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**Original Article**

# Evaluation of *Camelina sativa* Doubled Haploid Lines for the Response to Water-deficit Stress

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## Abstract

Camelina is low input and re-emergent medicinal and oilseed crop that it needs a lot of research to make the most of its genetic potential. This study aimed to evaluate the water deficit tolerance of six genotypes (Soheil cultivar and DH083, DH095, DH111, DH116, and DH133 doubled haploid lines) of camelina for some traits in the greenhouse and laboratory conditions. In the greenhouse condition, the response of camelina genotypes to irrigating regime (once every 4, 7 and 10 days) was investigated for some agro-morphological traits. This experiment was done in a randomized complete block design. Analysis of variance showed that the studied genotypes, as well as drought stress levels, were significantly different in terms of plant height, root length, root weight, grain yield, biological yield and harvest index. Mean comparison results also showed that the Soheil cultivar had the highest grain yield (3.03 g/m$^2$). At the laboratory section and the cotyledon culture and callus induction experiment, six lines were evaluated by four levels of PEG 6000 (0, 10, 20 and 30%) for water-deficit stress in a randomized completely design. The callus growth rate, relative callus growth rate and relative callus water content under stress conditions were also studied. There was a significant difference between studied genotypes in studied traits under stress conditions and between different stress levels. In this section, the Soheil cultivar was identified as high tolerant cultivar among other genotypes due to its relative water content (8.3%) and higher callus growth rate (4.2%). So the cultivar Soheil is considered as the least susceptible to dehydration in terms of greenhouse studies and callus induction.

**Keywords**: Water deficit, Callus, *Camelina sativa* L., Cotyledon

## Introduction

*Camelina sativa* L. is a medicinal plant that belongs to the Brassicaceae family that has many properties and applications. In nutrition and health, its oil contains high levels of omega-3, which can prevent cancer and obesity. It is used in the industry as biofuel, for the production of resins, waxes, as well as for the production of sanitary and pharmaceutical cosmetics [1]. Stresses are the most important cause of crop decline and drought is a major factor limiting crop production [2] and is also a serious threat to the world food supply, so one of the ways to counter that is to develop drought-tolerant cultivars [3]. Drought causes water loss and reduces water potential, which at the same time results in a decrease in cell turbidity. One of the fastest drought-induced processes is the closure of the stomachs. Prolonged drought stress causes severe stress in the plant, leading to an increase in abscisic acid and its response to osmotic regulation, reduction of root growth, reorganization of metabolism and activation of the antioxidant system. Many of these terms are measurable and are used to describe the severity of drought [4,5]. Plants can adapt to varying degrees of stress, and the best strategy to reach the resistant plant is to examine the plants' ability to withstand environmental stresses. Numerous techniques have been used to select drought-tolerant genotypes in plants. In recent years, many efforts have been made to complement these techniques through

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tissue culture. The tissue culture system is useful for assessing tolerance to environmental stresses because environmental stresses are easily controlled under in vitro conditions. When the plant tissue is growing in the medium, it can become a whole plant if it is subjected to stress and as a result tissue that can survive in stress conditions [6]. Tissue and plant cell culture is a useful tool for studying stress tolerance mechanisms under in vitro conditions in a confined space and for a short period [7].

An experiment conducted on five almond genotypes at different concentrations of polyethylene glycol (PEG) showed that with increasing PEG in MS medium, a significant decrease in fresh weight and leaf growth was observed [8]. Investigating the effect of drought stress on two bean cultivars, the results showed that morphological traits were changed and dry weight, stem length, root length and root dry weight decreased in high values of stress [9].

The results of the study of the responses of different genotypes of safflower at different levels of stress showed a decrease in relative water content with increasing stress [10]. In another experiment to measure the effect of drought stress on two wheat genotypes with stressful materials, the results showed that at high concentrations, the relative growth of callus decreased and proline and soluble carbohydrate levels increased [11]. Zebarjadi *et al* studied of safflower callus on mannitol stress medium showed that proline content was significantly increased, while relative water content and relative growth rate of callus decreased significantly [12]. This study was conducted to obtain tolerant lines to dehydration under in vitro and in vitro conditions in the camelina plant.

