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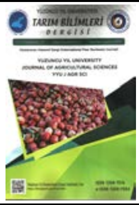

Araştırma Makalesi (Research Article)

Effects of Low Temperature and Sowing Date on the Germination and Seedling Characteristics of Forage Peas

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Abstract: In the study reported here, six forage pea cultivars Töre, Taşkent, Özkaynak, Ulubatlı, Ürünlü, and Gölyazı were examined at different temperatures 20 °C (i.e., control), 15 °C, and 10 °C and sowing dates October 1, October 15, and November 1 in Eskişehir condition, to determine their cold tolerance during germination and as early seedling stages. Results included that survival rates varied among cultivars and by sowing date, with the highest rate achieved by Töre (98.2%) and the lowest (87.9%) by Ulubatlı; however, the high survival rates of the Töre, Taşkent, Özkaynak, Ürünlü, and Gölyazı varieties did not differ significantly. Early sowing negatively affected the survival rates of the Ulubatlı and Gölyazı cultivars more than the others. Survival rate correlated negatively with plant height and number of nodes but correlated positively with SPAD value and leaf relative water content. Gölyazı achieved the highest germination rate (92.8%) and Ulubatlı the lowest (81.8%), although the high rates of the Gölyazı, Töre, Taşkent, and Özkaynak cultivars did not significantly differ. Low temperatures adversely affected all observed characteristics, and seedling lengths highly paralleled survival rates obtained in field conditions. Taken together, such findings suggest that purple-flowered cultivars tolerate low temperatures better than white-flowered ones.

Yem Bezelyesinin Çimlenme ve Fide Özellikleri Üzerine Düşük Sıcaklık ve Ekim Tarihlerinin Etkileri

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Anahtar kelimeler

Soğuk stresi,
Yem bezelyesi,
Çimlenme,
Düşük sıcaklık,
Ekim zamanı.

Öz: Bu çalışmada, 6 yem bezelyesi çeşidinin Eskişehir koşullarında Töre, Taşkent, Özkaynak, Ulubatlı, Ürünlü, ve Gölyazı çimlenme ve erken fide dönemlerinde soğuğa toleransını belirlemek için farklı sıcaklıklar 20 °C (kontrol), 15 °C ve 10 °C ve ekim tarihleri 1 Ekim 15 Ekim ve 1 Kasım incelenmiştir. Sonuç olarak; sağkalım oranlarının çeşitlere ve ekim zamanlarına göre değiştiği, en yüksek (% 98,2) ve en düşük (% 87,9) sağkalım oranlarının sırasıyla Töre ve Ulubatlı çeşitlerinde görüldüğü; ancak Töre, Taşkent, Özkaynak, Ürünlü ve Gölyazı çeşitlerinin sağkalım oranları arasında önemli bir farklılığın olmadığı belirlenmiştir. Erken ekim Ulubatlı ve Gölyazı çeşitlerinin sağkalım oranlarını diğerlerinden daha fazla olumsuz etkilemiştir. Sağkalım oranı bitki boyu ve boğum sayısı ile negatif, SPAD değeri ve yaprak nispi su içeriği ile pozitif korelasyon göstermiştir. Çimlenme oranı en yüksek çeşit (% 92.8) Gölyazı ve en düşük çeşit (% 81.8) Ulubatlı olmasına rağmen Gölyazı, Töre, Taşkent ve Özkaynak çeşitlerinin çimlenme oranları arasında önemli bir farklılık oluşmamıştır. Düşük sıcaklıklar, gözlemlenen tüm özellikleri olumsuz etkilemiş ve fide uzunluğu değerleri tarla koşullarında elde edilen sağkalım oranlarıyla yüksek oranda paralellik göstermiştir. Birlikte ele alındığında, bu bulgular mor çiçekli çeşitlerin düşük sıcaklıkları beyaz çiçeklilerden daha iyi tolere ettiğini göstermektedir.

