant and positive correlation with all below ground parameters except for RB and RAD. TRSA had significant and positive correlation with all below ground traits with the exception of RB, RLD and RAD. Significant and positive correlation existed between RLD and the other below ground traits except for TRSA and RAD. However, RB was only significantly and positively correlated with RLD (r= 0.59, $P \leq 0.05$) and TNON (r= 0.54, $P \leq 0.05$). The strongest correlation between below ground parameters was observed between TRL and TRSA (r= 0.95, $P \leq 0.0001$). The weakest correlation was observed between TNORF and TRL (r= 0.47, $P \leq 0.05$). Regarding correlation between above and below ground traits, the strongest significant and positive correlation was observed between RB and TNOL (r= 0.68, $P \leq 0.01$) and the weakest correlation existed between SB and TNORT (r= 0.41, $P \leq 0.05$). Shoot biomass had significant and positive correlation with all below ground traits with the exception for RLD and RAD. RB had the strongest significant and positive correlation with shoot biomass, (r= 0.65, $P \leq 0.01$).

**Table 6.16** Pearson correlation coefficients for plant height, total number of leaflets, SPAD value, shoot biomass, root biomass, total root length, root length density, total root surface area, root average diameter, total number of root tips, total number of root forks and total number of nodules of seven lentil genotypes grown in Sunshine Mix # 4 under fully-watered conditions

| **Characters** | TNOL 2 | SPAD 3 | SB 4 | RB 5 | TRL 6 | RLD 7 | TRSA 8 | RAD 9 | TNROT 10 | TNROF 11 | TNON 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PH (1) | 0.427$^{*}$ | -0.456* | 0.420* | 0.481** | 0.474* | 0.195$^{ns}$ | 0.418* | -0.147$^{ns}$ | 0.113$^{ns}$ | -0.068$^{ns}$ | 0.029$^{ns}$ |
| TNOL (2) | | -0.463* | 0.540** | 0.680** | 0.207$^{ns}$ | 0.456* | 0.086$^{ns}$ | 0.055$^{ns}$ | 0.135$^{ns}$ | -0.092$^{ns}$ | 0.502* |
| SPAD (3) | | | -0.332$^{ns}$ | -0.366$^{ns}$ | -0.098$^{ns}$ | 0.270$^{ns}$ | -0.122$^{ns}$ | 0.249$^{ns}$ | 0.101$^{ns}$ | 0.328$^{ns}$ | 0.070$^{ns}$ |
| SB (4) | | | | 0.655** | 0.514* | 0.381$^{ns}$ | 0.537* | -0.046$^{ns}$ | 0.412* | 0.209$^{ns}$ | 0.593** |
| RB (5) | | | | | 0.233$^{ns}$ | 0.596** | 0.247$^{ns}$ | -0.077$^{ns}$ | 0.258$^{ns}$ | 0.154$^{ns}$ | 0.544** |
| TRL (6) | | | | | | 0.507* | 0.958*** | -0.088$^{ns}$ | 0.798*** | 0.475* | 0.579** |
| RLD (7) | | | | | | | 0.339$^{ns}$ | -0.041$^{ns}$ | 0.794*** | 0.477* | 0.687** |
| TRSA (8) | | | | | | | | -0.108$^{ns}$ | 0.814*** | 0.574** | 0.615** |
| RAD (9) | | | | | | | | | 0.075$^{ns}$ | -0.047$^{ns}$ | -0.028$^{ns}$ |
| TNRT (10) | | | | | | | | | | 0.824*** | 0.500* |
| TNRF (11) | | | | | | | | | | | 0.306$^{ns}$ |

PH (1): Plant height
TNOL (2): Total number of leaflets
SPAD (3): SPAD value
SB (4): Shoot biomass
RB (5): Root biomass
TRL (6): Total root length
RLD (7): Root length density
TRSA (8): Total root surface area
RAD (9): Mean root diameter
TNORT (10): Total number of root tips
TNORF (11): Total number of root forks
TNON (12): Total number of root nodules

*, ** and *** indicate significance at $P \leq 0.05$, 0.01 and 0.0001, respectively; ns: non-significant

### 6.17 Correlation between shoot and root traits of lentil genotypes grown in SSM4 under drought conditions

All above-ground traits were significantly and positively correlated with each other except for TNOL. The strongest significant correlation among all other above- ground was between PH and SB (r= 0.73, P ≤ 0.0001) with the weakest significant correlation between SPAD and SB (r= 0.63, P ≤ 0.0001). Most below-ground traits were significantly and positively correlated with each other. For instance, TRL, TRSA, TNORT, TNORF and TNON have significant positive correlation with each other but they were not significantly correlated to RAD and RLD. RB and RLD were not significantly correlated to each other, and both of them were not correlated to RAD. The strongest significant correlation among below-ground traits under drought was observed between TNORT and TRL (r= 0.87, P ≤ 0.0001) meanwhile the weakest significant correlation was observed between TNORT and RB (r= 0.43, P ≤ 0.05). For above and below-ground traits, PH and SB had significant and positive correlation with all below-ground traits except for RLD and RAD. SPAD was significantly and positively correlated with most of below-ground traits except for RLD, RAD and TNORF. SB had the strongest significant correlation with TNON (r= 0.72, P ≤ 0.0001) and the weakest with TRSA (r= 0.53, P ≤ 0.05) (Table 6.17).

