

Impacts of cold stress on some physio-biochemical characteristics of three lines/varieties of lentils

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Abstract

Lentil is one of the arctic legumes which can be damaged by the severe cold. In this regard, determination of the cold tolerance among different lentil lines or varieties has considerable importance. To evaluate Bilesovar and Precoz varieties and ILL-2580 line of lentils for cold tolerance and seed yield, a field experiment was carried out as a randomized complete block design with three replications. Also, to study the physiological and biochemical responses of the lentil plants to cold conditions, a factorial experiment was conducted in the greenhouse based on a completely randomized design. The factors were cold conditions and lentil genotypes. The results showed that the highest plant height, 100-seed weight, seed yield, and the shoot dry and fresh weight were observed in the Bilesovar variety followed by Precoz. Also, the highest percentage of cold damage was observed in the ILL-2580 line. The cold damage was 80% in ILL-2580 but it was about 41 and 32% for the Bilesovar and Precoz, respectively. The results also indicated that the chlorophyll index (SPAD) declined in Bilesovar and ILL-2580 under the cold stress conditions, whereas it enhanced in Precoz (252% over the control). Moreover, the proline content was significantly increased by about 300% in the cold-treated Precoz plants as compared to the control plants. The activity of the catalase, as an antioxidant enzyme, drastically increased in Precoz and Bilesavar under cold treatment (20 and 73%, respectively over the related controls). Bilesovar showed a significant increase in total flavonoids and anthocyanin content of the leaves at cold conditions. The results of SDS PAGE analysis showed that in Bilesavar and Precoz, some characteristic bands with molecular weights of 30-40 and 80-90 KD appeared in the cold-treated plants that were absent in the control plants. It can be concluded that the Bilesovar of lentils might be considered a cold-tolerant variety. On the other hand, Precoz and ILL-2580 can be regarded as semi-tolerant and cold-sensitive genotypes, respectively.

Keywords: Biochemical characteristics; Cold tolerance; Lentil; Physiology

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Introduction

Cold stress is one of the limiting factors in the production of agricultural products. As plants can't change their environment, they respond to the cold stress by varying their gene expression patterns reflected in the physiological or biochemical process giving rise to cold tolerance (Heidarvand and Maali Amiri 2010). Cold-tolerant genotypes have effective physio-biochemical process to undergo chilling and freezing conditions. However, cold-sensitive

plants not only may be injured in freezing conditions but also may be damaged by chilling. Different phenological stages of the lentil from seed germination/seedling establishment to seed production may be affected by cold. Cold weather is one of the main climate factors determining the spread of plant species all over the world (Rihan *et al.* 2017). Depending on the sensitivity of the plants to the low temperatures, the low-temperature stress can be divided into two types: chilling stress with the temperature range of 0-15

°C and the freezing stress at temperatures below zero degrees centigrade (Banerjee *et al.* 2013).

The winter planting of the lentil tends to 2-6 fold more yield than the spring cultivation. However, in the northwest Iran, seed sowing of lentils at low soil temperature in early winter or autumn is a critical problem for this kind of cultivation. Most commercial cultivars of lentils do not tolerate the low temperatures of winter (Kumar *et al.* 2013). Freezing temperatures below -20 to -25 °C may damage the lentil plants (Ozdemir 2002) so that the plant can't be cultivated in very cold climates. Therefore, determining cold-resistant or cold-tolerant genotypes of lentils for cultivation in the highlands with hard winters is a useful strategy to enhance the lentil yield.

The study of the physiological and biochemical response of lentil plants to cold stress can be used to identify tolerance or acclimation mechanisms of the plants against cold conditions. Through the evaluation of the chlorophyll fluorescence, one can assess photosynthesis (Kumagai *et al.* 2016) and the cold-caused damage in the plants. Photosystem II efficiency is one of the important parameters of chlorophyll fluorescence (Poormohammad Kiani *et al.* 2008).

This research was conducted to assess the cold tolerance of three lentil genotypes through seed yield and some physiological and biochemical characteristics such as chlorophyll index, compatible solutes, antioxidant enzymes activity, phenolic compounds, and electrophoretic patterns of protein bands.