1. Introduction

In arable regions worldwide, cold stress can limit the production of plants by up to 15% (Blum and Jordan, 1985). For plants in those areas, extremely low temperatures represent an environmental factor that affects germination, growth and development, reproductive organs, and post-harvest storage periods (Wang, 1990). Exposure to such temperatures and, in turn, cold stress prevent plants from reaching their genetic potential and achieving efficient productivity. Symptoms of cold damage occurring in plants vary depending on duration of exposure to the cold, plant genotype and developmental stage, the plant tissue (e.g., leaves, roots, and flowers) exposed to the cold, and environmental conditions such as water, wind and light (Saltveit and Morris, 1989; Mahajan and Tuteja, 2005).

Forage peas are good fodder plants for cool climates that can adapt to nearly all climatic and soil conditions in Turkey (McKenzie and Sponer, 1999; Avcioglu et al., 2009). Including forage peas to crop rotations can benefit both animal nutrition and soil composition, for its hay and seeds offer a tasty, nutritious, protein-rich food for livestock and it supplies a significant source of added nitrogen and organic matter for soils (Acikgoz et al., 1985; Parr et al., 2011).

Winter-resistant or -tolerant varieties of forage peas are important in winter cultivation as primer crops, especially during bare periods between the cycles of two main crops. On that topic, researchers have investigated the effects of low temperatures on different pea genotypes at different developmental stages under controlled conditions in laboratories (Bourion et al., 2003; Sincik et al., 2005; Raveneau et al., 2011), under field conditions (Kadıoğlu and Tan, 2014; Homer et al., 2016; Karaköy et al., 2016; Davies and Pham, 2017), and under controlled conditions in both a greenhouse and a laboratory (Shafiq et al., 2012). Nevertheless, the responses of different forage pea genotypes to low temperatures should also be determined under field and laboratory conditions in different ecological conditions.

Thus, in the study reported here, six forage pea varieties were planted on different sowing dates in field as well as controlled condition in laboratory in Eskişehir, Turkey, in order to determine their response to low temperatures.

2. Materials and Methods

Purple-flowered (i.e., Töre, Özkaynak, and Taşkent) and white-flowered (i.e., Ulubatlı, Ürünlü, and Gölyazı) forage pea (*Pisum sativum* var. *arvense* L. Poir.) cultivars were used as materials. Field experiment was carried out under the ecological conditions of Eskişehir, Turkey. Monthly mean, minimum, and maximum temperatures at the experimental site between October 1, 2018, and March 31, 2019, are presented in Table 1. Minimum temperatures decreased from October to January and increased thereafter, with the lowest minimum temperature measured as -13.5 °C in January 2019.

Table 1. Changes and trends in maximum, minimum and mean temperatures in experimental site

	Temperatures		
Dates	Mean	Min	Mak.
Oct. 2018	13.3	-1.3	26.5
Nov. 2018	7.6	-3.8	24.9
Dec. 2018	2.3	-8.3	10.4
Jan. 2019	1.2	-13.5	12.8
Feb. 2019	3.4	-8.6	15.4
Mar. 2019	6.3	-6.8	22.2

All data were provided by Turkish State Meteorological Service.

The field experiment was arranged in split plots in a randomized complete block design with four replications. The primary plots corresponded to sowing dates (i.e., October 1, October 15, and November 1), whereas the sub-plots corresponded to the different forage pea cultivars. The controlled experiment in laboratory was performed in incubators in a two-factor arrangement with a completely randomized design involving four replications. The first factor was temperature 20 °C (i.e., control), 15 °C, and 10 °C while the second factor was forage pea cultivars.