## Material and Methods

### Plant Material and Experimental Design

In this study, the plant material contained six genotypes of camelina, including Soheil cultivar and DH083, DH095, DH111, DH116, and DH133 doubled haploid lines. The studied genotypes were produced via hybridization and haplodiplodization by another culture and they obtained from the Biston Shafa Company. These genotypes were evaluated in greenhouses to investigate agro-morphological traits under different irrigating regimes (once every 4, 7 and 10 days). Then the experiment in the greenhouse condition was as factorial with two factors (genotypes and irrigating regime). This experiment was carried out in a randomized complete block design. Also, the genotypes were compared to callus induction in the presence of PGA through cotyledon culture. The experiment was conducted at the Research Greenhouse and the Tissue Culture Laboratory of Razi University Campus of Agriculture and Natural Resources.

### Seed Culture and Callus Induction

The camelina seeds were disinfected with 2% sodium hypochlorite for 10 m. Then, they were rinsed with sterile distilled water three times. Twenty disinfected camelina seeds were placed on MS medium containing 3% sucrose, 0.8% agar and pH 5.8 under the laminar hood. The cultured seeds were incubated at 25 °C at 16 h photoperiod and 8 h dark conditions. After 14 days, the cotyledon explants (about 1 cm) were separated from the seedlings and placed on the callus induction medium.

Callus culture experiments were performed in a 4×6 factorial based on a randomized complete design. Five samples were used in each petri dish. Polyethylene glycol (PEG6000) was used in four levels of zero (as control), 10, 20 and 30% for drought stress, which were equal to 0, -0.4, -0.8 and -1.2 MPa, respectively.

The MS callus induction medium containing 0.5 mg/l kinetin, 2 mg/l 2,4-D, 3% sucrose, 0.8% agar and pH 5.8. Samples were subcultured every two weeks to the same medium and calli were formed after 4 weeks.

Then the calli were transferred to the same medium containing different concentrations of PEG and the desired traits related to calli were measured.

### Traits measured in cotyledon culture experiment

1. Relative growth rate (RGR) of callus

RGR = [Initial Weight / (Initial Weight - Final Weight)] × 100

2. Callus growth rate (CGR)

CGR = Average Callus Diameter/ Time

3. Relative water content (RWC) of callus

RWC = [(fresh weight- dry weight)/ dry weight)] × 100 [13].

### Measured Traits in the Greenhouse

After planting, all pots were irrigated to achieve a uniform green level. Subsequently, irrigation was not performed in some pots under drought stress (7 and 10 days) and other pots were considered as control (4 days). Finally, some morphological traits including plant height, root length, root weight, seed yield, biological yield and harvest index were studied. The greenhouse experiment was also carried out as randomized complete block design.

Statistical Calculations

To determine the effect of treatments, data from each experiment were statistically analyzed. Analysis of variance and mean comparison with Duncan's least significant difference test were performed using SPSS software. Also, graphs were drawn by EXCEL software and correlation analysis was performed using SPSS software.

## Results

Results of Analysis of Studied Traits in Callus Culture

The results of the analysis of variance of related traits in callus culture (Table 1) has been shown that there was a significant difference between the studied genotypes for relative callus water content and relative callus growth. There were significant differences between the different levels of drought in terms of relative callus water content, callus growth rate and relative callus growth (P<0.01). Also, genotype and drought interactions were not significant for relative callus water content and callus relative growth traits. Results of analysis of variance of related traits at different drought levels in a greenhouse (Table 2) showed that among the studied genotypes as well as different drought stress levels there was a significant difference for plant height, root length, root weight, biological yield, seed yield and harvest index (P<0.01), indicating that these traits were altered by drought stress.

The results of the mean comparison of genotype in drought interaction on callus induction and morphological traits are presented (Tables 3 and 4). The results showed that there was a significant difference between levels of drought stress for relative callus water content. Also, the highest relative callus water content was related to genotype 83 (14.31%) and the lowest value was related to genotype 116 (7.57%).

