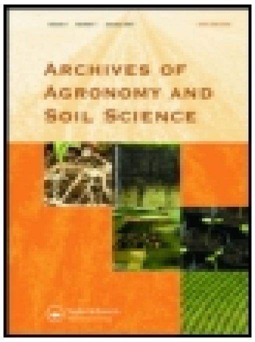

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Screening lentil (*Lens culinaris* Medik.) genotypes for fall sowing and low temperature tolerance

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ABSTRACT

Lentil is often exposed to drought stress during the reproductive phase in arid and semi-arid regions. Shifting the sowing date of lentil plants from spring to fall prevents the reproductive phase from exposure to drought stress. Nevertheless, winter frosts limit lentil productivity in the fall sowing. Twenty lentil genotypes were studied in two locations (Mashhad and Jolgeh Rokh, Iran) during 2018–2019. The results showed a 100% survival in all genotypes in Mashhad due to the lack of extreme cold. While the temperature of −12.4°C in Jolgeh-Rokh in February resulted in variable plant survival rates; the highest survival rates were observed in MLC103 (45.2%). In Mashhad, plant height and the number of lateral branches of genotypes were ~15 and 52%, respectively, greater than those in Jolgeh-Rokh. The greatest biological and grain yield was observed in MLC33 and MLC70, respectively, in Mashhad and Jolgeh-Rokh. However, none of the genotypes could produce more than 1000 kg ha^{-1} grain yield, which might be due to the Ascochyta blight disease in Mashhad and extreme cold in Jolgeh-Rokh. Cluster grouping showed that the MLC409 and MLC70 were selected in cold regions, while the MLC33, MLC47, MLC409, and MLC454 were recommended for temperate climates.

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KEYWORDS
Cold tolerance; cluster analysis; genetic diversity; mechanized harvesting; survival rate

Introduction

Legumes, as an abundant and inexpensive source of protein, are an integral part of the human diet and food security (Reddemma et al. 2019). Lentil (*Lens culinaris* Medik.) is an annual legume with high nutritional value, which is alternated with cereals in the cropping systems due to its ability to fix nitrogen (Gan et al. 2017). Lentil grains are a source of proteins and minerals and are used to feed humans and its straw is used to feed livestock (Ghanem et al. 2015). According to the Food and Agriculture Organization of the United Nations (FAO), the cultivation area of lentils in Iran was 1,468,000 ha and the grain yield was ~600 kg ha^{-1} in 2018, which is 49% less than the global production average (FAO 2018). The lower production was mainly due to the lack of high-quality seeds, improved cultivars, poor weed management, and lack of mechanized harvesting (Jawad et al. 2019).

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Planting date is one of the most important determinants of lentil growth and yield (Sen et al. 2016). The proper planting date of crops leads to achieving the highest economic performance by improving the growth potentials (Ahmad et al. 2018). The planting date largely affects the vegetative and reproductive growth and ripening of crops (Silim et al. 1991; Khalil Shad et al. 2010). In Iran, lentil is mostly cultivated in arid and semi-arid regions under the rainfed cropping systems, which enhances the risk of high temperature and drought stresses during the late spring and summer. While spring is a common season for lentil production in Iran, shifting the planting date to fall and winter could be an effective managerial factor in the lentil yield improvement. Such cropping date could lead to effective use of lentils from humid winter conditions during the growing season, as well as better adaptation to conservative tillage and less soil compaction, prevention of spring crop delay, development of the area under cultivation, and compliance with crop rotations (Ouji and Mouelhi 2017; Karimzadeh-Soureshjani et al. 2021).

In the spring cropping, lentil crop yield significantly decreases due to a shorter vegetative growth period and the dry and hot weather conditions at the end of the growth period. Moosavi et al. (2014) found that a delayed planting date decreased the lentil number of pods per plant, grain yield, and harvest index by 16%, 15%, and 13%, respectively. A diverse grain yield has been reported in the fall and spring planting dates in lentil genotypes; the crop yield improved by 67% in the fall compared to the spring planting date, which indicates the positive crop reaction to the fall planting dates (Barrios et al. 2016). Furthermore, the delayed planting date decreased the number of pods and the crop yield due to the reduction in flowering and ripening days. Nearly all lentils planted in November produced higher grain yields compared to those grown in February (Ouji and Mouelhi 2017). The branch number per plant was significantly higher in the fall planting owing to the appropriate use of humidity and temperature during vegetative growth compared to the spring planting date. A positive correlation was also observed between the branch number per plant and grain yield since the increased number of branches increased the number of pods and grain yield (Zafaranieh 2015).

Despite several benefits of lentil cultivation in fall and early winter, extreme frosts and freezing temperatures may severely damage the crop. Freezing stress is a limitation in crop distribution and production (Barrios et al. 2017). Most lentil fall cultivation areas are affected by winter frosts. Despite the benefits of fall planting, success requires cultivars that are tolerant to low temperatures, especially freezing stress (Mikić et al. 2011). The field plant survival index is used to assess a plant cold tolerance after the onset of winter cold. No significant difference has been reported in lentil genotypes up to −3°C, and almost no damage was observed to the plant leaves and meristem (Barrios et al. 2017). However, the plant survival of lentil genotypes differed following a decline in temperature up to −15°C. Regarding the benefit of fall planting and the importance of lentils as a valuable source of protein in human nutrition, and its role in soil fertility (Hojjat and Galstyan 2014), the present study was carried out in two locations aimed to select the most freezing-tolerant lentil genotypes under the field conditions.

Material and methods

The experiment site and procedure

The cold tolerance, yield, and yield components of 20 lentil genotypes (*Lens culinaris* Medik) were evaluated in a completely randomized block design with three replications. The characteristics and specific names of the genotypes are presented in Table 1. The genotypes were studied under the field conditions at two locations in Khorasan Razavi province, Iran: (1) the Faculty of Agriculture Research Station, Ferdowsi University of Mashhad, Mashhad, and (2) Jolgeh Rokh Agriculture Research Station, Torbat-e Heydarieh in 2018–2019. Rainfall and minimum absolute temperature during the growing season were 331 mm and −5°C, respectively, having below zero temperatures for 26 days at Mashhad station. In Jolgeh Rokh, however, rainfall and minimum absolute temperature during the growing season were 304 mm and −12.4°C, respectively, with below zero temperatures for 89 days without snow coverage (Figure 1).

Table 1. The lentil genotype names and origin.