**Table 6.17** Pearson correlation coefficients for plant height, total number of leaflets, SPAD value, shoot biomass, root biomass, total root length, root length density, total root surface area, root average diameter, total number of root tips, total number of root forks and total number of nodules of seven lentil genotypes grown in Sunshine Mix #4 under drought conditions

| **Characters** | TNOL 2 | SPAD 3 | SB 4 | RB 5 | TRL 6 | RLD 7 | TRSA 8 | RAD 9 | TNORT 10 | TNROF 11 | TNON 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PH (1) | -0.152$^{ns}$ | 0.682** | 0.743*** | 0.738*** | 0.762*** | 0.249$^{ns}$ | 0.818*** | -0.175$^{ns}$ | 0.760*** | 0.423$^{ns}$ | 0.675** |
| TNOL (2) | | -0.083$^{ns}$ | 0.176$^{ns}$ | 0.354$^{ns}$ | 0.186$^{ns}$ | -0.315$^{ns}$ | 0.085$^{ns}$ | 0.316$^{ns}$ | 0.228$^{ns}$ | 0.210$^{ns}$ | 0.137$^{ns}$ |
| SPAD (3) | | | 0.634*** | 0.556** | 0.714*** | -0.098$^{ns}$ | 0.738*** | 0.030$^{ns}$ | 0.503* | 0.210$^{ns}$ | 0.738*** |
| SB (4) | | | | 0.666*** | 0.608** | 0.000 | 0.535* | 0.000 | 0.543** | 0.547** | 0.721*** |
| RB (5) | | | | | 0.787** | 0.286$^{ns}$ | 0.445* | 0.007$^{ns}$ | 0.431* | 0.723*** | 0.476** |
| TRL (6) | | | | | | 0.590* | 0.803*** | -0.047$^{ns}$ | 0.871*** | 0.745*** | 0.691*** |
| RLD (7) | | | | | | | 0.677*** | 0.051$^{ns}$ | 0.681*** | 0.634** | 0.529** |
| TRSA (8) | | | | | | | | 0.012$^{ns}$ | 0.755*** | 0.857*** | 0.574** |
| RAD (9) | | | | | | | | | 0.082$^{ns}$ | -0.148$^{ns}$ | 0.350$^{ns}$ |
| TNRT (10) | | | | | | | | | | 0.737** | 0.793*** |
| TNRF (11) | | | | | | | | | | | 0.454* |

PH (1): Plant height
TNOL (2): Total number of leaflets
SPAD (3): SPAD value
SB (4): Shoot biomass
RB (5): Root biomass
TRL (6): Total root length
RLD (7): Root length density
TRSA (8): Total root surface area
RAD (9): Mean root diameter
TNORT (10): Total number of root tips
TNORF (11): Total number of root forks
TNON (12): Total number of nodules

*, ** and *** indicate significance at P ≤ 0.05, 0.01 and 0.0001, respectively; ns: non-significant

## 6.18 Correlation between shoot and root traits of lentil genotypes grown in GG under fully watered conditions

Most of above ground traits were significantly and positively correlated with each other except for TNOL, which only had significant and positive correlation with SB (r= 0.42, P ≤ 0.0001). For below-ground traits, RB, TRL, TNORF and TNON were significantly and positively correlated with all other below-ground traits except for RLD and RAD. TRSA and TNORT had significant and positive correlation with almost all root traits apart from RLD and RAD. TRSA and TNORT were not significantly correlated with each other, nor were correlated to RLD and RAD. The strongest correlation among below ground traits existed between TRL and TNRF (r= 0.93, P ≤ 0.0001), while the weakest significant correlation was observed between RB and TNORF (r= 0.59, P ≤ 0.05). Regarding correlation among below and above-ground traits, most of them were significantly and positively correlated with each other except for TNOL which was only significantly and positively correlated to RB, TRSA and TNORF. The strongest significant correlation existed between SB and RB (r= 0.99, P ≤ 0.0001), while TNOL and TNORT had the weakest significant correlation (r= 0.41, P ≤ 0.05) (Table 6.18).

**Table 6.18** Pearson correlation coefficients for plant height, total number of leaflets, SPAD value, shoot biomass, root biomass, total root length, root length density, total root surface area, root average diameter, total number of root tips, total number of root forks and total number of nodules of seven lentil genotypes grown in Greens Grade under fully-watered conditions

| **Characters** | TNOL 2 | SPAD 3 | SB 4 | RB 5 | TRL 6 | RLD 7 | TRSA 8 | RAD 9 | TNORT 10 | TNORF 11 | TNON 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PH (1) | $0.049^{ns}$ | 0.629*** | 0.822*** | 0.808*** | 0.732*** | 0.475* | 0.770*** | $-0.229^{ns}$ | 0.723*** | 0.581** | 0.520* |
| TNOL (2) | | $0.238^{ns}$ | 0.428* | 0.430* | $0.219^{ns}$ | $-0.294^{ns}$ | 0.526* | $0.190^{ns}$ | 0.418* | $0.080^{ns}$ | $0.242^{ns}$ |
| SPAD (3) | | | 0.689** | 0.707** | 0.775** | $0.019^{ns}$ | 0.716** | $-0.230^{ns}$ | 0.755** | 0.782*** | 0.730** |
| SB (4) | | | | 0.991*** | 0.891*** | $0.265^{ns}$ | 0.876*** | $-0.102^{ns}$ | 0.909*** | 0.614** | 0.697** |
| RB (5) | | | | | 0.905*** | $0.240^{ns}$ | 0.864*** | $-0.150^{ns}$ | 0.922*** | 0.599** | 0.741*** |
| TRL (6) | | | | | | $0.44^{ns}$ | 0.788*** | $-0.233^{ns}$ | 0.922*** | 0.930*** | 0.717** |
| RLD (7) | | | | | | | $0.198^{ns}$ | -0.488* | $0.423^{ns}$ | $0.410^{ns}$ | $-0.007^{ns}$ |
| TRSA (8) | | | | | | | | $0.030^{ns}$ | $0.863^{ns}$ | 0.611** | 0.690*** |
| RAD (9) | | | | | | | | | $-0.287^{ns}$ | $-0.276^{ns}$ | $-0.097^{ns}$ |
| TNRT (10) | | | | | | | | | | 0.792*** | 0.669*** |
| TNRF (11) | | | | | | | | | | | 0.594** |