In the field experiment, each plot consisted of four rows with 50 seeds sown in each. Inter- and intra-row spacings were 30 × 4 cm, and sowing depth was 3 cm. Irrigation was performed to germinate the seeds, and weeds were removed by hand as necessary. To determine the survival rate of the different varieties, the number of plants emerging in each row before the first frost and the number of seedlings that survived the winter into March were recorded. Height, number of nodes, SPAD value measured with a chlorophyll meter (SPAD-502 Plus), dry matter, and leaf relative water content (LRWC), calculated according to formula (1), were determined in the seedlings before winter began.

$$\text{LWRC} = (\text{Fresh weight} - \text{dry weight}) / (\text{Turgor weight} - \text{Dry weight}) \times 100 \qquad (1)$$

To gauge the low-temperature germination of the forage pea varieties, cool and cold tests were performed by following Hampton and Tekrony's (1995) method. In the cold test, seeds were kept at 10 °C for 7 d, then at 25 °C for another 7 d, and germination percentages were determined by counting the seeds that had germinated by the end of Day 14. In the cool test, by contrast, the seeds were kept in a dark incubator at 15 °C for 14 d, and ones that germinated were counted in order to determine the germination percentage.

Germination was performed in four replicates of 50 seeds each, all placed on filter papers in plastic zipper-sealed bags (ISTA, 2018). To the filter papers, 7 mL of pure water was added and replenished as necessary, which required checking the papers every 2 days. Seeds were counted every day, and when roots had extended at least 2 mm, the seeds were considered to have germinated. At the end of laboratory experiments, germination percentage, length of seedlings, fresh and dry weight of seedlings, and mean germination time (MGT), calculated as described by Ellis and Roberts (1980), were examined.

Data were analyzed in SPSS 16.0 and MSTAT. Arcsin $\sqrt{x}$ transformation was applied to percentage values (Sokal and Rolf, 1981), and Duncan's multiple comparison test was performed to determine the differences between mean values.

3. Results

Forage pea cultivars and sowing dates significantly affected the characteristics of seedlings in the field, except regarding the number of nodes, dry matter weight, and LRWC (Table 2). The interaction effect of cultivar and sowing date on all characteristics were also significant.

The survival rate by cultivars ranged from 87.9% to 98.2%, with the highest rate achieved by Töre and the lowest by Ulubatlı (Table 2). However, the high survival rates of the Töre, Taşkent, Özkaynak, Ürünlü, and Gölyazı cultivars did not differ significantly, nor did the low rates of the Ulubatlı and Gölyazı cultivars. From highest to lowest survival rate, the cultivars could be ordered as Töre, Taşkent, Özkaynak, Ürünlü, Gölyazı, and Ulubatlı. The early sowing date (i.e., October 1) negatively affected survival rates more than the late sowing dates (i.e., October 15 and November 1). Among those planted on October 1, the Ulubatlı and Gölyazı cultivars had especially low survival rates compared to the other cultivars (Table 3).

The longest seedling length and highest number of nodes were observed in Gölyazı cultivars sown on October 1 (Table 3). For all cultivars, the early sowing dates (i.e., October 1 and 15) facilitated longer seedlings and more nodes than the late sowing date (i.e., November 1).

The SPAD value and dry matter ratio among cultivars were highly similar (Table 2); both values were especially high for the Taşkent and Özkaynak and especially low for the Ulubatlı. In all cultivars, SPAD values increased with later sowing dates (Table 3). As shown in Table 2, the LRWC percentage in the cultivars ranged from 73.33% (i.e., Gölyazı) to 85.57% (i.e., Töre). The highest LRWC values in Töre, Özkaynak, and Ulubatlı did not significantly differ, nor did the lowest in Gölyazı, Ürünlü, Ulubatlı, and Taşkent.

