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***Original article***

# Physiology of lentil (*Lens culinaris* Medik.) genotypes against freezing stress

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## *Extended abstract*

### Introduction

Lentil is usually sowned in late winter and early spring. In this conditions, flowering and seed filling stage usually coincide with late drought stress and high temperature which reduce seed yield. Therefore, supplemental irrigation at the end of the growth season is necessary to overcome this problem. But since in Iran, lentil is mostly cultivated as a rainfed crop in mountain areas and slopes, supplemental irrigation is impossible. By autumn cultivation, plant maturity could be accelerated and higher efficiency of precipitation use, coincidence of growth stages with favorable weather conditions and finally increasing plant height and yield could be achieved. On the other hand, autumn cultivation exposes the plants to sever winter coldness; so, using freezing tolerant cultivars is necessary. The aim of the present study was to identify the role of antioxidants and physiological parameters in improving the cold tolerance of lentil genotypes in controlled conditions.

### Materials and methods

The study was conducted in autumn and winter of 2018 in research greenhouse of research center for plant sciences, Ferdowsi University of Mashhad, Mashhad, Iran. the experiment was conducted as factorial based on completely randomized design with three replications in controlled conditions. Experimental factors were consisted of 20 lentil genotypes and three freezing temperatures (0, -18 and -20$^0$C). Seeds were sown in October and Seedlings were kept in a natural environment for four months and then were transferred to a thermogradiant freezer. Gas exchange parameters (photosynthesis rate, evapotranspiration, substomatal CO2 concentration, stomatal and mesophilic conductance) as well as water use efficiency, relative water content of leaf, photosynthetic pigments, DPPH radical scavenging activity, Anthocyanin, total phenol and soluble carbohydrates were measured before the plants were exposed to freezing temperatures. Survival percentage, plant height, leaf area and plant dry weight were measured four weeks after freezing stress. Lethal temperature 50% of plants according to the survival

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percentage (LT50su), Reduced temperature 50% of height plant (HT50), Reduced temperature 50% of leaf area (RLAT50) and Reduced temperature 50% of dry matter (RDMT50) were also determined.

**Results**

Results indicated that in -18ºC, 14 genotypes had 100% survival while in -20ºC, all the genotypes were killed. Based on the studied parameters, lentil genotypes were divided in four groups by cluster analysis. Survival percentage in first, second and fourth group was superior compared to the total average. According to the antioxidant activity, metabolites and pigments concentration, plant height, leaf area and dry weight, genotypes in the first (MLC84, MLC407, MLC454) and second (MLC38, MLC303, MLC74, MLC334) groups were relatively superior compared to the other groups. Principal component analysis (PCA) showed that the first component explained 32.77% of changes in water use efficiency, carotenoids, chlorophyll a/b ratio, mesophilic conductance, photosynthesis, survival percentage, plant height, leaf area and dry weight while the second component explained 16.31% of changes in the concentration of chlorophylls a and b, total pigments, soluble carbohydrates, DPPH radical scavenging activity, phenol, anthocyanin, osmotic potential, evapotranspiration and stomatal conduction. Genotypes of MLC74, MLC334, MLC11, MLC84, MLC454 and MLC407 were better in terms of antioxidant capacity and metabolites and MLC303, MLC17, MLC38, MLC286and MLC469 were superior according to the survival percentage and regrowth.

**Conclusion**

The correlation between concentration of chlorophyll b, carotenoids and photosynthesis rate with survival percentage showed that these traits are suitable indicators for determining cold tolerance of lentil genotypes before exposing to freezing stress. Results of cluster analysis and group mean comparison also indicated the relative superiority of MLC84, MLC407, MLC454 and second MLC38, MLC303, MLC74, MLC334 in most studied parameters. Generally, these genotypes are recommended to be used for complementary studies of freezing tolerance in field conditions in cold regions.

***Keywords*:** Antioxidant activity, Metabolites, Osmotic potentials, Survival

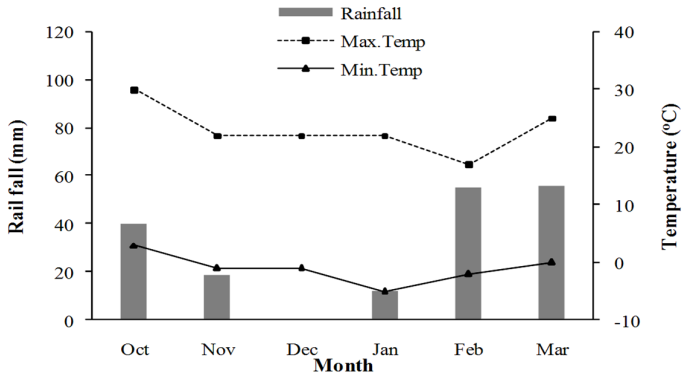


**Fig. 1. Rainfall, minimum and maximum temperature during fall and winter in 2018-2019, Mashhad**

**Table 1. Interaction effects of genotype and freezing temperature on survival percentage of lentil genotypes after freezing under controlled conditions**

| Genotype | Survival percentage | | |
|---|---|---|---|
| | Freezing temperature (°C) | | |
| | 0 | -18 | -20 |
| **MLC8**† | 100[a] | 53.0[c] | 0.00[d] |
| **MLC11** | 100[a] | 100[a] | 0.00[d] |
| **MLC13** | 100[a] | 100[a] | 0.00[d] |
| **MLC17** | 100[a] | 100[a] | 0.00[d] |
| **MLC33** | 100[a] | 100[a] | 0.00[d] |
| **MLC38** | 100[a] | 100[a] | 0.00[d] |
| **MLC47** | 100[a] | 100[a] | 0.00[d] |
| **MLC70** | 100[a] | 100[a] | 0.00[d] |
| **MLC74** | 100[a] | 67.3[b] | 0.00[d] |
| **MLC83** | 100[a] | 0.00[d] | 0.00[d] |
| **MLC84** | 100[a] | 100[a] | 0.00[d] |
| **MLC103** | 100[a] | 0.00[d] | 0.00[d] |
| **MLC286** | 100[a] | 100[a] | 0.00[d] |
| **MLC303** | 100[a] | 100[a] | 0.00[d] |
| **MLC334** | 100[a] | 100[a] | 0.00[d] |
| **MLC407** | 100[a] | 100[a] | 0.00[d] |
| **MLC409** | 100[a] | 0.00[d] | 0.00[d] |
| **MLC454** | 100[a] | 64.0[b] | 0.00[d] |
| **MLC469** | 100[a] | 100[a] | 0.00[d] |
| **MLC472** | 100[a] | 100[a] | 0.00[d] |
| **S.O.V** | **df** | **Mean squares** | |
| **Genotype (G)** | 19 | 1363[**] | |
| **Temperature** | 2 | 167091[**] | |
| **G×T** | 38 | 1364[**] | |
| **Error** | 120 | 33.4 | |
| **C.V (%)** | - | *9.67* | |

† MLC: Mashhad Lentil Collection

Means fallowed by the same letter are not significantly different ($p<0.05$). **:Significant ($P\leq 0.01$), C.V: Coefficient Variation.