PH (1): Plant height
TNOL (2): Total number of leaflets
SPAD (3): SPAD value
SB (4): Shoot biomass
RB (5): Root biomass
TRL (6): Total root length
RLD (7): Root length density
TRSA (8): Total root surface area
RAD (9): Root average diameter
TNORT (10): Total number of root tips
TNORF (11): Total number of root forks
TNON (12): Total number of nodules

*, ** and *** indicate significance at P ≤ 0.05, 0.01 and 0.0001, respectively; ns: non-significant

### 6.19 Correlation between shoot and root traits of lentil genotypes grown in GG under drought conditions

Above-ground traits (PH, SPAD and SB) had significant and positive correlation with each other except for TNOL, which had no significant correlation with other above-ground traits. The strongest significant correlation among above-ground traits was observed between PH and SB ($r = 0.74$, $P \leq 0.0001$), while SPAD and SB had the weakest significant correlation ($r = 0.63$, $P \leq 0.0001$). With respect to among below-ground traits, TRL, TNORT and TNON had significant and positive correlation with most of the below-ground traits except for RLD, RAD and TNORF. RB also had significant and positive correlation with most below-ground traits with the exception of RLD, TRSA and RAD. TRSA was significantly and positively correlated with TRL, TNORF and TNON. TNORF was only correlated to RB ($r = 0.54$, $P \leq 0.01$). The strongest correlation among below ground traits was observed between TRL and TNORT ($r = 0.89$, $P \leq 0.0001$), while the weakest significant correlation was observed between RB and TNORF ($r = 0.54$, $P \leq 0.01$). With regard to correlation between above and below-ground traits, PH, SPAD and SB had significant and positive correlation with most of below-ground traits except for RLD, RAD and TNORF. TNOL did not have significant correlation with any of below-ground traits. SB and RB ($r = 0.96$, $P \leq 0.0001$) along with SPAD and TNRT ($r = 0.50$, $P \leq 0.05$) had the strongest and weakest correlation among above and below-ground traits, respectively.

**Table 6.19** Pearson correlation coefficients for plant height, total number of leaflets, SPAD value, shoot biomass, root biomass, total root length, root length density, total root surface area, root average diameter, total number of root tips, total number of root forks and total number of nodules of seven lentil genotypes grown in Greens Grade under drought conditions

| **Characters** | TNOL 2 | SPAD 3 | SB 4 | RB 5 | TRL 6 | RLD 7 | TRSA 8 | RAD 9 | TNORT 10 | TNORF 11 | TNON 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PH (1) | -0.152$^{ns}$ | 0.682** | 0.743*** | 0.738*** | 0.762*** | 0.249$^{ns}$ | 0.818*** | -0.175$^{ns}$ | 0.760*** | 0.423$^{ns}$ | 0.675** |
| TNOL (2) |  | -0.083$^{ns}$ | 0.176$^{ns}$ | 0.354$^{ns}$ | 0.186$^{ns}$ | -0.315$^{ns}$ | 0.085$^{ns}$ | 0.316$^{ns}$ | 0.228$^{ns}$ | 0.210$^{ns}$ | 0.137$^{ns}$ |
| SPAD (3) |  |  | 0.634*** | 0.556** | 0.714*** | -0.098$^{ns}$ | 0.738*** | 0.030$^{ns}$ | 0.503* | 0.210$^{ns}$ | 0.738*** |
| SB (4) |  |  |  | 0.965*** | 0.637*** | -0.163$^{ns}$ | 0.645*** | 0.115$^{ns}$ | 0.561** | 0.265$^{ns}$ | 0.633*** |
| RB (5) |  |  |  |  | 0.895*** | -0.174$^{ns}$ | 0.801$^{ns}$ | 0.035$^{ns}$ | 0.880*** | 0.546** | 0.643** |
| TRL (6) |  |  |  |  |  | 0.095$^{ns}$ | 0.873*** | -0.202$^{ns}$ | 0.899*** | 0.312$^{ns}$ | 0.735*** |
| RLD (7) |  |  |  |  |  |  | -0.097$^{ns}$ | -0.69*** | 0.132$^{ns}$ | 0.304$^{ns}$ | -0.147$^{ns}$ |
| TRSA (8) |  |  |  |  |  |  |  | -0.190$^{ns}$ | 0.807*** | 0.147$^{ns}$ | 0.863*** |
| RAD (9) |  |  |  |  |  |  |  |  | -0.270$^{ns}$ | -0.074$^{ns}$ | -0.073$^{ns}$ |
| TNRT (10) |  |  |  |  |  |  |  |  |  | 0.354$^{ns}$ | 0.588** |
| TNRF (11) |  |  |  |  |  |  |  |  |  |  | 0.182$^{ns}$ |

PH (1): Plant height
TNOL (2): Total number of leaflets
SPAD (3): SPAD value
SB (4): Shoot biomass
RB (5): Root biomass
TRL (6): Total root length
RLD (7): Root length density
TRSA (8): Total root surface area
RAD (9): Root average diameter
TNORT (10): Total number of root tips
TNORF (11): Total number of root forks
TNON (12): Total number of nodules

*, ** and *** indicate significance at $P \leq 0.05$, 0.01 and 0.0001, respectively; ns: non-significant