Row	Genotype	Origin	Row	Genotype	Origin
1.	MLC8	Iran	11.	MLC84	ILL7723
2.	MLC11	Iran	12.	MLC103	ILL5698
3.	MLC13	Iran	13.	MLC286	ILL8617
4.	MLC17	Iran	14.	MLC303	Iran
5.	MLC33	Iran	15.	MLC334	Iran
6.	MLC38	Iran	16.	MLC407	Iran
7.	MLC47	Iran	17.	MLC409	Iran
8.	MLC70	ILL7679	18.	MLC454	Iran
9.	MLC74	ILL7681	19.	MLC469	Iran
10.	MLC83	ILL7618	20.	MLC472	Iran

MLC: Mashhad Lentil Collection, ILL: International Legume Lentil.

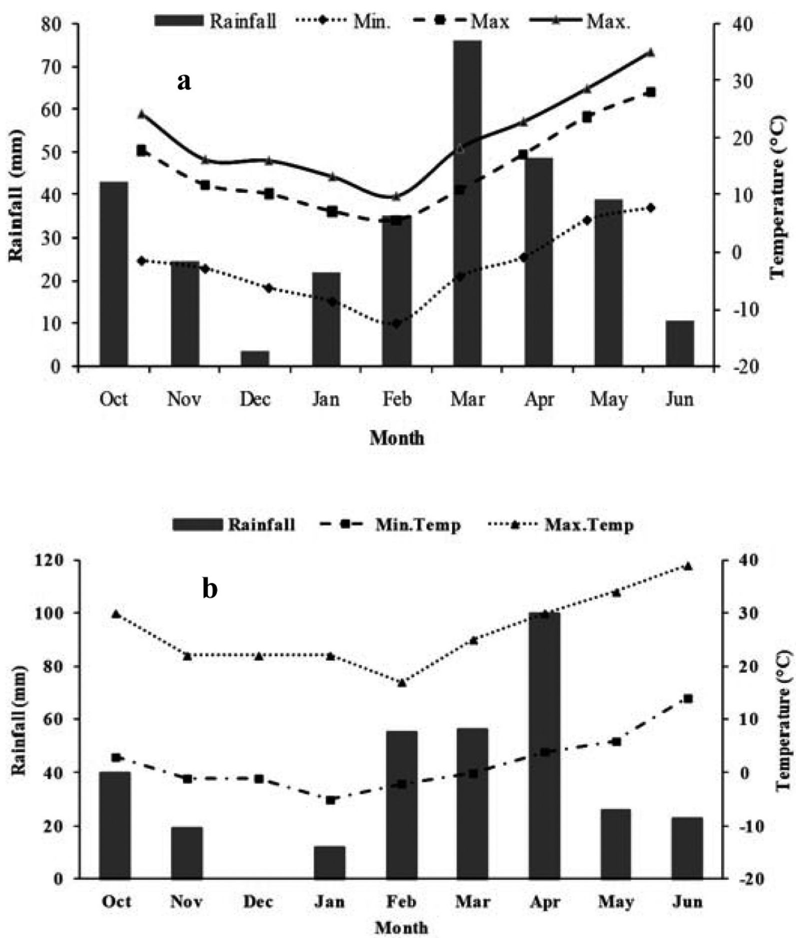

Figure 1. Daily minimum and maximum temperature and average monthly rainfall of (a) Jolgeh Rokh and (b) Mashhad during lentil growing season in 2018–2019.

Table 2. Main chemical properties of the soil at the Mashhad and Jolgeh Rokh experimental sites.

Mashhad					Jolgeh Rokh				
Soil texture	K(mg kg^{-1})	P(mg kg^{-1})	N(%)	pH	Soil texture	K(mg kg^{-1})	P(mg kg^{-1})	N(%)	pH
Silty Loam	397	29.8	0.07	7.8	Loam	292	10.6	0.065	7.8

Field preparation and sowing

Uniform lentil seeds (*cv*. Chase) were hand-sown on October 10th and 14th in Mashhad and Jolgeh Rokh locations, respectively, by a distance of 1 cm in 2 cm soil depth and four rows with 50 cm apart and two meters long in plots of 4 m^{-2}. The experimental soil characteristics are presented in Table 2. Weeding was done twice per month by hand. The field was surface irrigated right after sowing, and the plants were then irrigated weekly avoiding drought stress exposure at any growth stage.

Measurements

Ascochyta blight estimation

The Ascochyta blight damages were measured at ~45-50 days after planting. Various genotypes were scored from zero (resistant) to 10 (extremely sensitive) to determine the level of Ascochyta blight tolerance (Pande et al. 2013).

Plant survival rate

Plant survival rate was calculated using Eq. (1) to determine the 30-day winter survival rate.

$$Survival\ Rate = \frac{Number\ of\ Plants\ before\ Winter\ (after\ emergence)}{Number\ of\ alive\ Plants\ after\ Winter} \times 100 \quad (1)$$

Plant growth, yield, and yield components

At the end of the growing season, when all genotypes were physiologically ripened, five plants were harvested randomly from each plot considering the marginal effects and plant height, number of branches per plant, and yield and yield components (number of pods and seed weight per plant) were measured. The height of the lowest pod was measured using a ruler from the soil surface. To determine the biological and grain yield, plants from the two middle rows of each plot were harvested considering the marginal effects and were weighted after drying in the open air. The biological yield (aerial parts) was measured by harvesting the plants from the soil surface and weighed. Total yield per plot was calculated as the sum of the yield of the harvested plants and the five subsampled plants. Harvest index (HI) was estimated using Eq. (2).

$$Harvest\ Index = \frac{Biomass}{Grain\ weight} \times\ 100 \quad (2)$$

Statistical analysis

Data were subjected to combined analysis of variance using SAS 9.4, and means were compared using Duncan's test $_{p \le 0.05}$. Since the location × genotype interaction was significant, the means of the two locations were analyzed separately. In addition, JMP4 software and ward technique were used to estimate the correlations and cluster analysis.

Results

Ascochyta blight

Ascochyta blight was broadly detected in Mashhad due to rainfalls and weather conditions. Accordingly, the three genotypes of MLC33, MLC303, and MLC454 showed the highest resistance to Ascochyta blight, and the MLC47, MLC83, and MLC409 genotypes had a relatively high tolerance to the disease with minor damages (Table 3). On the other hand, the most sensitive genotypes to Ascochyta blight were MLC13, MLC286, MLC407, and MLC472 (Table 3).