**Table 2. Photosynthetic properties and mesophyll conductance in lentil genotypes before freezing stress under control condition**

| Genotype | | Photosynthetic rate | Evapotranspiration rate | Stomatal conductance | $CO_2$ substomatal | Mesophyll conductance |
|---|---|---|---|---|---|---|
| | | ($\mu mol.m^{-2}s^{-1}$) | ($mmol.m^{-2}s^{-1}$) | ($mol.m^{-2}s^{-1}$) | (ppm) | ($mol.m^{-2}s^{-1}$) |
| **MLC8**† | | $17.6^{f}$ | $3.25^{c-g}$ | $0.394^{i-l}$ | $393^{a}$ | $0.051^{b-e}$ |
| **MLC11** | | $15.3^{gh}$ | $1.70^{gh}$ | $0.364^{j-k}$ | $386^{a}$ | $0.034^{e-g}$ |
| **MLC13** | | $11.0^{jk}$ | $2.04^{d-h}$ | $0.516^{g-i}$ | $430^{a}$ | $0.022^{g}$ |
| **MLC17** | | $18.6^{ef}$ | $2.80^{c-h}$ | $0.671^{c-f}$ | $436^{a}$ | $0.041^{d-g}$ |
| **MLC33** | | $16.5^{f-h}$ | $2.94^{c-h}$ | $0.679^{c-f}$ | $418^{a}$ | $0.034^{e-g}$ |
| **MLC38** | | $30.7^{a}$ | $7.47^{a}$ | $1.44^{a}$ | $429^{a}$ | $0.064^{ab}$ |
| **MLC47** | | $14.8^{hi}$ | $2.52^{d-h}$ | $0.489^{h-j}$ | $406^{a}$ | $0.028^{fg}$ |
| **MLC70** | | $17.9^{f}$ | $1.39^{h}$ | $0.434^{i-l}$ | $402^{a}$ | $0.044^{c-f}$ |
| **MLC74** | | $16.9^{fg}$ | $5.54^{b}$ | $0.789^{bc}$ | $420^{a}$ | $0.043^{c-f}$ |
| **MLC83** | | $12.0^{j}$ | $3.62^{cd}$ | $0.844^{b}$ | $430^{a}$ | $0.034^{e-g}$ |
| **MLC84** | | $25.0^{c}$ | $3.44^{c-e}$ | $0.635^{e-g}$ | $394^{a}$ | $0.064^{ab}$ |
| **MLC103** | | $12.4^{j}$ | $2.85^{c-h}$ | $0.441^{i-k}$ | $427^{a}$ | $0.050^{b-e}$ |
| **MLC286** | | $17.9^{f}$ | $2.32^{d-h}$ | $0.655^{d-f}$ | $421^{a}$ | $0.069^{ab}$ |
| **MLC303** | | $29.0^{a}$ | $3.38^{c-f}$ | $0.784^{b-d}$ | $386^{a}$ | $0.071^{a}$ |
| **MLC334** | | $14.6^{hi}$ | $4.18^{bc}$ | $0.752^{b-e}$ | $409^{a}$ | $0.033^{e-g}$ |
| **MLC407** | | $27.0^{b}$ | $3.16^{c-g}$ | $0.601^{f-h}$ | $383^{a}$ | $0.072^{a}$ |
| **MLC409** | | $9.19^{k}$ | $2.19^{d-h}$ | $0.306^{l}$ | $382^{a}$ | $0.023^{g}$ |
| **MLC454** | | $20.8^{d}$ | $3.02^{c-g}$ | $0.423^{i-l}$ | $384^{a}$ | $0.061^{a-c}$ |
| **MLC469** | | $13.0^{ij}$ | $1.91^{e-h}$ | $0.442^{i-k}$ | $417^{a}$ | $0.035^{d-g}$ |
| **MLC472** | | $19.9^{de}$ | $1.78^{f-h}$ | $0.346^{kl}$ | $442^{a}$ | $0.053^{a-d}$ |
| **S.O.V** | **df** | | | **Mean squares** | | |
| **Genotype** | 19 | $107^{**}$ | $5.97^{**}$ | $0.196^{**}$ | $1159^{ns}$ | $0.001^{**}$ |
| **Error** | 40 | 1.26 | 0.693 | 0.005 | 1268 | 0.001 |
| **C.V (%)** | - | *6.24* | *27.1* | *11.6* | *8.69* | *16.0* |

† MLC: Mashhad Lentil Collection

Means fallowed by the same letter are not significantly different ($p<0.05$). ns: non- significant, **:Significant ($P\leq 0.01$), C.V: Coefficient Variation

**Table 3. Water use efficiency, relative water content, and osmotic potential in lentil genotypes before freezing stress under control condition**.

