



## Identification of Morpho-physiological Traits Affecting White Sugar Yield in Sugar Beet

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### Abstract

This research was carried out to evaluate the effects of some morphophysiological traits on white sugar yield of 10 commercial sugar beet (*Beta vulgaris* L.) varieties. All experiments were conducted in four important sugar beet growing areas in Iran, i.e. Moghan, Khoy, Karaj and Zarghan in 2006 and 2007. The experiment was arranged as a randomized complete block design with three replications in each environment. Total leaf production and cumulative number of senescent leaves were affected more by location than by genotype. There were significant differences among varieties for white sugar yield. Multiple regression analysis indicated that leaf death rate at the crop development, mid-season and late-season stages; leaf appearance rate and petiole dry weight at the crop development stage; and root dry weight at the late-season stage affected white sugar yield. Leaf death rate had the highest positive effect on white sugar yield at the late-season stage and the highest negative effect at the mid-season stage. In conclusion, leaf death rate at the crop mid-season stage and root dry weight at the late-season stage contributed the most to white sugar yield of sugar beet varieties.

**Keywords:** Leaf appearance rate; Leaf death rate; Leaf production; Senescent leaves; Sugar beet

### Introduction

At the early growth stage, growth rate has a direct relationship with the amount of light intercepted by the leaves (Biscoe and Gallagher 1977; Monteith 1977; Gallagher and Biscoe 1978). For sugar beet, biomass production corresponds with the ratio of absorbed light by the canopy during the growth period under normal, stress-free conditions (Jaggard and Qi 2006). Leaf area controls light absorption and its expansion is important until full leaf canopy cover is attained. In the case of availability of adequate water and nutrition after emergence, sugar beet needs 900 growing degree days (GDD) above the base temperature (3°C) to reach 85 percent canopy coverage (Werker and Jaggard 1997; Malnou *et*

*al.* 2006). Therefore, any factor that limits leaf expansion rate decreases final yield. Increasing the leaf area index depends on the leaf appearance and expansion rate, leaf full size and leaf area duration. All of these parameters depend upon environment such as climate, irrigation, nutrition etc. (Milford *et al.* 1985a). Furthermore, changes in leaf photosynthesis activities could be the result of internal phenomena, such as leaf senescence, which has an impact on the contribution of each leaf in the plant and canopy photosynthesis (Acock *et al.* 1978). Vegetative growth increases total dry weight and final root yield (Patterson and Moss 1979). According to Fick *et al.* (1975), leaf appearance rate, leaf life span and the required time to reach final leaf area are affected by

environment and genotype. Hodáňová (1981) considered photosynthetic capacity and senescence of sugar beet leaves and concluded that the changes in leaf photosynthesis, e.g. leaf appearance rate, duration of leaf expansion and the length of leaf developmental time, depend on the environment. Milford *et al.* (1985b) reported that leaf appearance and expansion rate and duration of leaf expansion depend on temperature. Milford *et al.* (1985a) also indicated that sugar beet leaf size depends on its position on the crown, sowing date, nitrogen, plant density and water stress. In addition, leaf senescence rate depended on intercepted heat.

Sugar beet growth is characterized by the continuous dying of old leaves and initiation of new leaves. If the photosynthetic activity of leaves can be extended, fewer leaves may be needed and more photosynthate could be translocated to the root for sucrose production. Doney and Martens (1994) reported that selection for extended leaf duration did not affect root and canopy dry matter, but root dry matter and total dry matter accumulations are reduced by the selection for reduced leaf duration. Slafer *et al.* (1994) observed that genotype and photoperiod affect leaf number in bread wheat (*Triticum aestivum* L.). Also, Ceppi *et al.* (1987) noted that leaf senescence was affected by genotype in maize (*Zea mays* L.).

In the plant growth modelling, the relationship between the source and sink and its effect on biomass and production is important. Thus, the objective of the current study was to identify some morpho-physiological traits affecting final sugar beet yield.