## 7.0 Discussion and conclusions

### 7.1 The effects of growth media and moisture levels on lentil genotypes

Growth media significantly influenced growth characteristics of lentil genotypes. The response of above and below ground traits to growth media was different. In SSM4, most above ground traits were significantly higher compared to GG. Most lentil genotypes grown in SSM4 had significantly higher PH, SPAD value, SB, TR and TNON compared to GG. This was likely related to the higher initial total available nitrogen in this media compared to GG (Table 2.1) and higher N mineralization (Conversion of organic to inorganic plant available form) in SSM4 compared to GG. Similar results have been reported by Fatima *et al*. (2013), who showed that there was an increase in PH and TNON in the lentil variety, Shalimar Masoor- I, exposed to increased N fertilizer of up to 45 kg ha$^{-1}$. Voisin *et al*. (2002), also reported increased SB of pea (*Pisum sativum* L. cv Baccara) in response to increased N application. The SPAD value was another above ground trait with increased response to higher N supply and has been shown to have a strong correlation with leaf chlorophyll content and N level in fig (*Ficus benjamina*) and cottonwood (*Populus deltoides*) (Seeman *et al*., 1987). Greater canopy of plants in SSM4 (Fig. 6.5) also contributed to higher transpiration of plants when grown in SSM4. According to findings of Gorim *et al*. (2017), shoot and root data observed on SSM4 was more similar to soil compared to GG.

Preliminary assessment indicates that GG is an inhibiting growth medium for above ground traits, which is not in agreement with findings of Becker *et al*. (2015), who found GG as a good growth medium for growing synthetic hexaploid wheat (SHW) lines and wheat cultivars under both fully-watered and drought conditions. Despite adverse effects of GG on above ground traits, most lentil genotypes had significantly higher values for below ground traits when grown in the GG medium. This mass root system corresponded to that demonstrated when soil additives such as Geohumus were added as ameliorates to soil (Duong, 2012). These substances were shown to trap nutrients making them unavailable to plants, suggesting that GG may have been also trapped the added nutrients, forcing lentils to allocate resources underground at the expense of shoot and nodulation. Greater root system observed in GG could also be explained by lower porosity and smaller pore space in this growth medium compared to SSM4, induced development of finer roots, greater RL and RSA, as these root traits are mainly made up of fine roots (Zobel *et al*., 2005; 2007).

Number of nodules was one of the root traits with significantly higher value in SSM4 compared to GG despite the adverse effect of high nitrogen concentration in SSM4 (676 mg/kg) (Table 2.1), which inhibits symbiotic $N_2$ fixation in legumes (Carroll *et al*., 1985). This might have been related to the ability of commercial rhizobium (*Rhizobium leguminosarum* biovar *viceae* strain 1435) to manage environmental stress (Agro BASF, 2018, Issue 1.0), allowing it to tolerate acidic condition in SSM4 and to cause high nodulation of lentil genotypes grown in this growth medium (Fig. 6.14). Also, greater photo-assimilate proportion in shoot biomass of genotypes grown in SSM4 (Fig. 6.5) might have supplied high photoassimilate to *Rhizobium leguminosarum* biovar *viceae* strain 1435 and allowed nodulation formation despite acidic condition of SSM4.

Moisture levels had an effect on growth characteristics of lentil genotypes in both media. For most above and below ground traits, lentil genotypes had significantly higher values under fully-watered compared to drought conditions. For example, significantly higher number of nodes, TNOL, SB, TRL, TNORF and TR were observed under fully-watered conditions irrespective of growth media, which is in agreement with findings of Idrissi *et al*. (2015), who reported significantly higher root and shoot traits in lentil genotypes grown under fully-watered compared to drought conditions. Reduced value in above and below ground traits of lentil genotypes under drought condition was likely associated with reduced carbon assimilate as a function of reduced photosynthesis under dry conditions. Ashraf *et al*. (2016), and Leport *et al*. (1998), reported decreased photosynthesis of wheat and lentil, respectively, under dry conditions. Reduced TR might have also been related to stomatal closure of plants in response to drought. Gorim and Vandenberg (2017), observed significant reduction in TR of lentil genotypes when grown under dry conditions. Although, drought did not cause significant reduction in some below ground traits. For example, RAD had similar value in both moisture levels regardless of growth media. This might have been associated with small diameter of lentil genotypes under both fully-watered and drought conditions, which did not vary in response to drought. This result was in contrast to findings of Wasson *et al*. (2012) and Idrissi *et al.* (2015), who reported reduced RAD in wheat and lentil genotypes, respectively, under dry conditions.

### 7.2 Response of cultivated and wild lentil genotypes to growth media and moisture levels

Cultivated lentil genotypes responded differently to growth media and had significantly higher above and below ground biomass compared to wild genotypes. However, there was at least one wild genotype producing similar biomass to either *L. culinaris* Eston or *L. culinaris* CDC Greenstar in both media. For example, *L. odemensis* IG 72623 had similar TRL, TRSA, SB and RB to *L.* CDC Greenstar when grown in SSM4, while the rest of the wild lentil genotypes had significantly lower above and below ground biomass. *Lens odemensis* IG 72623 had significantly lower root biomass reduction compared to all other genotypes under dry conditions (Fig. 9.3). This observation was of immense importance as plant resistance against drought is related to root biomass production. Saxena (1987), related higher yield of ICC 4958 (chickpea genotype) compared with Annigeri (standard cultivar) to higher RDW of this genotype when grown under drought conditions. Another interesting observation on *L. odemensis* IG 72623 was number of nodules in this genotype. *Lens odemensis* IG 72623 had significantly lower number of nodules compared with *L. culinaris* CDC Greenstar, suggesting that *L. odemensis* IG 72623 may have nodules that are better nitrogen fixers than *L. culinaris* CDC Greenstar as reported by (Gorim and Vandenberg, 2017). Yang *et al*. (2017), found no correlation between number of nodule on pea and fixed $N_2$. Higher number of nodules of cultivated compared to wild lentil genotypes might have been associated with a good relationship established between cultivated genotypes and commercial rhizobium (*Rhizobium leguminosarum* biovar *viceae* strain 1435), as this rhizobium had been used in the Prairies to inoculate cultivated lentils over the last few years. Laguerre *et al*. (2003), reported that host legumes vary in preference for specific rhizobium strain.

