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# The response of soybean growth parameters to different light intensities under cold stress conditions

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## Abstract

The present experiment designed to evaluate the response growth characteristics of two soybean cultivars (032 and BP) to cold stress (5 °C) at two light intensity levels [normal (8000 lux) or low light (2000 lux) intensity]. Treatments were arranged in a factorial experiment based completely randomized design with three replicates under controlled conditions. The results showed that root volume and nodule number of root in soybean seedlings significantly decreased (38 and 32 %, respectively) when grown at cold stress as compared to those grown under control conditions. Cold stress, also, reduced leaf and root fresh and dry weights and shoot dry weight in both light intensities, however, this effect was more noticeable at low light intensity for root dry weight (53%) and at normal light intensity for leaf and shoot fresh and dry weights (47% ). Cold stress, also, significantly suppressed stem fresh weight in both cultivars (34% in 032 and 27% in BP). BP cultivar when grown at 8000 lux had nearly 16% lower leaf dry weight than plants grown at, 2000 lux. However, 032 cultivar in normal light intensity showed no significant response in growth parameters to low light intensity. In both light intensities, cold stress significantly reduced the root fresh and dry weights. Although two soybean cultivars showed slightly contrast behaviors, the rate of cold stress damages decreased when soybean plants grown under lower light intensity.

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## Introduction

Soybean, *Glycine max* [L.] Merr, is one of the important oil and protein crops throughout the world which grown under various environmental conditions. Change in climatic factors such as temperature, photoperiod and moisture could exert a detrimental effect on plant growth and metabolism (Khan *et al.*, 2007 and Sadeghi *et al.*, 2014). Low temperature or cold stress is a major factor limiting the growth, productivity and geographical distribution of many species, including important agricultural crops (Allen and Ort, 2001; Aghaee *et al.*, 2011 and Lee *et al.*, 2007). Like many other warm-climate crop species (e.g. cucumber [*Cucumis sativus L.*], tomato [*Solanum lycopersicum L.*] and maize [*Zea mays*]), soybean is sensitive to suboptimal growth temperatures (Van Heerden *et al.*, 2008). Optimal temperature for soybean growth, development and symbiotic activity is about 25-30 °C (Zhang *et al.*, 1995 and Stępiński, 2002). It has been well documented that cold stress could disrupt the cell membranes (Xing and Rajashekar, 2001), reduce the cellular respiration (Lee *et al.*, 1997), increase the abscisic acid (ABA) levels (Nayyar *et al.*, 2005), and increase the reactive oxygen species (ROS) (Noriega *et al.*, 2012; Lee *et al.*, 1997).

Under field conditions, however, plants often are exposed simultaneously to more than one environmental stress factor (van Heerden and Kruger, 2002). For example, soybean is particularly sensitive to dark chilling (Gass *et al.*, 1996). Night temperatures below 15 °C limit a wide range of physiological processes including photosynthesis (Caulfield and Bunce, 1988 and van Heerden and Kruger, 2002), and temperature of 10 °C is the lowest allowing for vegetation of some cultivars of soybean (Jasińska and Kotecki, 1993 and Stępiński; 2002).

Cold stress more rapidly and severely inhibits the photosynthesis if occurs concurrent with illumination (Martin and Ort, 1985 and Wise and Ort, 1989). In this regard, Balestrasse *et al.* (2010) reported that the combination of high light intensities and low temperatures, such as those experienced on cold but sunny mornings in spring, can cause irreversible damage to young soybean seedlings. Accordingly, the purpose of this study was to investigate the jointed effects of cold stress and light intensity on some growth characteristics in two soybean cultivars.

## Material and methods

*Experimental Design and Treatments*

This study was conducted at Sari Agricultural Sciences and Natural Resources University in spring 2013. A completely randomized design in a factorial arrangement with three replicates was used. The treatments were two soybean cultivars (032 and BP), two levels of temperature i.e. control (28 °C) and cold stress (5 °C) and tow light intensity levels [normal light (8000 lux) or low light (2000 lux)].