## Material and Methods

### Sites and experiments

All experiments were conducted in four important sugar beet growing areas in northwest, west and central Iran, i.e. Moghan (39°39' N; 47°55' E; 76 m altitude; 332 mm mean annual precipitation), Khoy (38°37' N; 45°15' E; 1145 m altitude; 240 mm mean annual precipitation), Karaj (35°48' N; 50°57' E; 1300 m altitude; 244 mm mean annual precipitation) and Zarghan (29°46' N; 52°44' E ; 1604 m altitude; 230 mm mean annual precipitation) in two successive years (2006 and 2007). The experimental design was randomized complete block design with three replications and 10 commercial varieties at each location. The seeds were obtained from three seed companies (Table 1). All varieties were of monogerm type and diploid which had been chosen with respect to maximum differences in morphological traits, based on their variety descriptions. Phosphorous, nitrogen and potash were applied to the soil before sowing based on soil test. Plot size and row spacing were 10 × 3 m<sup>2</sup> and 0.50 m (six rows), respectively. The seeds were sown in the early spring (Table 2) and then the irrigation was performed. After two or three irrigations, seedlings were thinned to a within-row spacing of 16 cm at the four-leaf stage. Irrigations were scheduled on the basis of 80 mm evaporation from class-A evaporation pan. Weeds, insects and pathogens were controlled accordingly during the growing season if needed.

### Traits measured

Approximately one month after the first irrigation, the leaves in one square meter of each plot (about 10 plants) were counted and the petiole of the

**Table 1. Means of white sugar yield (WSY) for 10 sugar beet varieties at different locations averaged over two years (2006 and 2007). Due to heterogeneity among errors of experiments, Karaj, Khoy, and Moghan data were combined and Zarghan data was analysed individually.**

Number	Variety		White sugar yield (t.ha <sup>-1</sup> )	
	Name	Company	Zarghan	Khoy, Karaj and Moghan
1	216	SBSI	7.35 g <sup>+</sup>	7.24 e
2	Persia	Syngenta	8.59 de	8.51 bcd
3	Rasta	Syngenta	10.19 b	10.55 a
4	Isella	KWS	11.61 a	10.08 ab
5	Shirin	SBSI	8.92 cd	8.26 cde
6	Linda	KWS	8.93 cd	7.82 de
7	Dorotea	Syngenta	7.85 f	7.50 de
8	426	SBSI	9.21 c	8.15 cde
9	Brigitta	KWS	10.62 b	9.41 abc
10	Zarghan	SBSI	8.28 ef	8.64 bcd

<sup>+</sup>Means followed by the same letters are not significantly different according to Duncan's multiple range test ( $P \leq 0.05$ ).

**Table 2. Sowing and harvesting dates of Khoy, Karaj, Moghan and Zarghan experiments in 2006 and 2007 growing seasons**

Year	Location	Sowing date	Harvesting date
2006	Karaj	4 May	22 November
	Khoy	17 April	26 September
	Moghan	13 May	14 October
	Zarghan	21 May	23 November
2007	Karaj	27 May	3 November
	Khoy	7 May	23 September
	Moghan	7 April	14 October
	Zarghan	31 May	24 November

newest leaf was marked with a plastic string. Afterwards, the number of new leaves was counted every 10 days prior to harvesting time and the marking strings were moved to the newest leaf. All leaves with a length greater than 6 cm were counted (Rinaldi 2003). Total leaf production (TLP), leaf appearance rate (LAR), cumulative number of senescent leaves (CSL) and leaf death rate (LDR) were calculated using the equations proposed by Lee and Schmehl (1988). The only modification was the replacement of the “day-based rate” with a “GDD”-based rate. GDD was calculated by taking the average of the daily maximum ( $T_{max}$ ) and minimum temperatures ( $T_{min}$ ) compared with a base temperature,  $T_{base}$ , (3 °C) as follows:

$$GDD = \frac{T_{max} + T_{min}}{2} - T_{base}, \text{ If } T_{min} \leq 3 / T_{max} \geq 40,$$

$$GDD = 0.$$

Measurements at the crop development (about 50 days after the first irrigation, late June), mid-season (about 96 days after the first irrigation, early August) and late-season (about 141 days after the first irrigation, late September), stages according to sugar beet growth stages (Anonymous 2013), required destructive sampling from almost one square meter per plot. At these stages, plants had received about 1040, 2149 and 3054 GDD, respectively. The traits measured at each stage were leaf dry weight (LDW), petiole dry weight (PDW) and storage root dry weight (RDW). Then, using the above measurements,

shoot (LDM+ PDM) to root (RDM) ratio and total dry weight (TDW= LDW+ PDW+ RDW) were calculated.