Cultivated lentil genotypes also had higher above and below ground biomass compared to wild ones when grown in GG. However, there was also at least one wild genotype with similar above and below ground biomass to cultivated lentil genotypes in this medium. For example, *L. lamottei* IG 110813 had similar SB, RB and TRSA compared to *L. culinaris* Eston. Nonetheless, number of nodules in *L. lamottei* IG 110813 was significantly lower compared to *L. culinaris* Eston. *Lens orientalis* IG 72611, which falls between *L. culinaris* and *L. tomentosus* (Wong *et al*., 2015), had the lowest above and below ground biomass in both growth media. Agronomic traits of *L. orientalis* IG 72611 such as PH, SB and RB were similar to *L. tomentosus.*

Cultivated lentil genotypes responded differently to moisture levels compared to wild genotypes and had significantly higher above and below ground traits under both moisture levels. However, root traits of some wild genotypes were similar or even higher compared to cultivated genotypes. For example, *L. lamottei* IG 110813 had similar RB and RAD but higher RSR compared to cultivated genotypes under drought condition. This high RSR is in agreement with reports by Gorim and Vandenberg (2017) who found under drought conditions, *L. lamottei* IG 110813 had the highest RSR compared to both cultivated and wild lentil genotypes. Furthermore, this behavior has also been reported in common bean (Aswaf and Blair 2012). *Lens odemensis* IG 72623 was another wild genotype with comparable RB and RV to cultivated genotypes under drought conditions. These similar root traits resulted in similar above ground biomass in *L. odemensis* IG 72623 compared to cultivated genotypes.

In conclusion, SSM4 was a better growth medium compared to GG as most lentil genotypes grown in SSM4 were significantly taller, greener and healthier (higher SPAD values) with higher SB and TNON. GG was not the optimal medium for investigating shoot traits of lentil genotypes as it restricted plant nutrient uptake and caused plants to allocate higher carbon assimilate on root rather than shoot. Using GG as a growth medium for lentil is not advisable given the restricted above ground growth observed, despite its successful use in studies of drought effects on wheat and growing grass on sand-based sports fields. Drought caused significant reduction in most traits including number of nodes, TNOL, SB, TRL, TNORT, TNORF and TR in both SSM4 and GG. Interaction of growth media and moisture levels was found significant for traits like number of nodes on the main stem, TNOL, RB and TR. However, PH, SPAD, SB, RSR and TNON remained unaffected. Significantly greater above-ground biomass was observed in cultivated lentil genotypes regardless of media. Although, some wild genotypes like *L. odemensis* IG 72623 had significantly lower root biomass reduction compared with cultivated lentil genotypes (Fig. 9.3), which makes it ideal candidate for introgression into cultivated lentils in breeding programs where drought tolerance is targeted. *Lens odemensis* was considered drought tolerant species according to Hamdi and Erskine (1996), who investigated morphological and phenological evaluation of *Lens* germplasm collection at ICARDA. Center of origin (Turkey) of this species might have played a role in drought tolerance observed in *Lens odemensis.* According to Ladzinsky (1993), *Lens odemensis* had grown in calcareous soil, where water drained fast. Fast drainage of water might have caused little water to be left for the plants, inducing greater proportion of root production in search of water. Kumar *et al*, (2012), reported relationship between RB and high yield of lentil genotypes when grown in semi-arid to sub-humid regions. The importance of little reduction in root biomass of *L. odemensis* IG 72623 was observed in SPAD value of this genotypes (Fig. 6.4), which was significantly higher compared to all other lentil genotypes. Significant and positive correlation was reported (r = 0.45**) between SPAD value and root dry weight (RDW) in an experiment involving 43 lentil genotypes (Kumar *et al.*, 2012).

With respect to comparison of cultivated lentil genotypes, despite higher shoot biomass observed in *L. culinaris* Eston (Fig. 6.5), root biomass reduction in *L. culinaris* CDC Greenstar was significantly lower compared to *L. culinaris* Eston (Fig. 9.3), showing greater tolerance of *L. culinaris* CDC Greenstar to drought as this genotype had been grown in southern parts of Saskatchewan like Swift current with lower precipitation compared to northern areas in the province.

The investigation of other growth media as well as natural soil is recommended as the experiments discussed above were limited to responses of lentil genotypes to two growth media. Specific growth media designed for lentil with sufficient N content, good aeration, neutral pH, and low bulk density, providing easy and rapid separation of root samples from the medium might be a better growing condition for growing lentil in environmentally controlled growth chambers. Also, investigation of nodule size and activity of lentil genotypes is required as high numbers of nodules may not contribute to improved performance of plants unless they have efficient activity.