| Genotype | | WUE | RWC | Osmotic potential |
|---|---|---|---|---|
| | | ($\mu molCO_2.mMol\ H_2O$) | (%) | (MPa) |
| **MLC8**† | | 5.84$^{c-f}$ | 75.7$^{ab}$ | -2.80$^{d-f}$ |
| **MLC11** | | 8.53$^{b}$ | 69.0$^{a-e}$ | -3.65$^{bc}$ |
| **MLC13** | | 5.49$^{c-f}$ | 76.0$^{ab}$ | -2.02$^{f}$ |
| **MLC17** | | 6.02$^{c-e}$ | 70.7$^{a-e}$ | -2.64$^{d-f}$ |
| **MLC33** | | 5.95$^{c-e}$ | 77.0$^{ab}$ | -2.54$^{d-f}$ |
| **MLC38** | | 4.16$^{e-g}$ | 79.0$^{a}$ | -2.90$^{c-e}$ |
| **MLC47** | | 6.53$^{b-e}$ | 73.7$^{a-c}$ | -3.14$^{b-d}$ |
| **MLC70** | | 13.4$^{a}$ | 76.7$^{ab}$ | -2.27$^{ef}$ |
| **MLC74** | | 3.06$^{g}$ | 59.7$^{e}$ | -3.09$^{b-e}$ |
| **MLC83** | | 2.90$^{g}$ | 68.7$^{a-e}$ | -2.75$^{d-f}$ |
| **MLC84** | | 7.32$^{b-d}$ | 68.3$^{a-e}$ | -2.78$^{d-f}$ |
| **MLC103** | | 4.37$^{e-g}$ | 75.0$^{ab}$ | -2.53$^{d-f}$ |
| **MLC286** | | 6.37$^{b-e}$ | 67.7$^{a-e}$ | -2.66$^{d-f}$ |
| **MLC303** | | 8.68$^{b}$ | 68.0$^{a-e}$ | -5.05$^{a}$ |
| **MLC334** | | 3.55$^{fg}$ | 66.0$^{b-e}$ | -2.79$^{d-f}$ |
| **MLC407** | | 7.60$^{bc}$ | 61.3$^{de}$ | -2.47$^{d-f}$ |
| **MLC409** | | 4.60$^{e-g}$ | 62.7$^{c-e}$ | -3.81$^{b}$ |
| **MLC454** | | 6.44$^{b-e}$ | 78.0$^{a}$ | -2.92$^{c-e}$ |
| **MLC469** | | 5.10$^{d-g}$ | 74.0$^{ab}$ | -2.78$^{d-f}$ |
| **MLC472** | | 13.9$^{a}$ | 71.7$^{a-d}$ | -2.30$^{ef}$ |
| **S.O.V** | **df** | | **Mean squares** | |
| **Genotype** | 19 | 26.2$^{**}$ | 96.9$^{**}$ | 1.31$^{**}$ |
| **Error** | 40 | 1.52 | 33.3 | 0.175 |
| **C.V (%)** | - | 19.0 | 8.14 | 14.5 |

† MLC: Mashhad Lentil Collection

Means fallowed by the same letter are not significantly different ($p<0.05$). **:Significant ($P\leq 0.01$), C.V: Coefficient Variation

**Table 4. Photosynthesis pigments in lentil genotypes before freezing stress under control condition**

| Genotype | | Cha | Chb | Cartenoieds | Cha/Chb | Total pigment |
|---|---|---|---|---|---|---|
| | | ----------mg.gfw$^{-1}$---------- | | | | mg.gfw$^{-1}$ |
| **MLC8**† | | 0.768$^{j}$ | 0.473$^{ij}$ | 0.205$^{f}$ | 1.86$^{a}$ | 1.55$^{h-j}$ |
| **MLC11** | | 1.27d$^{-f}$ | 0.690$^{ef}$ | 0.388$^{b-d}$ | 1.84$^{a}$ | 2.34$^{c-f}$ |
| **MLC13** | | 0.847$^{ij}$ | 0.460$^{ij}$ | 0.292$^{e}$ | 1.78$^{a}$ | 1.43$^{ij}$ |
| **MLC17** | | 1.15$^{e-h}$ | 0.745$^{de}$ | 0.354$^{d}$ | 1.93$^{a}$ | 2.34$^{c-f}$ |
| **MLC33** | | 0.858$^{h-j}$ | 0.482$^{ij}$ | 0.289$^{e}$ | 2.04$^{a}$ | 1.54$^{h-j}$ |
| **MLC38** | | 1.18$^{e-g}$ | 0.760$^{cd}$ | 0.386$^{b-d}$ | 2.00$^{a}$ | 2.61$^{cd}$ |
| **MLC47** | | 0.987$^{f-j}$ | 0.475$^{ij}$ | 0.380$^{cd}$ | 1.83$^{a}$ | 1.84$^{f-i}$ |
| **MLC70** | | 1.09$^{e-i}$ | 0.621$^{gh}$ | 0.381$^{cd}$ | 2.23$^{a}$ | 1.97$^{e-h}$ |
| **MLC74** | | 1.48$^{cd}$ | 0.673$^{fg}$ | 0.443$^{b}$ | 2.07$^{a}$ | 2.75$^{bc}$ |
| **MLC83** | | 0.965$^{g-j}$ | 0.351$^{k}$ | 0.296$^{e}$ | 1.96$^{a}$ | 1.30$^{j}$ |
| **MLC84** | | 1.81$^{b}$ | 0.837$^{b}$ | 0.495$^{a}$ | 1.85$^{a}$ | 3.13$^{b}$ |
| **MLC103** | | 0.884$^{g-j}$ | 0.440$^{j}$ | 0.235$^{f}$ | 1.85$^{a}$ | 1.74$^{g-j}$ |
| **MLC286** | | 1.34$^{c-e}$ | 0.647$^{f-h}$ | 0.394$^{b-d}$ | 2.10$^{a}$ | 2.37$^{c-e}$ |
| **MLC303** | | 1.12$^{e-i}$ | 0.609$^{h}$ | 0.392$^{b-d}$ | 1.99$^{a}$ | 2.13$^{d-g}$ |
| **MLC334** | | 1.02$^{f-j}$ | 0.734$^{de}$ | 0.301$^{e}$ | 1.79$^{a}$ | 2.32$^{c-f}$ |
| **MLC407** | | 1.85$^{b}$ | 0.928$^{a}$ | 0.546$^{a}$ | 2.33$^{a}$ | 3.77$^{a}$ |
| **MLC409** | | 1.15$^{e-h}$ | 0.512$^{i}$ | 0.295$^{e}$ | 2.20$^{a}$ | 2.30$^{c-f}$ |
| **MLC454** | | 2.35$^{a}$ | 0.801$^{bc}$ | 0.426$^{bc}$ | 2.14$^{a}$ | 3.84$^{a}$ |
| **MLC469** | | 1.60$^{bc}$ | 0.665$^{f-h}$ | 0.541$^{a}$ | 2.43$^{a}$ | 2.80$^{bc}$ |
| **MLC472** | | 1.10$^{e-i}$ | 0.468$^{ij}$ | 0.384$^{b-d}$ | 2.35$^{a}$ | 1.92$^{e-i}$ |
| **S.O.V** | **df** | | | **Mean squares** | | |
| **Genotype(G)** | 19 | 0.481$^{**}$ | 0.073$^{**}$ | 0.025$^{**}$ | 0.118$^{ns}$ | 1.50$^{**}$ |
| **Error** | 40 | 0.024 | 0.001 | 0.001 | 0.113 | 0.073 |
| **C.V (%)** | - | 12.6 | 4.89 | 10.4 | 16.6 | 11.7 |

