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## Effects of sowing date on bolting and frost damage to autumn-sown sugar beet (*Beta vulgaris* L.) in temperate regions

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Abstract. Sugar beet is mostly cultivated in relatively cool regions of the temperate zones by sowing in spring, but in some Mediterranean areas with mild winters, the crop is sown in autumn. Due to global warming, autumn cultivation of sugar beet is gradually extending towards new areas that are still characterized by relatively cold winters. Seedling loss and bolting are two factors limiting the adoption of autumn sugar beet cultivation in new areas. The objectives of this study were to determine the effects of sowing date on (i) duration and rate of field emergence, (ii) phenological stages of sugar beet during early growth, (iii) quantitative traits of seedlings and (iv) bolting occurrence as well as frost killing of autumn-sown sugar beet. Field experiments were conducted in a randomized complete block design to determine the appropriate sowing date for autumn-sown sugar beet in 2017/18 and 2018/19 in the Karaj and Mashhad regions of Iran, which are both characterized by relatively cold winters. The experiment was conducted with a bolting-resistant cultivar and six sowing date treatments. The results showed that to reach plant growth stages of cotyledon, 2, 4, 6, 8, 10, 12, 14 and 16 leaves, 163, 200, 321, 418, 500, 600, 639, 700 and 757 growing degree days (GDD), respectively, were required. The average duration and speed of seedling emergence increased and decreased, respectively, with delay in sowing. The results suggest adjusting the sowing date of winter sugar beet so that when temperatures effective for bolting (6-8 °C) occur, the plant has already received about 300 to 400 GDD. At this time, the growth stage and the largest root diameter of sugar beet are approximately 4-6 leaves and 0.11-0.27 cm, respectively. After 14- to 16-leaf stage  $(\geq 700-750 \text{ GDD})$ , the percentage of killed plants due to low temperatures were negligible. Although, the risk for frost losses is higher at 4- to 6-leaf stage (300-400 GDD), accepting higher losses is justified by a lower probability of bolting.

Keywords: bolting, growing degree days, killed plants, sowing date.

## HIGLIGHTS:

Autumn sugar beet growth up to the 16-leavestage is modeled using growing degree days.

- Delayed autumn seeding delayed emergence but increased the rate of emergence.
- The age of the plants affected bolting more than the number of days with effective temperatures on bolt-ing.
- Linear regression model can predict the frost damage at different growing degree days.
- Proper sowing date allows for successful cultivation of autumn sugar beet in relatively cold areas.

## INTRODUCTION

Sugar beet (*Beta vulgaris* L.) and sugarcane (*Saccharum officinarum* L.) are the two sources of sucrose in the world (Mohammadian and Baghani, 2020). Sugar beet is mostly cultivated as spring crop in temperate zones of the world, but in some Mediterranean regions (such as southwestern Spain, northern Egypt and Morocco) sugar beet is sown in autumn (Jaggard and Qi, 2006). Up to the 1960ies, sugar beet has also been sown in autumn and harvested in mid-spring the next year in the Khuzestan province of Iran (located in the southwest of Iran, latitude: 30-33°, Max altitude: 143 m above sea level).

The important advantage of autumn-sown sugar beet is the use of the rainfall in autumn and winter, implying lower water consumption for irrigation than in spring-sown sugar beet (Mohammadian and Baghani, 2020; Jaggard and Qi, 2006). For example, in the Khuzestan province of Iran, the water consumption for the irrigation of autumn-sown sugar beet is about 400 mm, while for spring-sown sugar beet in provinces at higher latitudes the consumption is approximately 900 mm (Mohammadian and Baghani, 2020). It has been found that water use efficiency for sucrose production of autumn-sown sugar beet is higher than that of springsown sugar beet (Rinaldi and Vonella, 2006). In view of global warming and depletion of water resources in recent decades, there is growing interest for the possibility of autumn cultivation of sugar beet in areas of higher latitudes (more than 33° N), but due to environmental limitations and lack of information, autumn cultivation of sugar beet is currently adopted only in a few areas.