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## 9.0 Appendix

**Table 9.1.** Effects of genotype, media and moisture level on plant height and number of nodes of 7 lentil genotypes.

| | Plant height | | | | Number of nodes | | | |
|---|---|---|---|---|---|---|---|---|
| | SSM4 | | GG | | SSM4 | | GG | |
| Genotype | Fw | Dt | Fw | Dt | Fw | Dt | Fw | Dt |
| *L. culinaris* Eston | 40.5$^{a}$ | 36.6$^{a}$ | 34.0$^{b}$ | 29.5$^{a}$ | 16.0$^{a}$ | 14.0$^{a}$ | 22.0$^{b}$ | 19.0$^{ab}$ |
| *L. culinaris* CDC Greenstar | 39.0$^{a}$ | 31.1$^{bc}$ | 43.5$^{a}$ | 28.9$^{a}$ | 16.0$^{a}$ | 12.0$^{a}$ | 28.0$^{a}$ | 20.0$^{a}$ |
| *L. orientalis* IG 72611 | 24.6$^{d}$ | 18.6$^{e}$ | 13.8$^{cd}$ | 9.0$^{d}$ | 10.0$^{b}$ | 7.0$^{c}$ | 9.0$^{e}$ | 5.0$^{e}$ |
| *L. tomentosus* IG 72805 | 33.1$^{bc}$ | 25.2$^{d}$ | 9.5$^{d}$ | 7.0$^{d}$ | 15.0$^{a}$ | 10.0$^{b}$ | 6.0$^{f}$ | 3.0$^{f}$ |
| *L. odemensis* IG 72623 | 29.6$^{c}$ | 27.1$^{cd}$ | 17.3$^{c}$ | 14.3$^{c}$ | 12.0$^{b}$ | 10.0$^{b}$ | 12.0$^{d}$ | 9.0$^{d}$ |
| *L. lamottei* IG 110813 | 39.1$^{a}$ | 34.6$^{ab}$ | 33.2$^{b}$ | 31.5$^{a}$ | 16.0$^{a}$ | 13.0$^{a}$ | 21.0$^{b}$ | 18.0$^{b}$ |
| *L. ervoides* L01-827A | 37.8$^{ab}$ | 34.8$^{ab}$ | 29.7$^{b}$ | 22.0$^{b}$ | 15.0$^{a}$ | 13.0$^{a}$ | 20.0$^{c}$ | 14.0$^{c}$ |

Means within columns followed by the same letter are not significantly different according to LSD P 0.05.

SSM4: Sunshine Mix # 4; GG: Greens Grade. Fw: Fully-watered; Dt: Drought.

**Table 9.2.** Effects of genotype, media and moisture level on total number of leaflets and SPAD of 7 lentil genotypes.

| | Total number of leaflets | | | | SPAD | | | |
|---|---|---|---|---|---|---|---|---|
| | SSM4 | | GG | | SSM4 | | GG | |
| Genotype | Fw | Dt | Fw | Dt | Fw | Dt | Fw | Dt |
| *L. culinaris* 'Eston' | 324.0$^{a}$ | 189.0$^{a}$ | 344.0$^{ab}$ | 169.0$^{bc}$ | 37.8$^{ab}$ | 36.1$^{ab}$ | 37.6$^{a}$ | 49.6$^{a}$ |
| *L. culinaris* 'CDC Greenstar' | 230.0$^{b}$ | 178.0$^{ab}$ | 255.0$^{bc}$ | 97.0$^{cd}$ | 31.8$^{ab}$ | 34.1$^{b}$ | 23.6$^{a}$ | 28.9$^{b}$ |
| *L. orientalis* IG 72611 | 185.0$^{c}$ | 128.0$^{cd}$ | 212.0$^{c}$ | 129.0$^{bcd}$ | 42.0$^{a}$ | 41.9$^{ab}$ | 4.4$^{d}$ | 7.9$^{cd}$ |
| *L. tomentosus* IG 72805 | 177.0$^{c}$ | 102.0$^{d}$ | 308.0$^{b}$ | 148.0$^{bcd}$ | 41.8$^{ab}$ | 39.4$^{ab}$ | 2.5$^{d}$ | 1.9$^{d}$ |
| *L. odemensis* IG 72623 | 204.0$^{bc}$ | 126.0$^{cd}$ | 429.0$^{a}$ | 301.0$^{a}$ | 44.7$^{a}$ | 50.6$^{a}$ | 16.8$^{ab}$ | 4.7$^{cd}$ |
| *L. lamottei* IG 110813 | 305.0$^{a}$ | 147.0$^{bc}$ | 405.0$^{a}$ | 201.0$^{b}$ | 29.1$^{b}$ | 33.6$^{b}$ | 14.5$^{c}$ | 8.7$^{c}$ |
| *L. ervoides* L01-827A | 129.0$^{d}$ | 90.0$^{d}$ | 113.0$^{d}$ | 81.0$^{d}$ | 44.2$^{a}$ | 36.8$^{ab}$ | 3.4$^{d}$ | 2.8$^{cd}$ |

Means within columns followed by the same letter are not significantly different according to LSD at P 0.05.

SSM4: Sunshine Mix # 4; GG: Greens Grade. Fw: Fully-watered; Dt: Drought.

**Table 9.3.** Effects of genotype, media and moisture level on shoot biomass and root biomass of 7 lentil genotypes.

| Shoot biomass | | | | | Root biomass | | | |
|---|---|---|---|---|---|---|---|---|
| SSM4 | | | GG | | SSM4 | | GG | |
| Genotypes | Fw | Dt | Fw | Dt | Fw | Dt | Fw | Dt |
| *L. culinaris* Eston | $6.89^{a}$ | $4.80^{a}$ | $2.81^{b}$ | $1.13^{a}$ | $1.83^{a}$ | $0.90^{ab}$ | $3.49^{b}$ | $1.20^{a}$ |
| *L. culinaris* CDC Greenstar | $3.72^{b}$ | $2.86^{b}$ | $4.01^{a}$ | $1.13^{a}$ | $1.13^{b}$ | $1.00^{a}$ | $4.69^{a}$ | $1.23^{a}$ |
| *L. orientalis* IG 72611 | $1.81^{c}$ | $0.97^{d}$ | $0.51^{d}$ | $0.13^{b}$ | $0.40^{c}$ | $0.30^{c}$ | $0.40^{e}$ | $0.17^{b}$ |
| *L. tomentosus* IG 72805 | $3.15^{b}$ | $1.52^{c}$ | $0.47^{d}$ | $0.13^{b}$ | $0.80^{b}$ | $0.60^{bc}$ | $0.50^{e}$ | $0.18^{b}$ |
| *L. odemensis* IG 72623 | $4.00^{b}$ | $2.80^{b}$ | $1.52^{c}$ | $0.86^{a}$ | $0.76^{b}$ | $0.73^{abc}$ | $1.57^{d}$ | $1.00^{a}$ |
| *L. lamottei* IG 110813 | $2.87^{bc}$ | $1.26^{cd}$ | $2.10^{c}$ | $0.92^{a}$ | $0.82^{b}$ | $0.47^{bc}$ | $2.32^{c}$ | $1.18^{a}$ |
| *L. ervoides* L01-827A | $3.12^{bc}$ | $1.53^{c}$ | $0.70^{d}$ | $0.35^{b}$ | $0.41^{c}$ | $0.30^{c}$ | $0.75^{e}$ | $0.42^{b}$ |

Means within columns followed by the same letter are not significantly different according to LSD P 0.05.