† MLC: Mashhad Lentil Collection

Means fallowed by the same letter are not significantly different ($p<0.05$). ns: non- significant, **:Significant ($P\leq 0.01$), C.V: Coefficient Variation

**Table 5. DPPH, antocyanin, phenol and soluble carbohydrates in lentil genotypes before freezing stress under control condition**

| Genotype | DPPH<br>DPPH | Antocyanin | Phenol | Soluble carbohydrates |
|---|---|---|---|---|
| | mg.gfw$^{-1}$ | mmol.gfw$^{-1}$ | ----------mg.gfw$^{-1}$---------- | |
| [1]**MLC8** | 1.180$^{c}$ | 1.400$^{b}$ | 159$^{d-h}$ | 0.109$^{i}$ |
| **MLC11** | 1.060$^{d}$ | 1.640$^{a}$ | 174$^{c-g}$ | 0.250$^{de}$ |
| **MLC13** | 0.599$^{g}$ | 0.782$^{ef}$ | 99$^{j}$ | 0.116i |
| **MLC17** | 0.960$^{de}$ | 0.585$^{gh}$ | 127$^{h-j}$ | 0.161$^{gh}$ |
| **MLC33** | 0.579$^{g}$ | 0.486$^{hi}$ | 105$^{j}$ | 0.117$^{i}$ |
| **MLC38** | 0.670$^{g}$ | 0.566$^{gh}$ | 136$^{g-j}$ | 0.236$^{e}$ |
| **MLC47** | 0.967$^{de}$ | 0.758$^{ef}$ | 187$^{b-f}$ | 0.132$^{hi}$ |
| **MLC70** | 1.010$^{d}$ | 0.630$^{f-h}$ | 165$^{c-h}$ | 0.120$^{i}$ |
| **MLC74** | 1.890$^{a}$ | 0.973$^{d}$ | 162$^{d-h}$ | 0.331$^{b}$ |
| **MLC83** | 0.826$^{f}$ | 0.881$^{de}$ | 222$^{b}$ | 0.132$^{hi}$ |
| **MLC84** | 0.834$^{f}$ | 0.652$^{fg}$ | 160$^{d-h}$ | 0.338$^{b}$ |
| **MLC103** | 0.452$^{h}$ | 0.334$^{i}$ | 110$^{ij}$ | 0.121$^{i}$ |
| **MLC286** | 0.871$^{ef}$ | 0.659$^{fg}$ | 149$^{f-i}$ | 0.110$^{i}$ |
| **MLC303** | 0.982$^{de}$ | 0.351$^{i}$ | 206$^{bc}$ | 0.188$^{fg}$ |
| **MLC334** | 1.680$^{b}$ | 1.530$^{ab}$ | 201$^{b-d}$ | 0.293$^{c}$ |
| **MLC407** | 1.051$^{d}$ | 1.140$^{c}$ | 225$^{ab}$ | 0.448$^{a}$ |
| **MLC409** | 0.623$^{g}$ | 0.481$^{hi}$ | 173$^{c-g}$ | 0.282$^{cd}$ |
| **MLC454** | 0.595$^{g}$ | 0.758$^{ef}$ | 261$^{a}$ | 0.352$^{b}$ |
| **MLC469** | 0.813$^{f}$ | 0.947$^{d}$ | 196$^{b-e}$ | 0.217$^{ef}$ |
| **MLC472** | 0.636$^{g}$ | 0.361$^{i}$ | 156$^{e-h}$ | 0.126$^{hi}$ |
| **S.O.V** | **df** | **Mean squares** | | |
| **Genotype** | 19 | 0.384** | 0.435** | 5357** | 31225** |
| **Error** | 40 | 0.005 | 0.008 | 492 | 406 |
| **C.V (%)** | - | *7.66* | *11.2* | *13.2* | *9.65* |

[†] MLC: Mashhad Lentil Collection

Means fallowed by the same letter are not significantly different ($p<0.05$). **:Significant ($P\leq 0.01$), C.V: Coefficient Variation

**Table 6. Interaction effects of genotype and freezing temperature on Plant height, Leaf area and dry weight of lentil genotypes after freezing under controlled conditions**