*Soil and Seed Preparation*

Experimental soil was taken from the research fields of Sari Agricultural Sciences and Natural Resources University and mixed with sand [2/1, v/v]. The main properties of soil were the following: sand: 22%; silt: 35.8%; clay: 42.2%; soil texture: sandy loam; N: 0.21%; P: 14.5 mg $kg^{-1}$; K: 270 $mgkg^{-1}$; pH: 7.65; EC: 1.52 $dSm^{-1}$; SP: 35%; organic matter: 2.37%. One kg air dried soil was filled in the pots. Seeds were sterilized and disinfected in sodium hypochlorite %1 (V/V) for 15 minutes and then washed twice with distilled water.

*Planting and Growth Conditions*

The six seeds were planted in each pot. The pots were maintained in the greenhouse for 20 days under controlled conditions of temperature (28°C) and light photoperiod (15h). The plants then transferred to growth chambers for 12 days to apply the treatments.

*Estimated characters*

After treatment, all plants harvested and shoot parameters such as leaf, stem and shoot fresh and dry weights were determined. Six plants gently up-rooted completely from the pots, washed with tap water carefully and some root related parameters such as nodule numbers of root, volume, length, fresh and dry weights were recorded. The plant root and shoot parts were dried at 70°C for 48 hours.

*Statistical analysis*

The data were subjected to analysis of variance using the SAS statistical software package (version 9.1) and mean comparison was performed using Fisher's protected least significant difference (FLSD) test at $P<0.05$.

## Results and discussion

*Shoot characteristics*

The effect of cold stress (C) on leaf, stem and shoot fresh and dry weights is shown in Table 1. Analysis of variance (ANOVA) showed that the effect of cold stress was highly significant ($P<0.01$) while light intensity main effect (L) was significant only for stem and shoot dry weights ($P<0/05$). There was a significant variations between two cultivars (V) in terms of stem fresh and dry weights ($P<0.01$). Interaction effects of C×L was significant for leaf fresh weight ($P<0.05$) and leaf and shoot dry weights ($P<0.01$). There was a significant effect on the stem fresh weight and leaf dry weight due to interaction of C×V and L×V, respectively.

**Table 1.** The effect of cold stress and light intensity on some shoot.

| Source | df | Fresh weight | | | Dry weight | | |
|---|---|---|---|---|---|---|---|
| | | Leaf | Stem | Shoot | Leaf | Stem | Shoot |
| Cold stress (C) | 1 | 0.700 ** | 1.739 ** | 4.646 ** | 0.0793 ** | 0.1878** | 0.519** |
| Light intensity (L) | 1 | 0.002 ns | 0.002 ns | 0.008 ns | 0.0043 ns | 0.0054 * | 0.019 * |
| Cultivar (V) | 1 | 0.023 ns | 0.406 ** | 0.236 ns | 0.0033 ns | 0.0079** | 0.001 ns |
| C×L | 1 | 0.160 * | 0.040 ns | 0.042 ns | 0.0204 ** | 0.0022 ns | 0.037 ** |
| C×V | 1 | 0.004 ns | 0.070 * | 0.043 ns | 0.0001 ns | 0.0037 ns | 0.002 ns |
| L×V | 1 | 0.086 ns | 0.004 ns | 0.126 ns | 0.0054 * | 0.0022 ns | 0.015 ns |
| C×L×V | 1 | 0.035 ns | 0.028 ns | 0.126 ns | 0.0028 ns | 0.0010 ns | 0.006 ns |
| Error | 16 | 0.024 | 0.015 | 0.063 | 0.0011 | 0.0008 | 0.003 |
| CV (%) | | 12.68 | 8.24 | 9.34 | 12.83 | 9.40 | 10.31 |

** Significant at $P<0.01$ level; *Significant at $P<0.05$; ns: Non-significant.
characteristics in two soybean cultivars.

One of the symptoms of cold stress in plants is reducing carbon exported from the leaf that occurs due to disruption of the carbon cycle (Takeoka *et al.*, 1992). Therefore this soluble carbohydrate increased and prevents photosynthesis, resulting in reduced fresh and dry weight. The results showed that when the temperature decreased from 28 to 5 °C, shoot fresh weight and stem dry weight were decreased by 28 and 45%, respectively.

Also, when light intensity decreased from 8000 to 2000 lux, stem dry weight increased by 10%. As shown in Table 2, stem dry weight in 032 cultivar was about 14% more than BP. Decrease in growth parameters like shoot dry weight due to cold stress has also been reported previously by Sheng Xiang (1995) and Gorbani *et al.* (2011) in rice plants.