SSM4: Sunshine Mix # 4; GG: Greens Grade. Fw: Fully-watered; Dt: Drought.

**Table 9.4.** Effects of genotype, media and moisture level on root/shoot and number of nodules of 7 lentil genotypes.

| Root/shoot ratio | | | | | Number of nodules | | | |
|---|---|---|---|---|---|---|---|---|
| SSM4 | | | GG | | SSM4 | | GG | |
| Genotypes | Fw | Dt | Fw | Dt | Fw | Dt | Fw | Dt |
| *L. culinaris* Eston | $0.27^{b}$ | $0.20^{d}$ | $1.22^{a}$ | $1.00^{c}$ | $813.00^{a}$ | $668.00^{a}$ | $261.00^{a}$ | $203.00^{a}$ |
| *L. culinaris* CDC Greenstar | $0.48^{a}$ | $0.28^{c}$ | $1.20^{a}$ | $1.08^{bc}$ | $577.00^{b}$ | $594.00^{a}$ | $319.00^{a}$ | $121.00^{b}$ |
| *L. orientalis* IG 72611 | $0.22^{c}$ | $0.32^{bc}$ | $0.95^{b}$ | $1.00^{c}$ | $283.00^{c}$ | $183.00^{c}$ | $39.00^{c}$ | $19.00^{c}$ |
| *L. tomentosus* IG 72805 | $0.26^{b}$ | $0.40^{a}$ | $1.06^{ab}$ | $1.47^{a}$ | $82.00^{d}$ | $62.00^{c}$ | $26.00^{c}$ | $15.00^{c}$ |
| *L. odemensis* IG 72623 | $0.19^{cd}$ | $0.27^{c}$ | $1.20^{a}$ | $1.07^{c}$ | $348.00^{c}$ | $363.00^{b}$ | $159.00^{b}$ | $142.00^{b}$ |
| *L. lamottei* IG 110813 | $0.28^{b}$ | $0.39^{ab}$ | $1.08^{ab}$ | $1.30^{ab}$ | $47.00^{d}$ | $15.00^{c}$ | $69.00^{bc}$ | $34.00^{c}$ |
| *L. ervoides* L01-827A | $0.16^{d}$ | $0.20^{d}$ | $1.07^{ab}$ | $1.20^{bc}$ | $25.00^{d}$ | $7.00^{c}$ | $40.00^{c}$ | $17.00^{c}$ |

Means within columns followed by same letters are not significantly different according to LSD at P 0.05.

SSM4: Sunshine Mix # 4; GG: Greens Grade. Fw: Fully-watered; Dt: Drought.

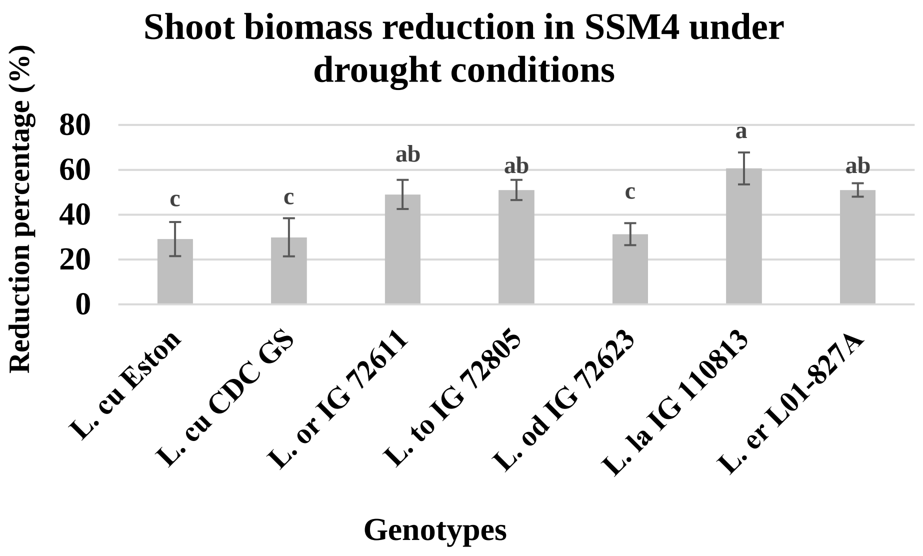

**Fig. 9.1.** Comparison of shoot biomass reduction among 7 lentil genotypes grown in Sunshine Mix # 4 (SSM4)

*L. cu*: *Lens culinaris*; *L. or*: *Lens orientalis*; *L. to*: *Lens tomentosus*; *L. od*: *Lens odemensis*; *L. la*: *Lens lamottei*; *L. er*: *Lens ervoides*].

Genotypes with same letters are not significantly different. Bars indicate standard error of means]. Subsequent figures have the same order of genotypes.