| | **Plant Height** (cm) | | | **Leaf area** ($mm^2.plant^{-1}$) | | | **Biomass** ($mg.plant^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Freezing temperature** (°C) | | | **Freezing temperature** (°C) | | | **Freezing temperature** (°C) | | |
| **Genotype** | 0 | -18 | -20 | 0 | -18 | -20 | 0 | -18 | -20 |
| **MLC8**† | 17.9$^{a-e}$ | 4.59$^{l}$ | 0.00$^{m}$ | 377$^{m-p}$ | 247$^{q}$ | 0.00$^{r}$ | 50.0$^{i-p}$ | 46.7$^{m-q}$ | 0.00$^{s}$ |
| **MLC11** | 16.8$^{b-i}$ | 17.1$^{b-g}$ | 0.00$^{m}$ | 382$^{m-p}$ | 584$^{b-g}$ | 0.00$^{r}$ | 68.9$^{c-e}$ | 47.5$^{l-p}$ | 0.00$^{s}$ |
| **MLC13** | 15.9$^{c-j}$ | 16.8$^{b-i}$ | 0.00$^{m}$ | 584$^{b-g}$ | 519$^{f-j}$ | 0.00$^{r}$ | 59.6$^{e-l}$ | 44.7$^{n-q}$ | 0.00$^{s}$ |
| **MLC17** | 16.9$^{b-h}$ | 17.8$^{a-f}$ | 0.00$^{m}$ | 571$^{b-h}$ | 591$^{b-g}$ | 0.00$^{r}$ | 92.9$^{a}$ | 58.9$^{e-m}$ | 0.00$^{s}$ |
| **MLC33** | 13.9$^{h-j}$ | 15.6$^{d-j}$ | 0.00$^{m}$ | 524$^{f-j}$ | 436$^{j-n}$ | 0.00$^{r}$ | 66.8$^{c-f}$ | 72.0$^{b-d}$ | 0.00$^{s}$ |
| **MLC38** | 14.6$^{g-j}$ | 15.1$^{d-j}$ | 0.00$^{m}$ | 351$^{n-p}$ | 508$^{g-k}$ | 0.00$^{r}$ | 60.9$^{d-j}$ | 57.9$^{e-m}$ | 0.00$^{s}$ |
| **MLC47** | 15.1$^{d-j}$ | 13.9$^{h-j}$ | 0.00$^{m}$ | 542$^{e-i}$ | 647$^{bc}$ | 0.00$^{r}$ | 48.6$^{j-p}$ | 35.7$^{q}$ | 0.00$^{s}$ |
| **MLC70** | 14.9$^{d-j}$ | 11.0$^{k}$ | 0.00$^{m}$ | 531$^{f-i}$ | 238$^{q}$ | 0.00$^{r}$ | 38.3$^{pq}$ | 51.8$^{i-o}$ | 0.00$^{s}$ |
| **MLC74** | 13.7$^{ij}$ | 6.75$^{l}$ | 0.00$^{m}$ | 656$^{b}$ | 229$^{q}$ | 0.00$^{r}$ | 48.0$^{k-p}$ | 18.8$^{r}$ | 0.00$^{s}$ |
| **MLC83** | 17.3$^{a-g}$ | 0.00$^{m}$ | 0.00$^{m}$ | 533$^{f-i}$ | 0.00$^{r}$ | 0.00$^{r}$ | 51.0$^{i-o}$ | 0.00$^{s}$ | 0.00$^{s}$ |
| **MLC84** | 20.2$^{a}$ | 18.8$^{a-c}$ | 0.00$^{m}$ | 331$^{p}$ | 341$^{op}$ | 0.00$^{r}$ | 35.9$^{q}$ | 60.1$^{d-k}$ | 0.00$^{s}$ |
| **MLC103** | 17.8$^{a-f}$ | 0.00$^{m}$ | 0.00$^{m}$ | 553$^{c-h}$ | 0.00$^{r}$ | 0.00$^{r}$ | 40.7$^{o-q}$ | 0.00$^{s}$ | 0.00$^{s}$ |
| **MLC286** | 15.0$^{d-j}$ | 19.7$^{ab}$ | 0.00$^{m}$ | 632$^{b-e}$ | 640$^{b-d}$ | 0.00$^{r}$ | 74.4$^{bc}$ | 75.3$^{bc}$ | 0.00$^{s}$ |
| **MLC303** | 13.9$^{h-j}$ | 14.9$^{d-j}$ | 0.00$^{m}$ | 654$^{b}$ | 424$^{k-o}$ | 0.00$^{r}$ | 53.9$^{g-n}$ | 74.3$^{bc}$ | 0.00$^{s}$ |
| **MLC334** | 15.8$^{c-j}$ | 14.7$^{f-j}$ | 0.00$^{m}$ | 587$^{b-g}$ | 370$^{m-p}$ | 0.00$^{r}$ | 53.5$^{h-n}$ | 66.5$^{c-f}$ | 0.00$^{s}$ |
| **MLC407** | 17.5$^{a-g}$ | 17.7$^{a-g}$ | 0.00$^{m}$ | 837$^{a}$ | 481$^{h-l}$ | 0.00$^{r}$ | 97.2$^{a}$ | 51.9$^{i-o}$ | 0.00$^{s}$ |
| **MLC409** | 17.7$^{a-g}$ | 0.00$^{m}$ | 0.00$^{m}$ | 630$^{b-e}$ | 0.00$^{r}$ | 0.00$^{r}$ | 61.4$^{d-i}$ | 0.00$^{s}$ | 0.00$^{s}$ |
| **MLC454** | 14.9$^{e-j}$ | 9.88$^{k}$ | 0.00$^{m}$ | 604$^{b-f}$ | 397$^{l-p}$ | 0.00$^{r}$ | 55.5$^{f-n}$ | 82.3$^{b}$ | 0.00$^{s}$ |
| **MLC469** | 17.3$^{a-g}$ | 18.1$^{a-d}$ | 0.00$^{m}$ | 508$^{g-k}$ | 573$^{b-h}$ | 0.00$^{r}$ | 56.2$^{f-n}$ | 65.6$^{c-h}$ | 0.00$^{s}$ |
| **MLC472** | 13.6$^{j}$ | 16.2$^{c-j}$ | 0.00$^{m}$ | 453$^{i-m}$ | 546$^{d-h}$ | 0.00$^{r}$ | 65.9$^{c-g}$ | 56.0$^{f-n}$ | 0.00$^{s}$ |

| **S.O.V** | **df** | **Mean squares** | | |
|---|---|---|---|---|
| **Genotype(G)** | 19 | 42.9** | 55799** | 1110** |
| **Temperature (T)** | 2 | 4244** | 4683986** | 59247** |
| **G×T** | 38 | 49.2** | 60235** | 789** |
| **Error** | 120 | 2.45 | 2358 | 39.7 |
| **C.V (%)** | - | *16.5* | *15.7* | *17.6* |

† MLC: Mashhad Lentil Collection

Means fallowed by the same letter are not significantly different ($p<0.05$). **:Significant ($P\leq 0.01$), C.V: Coefficient Variation.