Table 2. Analysis of variance and differences between mean values of survival rate and seedling characters in forage pea cultivars grown under different sowing dates

Factors	Survival rate (%)	Seedling length (cm)	Number of node	SPAD value	Dry matter (%)	LWRC (%)
Cultivars						
Töre	98.2[a†]	6.82[b]	4.35	47.53[b]	17.03[b]	85.57[a]
Taşkent	97.5[a]	5.10[c]	4.25	51.07[a]	20.79[a]	74.28[bc]
Özkaynak	97.0[a]	5.41[c]	4.20	50.82[a]	19.39[a]	81.67[ab]
Ulubatlı	87.9[b]	8.55[a]	4.58	40.65[c]	15.39[c]	79.76[a-c]
Ürünlü	96.5[a]	5.70[c]	4.11	46.02[b]	17.00[b]	77.47[bc]
Gölyazı	91.8[ab]	8.15[a]	4.45	47.16[b]	17.47[b]	73.33[c]
Sowing dates						
Oct. 1	88.8[b]	10.40[a]	5.55[a]	39.09[c]	17.55	79.73
Oct. 15	97.8[a]	6.32[b]	4.44[b]	46.47[b]	18.41	79.10
Nov. 1	97.9[a]	3.15[c]	2.97[c]	56.07[a]	17.57	77.19
Analysis of variance						
Cultivars (C)	**	**	ns	**	**	**
Sowing dates (S)	**	**	**	**	ns	ns
C × S	**	**	*	**	**	*

*, **: significant level of 5% and 1%, respectively, ns: non-significant. †: letters show different groups at 5% level.

Table 3. The effect of cultivars × sowing dates interaction on survival rate and some seedling characters

Cultivars	Sowing dates	Survival rate (%)	Seedling length (cm)	Number of node	SPAD value	Dry matter (%)	LWRC (%)
Töre	Oct. 1	98.5	10.67	5.55	38.97	16.32	85.27
	Oct. 15	97.5	7.55	4.70	45.47	16.47	85.27
	Nov. 1	98.5	2.25	2.80	58.15	18.30	86.17
Taşkent	Oct. 1	92.5	7.02	5.20	46.35	19.70	82.72
	Oct. 15	100.0	5.00	4.40	51.35	24.65	61.82
	Nov. 1	100.0	3.27	3.15	55.52	18.02	78.30
Özkaynak	Oct. 1	99.0	8.20	5.30	46.12	21.55	76.35
	Oct. 15	93.0	5.10	4.15	47.47	18.55	87.22
	Nov. 1	99.0	2.95	3.15	58.87	18.07	81.35
Ulubatlı	Oct. 1	69.5	12.27	5.40	32.85	15.50	81.60
	Oct. 15	96.0	9.75	5.30	40.15	15.12	82.95
	Nov. 1	98.0	3.62	3.05	48.97	15.55	74.75
Ürünlü	Oct. 1	95.5	9.07	5.40	35.55	15.65	84.25
	Oct. 15	100.0	4.92	4.20	47.21	17.84	78.67
	Nov. 1	94.0	3.12	2.75	55.32	17.52	69.50
Gölyazı	Oct. 1	77.5	15.15	6.50	34.70	16.57	68.22
	Oct. 15	100.0	5.60	3.90	47.21	17.84	78.67
	Nov. 1	98.0	3.70	2.95	59.57	18.00	73.10
$LSD_{\%5}$		10.68	1.52	0.84	4.11	2.05	8.77

A positive correlation was detected between survival rate and both SPAD value and LRWC; however, survival rate was more closely related to SPAD value than to LRWC (Table 4). Beyond that, survival rate demonstrated a strong negative correlation with seedling length and number of nodes as well as decreased as plant height and number of nodes increased.

Table 4. Correlation values among the examined characteristics of forage pea cultivars under field conditions

Characters	1	2	3	4	5	6
1. Survival rate	1.000					
2. Seedling length	-0.484**	1.000				
3. Number of node	-0.412**	0.896**	1.000			
4. SPAD value	0.299**	-0.855**	-0.774**	1.000		
5. Dry matter	-0.052^{ns}	-0.266*	-0.046^{ns}	0.376**	1.000	
6. LWRC	0.250*	0.032^{ns}	0.017^{ns}	-0.471**	-0.471**	1.000

*, **: significant level of 5% and 1%, respectively, ns: non-significant.