One of the major limitations for autumn-sown sugar beet is bolting. The sugar beet is a biennial plant and the cold weather in winter and subsequent long-day conditions in spring cause bolting (Milford et al., 2010). In autumn cultivation conditions, cold weather followed by long days may cause bolting in the first year. It has been reported that maximum daily temperatures effective for sugar beet vernalization are in the range 1-12 °C with an optimum range of 6-8 °C (Milford et al., 2010). The occurrence of bolting in the first year significantly reduces root and sugar yield, and causes problems in processing sugar beet roots due to the hardening and fibrosis of roots (Hoffmann and Klug-Severin, 2011), making them unsuitable for sugar factories. Consequently, for autumn cultivation of sugar beet in areas with cold winters, cultivars with very high resistance to bolting, as recently introduced by some companies, are needed (Bosemark, 2006).

Sowing date is a key agronomic factor affecting the occurrence of bolting and early sowing of sugar beet is expected increase the probability of bolting (Al-Jbawi et al., 2015). On the other hand, sowing date also determines the length of the growth period (thermal time), and is therefore one of the most important factors affecting the sugar yield of sugar beet (Hoffmann, 2019). Especially in years with suitable weather conditions a large increase in yield from timely cultivation was found (Esmaeili et al., 2022). Moreover, there is a positive correlation betweenroot yield and light energy intercepted during sowing and harvest period (Jaggard and Qi, 2006). Therefore, increasing the length of the plant growth period by earlier sowing date causes more radiation absorption by leaves resulting in greater yield of sugar beet.

Many problems have been reported for the survival of young autumn-sown sugar beet seedlings during frost periods (Albuquerque and Carvalho, 2003; Kockelmann and Meyer, 2006). Under Central European climates, there is a 10 to 35 percent risk of freezing stress in autumn-sown sugar beet (Reinsdorf and Koch, 2013). In view of the low resistance of young sugar beet seedlings to cold weather and frost (Deihimfard et al., 2019), autumn cultivation of sugar beet in colder northern regions to escape the summer heat may not be a favorable strategy even if the presence of a snow cover can partially protect plants from frost (Sokratov and Barry, 2002). Furthermore, results of field trials for autumnsown sugar beet in Germany showed that the survival rate depends more on the growth stage reached before frost than on the impact of weather conditions during winter (Loel and Hoffmann, 2014). The importance of reaching a minimum growth stage with respect to cold weather resistance underlines once more the importance of the sowing date for the success of autumn cultivation of sugar beet (Jaggard and Qi, 2006).

Due to the limited water resources and climate change, the prospect for continuing sugar beet cultivation in arid and semi-arid regions, such as Iran, is related to the possibility of developing autumn cultivation. Owing to the lack of knowledge in this regard, this study was conducted to evaluate the effects of phenological stages of autumn-sown sugar beet on cold and both frost tolerance as well as bolting. The specific objectives of this study were to determine the effects of sowing date on: (i) duration and rate of field emergence ; (ii) phenological stages of sugar beet in early growth; (iii) quantitative traits of seedlings just before starting winter; and, (iv) bolting occurrence as well as frost killing on autumn-sown sugar beet.

#### MATERIALS AND METHODS

#### Site characteristics

The experiments were conducted during the autumns of 2017 and 2018 in Iran, in the two regions of Karaj, Alborz province (latitude: 35°59'N and longitude: 51°6'E, altitude: 1320m above sea level) and Mashhad, Khorasan-Razavi province (latitude: 36°13'N and longitude: 59°40'E, altitude: 1050m above sea level). Climatic characteristics of the two experimental sites inferred from meteorological data for 2002-2016 are shown in Table 1. Although the average temperature at the two sites was similar, there were differences in terms

Table 1. Historical weather data of Karaj and Mashhad 2002-2016.

	Temperature °C								
Location	Min Max Mean of Max of Mean								
Karaj	-17	-17 42		22	16	239.0			
Mashhad	-24	43	10	23	16	219.8			

of absolute minimum and maximum temperatures, average minimum and the maximum of minimum temperatures. The minimum temperature observed in Mashhad was 7 °C lower than that in Karaj. However, the average minimum temperature in Mashhad was 1 °C higher than in Karaj. The data suggest that winter in Mashhad was somewhat warmer than Karaj.

Table 2 illustrates the climatic characteristics during the sugar beet growth period in two experimental years. In the first year of the trial, the amount of rainfall in Mashhad was higher than in Karaj. However, in the second year, there was no significant difference between the two locations in term of rainfall. The absolute minimum

Table 2. Climatic characteristics during the sugar beet growth periods addressed by this study.