Materials and Methods

Field evaluation

The seeds of three genotypes of lentil, Billesovar, Precos, and ILL-2580, were obtained from the Research Center for Agriculture and Natural Resources, Ardabil, Iran. To evaluate the crop yield of lentil lines and cold-caused damages in the field conditions, an experiment was carried out as a randomized complete block design with three replications at the research field of Agriculture and Natural Resource Research Center, Ardabil (38° 15' N/ 48°20' E) during 2016-2017. The mean annual maximum and minimum temperatures were 15.98 and 1.98 °C. The annual rainfall was 291.2 cm. The lentil seeds were planted manually on 30th October 2016. The planning was performed in 4-row plots of 4 m in length and with an average density of 200 seeds per m². After seedling establishment, the average density of seedlings was recorded as 50 seedlings in m² (50-55 plants in each row). The inter-row spacing was considered as 25 cm. The cold course began in late November, when the seedlings were 4-weeks old and continued for at least 60 days during the vegetative stage. The lowest temperature was recorded as -24 °C. At the ripening stage, the plant height, 100-seed weight, and seed yield were measured. The cold damage on the plant's vegetative parts was determined as the percentage of morphological signs based on the protocol of ICARDA (Anonymous 2011).

Greenhouse evaluation

To study the effects of cold conditions on physiological and biochemical characteristics of the lentil plants, a factorial experiment was conducted in a greenhouse based on a completely

randomized design with three replications. The factors were cold conditions and lentil genotypes. The lentil seeds were first disinfected and then transferred to pots containing peat. After the seedlings appeared, the pots were irrigated with the Hoagland nutrient solution. After 40 days, the cold-treated groups were transferred to the cold storage to be subjected to the cold treatment at 2 °C for 48 hours. For the measurement of growth characteristics, the roots and shoots of harvested lentil plants were separated and their fresh weight was measured. Then, the plant parts were put in an oven at 75 °C for 20 min for the dry-weight measurement.

Measurement of physiological characteristics

The relative chlorophyll content was determined in SPAD units, by a chlorophyll meter (CL-01 model, Hansatech instrument, UK). The chlorophyll fluorescence parameters were measured using the fluorescence monitoring system (PEA model, Hansatech instrument, UK).

To measure the proline content, frozen leaves (0.5 gr) were homogenized in 10 ml of sulphosalicylic acid (3%) and then centrifuged 10000g. The supernatant (0.5 ml) was mixed with 1 ml of ninhydrin (2.5%). The mixture was kept at 100 °C for 1 h and then the reaction was terminated by cooling the mixture in the ice bath. The reaction mixture was extracted by 2 ml of toluene and finally, the absorption was recorded at 520 nm. The proline concentration was calculated by using a standard curve (Bates *et al.* 1973).

Measurement of biochemical characteristics

The proteins of lentil leaves were extracted using 0.1 M phosphate buffer (pH 6.8) for the measurement of total proteins, enzyme activity, and SDS-PAGE electrophoresis. The protein concentration was determined using the Folin Ciocalteu reagent (Bradford 1976). Bovine serum albumin was used for the preparation of the standard curve. The absorption was measured at 540 nm using a spectrophotometer.

Catalase activity assay was recorded through the decomposition of H₂O₂ at 240 nm. The reaction mixture contained 50 mM K-phosphate buffer (pH= 7) and 0.3 ml of H₂O₂ 3%. The enzyme activity was determined at the extinction coefficient of 39.4 mM⁻¹ cm⁻¹ (Cakmak and Marschner 1992).

To measure the polyphenol oxidase activity, pyrogallol was used as a substrate. The reaction mixture consisted of 2.5 mM potassium phosphate buffer (pH= 6.8) and 0.2 mL of pyrogallol 14%. The mixture was incubated at 40 °C and then 0.2 ml of the enzyme extraction was added to the reaction. The absorbance was recorded at 430 nm. The enzyme activity was determined at the extinction coefficient of 2.47 mM⁻¹ cm⁻¹ (Raymond *et al.* 1993).

Ascorbate-peroxidation activity was measured according to the method of Nakano and Asada (1981) by reducing the concentration of ascorbate at 290 nm. The reaction mixture contained 0.05 M potassium phosphate buffer (pH 7.0), 0.6 mM ascorbate, and 0.2 ml of 3% hydrogen peroxide in a total volume of 6 ml. The reaction was initialized by adding hydrogen peroxide and the change in absorbance was

measured at 290 nm with the extinction coefficient of $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$.