At harvest, the roots from six square meters of each plot were weighed and their pulps were used to determine the sugar, potassium, sodium and nitrogen concentrations. White sugar percentage was estimated by the equation proposed by

Reinefeld *et al.* (1974). White sugar yield (WSY) was obtained as the product of root yield by white sugar percentage.

### Statistical analysis

For determining the effect of varieties and locations on TLP and CSL, after regression analysis, the following F test was used:

$$F = \frac{(SS_{\text{combined}} - SS_{\text{separate}}) / (DF_{\text{combined}} - DF_{\text{separate}})}{SS_{\text{separate}} / DF_{\text{separate}}}$$

Where  $SS_{\text{separate}}$  and  $DF_{\text{separate}}$  are the sum of squares and degrees of freedom from individual variety/location regression equation and  $SS_{\text{combined}}$  and  $DF_{\text{combined}}$  are sum of squares and degrees of freedom from the combined regression analysis, respectively (Adams 2011).

To test the differences between varieties for white sugar yield and also the interactions of varieties with locations and years, the combined analysis was carried out for this character. Before combined analysis of variances for WSY, homogeneity of error variances for experiments was tested by the  $K_{\text{max}}$  test (Lee *et al.* 2010). Due to the heterogeneity of error variances, the square root data transformation was applied. However, the data transformation was not useful in relation to the Zarghan location and therefore, the analysis for this location was carried out separately. Years were considered as a random and locations and varieties as fixed factors. White sugar yield of varieties were compared by the Duncan's multiple range test at the 5% probability level. These analyses were carried out by SAS software, version 8.02.

Before the multiple regression analysis, normality test of the data was carried out by the Shapiro-Wilk method using SPSS software (version 16.0.0). Afterwards, stepwise multiple regression analysis was carried out considering WSY of each variety (averaged over replications) in each location and year (n= 80) as the dependant variable, and LDW, PDW, RDW, TDW, shoot: root ratio, LAR and CSL at the crop development, mid-season and late-season stages as the independent variables. The traits remained in the regression model were subjected to path-analysis by the Amos software (Karl 2012).

## Results

### TLP and CSL trends

Differences among varieties for the regression equations of TLP and CSL on GDD were not significant ( $P > 0.05$ ), but the differences of regression equations among locations were significant ( $P \leq 0.01$ , Table 3). Table 4 shows regression equations of TLP and CSL in all locations and Figure 1 shows the differences among the trends. Overall, number of leaves

produced in Khoy and Zarghan were more than Karaj and Moghan during the growing season. The number of leaves produced until about 2850 GDDs were similar in Khoy and Zarghan. But, after that, TLP trends differed in these locations and the slope in Khoy was steeper than Zarghan. The trends for TLP were different in Karaj and Moghan. The number of leaves produced in Moghan was a little more than Karaj till about 2720 GDDs but then, the slope was increased in

Karaj as compared to Moghan. From beginning of the season, CSL in Khoy was different from the other three locations (Figure 1), however, the differences of slopes for CSL among the three mentioned locations were observed after about 2100 GDDs. Overall, CSL in Khoy was more than the other locations during the growing season. Figure 2 indicates that there were a lot of differences among the locations for GDD and average temperature during the growing season.

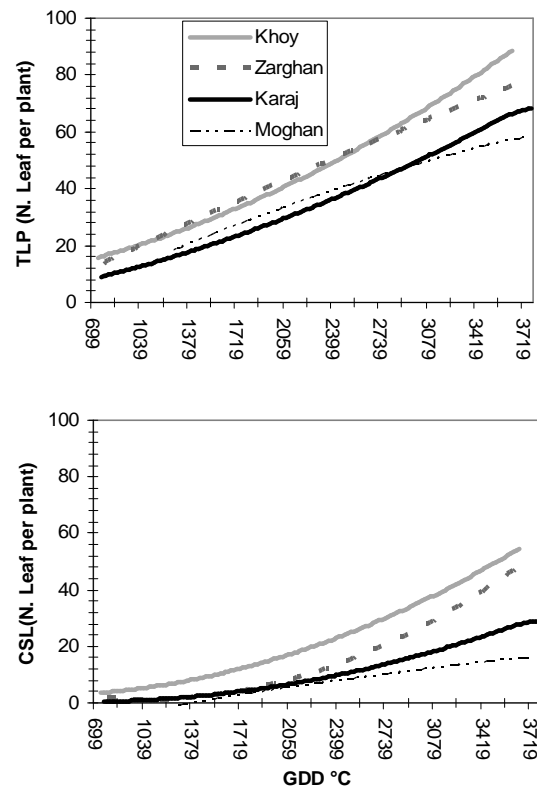
**Table 3. F test for comparing regression of total leaf production (TLP) and cumulative number of senescent leaves (CSL) on growing degree days (GDD) for 10 sugar beet varieties (mean of four locations and two years) and four locations (mean of 10 varieties and two years)**