It appears that the increase in shoot dry weight following the decrease in light intensity was related to the increase in the inter-plant competition over light and the disruption of the balance of growth regulators and therefore increases in plant height (Moosavi *et al.*, 2012).

**Table 2.** The effect of cold stress, light intensity and cultivar on shoot fresh weight and stem dry weights of soybean.

| Treatments | Shoot fresh weight | Stem dry weight |
|---|---|---|
| | (g plant$^{-1}$) | |
| Cold stress | | |
| Control | 3.13 a | 0.40 a |
| Cold (5 °C) | 2.25 b | 0.22 b |
| Light intensity | | |
| normal | 2.67 | 0.30 b |
| low | 2.71 | 0.33 a |
| Cultivar | | |
| 032 | 2.79 | 0.33 a |
| BP | 2.59 | 0.29 b |

In each column and for each treatment, means with the same letter(s) are not significantly different according to LSD test at $P<0.05$.

Table 3 showed that cold stress reduced the leaf fresh weight of soybean plants (approximately 34%) under normal light intensity. Also cold stress in both light treatments significantly reduced the leaf and shoot dry weight, which was more reduction in normal light intensity (47%). Inhibition of photosynthesis following exposure to low temperatures and high light has been observed frequently in plants of tropical origin (Byrd *et al.*, 1995). Chilling stress in combination with light is more inhibitor to photosynthesis than chilling in the low light intensity. Chilling-induced photosynthetic inhibition appears to be due to direct inhibition of the photosynthetic process in chilling-sensitive plants, and not simply to the limitation of $CO_2$ supply by chilling-decreased stomata conductance (Peeler and Naylor, 1988). In control conditions, reducing the light intensity decreased leaf and shoot dry weight (Respectively up 22% and 17%). Further reduction in fresh and dry weight, stem dry weight at 8000 lux light intensity can show an avoidance mechanism regulating the growth rate of the optical intensity.

When the temperature decreased to 5 °C (cold stress), the stem fresh weight reduced in both cultivars (approximately 34% in 032 and 27% in BP). Also reducing light intensity from 8000 to 2000 lux, significantly increased leaf dry weight in BP cultivar up to 20%. Gorbani *et al.* (2011) also reported a reduction in shoot and root fresh and dry weights due to cold stress in two rice cultivars. The decrease in plant weight may be attributed to limited water supply, nutrient supply by root and decline of net photosynthesis (Aghaee *et al.*, 2011).

**Table 3.** Interaction effect of cold stress× Light intensity, cold stress ×cultivar and cultivar ×light intensity on fresh and dry weights of soybean.

| Treatments | | Fresh Weight Leaf | Fresh Weight Stem | Dry Weight Leaf | Dry Weight Shoot |
|---|---|---|---|---|---|
| | | (g plant$^{-1}$) | | | |
| Cold Stress × Light intensity | | | | | |
| Control | normal | 1.48 a | 1.71 | 0.36 a | 0.79 a |
| | Low | 1.30ab | 1.73 | 0.28 b | 0.65 b |
| Cold Stress | normal | 0.98 c | 1.23 | 0.19 c | 0.42 c |
| | Low | 1.12bc | 1.16 | 0.22 c | 0.44 c |
| Cold Stress × Cultivar | | | | | |
| Control | 032 | 1.35 | 1.93 a | 0.31 | 0.74 |
| | BP | 1.43 | 1.56 b | 0.33 | 0.69 |
| Cold Stress | 032 | 1.03 | 1.28 c | 0.20 | 0.42 |
| | BP | 1.07 | 1.13 d | 0.21 | 0.43 |
| Cultivar × Light intensity | | | | | |
| 032 | normal | 1.14 | 1.60 | 0.25 b | 0.58 |
| | Low | 1.24 | 1.61 | 0.25 b | 0.58 |
| BP | normal | 1.32 | 1.36 | 0.25 b | 0.51 |
| | Low | 1.18 | 1.32 | 0.30 a | 0.61 |

In each column and for each interaction, means with the same letter(s) are not significantly different according to LSD test at $P$ <0.05.