**Table 1** Analysis of variance of genotype and drought effects on measured traits about callus induction in camelina.

| | | Mean squares | | |
|---|---|---|---|---|
| Source of variation | df | RWC | CGR | RGR |
| Genotype | 5 | 51.913 ** | 0.169 | 5.282 ** |
| Drought | 3 | 72.847 ** | 0.877 ** | 11.969 ** |
| Genotype* Drought | 15 | 44.203 ** | 0.094 | 2.160 ** |
| Error | 48 | 13.288 | 0.076 | 0.661 |

** Significant (P<0.01)

**Table 2** Analysis of variance of genotype and drought effects on morphological traits in the greenhouse in camelina.

| | | Mean squares | | | | | |
|---|---|---|---|---|---|---|---|
| Source of variation | Df | Plant height | Root length | Root weight | Biological yield | Grain yield | Harvest index |
| Genotype | 5 | 163.041 ** | 11.849 ** | 0.054 ** | 20.805 ** | 0.039 ** | 0.002 * |
| Drought | 2 | 309.130 ** | 9.310 ** | 0.042 ** | 25.879 ** | 0.267 ** | 0.010 ** |
| Replication | 2 | 18.907 | 3.685 * | 0.002 | 0.681 | 0.002 | 0.001 |
| Genotype* Drought | 10 | 24.619 ** | 1.555 | 0.005 | 5.272 ** | 0.038 ** | 0.002 ** |
| Error | 34 | 6.143 | 0.950 | 0.004 | 0.364 | 0.004 | 0.001 |

* and ** Significant (P<0.05 and P<0.01 respectively)

At the highest stress level, the Soheil cultivar had the highest relative water content of callus (8.32%) and genotype DH111 had the lowest relative water content of callus (6.11%). Also, the highest relative growth rate of callus at non-stress conditions (control) was found in genotype 83 (0.306). The lowest growth rate was related to genotype DH116 (0.30%). The highest rate of the relative growth rate of callus at the highest level of stress was related to cultivar Soheil (4.29%) and the lowest growth rate belonged to genotype DH133 (0.30%) (Table 3).

Kakaei *et al* (2013) by studying drought stress on two safflower cultivars showed that drought stress induced by polyethylene glycol can significantly decrease the growth and relative water content of callus. As well as by a study was carried out on the growth of cloves callus on different levels of stress with polyethylene glycol. The results showed that with increasing concentration of polyethylene glycol the relative water content index and growth rate decreased in cloves callus.

In the study of plant height, genotype AH95 showed the highest plant height (63.3 cm) at control condition and in the highest level of stress, the Soheil cultivar showed the highest height (58.6cm). As well as the Soheil cultivar showed the highest grain yield in control (10.100 $g/m^2$) and maximum water deficit stress (3.033 $g/m^2$). The highest biological yield belonged to the Soheil cultivar (0.700 $g/m^2$) at the control level, but genotype 116 had the highest biological yield (0.133 $g/m^2$) at water deficit stress. In the study of harvest index, the results showed that at the control level genotype DH95 (0.120 g) and the highest stress level, genotype DH83 (0.120 g) had the highest harvest index (Table 4).

Results of Correlation Analysis of Studied Traits in Callus and Greenhouse Culture

The results of the correlation analysis of studied traits for callus culture (Table 5) showed that none of the callus induction traits were significantly correlated. Correlation analysis in greenhouse conditions also indicated that there were significant and positive correlations between plant height with root length (0.528 **), root weight (0.408**), biological yield (0.434 **) and grain yield (0.357 **). Also, root weight (0.431 **) and biological yield (0.434 **) had a positive and significant correlation with root length.

Between biological yield (0.825 **) and grain yield (0.716 **) with root weight and also between grain yield with biological yield (0.820 **) and between harvest index and grain yield (0.332 *) was positively and significantly correlated (Table 6).

Jouyban *et al*. indicated that the harvest index and 1000-grain weight were significantly correlated with grain yield, so these traits could be used to improve grain yield [15]. Tabassam *et al*., 2014 showed a significant positive correlation between grain yield and plant height, the number of grains per spike, 1000-grain weight and harvest index [16].