Plant survival rate

The results indicated a difference between Mashhad and Jolgeh Rokh locations in plant survival rate. All genotypes had a 100% survival rate in Mashhad due to the lack of extreme cold (Table 4). Meanwhile, due to extreme cold in Jolgeh Rokh, all genotypes showed a plant survival rate lower than 50%; a 44% difference was observed between the highest and lowest survival rates. The majority of the genotypes' survival was observed within the range of 1–30%, which included 85% (n = 17) of the genotypes. The highest survival rate was observed in MLC103, MLC472, and MLC8, which had relative superiority over the other genotypes at a minimum temperature of −12°C with a higher grain yield (Table 5). Among the genotypes that were classified as the most tolerant against Ascochyta blight (Table 3), the highest survival rate belonged to MLC303 in Jolgeh Rokh (Table 5).

Plant height

The genotypes were significantly different in plant height at two locations (Tables 4 and 5). In Mashhad, the highest and lowest plant height with a 22 cm difference was observed in MLC33 and MLC286, respectively. While in Jolgeh Rokh, the highest and lowest plant height was observed in MLC70 and MLC38, respectively, with a 32 cm difference. In addition, the most significant difference in the plant height was observed in MLC33 and MLC38 in Mashhad, which were 24 and 21 cm higher than the same genotypes in Jolgeh Rokh.

Lowest pod height

The results of the present study indicated a significant difference between the genotypes in terms of the lowest pod height (Tables 4 and 5). Overall, the pod height was lower in all genotypes in Jolgeh Rokh compared with Mashhad. The lowest pod height in Mashhad and Jolgeh Rokh was on average 7.33–20.8 and 0.867–7.33 cm, respectively. Among the lentil genotypes, MLC409 and MLC33, and

Table 3. Classification resistance to Ascochyta blight in lentil genotypes in Mashhad.

Row	Genotype	Score	Row	Genotype	Score
1.	MLC8	3.33	11.	MLC84	5.00
2.	MLC11	6.00	12.	MLC103	5.00
3.	MLC13	7.00	13.	MLC286	7.00
4.	MLC17	4.00	14.	MLC303	0.00
5.	MLC33	0.00	15.	MLC334	3.67
6.	MLC38	2.00	16.	MLC407	7.00
7.	MLC47	1.33	17.	MLC409	1.33
8.	MLC70	3.33	18.	MLC454	0.00
9.	MLC74	3.33	19.	MLC469	6.00
10.	MLC83	1.33	20.	MLC472	7.00

MLC: Mashhad Lentil Collection. Resistant (0.0–1.9), tolerated (2.0–3.9), sensitive (4.0–6.9) and extremely sensitive (7.0–10)

Table 4. Survival percentage, morphological traits, and yield and yield components of lentil genotypes in fall planting during the growing season 2018–2019 in Mashhad.

Genotype	Survival	Plant height	Lowest pod height	Branch No.	Pod No. Plant^{-1}	Fertile pod	Dry weight (g	Grain (g	Biological yield	grain yield (kg	HI
MLC	(%)	(cm)	(cm)			(%)	plant^{-1})	plant^{-1})	(kg ha^{-1})	ha^{-1})	(%)
8	100^{a}	29.9^{cd}	10.7$^{d\text{-}f}$	5.27^{b}	52.0$^{g\text{-}i}$	59.3$^{a\text{-}e}$	16.9^{d}	1.99^{de}	1810$^{e\text{-}h}$	163de	8.67^{cd}
11	100^{a}	21.7$^{e\text{-}h}$	8.00^{f}	4.73^{b}	70.0$^{e\text{-}h}$	37.0^{e}	10.6$^{e\text{-}h}$	0.703^{gh}	1100$^{g\text{-}j}$	61$^{e\text{-}g}$	5.67$^{e\text{-}h}$
13	100^{a}	20.3^{gh}	9.53^{ef}	3.93^{b}	9.00^{i}	48.0$^{b\text{-}e}$	6.49^{gh}	0.055^{i}	352^{j}	9.78^{g}	3.00^{h}
17	100^{a}	23.7$^{d\text{-}h}$	10.1$^{d\text{-}f}$	4.73^{b}	102^{ef}	55.7$^{a\text{-}e}$	9.97$^{e\text{-}h}$	0.698^{gh}	983$^{h\text{-}j}$	62.9$^{e\text{-}g}$	6.67$^{d\text{-}g}$
33	100^{a}	40.7^{a}	15.7^{bc}	7.80^{a}	113de	67.3$^{a\text{-}d}$	22.2^{c}	5.43^{a}	7500^{a}	949^{a}	12.7^{a}
38	100^{a}	26.5$^{c\text{-}h}$	10.4$^{d\text{-}f}$	5.60^{b}	153^{cd}	40.0^{de}	15.2^{de}	1.55^{ef}	2580de	190^{d}	7.33^{de}
47	100^{a}	38.0^{ab}	12.9$^{c\text{-}e}$	5.40^{b}	178^{bc}	75.7^{a}	22.7^{bc}	2.48^{d}	4610^{c}	486^{c}	12.3^{ab}
70	100^{a}	29.0$^{c\text{-}e}$	16.8^{b}	4.40^{b}	32.0^{hi}	78.0^{a}	12.1$^{d\text{-}g}$	0.424^{hi}	3410^{d}	512^{c}	12.7^{a}
74	100^{a}	27.9$^{c\text{-}f}$	9.80$^{d\text{-}f}$	4.20^{b}	39.0^{hi}	36.7^{e}	10.3$^{e\text{-}h}$	0.453^{hi}	1540$^{f\text{-}i}$	74.5$^{d\text{-}g}$	5.00$^{e\text{-}h}$
83	100^{a}	27.3$^{c\text{-}g}$	10.3$^{d\text{-}f}$	4.20^{b}	42.0^{hi}	59.7$^{a\text{-}e}$	15.1^{de}	0.669^{gh}	2270^{ef}	151$^{d\text{-}f}$	6.67$^{d\text{-}g}$
84	100^{a}	26.7$^{c\text{-}g}$	10.1$^{d\text{-}f}$	4.27^{b}	51.7$^{g\text{-}i}$	47.0$^{b\text{-}e}$	10.4$^{e\text{-}h}$	0.524$^{g\text{-}i}$	1510$^{f\text{-}i}$	97.5$^{d\text{-}g}$	5.67$^{e\text{-}h}$
103	100^{a}	23.9$^{d\text{-}h}$	7.33^{f}	4.20^{b}	37.3^{hi}	62.3$^{a\text{-}e}$	9.74$^{e\text{-}h}$	0.593$^{g\text{-}i}$	4820^{c}	97.6$^{d\text{-}g}$	5.00$^{e\text{-}h}$
286	100^{a}	19.2^{h}	7.46^{f}	3.67^{b}	21.0^{hi}	71.7^{ab}	6.38^{h}	0.296^{hi}	726^{ij}	34.1^{fg}	4.33$^{f\text{-}h}$
303	100^{a}	38.6^{ab}	12.5^{de}	5.80^{b}	203^{b}	67.7$^{a\text{-}c}$	27.5^{ab}	3.79^{b}	6010^{b}	678^{b}	10.0^{bc}
334	100^{a}	26.3$^{c\text{-}h}$	9.67^{ef}	4.33^{b}	94.0$^{e\text{-}g}$	43.3$^{c\text{-}e}$	13.3$^{d\text{-}f}$	0.711^{gh}	2130$^{e\text{-}g}$	109$^{d\text{-}g}$	5.00$^{e\text{-}h}$
407	100^{a}	28.9$^{c\text{-}f}$	10.2$^{d\text{-}f}$	4.33^{b}	50.3$^{g\text{-}i}$	68.3$^{a\text{-}c}$	12.4$^{d\text{-}f}$	1.09^{fg}	810$^{h\text{-}j}$	57.1$^{e\text{-}g}$	7.00$^{d\text{-}f}$
409	100^{a}	32.9^{bc}	20.8^{a}	9.00^{a}	387^{a}	66.7$^{a\text{-}d}$	17.6^{cd}	3.83^{b}	4600^{c}	431^{c}	9.00^{cd}
454	100^{a}	39.1^{ab}	13.1^{cd}	8.20^{a}	176^{bc}	59.0$^{a\text{-}e}$	32.2^{a}	3.21^{c}	7200^{a}	917^{a}	13.0^{a}
469	100^{a}	21.5$^{f\text{-}h}$	9.13^{f}	4.07^{b}	32.3^{hi}	47.3$^{b\text{-}e}$	8.12$^{f\text{-}h}$	0.828^{gh}	731^{ij}	28.8^{g}	3.67^{h}
472	100^{a}	24.3$^{d\text{-}h}$	8.93^{f}	4.13^{b}	58.7$^{f\text{-}i}$	69.0$^{a\text{-}c}$	12.5$^{d\text{-}f}$	0.596$^{g\text{-}i}$	1190$^{g\text{-}j}$	47.1$^{e\text{-}g}$	4.00^{gh}
P-value	ns	**	**	**	**	**	**	**	**	**	**
C.V (%)	0.0	13.5	15.5	21.5	27.1	24.2	20.4	20.1	20.1	24.0	19.7