	Variety			Location		
	df	Mean squares		df	Mean squares	
		TLP	CSL		TLP	CSL
Combined	917	93143.95	50748.26	89	9017.58**	4963.66**
Separate	890	92795.31	50579	80	2077.60	347.67

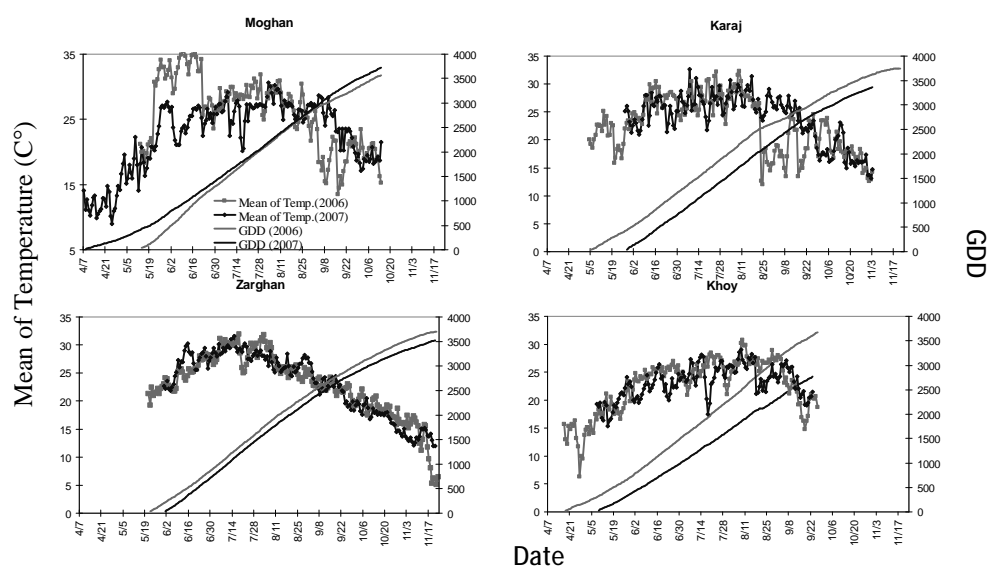
\*\*Significant at 0.01 probability level

**Table 4. Relationships of total leaf production (TLP) and cumulative number of senescent leaves (CSL) with growing degree days (GDD) at four locations averaged over two years (2006 and 2007)**

Location	Regression equation	R <sup>2</sup>
Khoy	TLP= 7.5985+ 0.0084 GDD+ 0.0000037 GDD <sup>2</sup>	0.9890
	CSL= 2.7034- 0.0021 GDD+ 0.0000044 GDD <sup>2</sup>	0.9820
Zarghan	TLP= -5.0225+ 0.0246 GDD- 0.0000007 GDD <sup>2</sup>	0.9310
	CSL= 8.8921- 0.0147 GDD+ 0.0000069 GDD <sup>2</sup>	0.9620
Karaj	TLP= 0.5738+ 0.0091GDD+ 0.0000024 GDD <sup>2</sup>	0.7700
	CSL= 1.5022- 0.0035 GDD+ 0.0000029 GDD <sup>2</sup>	0.9640
Moghan	TLP= -11.54726+ 0.02566 GDD- 0.0000019 GDD <sup>2</sup>	0.9807
	CSL= -13.1695+ 0.0104 GDD- 0.0000007 GDD <sup>2</sup>	0.8690



**Figure 1.** Pattern of total leaf production (TLP) and cumulative number of senescent leaves (CSL) of sugar beet at four locations (Khoy, Karaj, Moghan and Zarghan) averaged over two years (2006 and 2007)