		Karaj					Mashhad					
Year	Month		Temper	ature (°C)								
		Min	Max	Mean of Min	Mean	Rain (mm)	Min	Max	Mean of Min	Mean	Rain (mm)	
2017	9	9.7	31.0	13.4	21.0	0.0	5.8	31.0	11.6	19.2	0.1	
	10	4.0	29.0	10.6	17.0	4.8	3.6	31.0	8.9	16.9	0.0	
	11	-3.5	24.0	6.3	11.5	0.6	-1.7	31.0	5.2	12.1	14.5	
	12	-3.4	22.0	1.4	6.7	4.7	-6.5	24.0	-0.9	5.9	0.4	
2018	1	-11.7	16.0	-0.1	4.1	8.6	-11.9	21.0	-1.7	4.8	0.9	
	2	-8.5	15.0	1.7	6.5	16.6	-7.7	22.0	1.8	7.7	41.8	
	3	0.2	30.0	8.1	14.7	14.1	4.2	33.0	9.0	14.8	37.8	
	4	-0.3	26.0	7.8	13.6	26.8	1.0	32.0	8.9	14.4	33.5	
	5	6.2	32.0	12.1	18.4	57.1	9.1	36.0	13.7	20.6	56.8	
	6	13.0	35.0	16.2	24.2	7.2	17.3	40.0	19.5	26.6	2.1	
2018	9	13.4	31.0	15.7	21.7	0.0	10.9	29.0	13.0	21.0	0.0	
	10	5.9	30.0	11.2	16.6	29.1	2.6	31.0	8.0	15.0	41.9	
	11	0.0	16.0	5.4	9.1	65.9	-1.3	22.0	4.0	9.0	18.8	
	12	-0.8	18.0	4.0	11.0	33.8	-1.4	22.0	1.0	7.0	0.3	
2019	1	-5.5	16.0	0.4	4.5	50.5	-4.9	22.0	0.0	7.0	12.8	
	2	-3.6	14.0	0.8	5.3	12.2	-5.0	17.0	0.0	6.0	52.2	
	3	-2.4	18.0	3.3	8.1	70.7	-0.4	24.0	5.0	10.0	62.7	
	4	-1.1	24.0	6.6	12.5	41.9	3.4	25.0	9.0	14.0	77.1	
	5	5.8	32.0	12.8	20.5	12.0	8.6	34.0	14.0	21.0	47.7	
	6	16.2	37.0	18.0	26.4	0.0	13.4	36.0	17.0	25.0	10.0	

temperature recorded in each of the two years of the trial was not different between the two locations (about -12 and -5 °Cin the first and second years, respectively). Although, the mean temperature recorded in the winter season in Mashhad was higher than Karaj (about 1 °C). In summary, Mashhad had slightly warmer winters compared with Karaj.

#### Experimental design

The experiments were arranged as randomized complete block design in five replications with six sowing dates from mid-September to mid-November (except for the first year of the experiment in Karaj, where the sixth treatment was in the second half of December). They were conducted with a bolting-tolerant cultivar called "Jerra". Sowing dates are shown in Table 3. The dates were set based on 15-year meteorological statistics to generate different sugar beet growth stages before the onset of winter. Each experimental plot consisted of 6 eight-meters long rows, with 50 cm row spacing.

#### Agricultural operations

Urea, triple superphosphate and potassium sulfate were used as fertilizers. They were applied in accordance with the soil test results. Seeds were sown with a seeder with an in-row spacing of about 4.8 cm and 50 cm row distance. Furrow irrigation was applied immediately after sowing at intervals of about 4 days for safeguarding germination and emergence. Plants were thinned to about 5 plants per meter of row at the 4-6 leaf stage. Because of the weather conditions, further irrigation was not required until the end of the experiment (late April).

**Table 3.** Sowing date treatments in Karaj and Mashhad in 2017 and2018.

Treatment	Ka	raj	Mashhad			
sowing date	2017	2018	2017	2018		
1	19-Sep.	24-Sep.	23-Sep.	23-Sep.		
2	3- Oct.	2- Oct.	2- Oct.	1- Oct.		
3	10-Oct.	10-Oct.	9-Oct.	10-Oct.		
4	19-Oct.	20-Oct.	21-Oct.	20-Oct.		
5	2-Nov.	3-Nov.	1-Nov.	5-Nov.		
6	18-Dec.	19-Nov.	21-Nov.	26-Nov.		