The procedure of Brik *et al.* (1962) was used for the determination of protease activity. The reaction mixture consisted of 1 ml of 1% casein (prepared in 0.05 M phosphate buffer) and then incubated at 45 °C for 1h. The reaction was terminated by the addition of 0.1 mL of 40% trichloroacetic acid. Absorbance was recorded at 280 nm. The activity of the protease enzyme was calculated by using the extinction coefficient of $21.5 \text{ mM}^{-1} \text{ cm}^{-1}$.

Measurement of phytochemical characteristics

The flavonoid level of the extract was estimated using the colorimetric method with some modifications. A 0.2 g of the lentil leaves was extracted in 10 ml methanol. A 0.5 ml of the obtained extract was diluted with distilled water to reach a volume of 5 ml. Then, 0.3 ml of NaNO_2 (5%) and 0.5 ml AlCl_3 was added. Thereafter, 2 ml of NaOH (1M) and 2 ml of distilled water were added and the mixture was centrifuged. The absorption of the supernatant was recorded at the 510 nm wavelength. Flavonoids concentration was determined by plotting the quercetin standard curve (Toor and Savage 2005).

For the measurement of anthocyanin content, the plant samples were homogenized in acidic methanol. After centrifugation, the absorption of the supernatant was measured at 550 nm (Merzlyak *et al.* 2008).

SDS-PAGE procedure

Protein profiling was studied on 10% SDS-PAGE. By adding the sample buffer (5X) and the protein

samples in the 1:4 ratio, the samples were prepared for electrophoresis. The same preparation method was also repeated for the standard protein marker. Samples were subjected to the boiling water bath for 2 to 3 min and then were injected using a microsyringe into wells through the electrode buffer. The standard marker was also injected into the gel to identify the molecular weight of the bands. Each sample well was loaded with 100 µg of protein in 10 µl of the sample buffer containing bromophenol blue as the tracking dye. The prepared gel was run at 50 mA current and voltage of 150 V. Protein bands were detected by coomassie brilliant blue staining. Finally, the stained gel was transferred to the staining solution (methanol and glacial acetic acid in 5:1 proportion). SDS-PAGE destaining solution was used to destain the Coomassie dye from the gel (Ladizinsky and Hymowitz 1979).

Statistical analysis

All of the physiological and biochemical characteristics and the seed yield data were analyzed according to the respective experimental design, followed by the Duncan's Multiple Range Test, using the SPSS software, Version 21, at $p \leq 0.05$.

Results

Field evaluation showed that there were significant differences among the lentil genotypes for plant height, 100-seed weight, and seed yield. The highest plant height and 100-seed weight were observed in the Bilesovar variety followed by Precoz. However, the highest cold damage percentage was seen in the ILL-2580 line. The

cold damage was 80, 41, and 32% in ILL-2580, Bilesovar, and Precoz, respectively. Bilesovar showed the highest seed yield (1500 kg/ha) which was approximately 4.5 and 20 fold higher than Percoz and ILL-2580, respectively (Table 1).

At the greenhouse conditions, the fresh weight of aerial parts significantly decreased in all three genotypes under cold stress. ILL-2580 line showed the lowest decrease in shoot fresh weights followed by Precoz. The root fresh weight decreased significantly in the Bilesovar variety. In addition, the dry weight of both shoot and root underwent a significant increase in the ILL-2580 line (Table 2).

Evaluation of physiological characteristics indicated a significant difference between the control and cold-treated groups in each lentil

genotype. Whereas the chlorophyll index declined in the Bilesovar and ILL-2580 genotypes under cold stress conditions, it enhanced in the Precoz variety by about 252% over the control. Moreover, the proline content was significantly increased in the cold-treated Percoz and ILL-2580 genotypes for about 250-300% over the related controls. On the other hand, the photochemical efficiency of photosystem II (Fv/Fm) was significantly reduced in all three varieties under cold stress. The highest decrease was observed in the ILL-2580 line followed by Percoz (Table 3).