**Figure 2.** The rate of daily and cumulative growth degree-days (GDD) and average temperature during growing season in four locations (Khoy, Karaj, Moghan and Zarghan) averaged over two years (2006 and 2007)

### WSY

Combined analysis of variance showed no significant location  $\times$  variety, year  $\times$  variety and year  $\times$  location  $\times$  variety interactions when Khoy, Karaj and Moghan locations were used in the analysis ( $P > 0.05$ , Table 5). However, the difference among varieties was significant for WSY (Table 5). Rasta, Isella and Brigitta had higher WSY (10.5, 10 and 9.4 t.ha<sup>-1</sup>, respectively)

than the other varieties in Khoy, Karaj and Moghan (Table 1). Furthermore, Isella with a WSY of 11.61 t.ha<sup>-1</sup> in Zarghan showed higher white sugar yield than the other varieties and 216 in Zarghan produced the lowest WSY of about 7 t.ha<sup>-1</sup> (Table 1). In summary, varieties had different WSY and Isella produced more sugar than the other nine varieties in the average of all locations.

**Table 5.** Combined analysis of variance of white sugar yield (WSY) of 10 sugar beet varieties at different locations in 2006 and 2007 growing seasons. Due to heterogeneity among errors of experiments, Karaj, Khoy and Moghan data were combined and Zarghan data was analysed individually.

Khoy, Karaj and Moghan			Zarghan		
Source of variation	df	Mean squares	Source of variation	df	Mean squares
Location	2	13.48			
Year	1	0.20	Year	1	1.01**
Location $\times$ Year	2	6.57**			
Rep (Location $\times$ Year)	12	0.19	Rep (Year)	4	0.04
Variety	9	0.59**	Variety	9	0.26**
Location $\times$ Variety	18	0.18			
Year $\times$ Variety	9	0.08	Year $\times$ Variety	9	< 0.01
Location $\times$ Year $\times$ Variety	18	0.18			
Error	108	0.13	Error	36	0.03

\*\*Significant at 0.01 probability level, respectively

### Multiple regression

The traits retained in the final multiple regression model were LDR at all of the three growth stages (crop development, mid-season and late-season), LAR and PDW at the crop development stage and RDW at the late-season stage (Table 6). Coefficient of determination ( $R^2= 0.67$ ) of the final model implied that 67% of variation for WSY could be explained by these six traits.

### Discussion

It seems that variation among locations was higher than variation among genotypes for the regression of TLP and CSL on GDD. The differences among locations for TLP and CSL may be associated with the differences in climate and other environmental parameters which could impact TLP and CSL in the growing season. Many studies show that the appearance and death of leaves are affected by environmental conditions such as climate, irrigation and nutrition (Hodáňová 1981; Bůrky and Biscoe 1983; Milford *et al.* 1985a, b; Slafer *et al.* 1994). However, there are reports showing significant effect of genotype on appearance and senescence of leaves (Ceppi *et al.* 1987; Slafer *et al.* 1994).

The fact that leaf death rate remained in the final multiple regression model at all three growth stages, while leaf appearance rate remained only at the crop development stage, implies that the leaf life span at different growth stages was more important than the leaf appearance rate. Woledge and Leafe (1976) also reported that leaf senescence pattern can be useful for the selection of new plant varieties for the photosynthesis ability.

Leaf death rate at the late-season and mid-

season stages had the highest positive and negative effects on WSY, respectively (Table 6). Each standardized unit increase in LDR at the late-season stage resulted in 2.19 standardized units increase in WSY, while each standardized unit increase in LDR at the mid-season stage resulted in 1.86 standardized units decrease in WSY (Table 6). Sugar beet growth is characterized by the continuous shedding of old leaves and initiation of new leaves (Doney and Martens 1994). Assimilates are continuously transmitted from the leaves as the sources of photosynthetic production to sinks during the growth period (Cooke and Scott 1993), but the rate of this transmission is affected by the photosynthetic rate and sinks demand. In sugar beet, photosynthesis sinks are young leaves (Joy 1964), petiole and beet (Lemaire *et al.* 2009). The leaf death rate effect on WSY at the late-season stage could be related to an increase in assimilates transmission rate from leaves as a source to roots as a sink. Clearly, varieties which can transmit more assimilates from leaves to roots would be expected to have higher yield. On the other hand, when photosynthetic active radiation (PAR) is high (from June to August), any factor that decreases leaf area index and as a result increases LDR, may have a negative effect on WSY. Thus, the negative effects of high LDR on WSY at the mid-season stage, which coincides with high PAR, can be related to this phenomenon.