**Table 7. Lethal temperature 50% of plants according to the survival percentage ($LT_{50su}$), Reduced temperature 50% of height plant ($^{R}HT_{50}$), Reduced temperature 50% of leaf area ($RLAT_{50}$) and Reduced temperature 50% of dry matter ($RDMT_{50}$) in lentil genotypes after freezing stress under control condition**

| Genotype ژنوتیپ | $LT_{50su}$ | $RHT_{50}$ | $RLAT_{50}$ | $RDMT_{50}$ |
|---|---|---|---|---|
| **MLC8**† | -16.8$^{b}$ | -12.1$^{b}$ | -16.2$^{c}$ | -18.7$^{d}$ |
| **MLC11** | -19.0$^{c}$ | -18.8$^{d}$ | -18.4$^{c}$ | -18.5$^{d}$ |
| **MLC13** | -19.0$^{c}$ | -18.9$^{d}$ | -18.7$^{c}$ | -18.5$^{d}$ |
| **MLC17** | -19.0$^{c}$ | -18.9$^{d}$ | -19.0$^{c}$ | -18.3$^{d}$ |
| **MLC33** | -19.0$^{c}$ | -18.9$^{d}$ | -18.9$^{c}$ | -18.9$^{d}$ |
| **MLC38** | -19.0$^{c}$ | -18.8$^{d}$ | -19.0$^{c}$ | -18.8$^{d}$ |
| **MLC47** | -19.0$^{c}$ | -18.8$^{d}$ | -18.9$^{c}$ | -18.6$^{d}$ |
| **MLC70** | -19.0$^{c}$ | -17.8$^{d}$ | -18.9$^{c}$ | -19.0$^{d}$ |
| **MLC74** | -17.0$^{b}$ | -16.3$^{c}$ | -12.9$^{b}$ | -15.0$^{c}$ |
| **MLC83** | -9.00$^{a}$ | -9.03a | -9.03$^{a}$ | -9.07$^{a}$ |
| **MLC84** | -19.0$^{c}$ | -18.8$^{d}$ | -19.0$^{c}$ | -13.6$^{b}$ |
| **MLC103** | -9.00$^{a}$ | -8.97$^{a}$ | -11.3$^{ab}$ | -9.00$^{a}$ |
| **MLC286** | -19.0$^{c}$ | -19.0$^{d}$ | -16.2$^{c}$ | -18.9$^{d}$ |
| **MLC303** | -19.0$^{c}$ | -19.0$^{d}$ | -18.9$^{c}$ | -18.9$^{d}$ |
| **MLC334** | -19.0$^{c}$ | -18.8$^{d}$ | -19.0$^{c}$ | -18.9$^{d}$ |
| **MLC407** | -19.0$^{c}$ | -18.9$^{d}$ | -18.4$^{c}$ | -17.8$^{d}$ |
| **MLC409** | -9.00$^{a}$ | -9.20$^{a}$ | -10.0$^{ab}$ | -9.03$^{a}$ |
| **MLC454** | -17.5$^{bc}$ | -18.5$^{d}$ | -18.4$^{c}$ | -13.7$^{b}$ |
| **MLC469** | -19.0$^{c}$ | -19.0$^{d}$ | -18.9$^{c}$ | -19.0$^{d}$ |
| **MLC472** | -19.0$^{c}$ | -18.9$^{d}$ | -19.0$^{c}$ | -18.7$^{d}$ |
| **S.O.V** | df | Mean squares | | |
| **Genotype** | 19 | 39.1$^{**}$ | 41.4$^{**}$ | 44.1$^{**}$ | 40.3$^{**}$ |
| **Error** | 40 | 1.02 | 0.809 | 4.11 | 0.464 |
| **C.V (%)** | - | -5.87 | -5.33 | -12.0 | -4.11 |

† MLC: Mashhad Lentil Collection

Means fallowed by the same letter are not significantly different ($p<0.05$). **:Significant ($P\leq 0.01$), C.V: Coefficient Variation