MLC: Mashhad Lentil Collection, C.V: Coefficient Variation, ns: Non-significant, *: Significant at $p \le 0.05$, **: Significant at $p \le 0.01$, Means with at least one similar letter in each column are not significantly different based on the Duncan test at $p \le 0.05$.

MLC33, MLC47, MLC70, and MLC409 in Mashhad and Jolgeh Rokh, respectively, recorded the highest height of the first pod from the soil surface and were selected as the most appropriate genotypes for the mechanized lentil harvesting in those regions (Tables 4 and 5).

Number of lateral branches

A significant difference in the number of the lateral branches was observed between the genotypes grown in different locations; more than 90% of the genotypes had more than four branches in Mashhad. In comparison, all genotypes grown in Jolgeh Rokh had less than three lateral branches. Generally, MLC409, MLC454, and MLC33 genotypes in Mashhad and MLC74 in Jolgeh Rokh had the highest number of lateral branches (Tables 4 and 5).

Number of fertile pods

In Jolgeh Rokh, the genotypes had shorter vegetative and reproductive growth periods than Mashhad due to the long cold weather and lower temperatures. A significant difference was also observed between genotypes in the fertile pod percentage (Tables 4 and 5). A 41% difference was observed between the genotypes with the highest (MLC70) and lowest (MLC74) fertile pods in Mashhad, while the differences were estimated as 65% in Jolgeh Rokh. Sixty-five percent of the genotypes had more than 90% fertile pods and the fertility rate of pods was higher in all the genotypes in Jolgeh Rokh than Mashhad, except for the MLC38 and MLC407 (Tables 4 and 5).

Table 5. Survival percentage, morphological traits, and yield and yield components of lentil genotypes in fall planting during the growing season 2018–2019 in Jolgeh Rokh.