Leaf death rate increase at the crop development stage resulted in higher WSY. Each standardized unit increase in LDR at this stage caused 0.51 standardized units increase in WSY (Table 6). This could be the result of early



beginning of photosynthetic transmission from leaf to root and consequently, it would increase WSY at the harvest time. The linear correlation coefficient of LDR at the crop development stage with the shoot and root dry weight at this stage confirmed this theory (Figure 4). On the other hand, these plants had more leaves at this stage (Figure 4). The correlation showed that these varieties completed their vegetative growth faster than others.

Positive effects of LAR at the crop development stage, similar to LDR, caused an increase in WSY (Table 6). Although at this stage, the effect of LAR on WSY was lower than LDR (0.18 units per unit increase in LAR), achieving the leaf area index of about three for absorbing most of the intercepted light is necessary (Malnou *et al.* 2008). In addition, each factor that limits leaf area expansion rate directly decreases final yield. Milford *et al.* (1985a) showed that climate, irrigation, nutrition and sowing date affect the

time of attaining maximum leaf area index. LAR at the crop development stage could influence the time of achieving maximum leaf area index so it could affect WSY. Leaf production with a higher speed at this stage can result in earlier attainment of crop development stage and better use of environmental conditions for the improvement of WSY.

For each standardized unit increase in PDW at the crop development stage, WSY increased by 0.16 standardized units (Table 6). At the early growth stages of the sugar beet when the expansion of the roots is not complete and therefore, their demand for assimilates is low, petiole may be an important sink for assimilates. These assimilates could be used as a supply of growth material when required.

**Table 6. Multiple regression coefficient, standard error, t-value and p-value of the variables retained in the regression model for the white sugar yield as the dependent variable**

Variables	Standardized multiple regression coefficient	Multiple regression coefficient	Standard error (SE)	t	p-value
Intercept	-	2.564	0.970	2.643	0.008
Leaf appearance rate <sup>1+</sup>	0.182	3.382	1.787	1.892	0.062
Leaf death rate <sup>3</sup>	2.189	37.018	7.698	4.809	0.000
Petiole dry weight <sup>1</sup>	0.164	0.008	0.004	2.117	0.038
Leaf death rate <sup>2</sup>	-1.863	-58.239	13.405	-4.345	0.000
Root dry weight <sup>3</sup>	0.466	0.002	0.000	6.654	0.000
Leaf death rate <sup>1</sup>	0.507	20.895	8.664	2.412	0.018

R<sup>2</sup>= 0.671; Adjusted R<sup>2</sup>= 0.644

<sup>+</sup>Numbers 1, 2 and 3 implies crop development, mid-season and late-season stages, respectively



Root dry weight at the late-season stage had a positive effect on WSY and each standardized unit increase in RDW increased WSY by 0.47 standardized units (Table 6). At the ripening stage, high RDW can assure high root yield. It is reported that direct and indirect effects of root yield and sugar content on WSY were stronger than the effects of other traits at the harvest time (Ouda 2005).

The relations of LDR, LAR, PDW and RDW with white sugar yield are shown in Figure 3. The mean of these ranges are the decision borders for these relationships. Due to the positive correlation of LAR, PDW and LDR with WSY at the crop development stage and LDR and RDW at the late-season stage, the increase/decrease in these independent variables was followed by the increase/decrease in WSY of five, four, three, one and eight out of ten varieties studied, respectively. On the other hand, due to the negative correlation of LDR at the crop development stage with WSY, this trait showed negative correlation in eight out of ten varieties. Therefore, it can be concluded that LDR and RDW at the crop development and ripening stages, respectively, were more

dependent on the genotype as compared to the other four traits.

Understanding the physiological and morphological aspects of sugar beet at different growth stages can help to explain the differences between the varieties in terms of their economic yields. Thus, it is recommended to consider other attributes of available genetic resources of the crops in breeding programs in order to mitigate the risk of failure in these programs and to improve the final yield. Considering the results of this study, leaf death rate at the mid-season stage and root dry weight at the late-season stage were the most important traits which could be used in the breeding programs to improve final yield of sugar beet varieties.

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