Sequential path analysis of some yield and quality components in sugar beet grown in normal and drought conditions

Mehdi Baradaran Firouzabadi, Naser Farrokhi, Mahdieh Parsaeyan

Department of Agronomy and Plant Breeding, Shahrood University of Technology, Iran

Abstract

Through biometrical analyses of yield and its components selection indices can be generated and be used in future breeding programs. Sugar yield components were considered as the first order variables (FOV) in previous path analyses studies, while white sugar yield (WSY) and its related traits were the FOV here. Three lines of sugar beet (7219-P.69, BP-Karaj, 7112) were evaluated in drought and non-drought conditions. Two sequential path models were used for analysis of associations among WSY and its related traits by arraying the independent variables in first-, second-, and third-order paths on the basis of their maximum direct effects and minimal collinearity. Four first-order variables, namely root diameter, sugar yield, molasses content and sugar content, revealed highest direct effects on WSY under normal condition, while root length, α-amino-N, root yield, crown dry weight, water use efficiency and Na^+ were found to fit as second-order variables. Three first-order variables, namely sugar content, sugar yield and molasses content, revealed highest direct effects on white sugar yield under drought-stress condition. In this case, sugar yield had the highest direct effect on WSY. In general, the sequential path analysis efficiently demonstrated the effects of predictor variables.

Introduction

The yield components in sugar beet (*Beta vulgaris* L.) consist of biomass-, root, and sugar-yield of which the white sugar yield is concerned as the most important component (Mohammadi and Assad, 1997). Therefore, understanding the sugar yield interrelationships with other agronomic traits may lead in determination of selection indices for higher white sugar yield.

Sugar content, and both chemical and physical states of beet roots, known as beet quality, greatly affect sugar and its by-product yield. Beet quality is defined by the optimum condition that provides the higher sugar content next to the lower amounts of non-sugar compounds, mainly α-amino-N, glycine betaine, proline, sodium and potassium (Gzik, 1996). These impurities inhibit the crystallization of sucrose and increase the molasses production rate (Dunham and Clark, 1992; Harvey and Dutton, 1993). The rate of these non-sugar compounds usually increases upon drought stress, lowering the sugar beet quality (Clarke *et al.*, 1993).

Although correlation studies reveal the genetic gain of a trait upon selection, it would not be definitive in cases where numerous independent variables are involved in controlling the dependent trait (Agrama, 1996; Board *et al.*, 1997). To resolve this, path analysis would be a useful technique in breaking down the direct correlations of independent variables into their direct and indirect effects and establishing their relations to the dependent trait (Williams *et al.*, 1990). In path analysis, the variables that are most effective on dependent variable are being considered as the first-order predictors of response variable that is mainly yield in agricultural studies (Maity and Chattarzee, 1977; Gunel *et al.*, 1991; Gopal *et al.*, 1994; Yildirim *et al.*, 1997; Bhagowati and Saikia, 2003; Tuncturk and Çiftçi, 2005). However in places where the independent variables have correlation with each other, multicollinearity (combining effects of different correlated variables) obscures the interpretation of results (Milligan *et al.*, 1990; Hiar *et al.*, 1995). Samonte *et al.* (1998) developed a sequential path analysis and used it to determine the interrelationships of rice yield and its components considering minimizing the multicollinearity. Thus, sequential path analysis may improve the reliability of results by placing the predictor variables in different orders of relation to the response variable (usually up to three orders) and eventually eliminating the multicollinearity. Previous studies emphasize on the trustworthiness of sequential path analysis in contrast to single-step path analysis (Agrama, 1996; Mohammadi *et al.*, 2003; Asghari-Zakaria *et al*., 2007).

Here, correlation and two sequential path analyses (SPA) were used to reveal the predictor traits on white sugar yield (WSY) for sugar beet breeding grown under normal and drought-stressed conditions.

Materials and Methods

Field trial

The experiment was performed in Karaj, Iran in 2002. The soil was calcic brown silty clay (pH=7.6, EC=0.6). The average rainfall for a period of 35 years was 243 mm occurring mostly during late autumn till early spring providing a xerothermo Mediterranean climate.