Genotype MLC	Survival (%)	Plant height (cm)	Lowest pod height (cm)	Branch No.	Pod No. Plant^{-1}	Fertile pod (%)	Dry weight (g plant^{-1})	Grain (g plant^{-1})	Biological yield (kg ha^{-1})	Grain yield (kg ha^{-1})	HI (%)
8	38.0^{a}	23.4^{a-c}	5.07^{a-e}	2.27^{a-c}	29.2^{d-i}	95.1^{ab}	2.26^{c-f}	0.965 $^{f-j}$	441^{b}	159^{b}	38^{a-c}
11	24.0^{bc}	25.2^{ab}	4.67 $^{c-e}$	2.87^{ab}	48.3^{b-e}	90.0^{ab}	3.53^{b-e}	2.10^{b-d}	232 $^{c-e}$	68.0^{ef}	40^{a-c}
13	16.8^{c-e}	25.5^{ab}	4.33 $^{c-e}$	2.67^{ab}	39.3^{b-h}	94.1^{ab}	3.05^{b-e}	1.19^{e-i}	306 ^{c}	126 ^{c}	43^{ab}
17	12.1^{ef}	24.2^{a-c}	5.20^{a-e}	2.73^{ab}	42.3^{b-g}	92.2^{ab}	3.41^{b-e}	1.38 $^{c-h}$	143^{e-g}	47.3 $^{f-h}$	25 ^{c}
33	14.9^{de}	24.5^{a-c}	7.33^{ab}	2.67^{ab}	37.3 $^{c-h}$	81.2^{ab}	4.01^{bc}	1.69^{b-f}	135 ^{fg}	48.4 $^{f-h}$	39^{a-c}
38	0.450 ^{h}	5.67^{d}	0.867 ^{f}	0.700 ^{db}	9.47^{i}	30.5 ^{c}	0.678^{fe}	0.227 ^{j}	11.9 ^{h}	3.67^{i}	10^{d}
47	2.69^{gh}	26.9^{ab}	7.43^{a}	2.33^{ab}	61.7^{b}	96.1^{a}	4.72^{b}	1.59^{b-g}	17.3 ^{h}	15.5^{hi}	39^{a-c}
70	28.2^{b}	33.1^{a}	7.47^{a}	2.80^{ab}	103^{a}	94.9^{ab}	8.20^{a}	3.18^{a}	730^{a}	246^{a}	36^{a-c}
74	5.56 $^{f-h}$	22.4^{bc}	5.27^{a-e}	3.33^{a}	40.5^{b-h}	92.0^{ab}	3.73^{bc}	1.21^{e-i}	87.8 ^{gh}	73.6^{de}	38^{a-c}
83	4.71 $^{f-h}$	28.3^{ab}	6.53^{a-c}	2.47^{ab}	52.4^{b-d}	95.3^{a}	2.98^{b-e}	2.17^{bc}	158^{e-g}	62.5^{e-g}	40^{a-c}
84	9.46^{e-g}	20.7^{bc}	4.00^{de}	2.60^{ab}	18.1^{hi}	62.7^{a-c}	2.27 $^{c-f}$	1.46 $^{c-h}$	67.6^{gh}	30.4 $^{g-i}$	45^{a}
103	45.2^{a}	26.0^{ab}	4.40 $^{c-e}$	2.80^{ab}	41.8^{b-g}	91.3^{ab}	2.84^{b-e}	1.39 $^{c-h}$	506^{b}	218^{a}	41^{a-c}
286	3.59 $^{f-h}$	23.7^{a-c}	4.27 $^{c-e}$	2.40^{ab}	60.2^{bc}	92.5^{ab}	4.88^{b}	1.85^{b-e}	65.3 ^{gh}	28.8 $^{g-i}$	40^{a-c}
303	22.6^{b-d}	24.2^{a-c}	6.33^{a-d}	2.40^{ab}	27.3^{e-i}	80.5^{ab}	2.32 $^{c-f}$	0.781 $^{g-j}$	286 ^{c}	85.9^{de}	34^{a-c}
334	2.47^{gh}	26.4^{ab}	4.93^{b-e}	2.53^{ab}	42.5^{b-g}	93.8^{ab}	3.70^{b-d}	1.42 $^{c-h}$	84.7 ^{gh}	31.8 $^{g-i}$	37^{a-c}
407	3.29^{gh}	14.6 ^{c}	3.13^{e}	1.33 ^{cd}	24.1 $^{f-i}$	54.5^{bc}	2.00 $^{c-f}$	0.757 $^{h-j}$	15.0 ^{h}	5.97^{i}	26^{bc}
409	11.1^{e-g}	30.7^{ab}	6.67^{a-c}	2.47^{ab}	90.9^{a}	93.8^{ab}	7.15^{a}	3.13^{a}	186^{d-f}	164^{b}	45^{a}
454	8.74^{e-h}	26.3^{ab}	6.27^{a-d}	2.57^{ab}	38.2^{b-h}	87.0^{ab}	4.67^{b}	2.22^{b}	95.1 $^{f-h}$	32.9 $^{g-i}$	34^{a-c}
469	10.5^{e-g}	22.4^{bc}	5.50^{a-e}	2.10^{bc}	22.6 $^{g-i}$	85.8^{ab}	1.58^{d-f}	0.510^{ij}	23.4 ^{h}	7.70^{i}	34^{a-c}
472	38.7^{a}	24.7^{a-c}	5.87^{a-d}	2.53^{ab}	47.7^{b-f}	94.9^{ab}	3.61^{b-d}	1.35^{d-h}	264 ^{cd}	107 ^{cd}	42^{ab}
P-value	**	**	**	**	**	*	**	**	**	**	**
C.V (%)	29.7	22.1	23.5	22.9	27.9	24.0	31.8	27.0	27.1	23.7	23.4

MLC: Mashhad Lentil Collection, C.V: Coefficient Variation, *: Significant at $p \le 0.05$, **: Significant at $p \le 0.01$. Means with at least one similar letter in each column are not significantly different based on the Duncan test at $p \le 0.05$.

Plant dry weight (DW)

The results demonstrated a significant difference between lentil genotypes in the plant DW (Tables 4 and 5). In Mashhad, the highest and lowest plant DW was observed in MLC303 and MLC286 genotypes, respectively, with a 77% difference. While in Jolgeh Rokh, an 86% difference was observed between MLC286 and MLC38 genotypes, which had the highest and lowest plant DW, respectively (Tables 4 and 5).

Biological yield (BY)

The results of the present study demonstrated a significant difference between the genotypes in plant BY per unit area (Tables 4 and 5). Plant BY ranged from 7500 kg ha^{-1} in MLC33 (resistant to Ascochyta blight) to 352 kg ha^{-1} in MLC13. Overall, ~65% of the genotypes produced more than 1000 kg ha^{-1} plant BY in Mashhad (Table 4). Meanwhile, all genotypes had <1000 kg ha^{-1} plant BY in Jolgeh Rokh (Table 5).

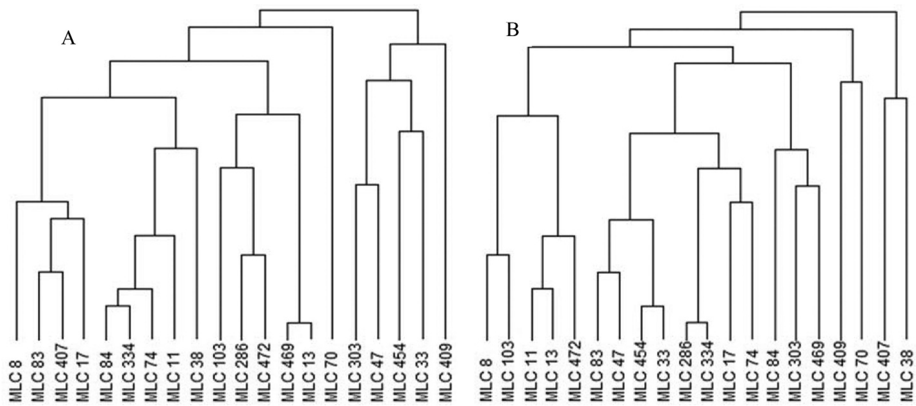

Figure 2. Cluster analysis of 20 lentil genotypes based on the morphological traits, yield, and yield components under field conditions in (a) Mashhad and (b) Jolgeh Rokh.

Grain yield (GY)

Lentil genotypes were different in grain yield; however, no significant difference in grain yield was observed between the two locations (Tables 4 and 5). The highest grain yield was observed in MLC33 and MLC70, respectively, in Mashhad and Jolgeh Rokh. The genotypes that were extremely sensitive to Ascochyta blight (*i.e.* MLC13 and MLC472) showed the lowest grain yield in Mashhad than Jolgeh Rokh. The survival rate of the genotypes was estimated as 17% and 39%, respectively, in Mashhad than Jolgeh Rokh. The most significant grain yield difference between the locations belonged to MLC47, with a > 97% grain yield in Mashhad compared with Jolgeh Rokh. The resistant genotypes to Ascochyta blight had low tolerance to cold in Jolgeh Rokh. Our findings also indicated that 80% of the genotypes had a lower grain yield than 500 kg ha^{-1} in Mashhad, while no genotypes had a higher grain yield than 500 kg ha^{-1} in Jolgeh Rokh.