Analysis of biochemical characteristics showed that the amount of total protein was not significantly changed in either of the lentil genotypes. The activity of the catalase enzyme in the Precoz, Bilesovar, and ILL-2580 genotypes

Table 1. Seed yield, plant height, 100-seed weight, and the percentage of old damage of three lentil genotypes

Traits	Genotypes		
	Bilesovar	Precoz	ILL-2580
Plant height (cm)	28.67±0.88a	23.67±1.33b	17.67±0.33c
100-seed weight (g)	6±0.15a	5.13±0.20b	3.36±1.76c
Cold damage (%)	41.67±4.41b	35.2±2.88b	80±5.77a
Seed yield (kg/ha)	1500.67±172.24a	356.67±48.83b	73±17.47c

The means with the similar letters in each row are not significantly different based on Duncan's Multiple Range Test at the 5% probability level

Table 2. The effects of cold stress on growth characteristics of three lentil genotypes

Traits	Genotypes					
	Bilesovar		Precoz		ILL-2580	
	Control	Stress	Control	Stress	Control	Stress
Shoot fresh weight (g)	0.29±0.01a	0.18±0.003c	0.24±0.006b	0.16±0.017c	0.17±0.01c	0.10±0.003d
Shoot dry weight (g)	0.05±0.004b	0.04±0.003b	0.03±0.001c	0.03±0.004c	0.02±0.004c	0.09±0.07a
Root fresh weight (g)	0.21±0.029a	0.13±0.014b	0.15±0.005b	0.14±0.020b	0.14±0.002b	0.18±0.014ab
Root dry Weight (g)	0.099±0.008c	0.016±0.001d	0.14±0.001ab	0.15±0.001a	0.11±0.002b	0.17±0.0009a

The means with the similar letters in each row are not significantly different based on Duncan's Multiple Range Test at the 5% probability level

Table 3. The effects of cold stress on physiological characteristics of three lentil genotypes

Traits	Genotypes					
	Billesovar		Precoz		ILL-2580	
	Control	Stress	Control	Stress	Control	Stress
Chlorophyll	7.09±2.01a	3.80±1.1b	3.89±0.42b	9.65±0.8a	1.85±0.2c	1.45±0.12d
Fo	352.67±11.26b	428.67±14.17a	356.67±2.02b	473.67±11.97a	367±20.79b	488.33±4.8a
Fm	2235.33±393a	1708.67±120bc	1787±17b	1314.33±194c	1917.6±100b	1108.33±101d
Fv/Fm	0.814±0.0005a	0.736±0.002b	0.79±0.003a	0.55±0.005c	0.80±0.001a	0.49±0.002d
Proline (mg/g FW)	204.44±4.5b	172.39±19.5c	113.3±5.7d	344.28±48.18a	66.68±6.7e	181±11.98c

The means with the similar letters in each row are not significantly different based on Duncan's Multiple Range Test at the 5% probability level

increased drastically under cold treatment (about 20, 73, and 12%, respectively, over the related control). Also, for the protease enzyme activity, a significant increase was observed under cold stress in the cold-treated Precoz plants as compared with the related controls. The activity of the polyphenol oxidase enzyme significantly increased by the cold treatment only in the Billesovar variety (Table 4). A significant increase was seen in total flavonoids and anthocyanin content only in the Billesovar variety under cold conditions (about 15 and 35% over the control, respectively). On the contrary, in Precoz and ILL-2580, an insignificant change or a significant decrease was observed under cold stress conditions (Table 5).

The results of SDS PAGE analyses showed that different lentil lines had different electrophoretic patterns of protein bands in the cold-treated plants as compared to the control conditions. The pattern of change was also different among the lentil genotypes. In Precoz, some characteristic bands with molecular weights of 30-40 and 80-90 KD appeared in the cold-treated plants but were absent in the control plants. A similar band with the molecular weight of 80-90 KD was seen in the cold-treated plants of Bilesavar but it was very slight in the related control plants. No important difference was seen between the cold-treated and control plants in the banding pattern of the ILL-2580 line.

Table 4. The effects of cold stress on some biochemical characteristics of three lentil genotypes

Traits	Genotypes					
	Billesovar		Precoz		ILL-2580	
	Control	Stress	Control	Stress	Control	Stress
Total protein (mg/L)	1.16±0.08a	1.19±0.03a	1.10±0.08a	1.15±0.01a	1.14±0.03a	1.08±0.023a
Catalase activity (µmol/mg.min)	0.0040±0.0003d	0.0071±0.0003b	0.0064±0.0002c	0.0077±0.00006b	0.0071±0.0001b	0.0080±0.00001a
Ascorbate peroxidase activity (µmol/mg.min)	0.082±0.007a	0.090±0.001a	0.019±0.003b	0.010±0.004b	0.015±0.007b	0.003±0.001b
Protease activity (µmol/mg.min)	0.0110±0.001b	0.0084±0.0009c	0.0146±0.0003b	0.0220±0.001a	0.0116 ±0.0008b	0.0111 ±0.001b
Polyphenol oxidase activity (µmol/mg.min)	0.053±0.001b	0.078±0.007a	0.050±0.003b	0.051±0.002b	0.051±0.004b	0.047±0.009b