#### Determination of duration and speed of seedling emergence

The number of emerging seedlings in a 0.5 m<sup>2</sup> area on each plot was counted at the two experimental sites in the second year and after the first irrigation. Counting was continued until reaching a maximum number of seedlings emerged, after which no new ones were observed. In each plot, there were 42 seeds sown per each meter of row length. Equations 1 and 2 (Ellis and Roberts (1980) for seed germination were used to calculate the mean time of emergence,  $\overline{D}$ , and the average daily emergence rate,  $\overline{R}$ , respectively:

$$\overline{D} = \frac{\sum D n}{\sum n}$$
(1)

$$\overline{R} = \frac{1}{\overline{D}} \tag{2}$$

where D is the number of days after the first irrigation date and is the number of seedlings appearing on dayD.

#### Phenological evaluation in the early growth stage

At both sites and for each treatment, plant phenological stages up the 16-leaf stage were recorded. A phenological stage was considered as achieved when about 50% of the plants were in this stage. Corresponding cumulated growing degree-days, GDD (°C d) were evaluated based on equation (3):

$$GDD = \sum T_{eff} \tag{3}$$

where the effective temperature is given by:

$$T_{eff} = \begin{cases} \frac{T_{min} + T_{max}}{2} - T_{base} & if \ T_{min} > 3^{\circ} C \& T_{max} < 40^{\circ} C \\ 0 & otherwise \end{cases}$$
(4)

In this equation,  $T_{max}$  and  $T_{min}$  are the maximum and minimum daily temperatures in °C, respectively, and  $T_{base}$  is the sugar beet base temperature, considered to be 3 °C.

Different regression models were fitted to express the relation between growth stages and thermal time for Karaj and Mashhad, separately, and for the average of the two regions. In all circumstances, the quadratic model had a higher coefficient of determination ( $\mathbb{R}^2$ ) compared to the linear, logarithmic, inverse, compound, power, s, growth, exponential, logistic and cubic models.

## Determination of seedling morphological traits

In the first and second year at the Karaj site, morphological traits of the seedlings, including the largest root diameter, and root, leaf and petiole dry weights, were measured in late November for the first four levels of seeding dates treatment. The amount of plan growth for the last two levels of the seeding dates treatment were very negligible in late November and so it could not be measured.

Plants in 2 m<sup>2</sup> area on each plot were harvested and counted. In the laboratory, after measuring the largest diameter of each root, the leaves, petioles, and roots of each plant were separated. The samples were placed in an oven for 48 hours at 75 °C, and then the dry matter of the samples was measured. Data for each trait were averaged according to the number of plants in each sample and evaluated on a single-plant basis. Next, regression models were fitted to express the size of the largest root diameter during the growing season as a function of thermal time. Among the models, the quadratic and cubic models had a higher coefficient of determination (R<sup>2</sup>) compared to the linear, logarithmic, inverse, compound, power, s, growth, exponential and logistic models, and the quadratic model was, therefore, retained for predicting the root diameter.

#### Bolting percentage assessment

In mid-spring, bolting percentage for each treatment was calculated by counting the total number of plants and the number of the bolted plants in a 4 m<sup>2</sup> area on each plot. Thereafter, we determined the number of days in which plants were exposed to temperatures effective for bolting, which we calculated as the number of days for which  $T_{max}$  was between 0 and 12 °C, and 6 and 8 °C (Milford et al. 2010). Next, we assessed the correlation between the percentage of bolting and the number of days with temperatures effective for bolting and the age of the plants (based on GDD) at the onset of bolting. Various regression models on bolting percentage of treatments over thermal time at the onset of bolting were fitted. Quadratic and cubic models exhibited the best fit (higher R<sup>2</sup>), and the quadratic model was retained.

## Cold and frost damage assessment

The number of plants was counted in a  $1 \text{ m}^2$  area on each plot at the beginning of winter (January 1) and also at the beginning of spring (early April), so that the percentage of plants lost due to cold climate and frost during the cold season could be calculated. Regression models were subsequently fitted to express the percentage of frost damage as a function of thermal time after January 1<sup>st</sup>. Since linear and logarithmic regression models had a higher coefficient of determinations compared to other models, the linear model was retained to predict the percentage of killed plants at different seedling ages during the winter.

## Statistical analysis

We carried out an analysis of variance (ANOVA) with respect to the duration and speed of field emergence as well as quantitative traits of sugar beet seedlings using the SAS software (version 9.1). The comparison of the means was conducted by the least significant difference test ( $P \le 0.05$ ).