**Table 3** Mean Comparison of camelina genotype - water deficit interactions effects on callus induction in stress condition (P<0.05).

| | | RWC (%) | | |
|---|---|---|---|---|
| Genotype | Control | -0.4 | -0.8 | -1.2 |
| DH83 | 14.316 cd | 13.110 bcd | 12.500 bcd | 7.196 abc |
| DH95 | 11.080 abcd | 10.143 abc | 6.026 ab | 7.853 abc |
| DH111 | 9.766 abc | 8.876 abc | 17.416 de | 6.110 ab |
| DH116 | 7.576 abc | 6.333 ab | 4.776 a | 7.863 abc |
| DH133 | 13.933 cd | 13.053 bcd | 11.000 abcd | 8.106 abc |
| Soheil | 8.640 abc | 7.750 abc | 22.466 e | 8.326 abc |
| | | RGR (%) | | |
| Genotype | Control | -0.4 | -0.8 | -1.2 |
| DH83 | 3.056 efg | 2.236 def | 0.480 ab | 3.353 fg |
| DH95 | 1.913 bcdef | 1.393 abcd | 0.230 a | 2.156 cdef |
| DH111 | 2.970 efg | 2.130 cdef | 0.596 abc | 1.213 abcd |
| DH116 | 0.306 a | 0.176 a | 0.413 ab | 2.290 def |
| DH133 | 0.860 abcd | 0.613 abc | 0.193 a | 0.406 ab |
| Soheil | 1.613 abcde | 0.920 abcd | 0.353 ab | 4.290 g |

The means in each column that common letters have no significant difference

**Table 4** Mean comparison of camelina genotype- water deficit stress interaction effects on plant height, grain yield, biological yield and harvest index using Duncan's method (P<0.05).

| | | Plant height (cm) | |
|---|---|---|---|
| Genotype | Control (4 day) | 7 day | 10 day |
| DH83 | 57.0 de | 48.3 b | 36.6 a |
| DH95 | 63.3 g | 59.3 efg | 56.0 de |
| DH111 | 57.0 de | 53.0 cd | 51.3 bc |
| DH116 | 58.3 ef | 55.3 cde | 51.0 bc |
| DH133 | 62.0 fg | 56.0 de | 52.3 bcd |
| Soheil | 60.0 efg | 58.6 ef | 58.6 ef |
| | | Grain yield (g/m$^2$) | |
| Genotype | Control (4 day) | 7 day | 10 day |
| DH83 | 3.166 fghi | 2.000 bcde | 0.833 a |
| DH95 | 2.533 cdefg | 2.233 bcde | 1.700 abcd |
| DH111 | 2.333 bcdefg | 1.966 bcde | 1.700 abcd |
| DH116 | 3.766 hi | 3.200 ghi | 2.033 bcdef |
| DH133 | 2.733 defgh | 1.500 abc | 1.233 ab |
| Soheil | 10.100 j | 4.200 i | 3.033 efgh |
| | | Biological yield (g/m$^2$) | |
| Genotype | Control (4 day) | 7 day | 10 day |
| DH83 | 0.166 | 0.100 a | 0.100 a |
| DH95 | 0.233 | 0.100 a | 0.100 a |
| DH111 | 0.233 | 0.100 a | 0.100 a |
| DH116 | 0.333 | 0.133 ab | 0.133 ab |
| DH133 | 0.233 | 0.100 a | 0.100 a |
| Soheil | 0.700 | 0.100 a | 0.100 a |
| | | Harvest index (g) | |
| Genotype | Control (4 day) | 7 day | 10 day |
| DH83 | 0.103 ef | 0.030 ab | 0.120 f |
| DH95 | 0.120 f | 0.040 ab | 0.040 ab |
| DH111 | 0.093 def | 0.053 abcd | 0.043 abc |
| DH116 | 0.086 cdef | 0.056 abcd | 0.040 ab |
| DH133 | 0.073 bcde | 0.070 bcde | 0.070 bcde |
| Soheil | 0.066 bcde | 0.030 ab | 0.020 a |

The means in each column that common letters have no significant difference

**Table 5** Correlation of studied traits in callus culture experiment in camelina.

| | RGR | CGR | RWC |
|---|---|---|---|
| RGR | 1 | - | - |
| CGR | 0.128 | 1 | |
| RWC | -0.103 | 0.126 | 1 |

**Table 6** Correlations of measured traits in six genotypes of camelina.

| | Plant height (cm) | Root length(cm) | Root weight (g) | Biological yield (g/m$^2$) | Grain yield (g/m$^2$) | Harvest index (g) |
|---|---|---|---|---|---|---|
| Plant height | 1 | - | - | - | - | - |
| Root length | 0.528 $^{**}$ | 1 | - | - | - | - |
| Root weight | 0.408 $^{**}$ | 0.431 $^{**}$ | 1 | - | - | - |
| Biological yield | 0.434 $^{**}$ | 0.459 $^{**}$ | 0.825 $^{**}$ | 1 | - | - |
| Grain yield | 0.357 $^{**}$ | 0.226 | 0.716 $^{**}$ | 0.820 $^{**}$ | 1 | - |
| Harvest index | 0.094 | -0.134 | -0.104 | 0.073 | 0.332 $^{*}$ | 1 |