*Root characteristics*

Effects of cold stress and light intensity on root parameters in two soybean cultivars are presented in Table 4. Results of ANOVA indicated that cold stress significantly affected all the measured root parameters except for root length, while light intensity main effect (L) was significant only for root nodule number ($P$<0/01). There was a significant variations between two cultivars (V) in volume, fresh and dry weights of root, shoot: root ratio ($P$<0.01) and nodule number of root ($P$<0.05). Interaction effects of C×L was significant for root fresh weight and shoot: root ratio ($P$<0.01) and root dry weight ($P$<0.05). There was a significant effect on the root length due to interaction of C×V and on the volume, fresh and dry weight of root due to interaction of V×L

($P$<0.01). Triple effect of cold stress, light intensity and cultivar was significant ($P$<0.01) on nodule number and fresh weight of root.

**Table 4.** Analysis of variance for the effect of cold stress and light intensity on some root characteristics in two soybean cultivars.

| Source | df | Root | | | | | Shoot: Root |
|---|---|---|---|---|---|---|---|
| | | Length | Volume | Nodule Number | Fresh Weigh | Dry Weight | |
| Cold stress (C) | 1 | 5.273 $^{ns}$ | 5.3865 $^{**}$ | 3.307 $^{**}$ | 5.17082 $^{**}$ | 0.1376 $^{**}$ | 0.73 $^{*}$ |
| Light intensity (L) | 1 | 0.750 $^{ns}$ | 0.0007 $^{ns}$ | 3.565 $^{**}$ | 0.00002 $^{ns}$ | 0.0026 $^{ns}$ | 0.29 $^{ns}$ |
| Cultivar (V) | 1 | 0.315 $^{ns}$ | 1.8315 $^{**}$ | 0.254 $^{*}$ | 1.41135 $^{**}$ | 0.0212 $^{**}$ | 2.13 $^{**}$ |
| C×L | 1 | 19.710 $^{ns}$ | 0.0155 $^{ns}$ | 0.189 $^{ns}$ | 0.44282 $^{**}$ | 0.0038 $^{*}$ | 1.71 $^{**}$ |
| C×V | 1 | 99.023 $^{**}$ | 0.0513 $^{ns}$ | 0.049 $^{ns}$ | 0.00001 $^{ns}$ | 0.0010 $^{ns}$ | 0.26 $^{ns}$ |
| L×V | 1 | 1.377 $^{ns}$ | 0.0543 $^{**}$ | 0.003 $^{ns}$ | 0.28602 $^{**}$ | 0.0056 $^{**}$ | 0.09 $^{ns}$ |
| C×L×V | 1 | 0.003 $^{ns}$ | 0.5320 $^{ns}$ | 0.988 $^{**}$ | 0.28602 $^{**}$ | 0.0001 $^{ns}$ | 0.36 $^{ns}$ |
| Error | 16 | 2.581 | 0.0272 | 0.046 | 0.02752 | 0.0006 | 0.13 |
| CV (%) | | 6.27 | 7.07 | 11.30 | 7.82 | 10.51 | 14.47 |

** Significant at $P$<0.01 level; * Significant at $P$<0.05; ns: Non-significant.

When the temperature decreased from 28 to 5 °C, root volume and nodule number in the roots of soybean plants were decreased by 38 and 32%, respectively. Also when light intensity decreased to 2000 lux, root nodule number significantly increased (up to 50%). As shown in Table 5, 032 cultivar had higher nodule number than BP cultivar. By contrast, the highest rate of shoot: root ratio was observed in cultivars BP (approximately 27 percent). Aghaee *et al.* (2011) also reported a reduction in shoot: root ratio when rice seedlings were exposed to low temperature. Shoot: root ratio was lower in 032 cultivar than the BP, mainly due to a higher root dry matter, which can be indicated a relative tolerance to environmental stresses.