Harvest index (HI)

Our findings showed that the genotypes had significantly different HI (Tables 4 and 5). Notably, the contribution of plant BY in seed filling was lower in Mashhad, with the highest HI as 13% (MLC454), which was relatively low. Only 25% of genotypes had a HI >10% in Mashhad (Table 4), while only 3 out of 20 genotypes had a HI less than 30% in Jolgeh Rokh (Table 5).

Cluster analysis

According to the results of cluster analysis, the genotypes were divided into three groups in Mashhad; Overall, fourteen, one, and five genotypes were grouped into groups one, two, and three, respectively (Figure 2). Meanwhile, the genotypes in Jolgeh Rokh were divided into five groups (Table 5). In Mashhad, the genotype in the second group (MLC70) was superior to the other genotypes in terms of the height of the first pod, the fertile pod percentage, and HI, while the genotypes of the third group were considered superior in the other traits (Table 8). The results of the deviation from the mean values in Jolgeh Rokh demonstrated the superiority of the first and fourth groups in the survival rate, the number of fertile pods, GY, and HI compared with the total mean value and other groups (Table 9). As for the other traits, the genotypes grouped in the fourth group were superior to the other groups. Despite the survival rate of >30% in the fourth group genotypes (*i.e.* MLC409 and MLC70), it seems that these genotypes remaining after the cold period were able to complete their reproductive stages under better temperature and humidity conditions, leading to a better growth using the optimal rainfall in fall and a prolonged vegetative growth period.

Discussion

Plant survival rate

Plant survival is considered the most important factor in freezing stress tolerance of plants in fall planting (Barrios et al. 2017). Maintaining a high survival rate under freezing stress conditions is an important criterion for the selection of genotypes. This important feature is affected by the minimum absolute temperature during cold periods. According to the results of controlled studies, the minimum duration of freezing stress of one hour at a given freezing temperature determines the tolerance to freezing stress at that temperature (Karimzadeh-Soureshjani et al. 2021). In addition to the minimum absolute temperature during the cold season in fall planting, it seems that the duration of exposure to temperatures below zero also affects the plant survival rate. Furthermore, studies demonstrated a decreased survival rate of lentils (*Lens culinaris*) with a temperature decline below −15°C (Barrios et al. 2017).

Nabati et al. (2021) observed a 100% survival rate in lentil (*Lens culinaris*) genotypes at −13°C, while the survival rate was estimated to be less than 75% and 50% at −15°C and −18°C, respectively. Nevertheless, our findings demonstrated a significant decrease in the plant survival rate of the same genotypes at the minimum temperature of −12°C in the Jolgeh Rokh location. In the present study, the temperatures below 0°C during the cold season was long (89 days), which increased the possibility of cold damage and the secondary freezing stress problems, including mechanical stress (damage to the crown due to soil freezing) and the development of pathogenic factors (*e.g. fusarium* wilt) due to the sensitivity of the crown.

Growth parameters, yield, and yield components

While plant height is affected by plant genetics, the results of the present study indicated that the weather conditions also significantly influenced plant height. Most of the genotypes had higher plant height in Mashhad compared with Jolgeh Rokh (Tables 4 and 5). Low temperatures are likely to delay plant growth by affecting plant physiology. Nabati et al. (2021) found that freezing temperatures adversely affected the photosynthetic and chlorophyll fluorescence parameters of faba bean (*Vicia faba* L.) plants, which lead to reduced plant growth. Therefore, plant height was higher in the location with higher average temperatures, *i.e.* Mashhad, compared to the colder location. Furthermore, considerable diversity has been observed in lentil (*Lens culinaris* Medik.) plant height

Table 6. Correlation matrix of morphological traits, yield, and yield components of 20 lentil genotypes after winter stress under the field conditions in Mashhad.

	Traits	1	2	3	4	5	6	7	8	9	10
1	Plant height	1									
2	Lowest pod height	0.59**	1								
3	Branch No	0.51**	0.50**	1							
4	Pod No.	0.57**	0.64**	0.64**	1						
5	Fertile pod	0.26*	0.21^{ns}	0.17^{ns}	0.10^{ns}	1					
6	Dry weight. $plant^{-1}$	0.81**	0.47**	0.53**	0.59**	0.20^{ns}	1				
7	Yield. $plant^{-1}$	0.78**	0.59**	0.72**	0.70**	0.27*	0.75**	1			
8	Biological yield	0.74**	0.56**	0.60**	0.54**	0.27*	0.76**	0.80**	1		
9	Seed yield	0.79**	0.63**	0.60**	0.51**	0.31*	0.80**	0.83**	0.92**	1	
10	HI	0.69**	0.60**	0.46**	0.48**	0.35**	0.69**	0.69**	0.86**	0.73**	1

ns, *and **: non-significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively. Due to 100% survival in all genotypes, the correlation of survival with other traits is not calculated

Table 7. Correlation matrix of morphological traits, yield, and yield components of 20 lentil genotypes after winter stress under the field conditions in Jolgeh Rokh.

	Traits	1	2	3	4	5	6	7	8	9	10	11
1	Survival percentage	1										
2	Plant height	0.25^{ns}	1									
3	Lowest pod height	0.16^{ns}	0.70**	1								
4	Branch No	0.26*	0.65**	0.54**	1							
5	Pod No.	0.05^{ns}	0.68**	0.51**	0.40**	1						
6	Filled pod	0.19^{ns}	0.70**	0.60**	0.66**	0.55**	1					
7	Dry weight. plant^{-1}	0.04^{ns}	0.71**	0.54**	0.47**	0.90**	0.45**	1				
8	Yield. plant^{-1}	0.04^{ns}	0.70**	0.49**	0.48**	0.84**	0.52**	0.85**	1			
9	Biological yield	0.77**	0.44**	0.24^{ns}	0.30*	0.42**	0.45**	0.37**	0.34**	1		
10	Seed yield	0.73**	0.46**	0.24^{ns}	0.28*	0.49**	0.40**	0.42**	0.42**	0.92**	1	
11	HI	0.25^{ns}	0.62**	0.50**	0.61**	0.38**	0.66**	0.40**	0.41**	0.30*	0.21^{ns}	1

$^{ns,\ *}$and **: non-significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Table 8. Mean and deviation from mean of groups in cluster analysis in lentil genotypes under the field conditions in Mashhad.