$^{*}$ and $^{**}$ Significant (P<0.05 and P<0.01 respectively)

Stepwise Regression Analysis

At this stage, grain yield was evaluated as a dependent variable against other traits and statistical analysis was performed. Results obtained under drought stress conditions (Table 7) showed that, in total, 4 independent variables including root length (-0.019), root weight (0.229), biological yield (0.055) and harvest index (1.684) were significant and justified for 85% of the total grain yield variation (Table 8).

Path Analysis of Traits under Stress onditions

According to Table 9, the most direct effect on grain yield is related to biological yield (0.735 g /$m^2$) and the most indirect effect belongs to root length through root weight (0.84 g).

**Table 7** Results of stepwise regression analysis for grain yield under drought stress conditions in camelina.

| | df | Mean squares | Correlation | Adjusted R Square | Std. error of the Estimate |
|---|---|---|---|---|---|
| Regression | 5 | 0.217 ** | 1.000 | 0.862 | 0.847 |
| Residual | 48 | 0.004 | - | - | - |
| Total | 53 | - | - | - | - |

* and ** Significant (P<0.05 and P<0.01 respectively)

**Table 8** Coefficient values for stepwise regression models of morphological traits under drought stress conditions in camelina.

| Independent variables | B | Standard error | Standardized Coefficients | T-test |
|---|---|---|---|---|
| Constant | -0.159 | 0.083 | - | -1.922 |
| Plant height | 0.002 | 0.002 | 0.097 | 1.482 |
| Root length | -0.019 | 0.006 | -0.193 | -2.904 ** |
| Root weight | 0.299 | 0.148 | 0.194 | 2.021 * |
| Biological yield | 0.055 | 0.007 | 0.735 | 7.531 ** |
| Harvest index | 1.684 | 0.235 | 0.389 | 7.165 ** |

* and ** Significant (P<0.05 and P<0.01 respectively)

**Table 9** Path analysis for traits entered in the inter regression model under drought stress conditions in camelina.

| Trait | Direct effect | | | Indirect effect | | Correlation |
|---|---|---|---|---|---|---|
| | | Plant height(cm) | Root length(cm) | Root weight(g) | Biological yield(g/m2) | |
| Plant height | 0.097 | - | -0.102 | 0.079 | 0.32 | 0.375 |
| Root length | -0.193 | 0.05 | - | 0.84 | 0.337 | 0.226 |
| Root weight | 0.194 | 0.039 | -0.083 | - | 0.606 | 0.716 |
| Biological yield | 0.735 | 0.042 | -0.088 | 0.16 | - | 0.820 |

## Discussion

At the maximum stress level, the Soheil cultivar had the highest RWC of callus (8.32%) and the genotype DH111 had the lowest RWC of callus (6.11%). Soheil cultivar showed higher RWC of callus (8.32%) rather than drought-sensitive genotypes [14]. The results showed that with increasing concentration of polyethylene glycol both RWC and growth rate decreased in cloves callus. Some significant positive correlations were observed between both traits of biological yield (0.825 **) plus grain yield (0.716 **) and root weight, between grain yield and biological yield (0.820 **), and lastly between harvest index and grain yield (0.332*) (Table 6). Joeban *et al.* indicated that the harvest index and 1000-grain weight are significantly correlated with grain yield, so these traits could be used to improve grain yield

[15]. The most direct effect on grain yield was related to biological yield (0.735 g /$m^2$), while the most indirect effect belongs to root length through root weight (0.84 g).

## Conclusion

The studied genotypes, as well as water deficit stress levels, were significantly different in terms of plant height, root length, root weight, grain yield, biological yield and harvest index. Soheil cultivar had the highest grain yield. There was a significant difference between studied genotypes in the callus growth rate, relative callus growth rate and relative callus water content under stress conditions and between different stress levels. Soheil cultivar was identified as high tolerant cultivar among other genotypes due to its relative water content and higher callus growth rate.

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