The statistical software SPSS (version 16) was used to fit Pearson correlations and regression models for phenological stages, root diameter, bolting percentage and killed plants percent over GDD.

Significant differences between regression models established separately for the two sites and the model inferred from the combined data sets were evaluated by the F-test (Motulsky,1999). The following formula was used:

$$F = \frac{\left(SS_{combined} - SS_{separate}\right) / \left(DF_{combined} - DF_{separate}\right)}{SS_{separate} / DF_{separate}}$$
(5)

Where SS denotes the total sum of squares and DF the total degrees of freedom for each of the settings (combined versus separated). As the results indicated that the F-tests were non-significant (P > 0.05), only the combined models were used (Mohammadian et al., 2014; Traversa, 2003).

#### RESULTS

#### Duration and rate of field emergence

The analysis of variance showed that the effect of sowing date on the mean duration and rate of field emergence was significant (P < 0.01). Overall, delay in sowing date, due to decreasing air temperature at the time of germination, caused the duration to increase and the rate of field emergence to decrease (Table 4).

## Phenological stages

Figure 1 shows the model that predicts the stages of sugar beet growth up to the 16-leaf stage as a function

**Table 4.** The effect of sowing date on duration and rate of field emergence of autumn sown sugar beet in 2018. DAS: days after seeding,  $\overline{D}$ : mean emergence time;  $\overline{R}$ : average rate of emergence. Lowercase letters next to numbers in each column indicate differences in significance based on the least significant difference test (P ≤ 0.0 5).

Treatment	DAG	Karaj			DAG	Mashhad			
	DAS	%Emergence	$\overline{D}$ (Day)	$\overline{R}$ (n. seed/ day)	DAS	%Emergence	Day	$\overline{R}$ (n. seed/ day)	
1	12	80.9	5.54e	0.18a	13	55.9	8.52d	0.12a	
2	12	71.4	7.06d	0.14b	16	35.7	9.51d	0.11a	
3	13	78.6	9.17c	0.11c	21	54.7	14.32c	0.07b	
4	11	66.7	9.38c	0.11c	23	39.3	16.16bc	0.06b	
5	25	51.2	15.07b	0.07d	32	67.3	17.88b	0.06b	
6	33	42.3	22.37a	0.04d	54	47.6	29.30a	0.03c	



**Figure 1.** Relationship between GDD and number of leaves (1: Cotyledon leaf stage, 2-16: Leaf stage) in the early growth stage of autumn sugar beet. Each dot represents the mean of five replications, 6 sowing dates and two years. The symbols  $\bigcirc$  and  $\blacklozenge$  represent results for Mashhad and Karaj, respectively.

of thermal time. According to the model, 163 GDD are required for cotyledon leaves to appear above the surface of the soil, while to reach stages 2, 4, 6, 8, 10, 12, 14 and 16 true leaves, 200, 321, 418, 500, 600, 639, 700 and 757 GDD are required, respectively.



**Figure 2.** The relationship between GDD and the size of largest root diameter of autumn sugar beet in late November. Each dot represents the mean of five replication.

## The effect of sowing date on quantitative traits of sugar beet seedlings

The effect of sowing date on leaf, shoot (leaf+ petiole) and root weight and root diameter of sugar beet plants was significant (P < 0.01) (Table 5). The comparison of means showed the greatest root diameter, root, leaf and shoot weight in 2017/18 and 2018/19 with 894 and 708 GDD, respectively; such values were achieved by the first

**Table 5.** The effect of sowing date on autumn sown sugar beet in 2017/18 and 2018/19 in Karaj. Lowercase letters next to numbers in each column indicate differences in significance based on the least significant difference test ( $P \le 0.05$ ).

			2017/18					2018/19			
Treatment	GDD	Root diameter (cm)	Root weight	Leaf weight	Shoot(leaf+ petiole) weight	GDD	Root diameter (cm)	Root weight	Leaf weight	Shoot(leaf+ petiole) weight	
			(g/plant)					(g/plant)			
1	894.2	1.92a	5.73a	7.11a	8.13a	708.8	1.43a	2.88a	4.36a	6.56a	
2	642.0	0.58b	0.24b	0.97b	1.15b	559.1	0.27b	1.20b	1.73b	2.83b	
3	560.9	0.37cb	0.08b	0.62b	0.70b	428.9	0.17c	0.67c	1.25c	1.92c	
4	441.1	0.14c	0.02b	0.08b	0.10b	295.1	0.001d	0.18d	0.49d	0.49d	