**Table 5**. The effect of cold stress, light intensity and cultivar on root volume and node number of soybean.

| Treatments | Root Volume (cm3) | Nodule number | Shoot: Root |
|---|---|---|---|
| Cold stress | | | |
| Control | 2.51 $^{a}$ | 2.27 $^{a}$ | 2.34 |
| Cold (5 °C) | 1.56 $^{b}$ | 1.53 $^{b}$ | 2.69 |
| Light intensity | | | |
| normal | 2.03 | 1.51 $^{b}$ | 2.62 |
| low | 2.04 | 2.28 $^{a}$ | 2.40 |
| Cultivar | | | |
| 032 | 2.31 | 2.00 $^{a}$ | 2.21 $^{b}$ |
| BP | 1.76 | 1.79 $^{b}$ | 2.81 $^{a}$ |

In each column and for each treatment, means with the same letter(s) are not significantly different according to LSD test at $P$<0.05.

According to Table 6, in both light intensities (8000 and 2000 lux), reducing of the temperature from 28 to 5 °C (cold stress), decreased the root fresh weight, which this reduction in normal light (8000 lux) was nearly 44% more than low light (2000 lux) intensity. Also cold stress in both light treatments significantly reduced the root dry weight, it appears that this trait more affected in low light intensity (nearly 53%). These results were similar to the results of Jafari *et al.* (2006) in tomato plants under cold stress. Also under control conditions, the plants grown at lower light intensity had more reduced the shoot:root ratio in soybean (up 31%). As shown in Fig. 3, two soybean cultivars respond differently to cold stress in terms of root length, whereas an increase was observed for 032 cultivar in cold stress (13%), while was resulted a significant decrease in BP cultivar (17%). High root length in 032 seemed to be correlated with chilling tolerance. Also when light intensity decreased from 8000 to 2000 lux, root volume decreased in both cultivars (32% in 032 and 16% in BP). Two soybean cultivars respond differently to light intensity in root fresh weight, so that decreasing light intensity, increased the root fresh weight by 9% in 032 cultivar, whereas decreased by 11% in BP cultivar. Decreasing light intensity caused a significant increase in root dry weight in 032 plants while BP plants exhibited slightly decrease (Table. 6). The highest root dry weight was observed in low light intensity in 032 cultivar. In maize (Koster and Leopold, 1988) and rice (Lee *et al.*, 2009) the osmotic protection was attributed to increase of cold stress tolerance.

**Table 6.** Interaction effect of cold stress × light intensity, cold stress × cultivar and cultivar × light intensity on some root parameters in soybean.

| Treatments | | Root Length (cm) | Root Volume (cm3) | Root Fresh weight (g plant-1) | Root Dry weight (g plant-1) | Shoot: Root |
|---|---|---|---|---|---|---|
| Cold Stress × Light intensity | | | | | | |
| Control | normal | 27.17 | 2.54 | 2.72 a | 0.29 b | 2.72 a |
| | low | 25.00 | 2.48 | 2.45 b | 0.34 a | 1.96 b |
| Cold Stress | normal | 24.41 | 1.54 | 1.52 d | 0.17 c | 2.53 a |
| | low | 25.87 | 1.58 | 1.79 c | 0.16 c | 2.85 a |
| Cold Stress × Cultivar | | | | | | |
| Control | 032 | 24.17 b | 2.83 | 2.83 | 0.35 | 2.15 |
| | BP | 28.00 a | 2.19 | 2.34 | 0.28 | 2.53 |
| Cold Stress | 032 | 27.29 a | 1.79 | 1.90 | 0.19 | 2.28 |
| | BP | 23.00 b | 1.33 | 1.41 | 0.14 | 3.09 |
| Cultivar × Light intensity | | | | | | |
| 032 | normal | 25.67 | 2.17 a | 2.25 b | 0.24 b | 2.39 |
| | low | 25.79 | 1.46 c | 2.47 a | 0.30 a | 2.86 |
| BP | normal | 25.92 | 1.92 b | 1.99 c | 0.22bc | 2.04 |
| | low | 25.08 | 1.60 c | 1.77 d | 0.21 c | 2.76 |

In each column and for each interaction, means with the same letter(s) are not significantly different according to LSD test at $P$ <0.05.

## Conclusion

The purpose of the current study was to determine the effects of light intensity in cold stress conditions in two soybean cultivars. Overall, results showed that under cold stress conditions, the plants grown at lower light intensity showed less damage than those plants grown in normal light conditions. Although two soybean cultivars showed slightly contrast behaviors, the rate of cold stress damage decreased when soybean plants grown under lower light intensity.

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