The means with the similar letters in each row are not significantly different based on Duncan's Multiple Range Test at the 5% probability level

Table 5. The effects of cold stress on some phytochemical characteristics of three lentil genotypes

Traits	Genotypes					
	Billesovar		Precoz		ILL-2580	
	Control	Stress	Control	Stress	Control	Stress
Total flavonoid (mg/g FW)	6.75±1.22a	7.80±1.82b	14.43±0.84a	10.69±1.68b	8.56±1.46b	8.92±1.61b
Anthocyanins (mM/g FW)	0.14±0.047b	0.19±0.003a	0.27±0.02a	0.21±0.008a	0.21±0.008a	0.10±0.02b

The means with the similar letters in each row are not significantly different based on Duncan's Multiple Range Test at the 5% probability level

Discussion

It is well known that cold stress causes serious physical damage and metabolic malfunctions in plants, which tends to a dramatic reduction in crop yield. Freezing temperatures in all crops and chilling temperatures in cold susceptible plants alter bio-membrane to a crystalline state and in the case of thylakoid membranes may lead to the destruction of photosystems. By damaging photosystem II and other electron transfer chains, environmental stresses inhibit or drastically reduce photosynthetic electron transfer. In such a situation, a greater portion of the absorbed light energy will be wasted as heat or fluorescence and photosynthetic yield will be declined. This expresses a significant reduction in photochemical efficiency of photosystem II reflected in the F_v/F_m ratio (Roháček *et al.* 2008). Our study showed a considerable diminish in photochemical efficiency of photosystem II in the ILL-2580 line of lentils which was more than the other two genotypes. This indicates the higher susceptibility of the ILL-2580 line to cold stress. Bilesovar exhibited a slight reduction in F_v/F_m ratio under cold treatment demonstrating its fair tolerance against cold stress. A decrease in the ambient temperature have reduced the efficiency of photosystem II in wheat (Hassan *et al.* 2021).

Along with direct physical damage, reactive oxygen species (ROS) production is another source of photosystems injury in plants under cold stress (Baek and Skinner 2012). ROS inhibits the biosynthesis of proteins and in particular, of the D1 protein, which are essential for the synthesis and recovery of photosystem II. Suppressing the reconstruction of the photosystem by ROS is hastened by the malfunction of the Calvin cycle that occurs when the availability of CO₂ is limited under any stressful conditions (Das and Roychoudhury 2014). Moreover, ROS promotes lipid peroxidation process in thylakoid membranes causing photosystems linkages with the membrane. All of these events tend to reduce the photosynthesis rate and the crop yield.

The results of the present work demonstrated that the seed yield of the Billesovar variety was much higher than other genotypes when planted in autumn. Hence, this variety might have had its own defense mechanisms against the harmful impacts of cold stress (Das and Roychoudhury 2014). Our results also indicated that potent antioxidant systems were utilized to scavenge ROS in Billesovar. We found that the activity of some antioxidant enzymes like catalase and polyphenol oxidase in Billesovar was raised at cold stress. This elevation was restricted to