	Group					
	1		2		3	
Genotypes (MLC)	8, 83, 407,17,84,334, 74, 11, 38, 103, 286, 472, 469,13		70		303, 47, 454, 33, 409	
Traits	Group mean	Deviation from mean	Group mean	Deviation from mean	Group mean	Deviation from mean
Survival (%)	100	0.00	100	0.00	100	0.00
Plant height (cm)	24.9	−3.46	29	0.670	37.9	9.54
Lowest pod height	9.40	−1.76	16.8	5.61	15.0	3.79
No. of branches (plant^{-1})	4.42	−0.700	4.40	−0.720	7.24	2.12
Pod No. (plant^{-1})	58.0	−37.1	32.0	−63.1	212	117
Filled pod%	53.2	−4.75	78.0	20.0	67.3	9.28
Dry weight (g plant^{-1})	11.2	−3.34	12.1	−2.45	24.4	9.85
Grain weight (g plant^{-1})	0.770	−0.730	0.42	−1.07	3.75	2.25
Biological yield (kg ha^{-1})	1610	−1180	3410	620	5980	3190
Grain yield (kg ha^{-1})	84.5	−173	512	254	692	434
Harvest Index (%)	5.55	−1.82	12.7	5.30	11.4	4.03

MLC: Mashhad Lentil Collection

(Ahamed et al. 2014). Another study showed a 65% difference between the highest and lowest plant height between lentil (*Lens culinaris*) genotypes (Jawad et al. 2019). Positive correlations were observed between the plant height and the morphological traits and yield in two locations (Tables 6 and 7). In fall planting, morphological traits such as plant height and the number of lateral branches, and consequently biomass production increase with the increased length of the vegetative growth period.

Following the variations in the plant height in Mashhad and Jolgeh Rokh, the lowest pod height was also varied; freezing stress reduced the lowest pod height due to a decrease in plant growth. The lowest pod height was higher than 10 cm above the soil surface in 67% of genotypes in Mashhad, which is considered a proper characteristic for mechanized harvesting (Table 4). Meanwhile, in Jolgeh Rokh, the lower pods were set below 10 cm from the soil surface, which made mechanized harvesting impossible (Table 5). Previous studies have reported a 92% variation between the highest and lowest height of the first pods in lentil (*Lens culinaris*) genotypes, which demonstrated the diversity of genotypes (Jawad et al. 2019). Mechanized harvesting reduces plant residuals and labor costs during harvesting.

Table 9. Mean and deviation from mean of groups in cluster analysis in Lentil genotypes under the field conditions in Jolgeh Rokh.

	`Group									
	1		2		3		4		5	
Genotypes (MLC)	8, 103, 11, 13, 472		83, 47, 454, 33, 286, 334, 17, 74		84, 303, 469		409, 70		407, 38	
Traits	Group mean	Deviation from mean	Group mean	Deviation from mean	Group mean	Deviation from mean	Group mean	Deviation from mean	Group mean	Deviation from mean
Survival (%)	32.5	17.4	6.85	−8.30	14.2	−0.960	19.6	4.48	1.87	−13.3
Plant height (cm)	25.0	1.02	25.3	1.39	22.4	−1.45	31.9	7.96	10.1	−13.8
Lowest pod height	4.88	−0.410	5.90	0.627	5.28	0.001	7.07	1.79	2.00	−3.28
No. of branches ($plant^{-1}$)	2.63	0.198	2.63	0.201	2.37	−0.062	2.63	0.205	1.02	−1.41
Pod No. ($plant^{-1}$)	41.2	−2.59	46.9	3.06	22.6	−21.2	96.9	53.0	16.8	−27.0
Filled pod%	93.1	8.16	91.3	6.36	76.3	−8.59	94.4	9.45	42.5	−42.4
Dry weight (g $plant^{-1}$)	3.06	−0.522	4.01	0.432	2.06	−1.52	7.68	4.10	1.34	−2.24
Grain weight (g $plant^{-1}$)	1.40	−0.129	1.69	0.162	0.916	−0.611	3.15	1.63	0.492	−1.04
Biological yield (kg ha^{-1})	349	157	98.2	−94.4	126	−67.0	458	265	13.5	−179
Grain yield (kg ha^{-1})	136	57.6	42.6	−35.5	41.3	−36.8	205	127	4.82	−73.3
Harvest Index (%)	40.9	4.52	36.6	0.191	37.5	1.13	40.8	4.39	18.2	−18.1

MLC: Mashhad Lentil Collection

A significantly positive correlation was observed between the number of lateral branches and the number of pods per plant in Mashhad (r^2 = 0.64**) and Jolgeh Rokh (r^2 = 0.40**) (Tables 6 and 7). In other words, the increased number of the lateral branches led to a higher number of pods per plant. In addition, a significant difference was observed between genotypes regarding the number of pods per plant. In Mashhad, MLC409 had the greatest number of pods per plant by 43 times more pods compared to MLC13 (Table 4). A 91% difference was recorded between the genotypes with the highest (MLC70) and lowest (MLC38) number of pods per plant in Jolgeh Rokh. Among the genotypes, only seven genotypes in Mashhad (MLC17, MLC33, MLC38, MLC47, MLC303, MLC409, and MLC454) and one genotype in Jolgeh Rokh (MLC70) had more than 100 pods per plant. Among the genotypes that were resistant to Ascochyta blight (MLC33, MLC47, MLC83, MLC303, MLC409, and MLC454), only MLC83 was unable to produce more than 100 pods per plant (Table 4). The diversity in the number of the lateral branches in lentils (*Lens culinaris*) and soybean (*Glycine max* Merr.) genotypes (1.7–3.3 lateral branches per plant) has also been reported in previous studies (Bakal et al. 2017; Maurya et al. 2018). More branches increase the biomass and number of pods per plant.

While the survival rate of MLC83 was lower than 20% in Jolgeh Rokh, the number of pods per plant and the fertility rate of the pods were higher compared to Mashhad. MLC47 and MLC409 produced 62 and 91 pods per plant, respectively, in Jolgeh Rokh, with a fertility rate of more than 90%. Variations in the number of pods per plant in lentil (*Lens culinaris*) varieties and an increased number of pods per plant have been reported in planning on November 1st, compared with November 30th (Sen et al. 2016). The results of the present study indicated positive correlations between the number of pods per plant and plant height, plant dry weight, grain weight, BY, and GY in both locations (Tables 6 and 7). Our results also indicated positive correlations between plant survival rate, BY, and GY (Table 7). In fall planting, it is possible to produce plants with more lateral branches and higher biomass due to the optimal environmental conditions (*e.g*. humidity and prolonged vegetative and reproductive growth), which is possible if plants survive and tolerate low temperatures.