			$0 < T_{max} \leq 12^{\circ} \mathrm{C}$		$6 \le T_{max}$	≤8°C	Datasí	Nul		
Location	Year	Treatment	Number of days	GDD	Number of days	GDD	- Date of counting	plants (m <sup>-2</sup> )	Bolting (%)	
Karaj	2017/18	1	62	894	13	894	6-May	12	81	
		2	62	642	13	642	26-May	12	29	
		3	62	561	13	561	26-May	7	0	
		4	62	441	13	441	26-May	2	0	
		5	62	230	13	230	26-May	1	0	
		6	41	58	9	58	26-May	3	0	
	2018/19	1	96	595	18	817	14-May	9	79	
		2	96	445	18	667	19-May	9	78	
		3	96	315	18	537	9-June	9	30	
		4	96	181	18	403	9-June	9	0	
		5	96	27	18	249	9-June	2	0	
		6	89	37	18	158	9-June	0	0	
Mashhad	2017/18	1	55	773	17	773	3-July	10	45	
		2	55	627	17	627	3-July	10	7	
		3	55	550	17	550	3-July	9	1	
		4	55	376	17	376	3-July	9	0	
		5	55	213	17	213	3-July	7	0	
		6	55	25	17	25	3-July	2	0	
	2018/19	1	73	252	9	713	23-June	10	79	
		2	73	112	9	573	23-June	9	34	
		3	72	230	9	454	23-June	9	1	
		4	72	131	9	355	23-June	10	0	
		5	64	59	9	201	23-June	8	0	
		6	58	18	9	67	23-June	7	0	

**Table 6.** Percentage of bolting, number of days with  $T_{max}$  between 0 and 12 °C, and 6 and 8 °C, respectively, and GDD on the first day these temperatures occurred.

**Table 7.** Pearson correlation coefficients between the percentage of bolting and the number of days (N days) with the temperatures effective on bolting and GDD on the first day these temperatures occurred for the treatments of sowing date in autumn sugar beet.\*, \*\*and ns: significant differences at P = 0.05 and 0.01, and non-significant, respectively.

sowing date. Figure 2 shows the regression model of root
diameter as a function of thermal time. According to this
model, the root diameter reaches 0.11, 0.27, 0.49, 0.84,
1.00, 1.29 and 1.59 cm at cumulated GDDs of 321, 418,
500, 600, 639, 700 and 757°C d, respectively.

. . .

	$0 < T_{max}$	, ≤12°C	$6 \le T_{max} \le 8 \ ^{\circ}\mathrm{C}$			
	N days	GDD	N. days	GDD		
Bolting%	0.332ns	0.524**	0.071ns	0.769**		

Evaluating the effect of sowing date on bolting

Table 6 shows the number of days with  $T_{max}$  between 0 and 12 °C, and 6 and 8 °C, respectively. In this table,

**Table 8.** Summary of the regression models for predicting bolting percentage as a function of GDD at the beginning of the effective temperature and killed plants as a function of GDD cumulated from January 1<sup>st</sup>.

			Model Summary					Parameter Estimates				
Independent variable	$T_{max}$ (C°)	Equation	R Square	F	df1	df2	Sig.	Constant	b1	b2		
% bolting	$0 < T_{max} \le 12 \text{ °C}$	Quadratic	0.285	4.176	2	21	0.030	3.762	0.022	4.935E-5		
% bolting	$6 \le T_{max} \le 8 \ ^{\circ}\mathrm{C}$	Quadratic	0.756	31.056	2	20	0.000	6.522	-0.094	0.000		
% killed plants	-	Linear	0.302	6.928	1	16	0.018	58.246	-0.071	-		



0

Poly. (Bolting%)

Linear (Killed Plant %),

.

0

0

GDD is the cumulated sum attained on the first day on which the above conditions were satisfied. In general, the percentage of bolting in Karaj was greater than that in Mashhad in both years. Also, compared with Mashhad, the number of days with  $T_{max}$  between 0 and 12 °C in Karaj was greater in both years. The minimum and maximum number of days with  $T_{max}$  between 0 and 12 °C were recorded in Mashhad in 2017/18 and in Karaj in 2018/19. However, the recorded number of days with  $T_{max}$  between 6 and 8 °C was different each year, and it was higher in the first year in Mashhad but in the second year in Karaj, respectively.