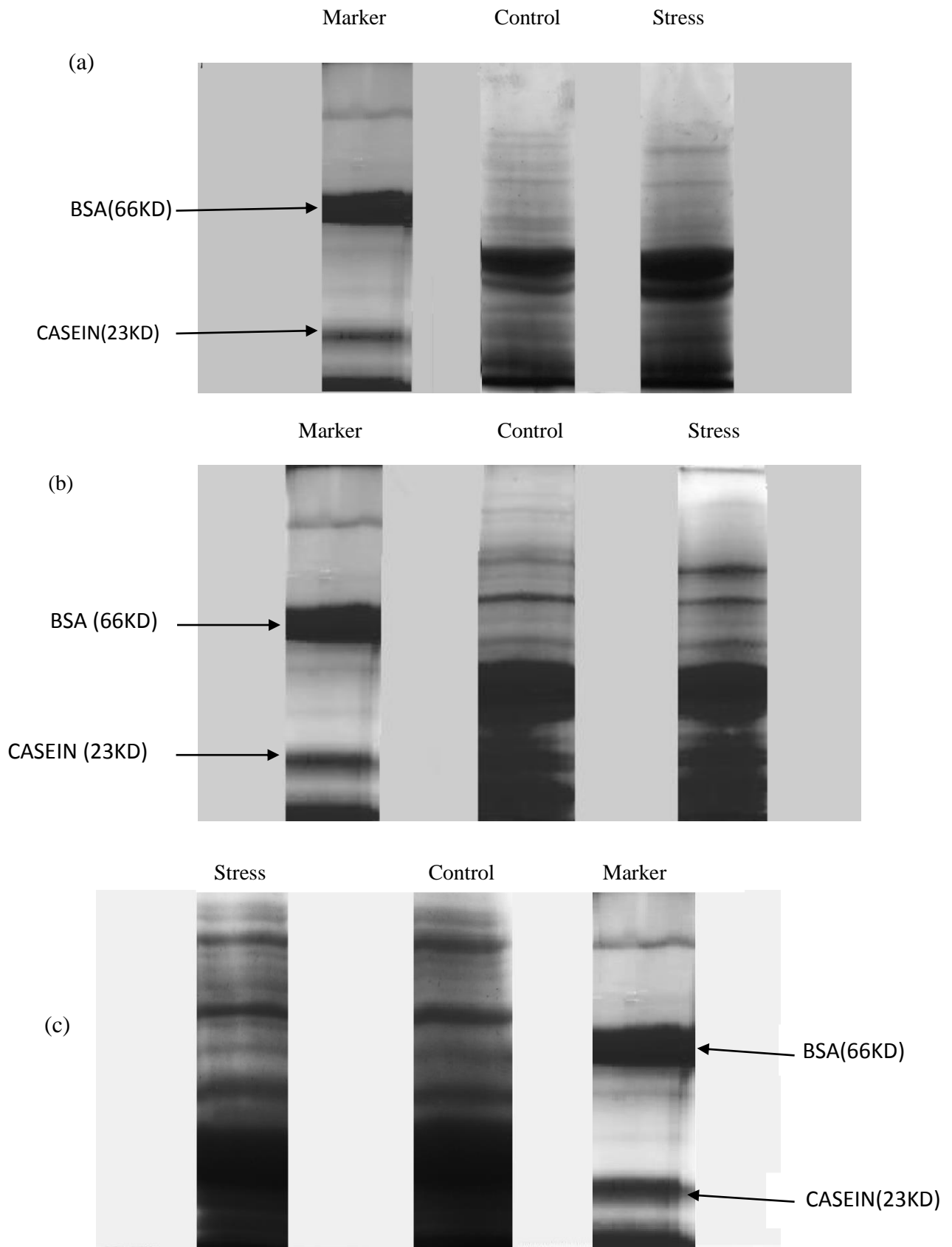


Figure 1. SDS PAGE electrophoretic patterns of the cold-treated and control plants of the three different lentil genotypes, Precoz (a), Billesovar (b), and ILL-2580 (c)

only the catalase enzyme in other tested genotypes with lower intensity. Moreover, non-enzymatic antioxidants such as flavonoids and anthocyanins increased in Billesovar causing its high potential to tolerate the cold stress.

The Percoz variety displayed some physiological aspects of cold tolerance in the greenhouse conditions. It was depicted from the results that chlorophyll index and proline content were elevated in cold-treated Percoz plants as compared to the control plants. Preserving and enhancing the photosynthetic pigments is an adaptive strategy to further exploit radiation at low temperatures at which the metabolic processes slow down (Kalisz *et al.* 2016). Proline, as a common compatible solute in plants, may also serve cryoprotectant function and prevents the plant cells from harmful impacts of low temperatures. It may play an antioxidant role to scavenge ROS from plant cells and act as chaperones by attaching to cell membranes and biomolecules like enzymes to prevent their denaturation at cold caused dehydration and related osmotic stress (Hayat *et al.* 2012).

The results of SDS-PAGE electrophoresis of proteins showed that in the Billesovar and Percoz varieties of lentils some bands appeared under cold stress. These electrophoretic bands belong to proteins with a molecular weight of 30-90 KD and should be related to heat shock proteins (HSPs). It has been previously pointed out that HSPs generally act as molecular chaperones protecting proteins against stressful conditions like cold

stress. HSPs regulate the folding, localization, and degradation of proteins in all plants specie (Al-Whaibi 2011).