Cultivars and temperatures significantly affected germination and seedling characteristics (Table 5). The interaction of cultivars and temperatures was also significant except concerning seedling length and fresh weight. Germination showed a downward trend as temperatures dropped (Table 5), and the greatest rate of germination (97.5%) occurred with Gölyazı at 15 °C (Table 6). In all cultivars, low temperatures steadily prolonged MGT as well. The most quickly germinating seeds, which sprouted in only 2.36 days, were of the Özkaynak at 20 °C.

The longest seedlings, at 9.97 cm, were observed in Töre, although seedling length did not differ significantly among Töre, Taşkent, Özkaynak, Ürünlü, and Gölyazı cultivars (Table 5). The responses of the cultivars to different temperatures in terms of seedling length, from longest to shortest, can be ranked as Töre, Özkaynak, Taşkent, Gölyazı, Ürünlü, and Ulubatlı. Last, the heaviest fresh weight occurred in the Gölyazı, at 183.50 mg/seedling, and lower temperatures were associated with lower fresh weights as well as seedling lengths. Although the heaviest dry weight, 27 mg/seedling, was observed in Gölyazı at 20 °C, the most light weight value, at 7.75 mg/seedling, emerged in Taşkent at 10 °C (Table 6). In low temperatures, the dry weight of forage pea seedlings decreased regularly across all cultivars.

Table 5. Analysis of variance and differences between mean values of germination and early seedling characters in forage pea cultivars grown under different temperatures

Factors	Germination (%)	MGT (day)	Seedling length (cm)	Fresh weight (mg/seedling)	Dry weight (mg/seedling)
Cultivars					
Töre	92.2^{ab†}	3.44^{cd}	9.97^{a}	149.67^{b}	14.66^{cd}
Taşkent	90.7^{ab}	3.69^{b}	9.37^{a}	149.75^{b}	14.00^{d}
Özkaynak	89.2^{ab}	3.38^{d}	9.47^{a}	147.58^{b}	14.00^{d}
Ulubatlı	81.8^{c}	4.43^{a}	8.31^{b}	145.17^{b}	16.33^{b}
Ürünlü	87.2^{b}	3.63^{bc}	8.95^{ab}	162.67^{ab}	15.58^{bc}
Gölyazı	92.8^{a}	3.55^{b-d}	9.39^{a}	183.50^{a}	18.16^{a}
Temperatures					
Control (20 °C)	90.2^{a}	2.62^{c}	13.38^{a}	240.58^{a}	22.04^{a}
15 °C	89.6^{a}	3.49^{b}	9.31^{b}	173.25^{b}	15.95^{b}
10 °C	87.2^{b}	4.95^{a}	5.04^{c}	71.04^{c}	8.37^{c}
Analysis of variance					
Cultivars (C)	**	**	**	*	**
Temperatures (T)	*	**	**	**	**
C × T	**	**	ns	ns	**

*, **: significant level of 5% and 1%, respectively, ns: non-significant. †: letters show different groups at 5% level.

Table 6. The effect of cultivars × temperatures interaction on germination and some early seedling characters

Cultivars	Temperatures	Germination (%)	Mean germination time (day)	Dry weight (mg/seedling)
Töre	Control (20 °C)	93.0	2.38	20.25
	15 °C	90.0	3.13	15.75
	10 °C	92.1	4.80	8.00
Taşkent	Control (20 °C)	95.0	2.71	21.00
	15 °C	90.0	3.74	13.25
	10 °C	87.0	4.62	7.75
Özkaynak	Control (20 °C)	88.0	2.36	18.50
	15 °C	92.5	3.14	14.75
	10 °C	87.0	4.65	8.75
Ulubatlı	Control (20 °C)	83.5	3.04	23.50
	15 °C	77.0	4.13	17.25
	10 °C	85.0	6.12	8.25
Ürünlü	Control (20 °C)	85.5	2.71	22.00
	15 °C	90.5	3.39	16.00
	10 °C	85.5	4.79	8.75
Gölyazı	Control (20 °C)	96.0	2.52	27.00
	15 °C	97.5	3.38	18.75
	10 °C	85.0	4.74	8.75
$LSD_{\%5}$		6.87	0.30	1.73