The correlation between the percentage of bolting and GDD, on the one side, and the correlation between the number of days with  $T_{max}$  between 0 and 12 °C, and 6 and 8 °C, respectively, and GDD, on the other side, were both positive (Table 7), but only the correlation between the percentage of bolting and GDD was significant (P < 0.01).

Table 8 shows regression models for predicting bolting percentage as a function of GDD. The coefficient of determination (R<sup>2</sup>) is considerably higher with respect to the beginning of the period with  $T_{max}$  between 6 and 8 °C (R<sup>2</sup>= 0.756) than with respect to the period with  $T_{max}$ between 0 and 12 °C ( $R^2 = 0.285$ ).

## The effect of cold and frost stress on sugar beet seedlings

In the present experiment, the lowest daily temperature in 2017/18 and 2018/19 in Karaj and in 2018/19 in Mashhad was about -12 °C (Table 2). Table 8 shows the model for the prediction of plant losses during the winter as a function of GDD. The results indicate a negative relationship between the percentage of plant loss during winter and plant age at the beginning of winter (Figure 3).

#### DISCUSSION

#### Duration and rate of field emergence

100

90

80

70 60

50

40

30

20

10

Killed plant %

The average duration of field emergence increased from about 7 to 26 days from the first sowing date (second half of September) to the sixth sowing date (second half of November). Conversely, the emergence rate decreased from 0.15 to 0.03 seeds per day. If soil moisture and structure are optimal, the field emergence of sugar beet largely depends on temperature (Esmaeili et al. 2022, Hoffmann et al., 2020). Germination increases with increasing temperature and reaches a maximum at 22-25°C (Campbell and Enz, 1991). The minimum temperature for sugar beet growth is 3 °C (Milford et al., 1985a, 1985b), but growth decreases at temperatures below 10 °C, so the critical growth period for seedlings is longer than estimated based alone on a critical threshold of 3 °C. Delay in autumn sowing of sugar beet leads to germination at temperatures below the desired level, increases the average duration, and decreases the emergence rate. Hongyong et al. (2007) reporter that wheat typically germinates in 7 days, but requires about 13 days in case of delayed sowing. In our experiment, the maximum delay in autumn cultivation of sugar beet (about two months) increased the time to reach emergence by about 31days (this value is the average for Mashhad and Karaj of the differences in DAS between treatment 6 and 1 in Table 4).

If the seeds are in the ground at temperatures below the desired level, the germination process proceeds very slowly and the germinating seeds and the seedlings remain vulnerable to soil-borne pests and diseases for a long time (Jaggard and Qi, 2006). Thus, in such conditions there is a high possibility of poor establishment in the field.

#### Phenological stages

It has been reported that the basal temperatures for leaf emergence and leaf expansion are 1°C and 3°C, respectively (Milford et al., 1985a). As the leaf area increases, so does the number of leaves (Milford et al., 1985b). On average, each of the growth stages until the 16-leaf stage required about 70 GDD. The highest partial GDD sum was required to reach the cotyledon stage. The time of onset of vegetative and reproductive stages and the number of the organs depend on temperature

100

90

80

70

60

40

30

20

10

**Bolting%** 50 and length of the day, but the subsequent survival and size of these organs depend on the availability of nutrients (Slafer, 2007). Our study evaluated sugar beet phenology up to the 16-leaf stage at two sites in Iran separated by 1 degree of latitude. As the relationship between number of leaves and GDD did not differ significantly between sites, we conclude that the growth stages were not affected by latitude. This is consistent with the finding reported by Krüger et al. (2012), that phenology stages of strawberry were independent from latitude.

# The effect of sowing date on quantitative traits of sugar beet seedlings

Quantitative traits of sugar beet seedlings, including leaf, shoot and root weight and maximum root diameter, were significantly reduced by delayed sowing dates. Hence, it is possible to have different levels of sugar beet growth stages before the onset of cold weather by adjusting the sowing date. It has been reported that in spring cultivation and in the presence of water stress in the early stages of growth, the genotypes that produce higher root and total dry weight early in the growing season, usually produce higher root and sugar yield at the end of the growing season (Mohammadian et al., 2005). It has also been found that the production of sugar beet leaves and roots in autumn until winter has a strong relationship with sowing date, and that more yield is obtained with earlier sowing dates (Hoffmann and Kluge-Severin, 2011). In addition, the yield of sugar beet directly depends on the amount of radiation received by the leaves from sowing to harvest. Therefore, management of sowing and harvesting dates plays an important role in determining the yield (Lee et al., 1987).