In summary, it looks like the Billesovar variety of lentils has a number of mechanisms for cold acclimation. It may have a detoxification system to alleviate the harmful effects of cold stress by scavenging ROS. This variety could exhibit also an osmoregulation process to maintain the plant cell water under cold-stress conditions.

Conclusions

It can be concluded that the Billesovar variety of lentils might be considered a cold-tolerant genotype. This genotype can be subjected to winter sowing and may be a good candidate for planting in cold regions of Iran. The yield of lentil genotypes in winter planting might be more than 2-4 times of spring planting. It is due to the usage of rainfall and escaping from drought and heat stress of the summer season. Percoz and ILL-2580 can be regarded as semi-tolerant and cold-sensitive genotypes, respectively.

Acknowledgments

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Conflict of Interest

The authors declare that they have no conflict of interest with any people or organizations concerning the subject of this manuscript.

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اثرات تنش سرما روی برخی ویژگی‌های فیزیوشیمیایی سه لاین - واریته از عدس

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چکیده

عدس از حبوبات بسیار قدیمی است که بسیار آسیب پذیر به سرما می‌باشد. از این رو، شناسایی روند تحمل به سرما در بین لاین‌ها یا واریته‌های عدس امری بسیار مهم می‌باشد. در این تحقیق، به منظور ارزیابی عملکرد دانه و میزان آسیب ناشی از سرما، یک آزمایش مزرعه‌ای در قالب طرح بلوک‌های کامل تصادفی در سه تکرار بر روی واریته‌های بیله‌سوار و پریکوز و لاین آی ال ال ۲۵۸۰ از عدس انجام گرفت. به علاوه، به منظور مطالعه پاسخ‌های فیزیولوژیکی و بیوشیمیایی این ژنوتیپ‌ها به سرما یک تحقیق گلخانه‌ای در قالب آزمایش فاکتوریل بر پایه طرح کاملاً تصادفی انجام گرفت. فاکتورهای آزمایش عبارت از شرایط سرما و ژنوتیپ‌های عدس بودند. بیشترین ارتفاع گیاه، وزن ۱۰۰ دانه، عملکرد دانه و وزن تر و خشک اندام‌های هوایی گیاه تحت تنش سرما در واریته بیله‌سوار و بعد از آن واریته پریکوز مشاهده شد. به علاوه بیشترین درصد آسیب سرمایی (۸۰٪) در لاین آی ال ال ۲۵۸۰ دیده شد. در حالی که آسیب سرمایی در واریته‌های بیله‌سوار و پریکوز بسیار کمتر و به ترتیب ۴۱ و ۳۲ درصد بود. نتایج همچنین نشان داد که میزان شاخص کلروفیل (اسپاد) در بیله‌سوار و آی ال ال ۲۵۸۰ در طی تنش سرمایی کاهش یافت ولی در واریته پریکوز در حد ۲۵۲ درصد نسبت به شاهد افزایش نشان داد. افزون بر این، محتوی پرولین پریکوز تحت تأثیر سرما در حد ۳۰ درصد افزایش پیدا کرد. فعالیت آنزیم آنتی‌اکسیدان کاتالاز در بیله‌سوار و پریکوز به ترتیب به میزان ۷۳ و ۲۰ درصد در شرایط تنش نسبت به شاهد افزایش نشان داد. میزان فلاونوئید کل و آنتوسیانین فقط در رقم بیله‌سوار در شرایط تنش سرما افزایش یافت. نتایج آنالیز SDS PAGE نیز مشخص کرد که در بیله‌سوار و پریکوز برخی باندهای پروتئینی شاخص با وزن مولکولی بین ۴۰-۳۰ و ۹۰-۸۰ کیلودالتون در شرایط تنش سرمایی پدیدار شدند که در نمونه‌های شاهد وجود نداشتند. در کل، می‌توان نتیجه‌گیری کرد که بیله‌سوار می‌تواند به عنوان رقم عدس متحمل به سرما در نظر گرفته شود. از طرف دیگر پریکوز و آی ال ال ۲۵۸۰ به ترتیب به عنوان ژنوتیپ‌های نیمه‌متحمل و حساس به سرما به حساب می‌آیند.

واژه‌های کلیدی: تحمل سرما؛ عدس؛ فیزیولوژی؛ ویژگی‌های بیوشیمیایی