Despite the low number of pods, the genotypes had a higher percentage of fertile pods in Jolgeh Rokh compared with Mashhad. The resistant genotype to Ascochyta blight (MLC409) had the highest number of pods per plant and more than 65% fertility rate in Mashhad and 90 pods per plant and fertility rate of more than 90% in Jolgeh Rokh. In other words, MLC409 could produce a proper number of pods with desired fertility rates in both locations. A positive correlation was observed between the fertile pod percentage and grain yield in both locations (Tables 6 and 7). The higher pod fertility rate per plant increased the grain yield per unit area.

Lentil plants had a longer growth period and produced a higher number of pods per plant due to the absence of extreme cold from March onwards under the weather conditions of Mashhad (Figure 1). In fall planting, more photoassimilates are allocated to fill the grains due to the increased length of growth periods and photosynthesis. However, an extremely low pod fertility percentage was observed in Mashhad despite a high number of pods per plant. High relative humidity and rainfall during flowering may be the reason for a decrease in fertilization and low fertility rate in Mashhad where the resources were limited to fill the seeds.

While no significant difference was observed between the single-plant dry weight and survival rate of genotypes, MLC38 had the lowest plant dry weight with a survival rate of less than 1%. Positive correlations were observed between the single-plant dry weight and plant height, the number of branches, the number of pods per plant, plant BY, and GY (Tables 6 and 7). Baidya et al. (2018) evaluated the impact of sowing dates on dry matter production, partitioning, and yield in twenty Lentil (*Lens culinaris* Medikus) and found that the most significant plant dry matter reduction (98%) was observed in the L-4076 genotype.

The increased plant height and the number of branches led to higher plant BY and the number of pods. A significant variation was observed between the genotypes in the grain weight per plant; in which the grain weight per plant varied from less than 0.1 g (MLC13) to 5.43 g per plant (MLC33). More than 60% of the genotypes grown in Mashhad had a grain weight of less than 1 g (Table 4). In Jolgeh Rokh, 25% of the genotypes had a grain weight of less than 1 g, while 50%, 15%, and 10% of the genotypes had grain weights of 1–2, 2–3, and >3 g, respectively (Table 5). In the present study, although positive correlations were observed between the grain weight and the morphological traits and yield components, no significant association was observed between the grain weight and survival rate (Tables 6 and 7). While the space allocated to each plant increases with a reduced survival rate, which ultimately increases the plant dry weight and grain yield per plant, this was not observed in the present study. Even in the genotypes with a survival rate of >20%, the plant dry weight and grain weight per plant were higher than the average of the genotypes (Table 5). The increased length of the plant growth period in the fall planting led to the increased plant height, BY, the number of branches and pods, providing an ample opportunity for the transfer of photosynthetic products to fill the grain.

The increased survival rate was associated with higher plant BY per unit area. Positive correlations were observed between the plant survival rate, plant height, and the number of branches with plant BY per unit area (Table 7). Fall planting of cold-tolerant genotypes could accelerate plant growth in spring by prolonging the growth period and optimal use of soil moisture. Furthermore, the plant reproductive stage is also exposed to favourable environmental conditions, which ultimately increase the plant BY.

According to the results of the present study, the resistant genotype to Ascochyta blight, *i.e.* MLC8, had a greater grain yield (>150 kg ha^{-1}) in Jolgeh Rokh despite a 38% survival rate, which was considered as one of the greatest grain yields in this area. Plant survival rate and grain yield were positively correlated (Table 7). Significantly Positive correlations were also observed between the plant height, fertile pod percentage, and plant BY with the GY in Mashhad and Jolgeh Rokh (Tables 6 and 7). Previous studies have also reported positive correlations between the grain yield and plant height, length of the plant phenological periods, total biomass, and harvest index of lentils (*Lens culinaris*) under the field conditions (Gupta et al. 2012; Majnoun Hosseini and Naghavi 2017). The grain yield showed the most positive correlation with the number of pods per plant. In the selection of lentil genotypes, the number of pods per plant seems crucial to obtain a higher grain yield, and a greater survival rate could also enhance morphological and growth characteristics, which in turn, increases the grain yield.

Agronomical (*e.g*. total biomass) and phenological characteristics (*e.g*. days from planting to flowering and ripening) affect various yield components, such as the number of pods per plant, number of seeds per pod, and 1000-grain weight, which in turn influence the ultimate grain yield. Despite the low number of pods in Jolgeh Rokh, a higher pod fertility percentage was observed in this location, indicating a higher photoassimilate allocation to the grains. A delay in planting from October to November decreased the plant BY, GY, and HI of all lentil (*Lens culinaris*) genotypes (Mandi et al. 2015). The increased vegetative growth period during fall leads to exposure of the sensitive reproductive stages of plants, *i.e*. flowering and pod filling, to high air temperatures.

Cluster analysis

The results, overall, indicated the superiority of the genotypes grouped in the third group in Mashhad (MLC33, MLC47, MLC303, MLC409, and MLC454) and the fourth group in Jolgeh Rokh (MLC409 and MLC70). Therefore, MLC409 and MLC70 were recommended for cultivation in case of long-term extreme cold exposure under field conditions. Considering the lack of extreme cold and the impact of Ascochyta blight on the yield and yield components of lentils, MLC33, MLC47, MLC303, MLC409, and MLC454 were suggested as the proper genotypes for temperate climates.

According to the evaluation of 110 lentil (*Lens culinaris*) genotypes over two years, those with a superior number of pods per plant, the number of grains per pod, and grain yield than the other genotypes were grouped into the same cluster, and their contribution in the breeding programs in the future was suggested (Roy et al. 2013). In the present study, the results indicated the similarity of Mashhad and Jolgeh Rokh locations in the superiority of MLC409, which was able to maintain its productivity despite a low survival rate in Jolgeh Rokh.

Conclusion

Our findings showed differences between the cold tolerance of lentil genotypes in the two locations. While all genotypes in Mashhad had a 100% survival rate due to the lack of temperature decline below −5°C, the extreme temperature decline below −12°C in Jolgeh Rokh and 89 days of the temperature below zero °C decreased the survival rate of all the genotypes by ~50%. However, the resistant genotypes to Ascochyta blight were not highly tolerant to cold. According to the results, MLC409 was the only genotype resistant to Ascochyta blight that could maintain its superiority in most traits regardless of its low survival rate in the cold region of Jolgeh Rokh, with the highest grain yield in the location.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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