**Table 8. Correlation matrix of lentil genotypes before freezing stress under controlled condition**

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | **Survival** | 1 | | | | | | | | | |
| **2** | **Photosynthetic rate** | 0.48$^{*}$ | 1 | | | | | | | | |
| **3** | **Evapotranspiration rate** | -0.04$^{ns}$ | 0.47$^{*}$ | 1 | | | | | | | |
| **4** | **$CO_2$ substomatal** | 0.03$^{ns}$ | -0.20$^{ns}$ | 0.18$^{ns}$ | 1 | | | | | | |
| **5** | **Stomatal conductance** | 0.17$^{ns}$ | 0.54$^{*}$ | 0.89$^{**}$ | 0.32$^{ns}$ | 1 | | | | | |
| **6** | **Mesophyll conductance** | 0.27$^{ns}$ | 0.85$^{**}$ | 0.31$^{ns}$ | -0.21$^{ns}$ | 0.31$^{ns}$ | 1 | | | | |
| **7** | **WUE** | 0.44$^{ns}$ | 0.29$^{ns}$ | -0.57$^{**}$ | -0.11$^{ns}$ | -0.40$^{ns}$ | 0.29$^{ns}$ | 1 | | | |
| **8** | **RWC** | 0.14$^{ns}$ | 0.02$^{ns}$ | -0.11$^{ns}$ | 0.26$^{ns}$ | 0.02$^{ns}$ | -0.07$^{ns}$ | 0.18$^{ns}$ | 1 | | |
| **9** | **Osmotic potential** | -0.07$^{ns}$ | 0.22$^{ns}$ | 0.07$^{ns}$ | -0.50$^{*}$ | 0.06$^{ns}$ | 0.15$^{ns}$ | -0.04$^{ns}$ | -0.34$^{ns}$ | 1 | |
| **10** | **Ch a** | 0.17$^{ns}$ | 0.39$^{ns}$ | 0.10$^{ns}$ | -0.42$^{ns}$ | -0.05$^{ns}$ | 0.50$^{*}$ | 0.05$^{ns}$ | -0.20$^{ns}$ | 0.04$^{ns}$ | 1 |
| **11** | **Ch b** | 0.49$^{*}$ | 0.61$^{**}$ | 0.29$^{ns}$ | -0.38$^{ns}$ | 0.25$^{ns}$ | 0.54$^{*}$ | 0.05$^{ns}$ | -0.26$^{ns}$ | 0.03$^{ns}$ | 0.76$^{**}$ |
| **12** | **Carotenoids** | 0.50$^{*}$ | 0.47$^{*}$ | 0.10$^{ns}$ | -0.22$^{ns}$ | 0.08$^{ns}$ | 0.49$^{*}$ | 0.23$^{ns}$ | -0.30$^{ns}$ | 0.09$^{ns}$ | 0.78$^{**}$ |
| **13** | **Ch a/Ch b** | 0.07$^{ns}$ | 0.14$^{ns}$ | -0.14$^{ns}$ | -0.04$^{ns}$ | -0.19$^{ns}$ | 0.26$^{ns}$ | 0.39$^{ns}$ | -0.12$^{ns}$ | -0.05$^{ns}$ | 0.43$^{ns}$ |
| **14** | **Total pigment** | 0.24$^{ns}$ | 0.50$^{*}$ | 0.23$^{ns}$ | -0.46$^{*}$ | 0.06$^{ns}$ | 0.54$^{*}$ | 0.01$^{ns}$ | -0.29$^{ns}$ | 0.08$^{ns}$ | 0.94$^{**}$ |
| **15** | **DPPH** | 0.20$^{ns}$ | 0.04$^{ns}$ | 0.31$^{ns}$ | -0.15$^{ns}$ | 0.16$^{ns}$ | -0.02$^{ns}$ | -0.18$^{ns}$ | -0.57$^{**}$ | 0.15$^{ns}$ | 0.03$^{ns}$ |
| **16** | **Anthocyanin** | 0.14$^{ns}$ | -0.17$^{ns}$ | 0.06$^{ns}$ | -0.33$^{ns}$ | -0.10$^{ns}$ | -0.19$^{ns}$ | -0.22$^{ns}$ | -0.24$^{ns}$ | -0.05$^{ns}$ | 0.08$^{ns}$ |
| **17** | **Phenol** | -0.10$^{ns}$ | 0.20$^{ns}$ | 0.06$^{ns}$ | -0.55$^{*}$ | -0.08$^{ns}$ | 0.28$^{ns}$ | 0.03$^{ns}$ | -0.30$^{ns}$ | 0.35$^{ns}$ | 0.60$^{**}$ |
| **18** | **Soluble carbohydrates** | 0.07$^{ns}$ | 0.36$^{ns}$ | 0.33$^{ns}$ | -0.54$^{*}$ | 0.11$^{ns}$ | 0.31$^{ns}$ | -0.18$^{ns}$ | -0.54$^{*}$ | 0.16$^{ns}$ | 0.77$^{**}$ |
| **19** | **Plant height** | 0.86$^{**}$ | 0.39$^{ns}$ | -0.13$^{ns}$ | -0.01$^{ns}$ | 0.08$^{ns}$ | 0.29$^{ns}$ | 0.30$^{ns}$ | 0.02$^{ns}$ | -0.11$^{ns}$ | 0.30$^{ns}$ |
| **20** | **Leaf area** | 0.73$^{**}$ | 0.27$^{ns}$ | -0.13$^{ns}$ | 0.02$^{ns}$ | 0.02$^{ns}$ | 0.22$^{ns}$ | 0.15$^{ns}$ | -0.11$^{ns}$ | 0.02$^{ns}$ | 0.28$^{ns}$ |
| **21** | **Dry weight** | 0.75$^{**}$ | 0.50$^{*}$ | -0.05$^{ns}$ | -0.07$^{ns}$ | 0.12$^{ns}$ | 0.45$^{*}$ | 0.29$^{ns}$ | 0.10$^{ns}$ | -0.02$^{ns}$ | 0.34$^{ns}$ |

**Table 8. Continued**

| | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **11 Ch b** | 1 | | | | | | | | | | |
| **12 Carotenoids** | 0.71$^{**}$ | 1 | | | | | | | | | |
| **13 Ch a/Ch b** | 0.20$^{ns}$ | 0.55$^{*}$ | 1 | | | | | | | | |
| **14 Total pigment** | 0.89$^{**}$ | 0.78$^{**}$ | 0.41$^{ns}$ | 1 | | | | | | | |
| **15 DPPH** | 0.29$^{ns}$ | 0.15$^{ns}$ | -0.16$^{ns}$ | 0.14$^{ns}$ | 1 | | | | | | |
| **16 Anthocyanin** | 0.26$^{ns}$ | 0.05$^{ns}$ | -0.25$^{ns}$ | 0.14$^{ns}$ | 0.62$^{**}$ | 1 | | | | | |
| **17 Phenol** | 0.35$^{ns}$ | 0.44$^{ns}$ | 0.3$^{2ns}$ | 0.54$^{*}$ | 0.26$^{ns}$ | 0.33$^{ns}$ | 1 | | | | |
| **18 Soluble carbohydrates** | 0.78$^{**}$ | 0.62$^{**}$ | 0.23$^{ns}$ | 0.87$^{**}$ | 0.33$^{ns}$ | 0.32$^{ns}$ | 0.55$^{*}$ | 1 | | | |
| **19 Plant height** | 0.58$^{**}$ | 0.58$^{**}$ | 0.04$^{ns}$ | 0.36$^{ns}$ | 0.01$^{ns}$ | 0.15$^{ns}$ | -0.09$^{ns}$ | 0.18$^{ns}$ | 1 | | |
| **20 Leaf area** | 0.41$^{ns}$ | 0.50$^{*}$ | 0.22$^{ns}$ | 0.34$^{ns}$ | 0.11$^{ns}$ | 0.07$^{ns}$ | 0.09$^{ns}$ | 0.13$^{ns}$ | 0.67$^{**}$ | 1 | |
| **21 Dry weight** | 0.56$^{*}$ | 0.40$^{ns}$ | 0.25$^{ns}$ | 0.41$^{ns}$ | -0.02$^{ns}$ | 0.09$^{ns}$ | 0.08$^{ns}$ | 0.16$^{ns}$ | 0.74$^{**}$ | 0.78$^{**}$ | 1 |

ns, * and **: non-significant and significant in the probability levels of 5%, and 1%, respectively

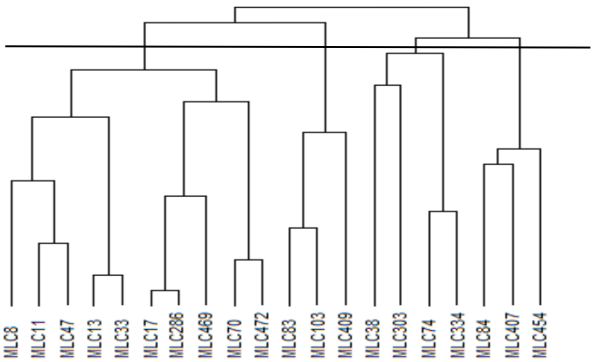


**Fig. 2. Cluster grouping of lentil genotypes based on studied characteristic under controlled conditions. MLC: Mashhad Lentil Collection**