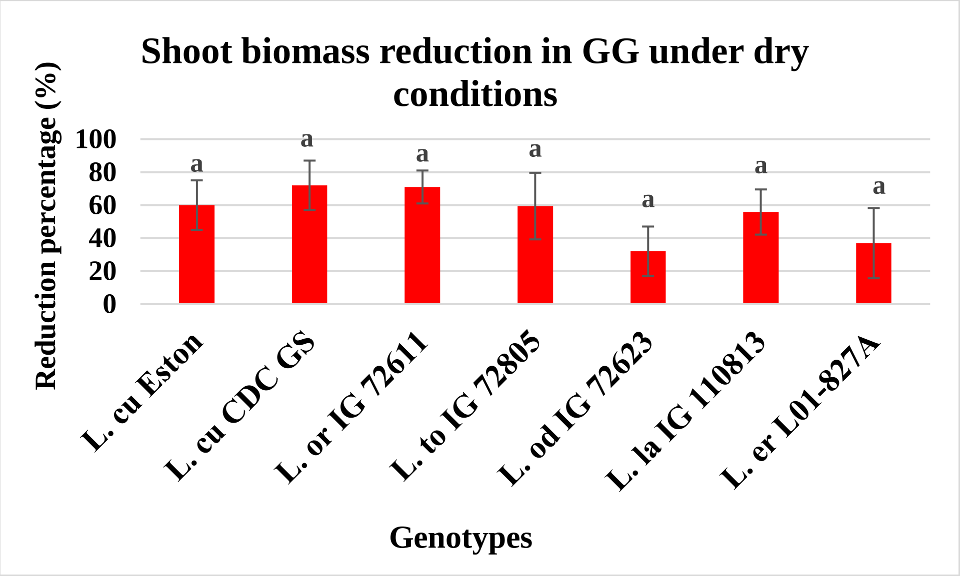

**Fig. 9.2.** Comparison of shoot biomass reduction among 7 lentil genotypes grown in Greens Grade® (GG)

Genotypes with same letters are not significantly different. Bars indicate standard error of means].

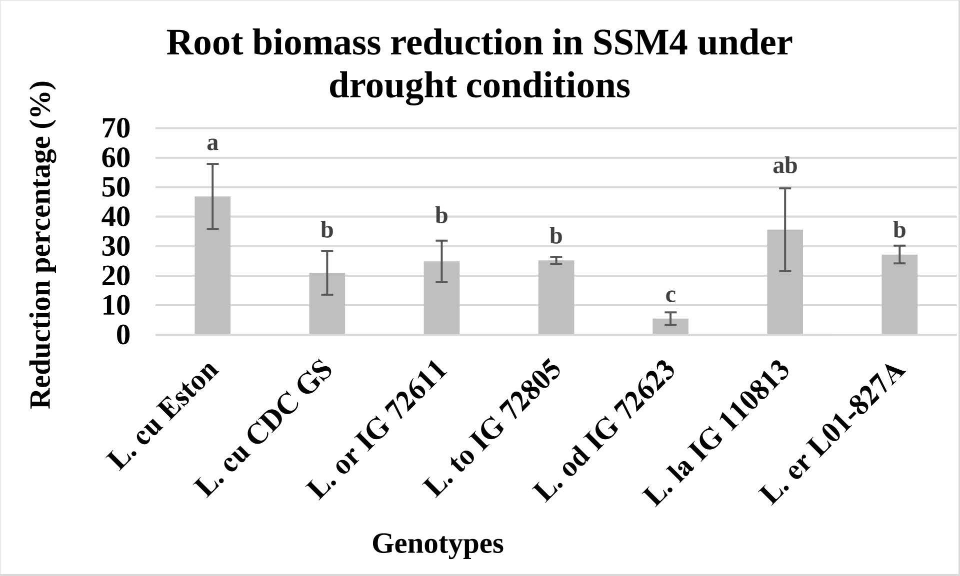

**Fig. 9.3.** Comparison of root biomass reduction among 7 lentil genotypes grown in Sunshine Mix # 4 (SSM4)
Genotypes with same letters are not significantly different. Bars indicate standard error of means].

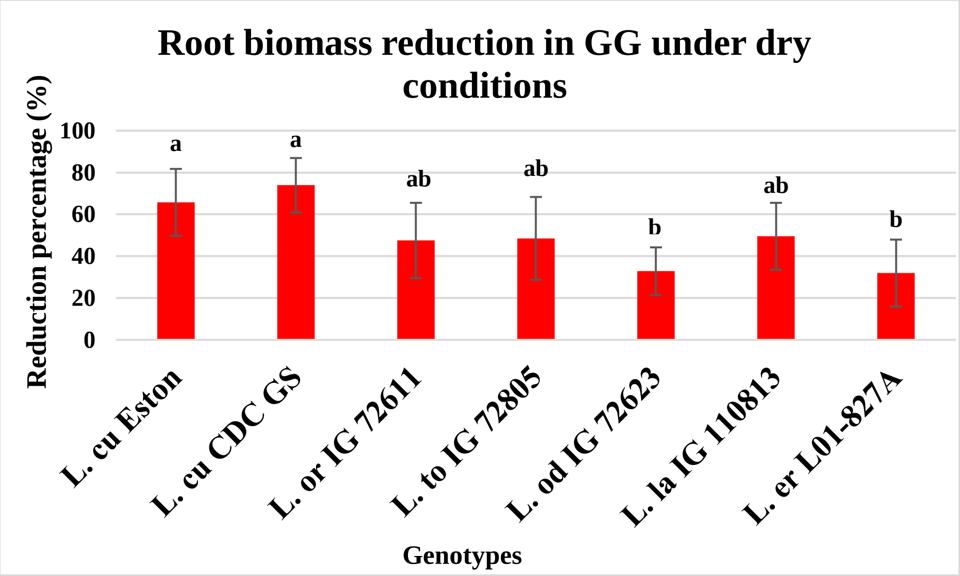

**Fig. 9.4.** Comparison of root biomass reduction among 7 lentil genotypes grown in Greens Grade® (GG)
Genotypes with same letters are not significantly different. Bars indicate standard error of means].