4. Discussion and Conclusion

In the experiment, the survival rates of the forage pea cultivars differed by sowing date. An earlier sowing increased the length of seedlings and the number of nodes, which made the cultivars more susceptible to cold stress due to the development of excess vegetative parts. Moreover, purple-flowered cultivars had greater cold tolerance than white-flowered ones. Annicchiarico and Iannucci (2007) found that having four to five leaves and rosettes reduces cold stress when temperatures are lowest in winter. Added to those findings, Kadıoğlu and Tan (2014) reported that purple-flowered cultivars of forage peas of the Özkaynak, Töre, and Taşkent varieties were the most winter-hardy; however, unlike in the experiment reported here, late sowing increased cold damage. In other studies, Karaköy et al. (2016) observed that pea genotypes collected from different regions had different levels of cold resistance, while Geren and Alan (2012) found that early sowing negatively affected the height of vegetation in peas.

Chlorophyll and dry matter content are important indicators of a plant’s growth (Pavlović et al., 2014). In the experiment, Özkaynak and Taşkent cultivars with high levels of chlorophyll and dry matter content demonstrated good survival rates, and Homer and Şahin (2015) reported that Taşkent and Özkaynak varieties with high chlorophyll content were the most tolerant to cold stress. According Wang et al. (2016), SPAD values of plants under freezing stress are another good metric to measure cold damage. That proposition was later confirmed by Tanaka and Nakano (2019), who found that later sowing dates increased SPAD values.

Leaf water and chlorophyll contents are widely used in determining water stress. In past research, total chlorophyll content decreased by 55% under high water stress compared to controls (Kirnak et al., 2001). By comparison, in the experiment presented here, a positive relationship between survival rate and both SPAD value and LRWC was observed. Contrary to those findings, Hao and Arora (2009) demonstrated in cold applications that the growth and LRWC of guava plants decreased while the accumulation of anthocyanins increased. The following year, Balestrasse et al. (2010) observed that the application of 5-aminolevulinic acid at low concentrations (i.e., 5–10 μM) increased chlorophyll content and LRWC, which provided significant protection against cold stress compared to what occurred in untreated plants.

Also in the experiment, germination and MGT varied by cultivars and temperatures under controlled conditions; germination decreased and MGT increased as temperatures dropped. Reporting similar results, Sincik et al. (2004) and Çaçan et al. (2014) found that germination and emergence rates in pea genotypes increased in direct proportion to the rise in temperature. In addition, Raveneau et al.

(2011) and Sincik et al. (2004) respectively demonstrated that pea germination was faster in winter than in spring genotypes and in purple-flowered than in white-flowered ones. For the different varieties of forage peas examined in the experiment, lower temperatures negatively affected seedling length as well as fresh and dry weights. Those findings were confirmed in studies on different pea genotypes conducted by Sincik et al. (2004), Stavang et al. (2005), and Raveneau et al. (2011).

In conclusion, in the experiment reported here, the responses of forage pea cultivars to cold stress were determined during the germination and early seedling stages. The most cold-tolerant and cold-sensitive cultivars were of the Töre and Ulubatlı cultivars, respectively. In general, purple-flowered cultivars were also more tolerant to cold conditions than white-flowered ones, although white-flowered genotypes seem able to tolerate cold conditions provided that they are sown after October 15. To determine the tolerance of the genotypes to cold, the reliable results were obtained from seedling length in controlled condition when compared with survival rate in the field. Although the cultivars were evaluated in early periods of development, it can be decided which cultivar should be sown in winter once the yield components of those cultivars are evaluated.

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