**Table 9. Mean and deviation from mean of groups in cluster analysis for traits in Lentil genotypes under controlled conditions**

| | Group | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | |
| Genotypes | MLC84, MLC407, MLC454 | | MLC38, MLC303, MLC74, MLC334 | | MLC83, MLC103. MLC409 | | MLC8. MLC11, MLC13, MLC33, MLC47, MLC17, MLC286, MLC469, MLC70, MLC472 | |
| Traits صفات | Group mean | Deviation from mean | Group mean | Deviation from mean | Group mean | Deviation from mean | Group mean | Deviation from mean |
| Survival (%) | 62.7 | 2.92 | 63.9 | 4.20 | 33.3 | -26.4 | 65.1 | 5.37 |
| Photosynthetic rate ($\mu mol.m^{-2}s^{-1}$) | 24.2 | 6.25 | 22.8 | 4.79 | 11.17 | -6.82 | 16.2 | -1.74 |
| Evapotranspiration rate ($mmol.m^{-2}s^{-1}$) | 3.34 | 0.157 | 5.14 | 1.96 | 3.06 | -0.123 | 2.38 | -0.796 |
| $CO_2$ substomatal (ppm) | 387 | -22.4 | 411 | 1.27 | 413 | 3.16 | 415 | 5.27 |
| Stomatal conductance ($mmol.m^{-2}s^{-1}$) | 0.553 | -0.047 | 0.940 | 0.340 | 0.530 | -0.070 | 0.499 | -0.101 |
| Mesophyll conductance ($mmol.m^{-2}s^{-1}$) | 0.066 | 0.020 | 0.052 | 0.006 | 0.034 | -0.012 | 0.041 | -0.005 |
| WUE ($\mu molCO_2.mMol\ H_2O$) | 7.12 | 0.625 | 4.86 | -1.64 | 3.96 | -2.54 | 7.73 | 1.23 |
| RWC (%) | 69.2 | -1.711 | 68.2 | -2.77 | 68.8 | -2.16 | 73.2 | 2.27 |
| Osmotic potential (MPa) | 2.70 | -0.224 | 3.46 | 0.534 | 3.03 | 0.107 | 2.75 | -0.179 |
| Cha ($mg.gfw^{-1}$) | 2.00 | 0.763 | 1.20 | -0.040 | 1.00 | -0.241 | 1.10 | -0.140 |
| Chb ($mg.gfw^{-1}$) | 0.855 | 0.237 | 0.694 | 0.075 | 0.434 | -0.184 | 0.573 | -0.046 |
| Carotenoids ($mg.gfw^{-1}$) | 0.489 | 0.118 | 0.381 | 0.009 | 0.275 | -0.096 | 0.361 | -0.010 |
| Cha/Chb | 2.11 | 0.080 | 1.96 | -0.066 | 2.01 | -0.023 | 2.04 | 0.009 |
| Total pigment ($mg.gfw^{-1}$) | 3.58 | 1.28 | 2.45 | 0.153 | 1.78 | -0.519 | 2.01 | -0.289 |
| DPPH ($mg.gfw^{-1}$) | 0.827 | -0.087 | 1.30 | 0.390 | 0.634 | -0.280 | 0.87 | -0.046 |
| Anthocyanin ($mmol.gfw^{-1}$) | 0.851 | 0.055 | 0.85 | 0.059 | 0.565 | -0.230 | 0.83 | 0.029 |
| Phenol ($mg.gfw^{-1}$) | 215 | 47.6 | 176 | 8.51 | 168 | 0.733 | 150 | -17.9 |
| Soluble carbohydrates ($mg.gfw^{-1}$) | 379 | 170 | 262 | 53.2 | 178 | -30.5 | 146 | -63.2 |
| Plant height (cm) | 11.0 | 1.52 | 9.12 | -0.366 | 5.86 | -3.63 | 10.3 | 0.78 |
| Leaf area ($mm^2.plant^{1-}$) | 332 | 22.1 | 315 | 4.66 | 191 | -120 | 338 | 27.4 |
| Dry weight ($mg.plant^{-1}$) | 42.5 | 6.78 | 36.1 | 0.39 | 17.0 | -18.8 | 39.2 | 3.44 |

MLC: Mashhad Lentil Collection

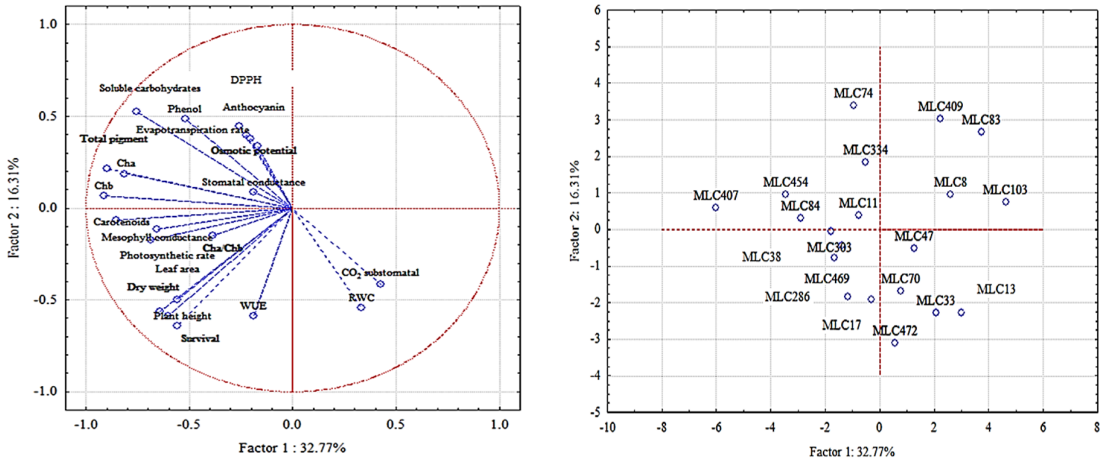


**Fig 3. Biplot based on two major principal component factors. MLC: Mashhad Lentil Collection**