## Evaluating the effect of sowing date on bolting

In general, the differences of bolting percentage for locations, seasons, and sowing dates are related to the number of cold days after sowing (Hoffmann et al., 2020). We found a higher percentage of bolting in Karaj than in Mashhad, which is explained by the greater number of cold days ( $T_{max}$  in the range 0 to 12 °C) at the former location. However, in each year and location, the bolting percentages differs depending on the sowing dates. The percentage of bolting was higher for early than late sowing dates (Table 6).

Moreover, our results showed that the age of the plants at the beginning of the period with temperatures effective for bolting had a larger impact on bolting percentage than the number of days with the suitable temperatures for bolting (Table 7). According to the coefficient of determination ( $R^2$ ) of the regression models predicting the percentage of bolting as a function of GDD, the temperatures 6 to 8 °C were found to be more effective than the temperatures 0 to 12 °C on the percentage of bolting (Table 8).

Figure 3 indicated that in order to minimize bolting percentage, it is necessary to adjust the sowing date so sugar beet is already at stage 4-6 leaves (300 to 400 GDD) at the time of occurrence of temperatures effective for bolting. At this time, the largest root diameter of sugar beet was 0.11 to 0.27 cm.

#### Effect of cold and frost stress on sugar beet seedlings

It has been shown that sugar beet roots are damaged by at temperatures below -6 °C (Reinsdorf and Koch, 2013). In addition, autumn sugar beet sowing in colder regions carries the risk of frost and freezing of the cell membrane and leakage of intracellular solutes (Baker and Rosenquist, 2004). In particular, Nezami et al. (2015) reports that, under controlled conditions, electrolyte leakage start to be effective when temperature drops below -7 °C.

Our findings indicate that there is a reverse relation between the degree of growth of sugar beet at the end of fall and cold tolerance and survival rate in winter (Fig. 3). In our experiment, we observed the least number of killed plants when the growth stage of sugar beet was beyond 16 leaves (more than 850 GDD). According to the equation presented in Figure 2, the largest diameter of the sugar beet root at 850 GDD is about 2cm. This is in line with the finding of a highest capacity to withstand temperatures below -7°C and maximum survival of sugar beet plants at 600-900 GDD after sowing (Loel and Hoffmann, 2014). Moreover, it has also been reported that the highest tolerance to sugar beet frost is when the root diameter reaches 1 to 2.5cm (Reinsdorf and Koch, 2013).

According to the results presented in Table 2 and Figure 3, we recommend for the region of Iran, in which our study took place, that sowing date for autumn cultivation are chosen so that the plants are able to reach the 14-16 leaf stage (700-750 GDD from sowing) before January. We expect that in these conditions, sugar beet plants will be able to tolerate cold temperatures which eventually will limit the percentage of killed plant to below 7%.

## CONCLUSION

Climate change has made it possible to grow sugar beet in the autumn in areas, where in the past only spring sowing was possible. However, unlike in warmer regions, in these areas autumn and early winter temperatures below the base temperature (3 °C) can delay growth, implying higher risk of frost damages. The results of our study showed that delayed sowing date led to an increase in the average duration of the early development phases, a decrease in the speed of field emergence, and reduced growth of sugar beet seedlings before the onset of winter in cold regions. This was associated with higher frost killing compared with earlier sowing dates.

The number of days with  $T_{max}$  in the range 6 to 8°C was more relevant for bolting than the number of days with  $T_{max}$  in the range 1 to 12°C. In our study, the bolting percentage depended on the growth stage achieved at the onset of the time period with temperatures effective for bolting (6 to 8°C). Assessing the effect of sowing date on bolting percentage is therefore of paramount importance for the success of autumn cultivation of sugar beet, even for cultivars with relatively high bolting tolerance. As a rule, sowing should be planned to allow sugar beet to accumulate 300 to 400 GDD at the time temperatures drop at levels effective for inducing bolting. In addition, 700 to 750 GDD at the time temperatures drops below 0°C are necessary to minimize the risk of frost killings. Considering 15 years of weather data allows obtaining a first-order estimate of the optimum time window for sowing. Finally, we would like to point out that in autumn cultivation of sugar beet it is better to sow the seed at higher densities than typically recommended for spring cultivation, because thinning can be performed after once the plants are successfully established.

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