The experiment occupied a surface of 1500 m^2 with 54 plots. Each

Correspondence: Dr. Naser Farrokhi, Faculty of Agriculture, Shahrood University of Technology, P.O. Box 316, Shahrood, Iran.
E-mail: nfarrokh@nigeb.ac.ir

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plot contained 6 lines (7 m in length) with 50 cm and 20 cm inter-row and intra-row, respectively. Experimental design was a split block-like (Hanks *et al.*, 1980) with Line-Source sprinkler irrigation system (three treatments) and three sugar beet lines in three replicates. According to Hanks *et al.* (1976; 1980) irrigation treatments were not randomized (Figure 1).

The Line-Source divided the field into two equal parts. Surrounding rows of the Line-Source were 18; the first six-rows were in normal (receiving 1272 mm), the middle six-rows in mild-stress (receiving 1141 mm), and the last six-rows in severe-stress (receiving 739 mm) conditions (Figure 1). The cultivars were tolerant (7219-P.69), semi-tolerant (Karaj-BP) and sensitive (7112) to drought condition. Following the complete establishment of plants, irrigation system was set and irrigation treatments were applied.

The soil leaching rate was measured at depths of 0-100 cm by the Time Domain Reflectometry (TDR, PALTIN International Inc., Maryland, USA). Delivered water volume per plot was measured by planted measuring containers within the center of each plot at 50 cm height.

Sampling and data collection

Destructive plant samples were collected during growth period at 170 and 210 days after sowing (DAF). The following traits were measured at 170 DAF; leaf dry weight (LDW), root dry weight (RDW), petiole dry weight (PDW), crown dry weight (CDW), total dry weight (TDW), leaf area index (LAI), root diameter (RD), root length (RL), water used (WU), and water use efficiency (WUE). Root yield (RY) and qualitative factors including potassium, sodium, and α-amino-N were measured at 210 DAF. After pulp preparation with VENEMA Line (KHBC Ltd., Poland), all the qualitative factors were analyzed with Betalyser (model OR-KERNCHEN). Betalyser is a software applet for automated routine analysis of sugar content and beet impurities including Na+, K+ and α-amino-N. The instrument measures potassium and sodium via its flame photometer, α-amino-N by double beam filter photometry using the blue number method (Stanek and Pavlas, 1934; Sheikh-Aleslami, 1997) and sugar content (SC) through its polarimeter. Molasses content (MC) was calculated with the following formula:

$$MC = 0.343\ (K + Na) + 0.094\ (\alpha\text{-amino-N}) - 0.31$$ (Reinefield *et al*., 1974)

Sugar yield (SY), and white sugar yield (WSY) were calculated after Reinefield *et al.* (1974) and according to the following formulas:

$$WSC = SC - MC$$
$$WSY = RY * WSC$$
$$SY = RY * SC$$

Alkaline level content (ALC) was calculated according to Sheikh-Aleslami (1997) using the following formulae:

$$ALC = (K + Na) / \alpha\text{-amino-N}$$

Statistical analyses

Normality test of the data was performed with SPSS. The data demonstrated to be normal. Simple correlations were calculated and stepwise regression was followed to determine interrelationship between independent and dependent variables and further to reveal the first-, second-, and third-paths of predictor variables.

The independent variables were grouped according to their contribution in yield considering minimal multicollinearity effect. Sequential path analysis was followed according to Samonte *et al.* (1998) to reveal the cause-effect relationship. The first order independent variables were the traits with highest regression coefficients in the stepwise regression. Consecutively, these independent traits were considered as dependent variables to the remaining traits and second stepwise regressions were performed to reveal the second order independent traits. These steps were followed to reveal the third order independent traits.

In order to compare single-step path analyses with sequential path analyses variance inflation factors (VIF) and tolerance values (TV) were measured via SPSS 11.5 (Williams *et al.*, 1990; Hiar *et al.*, 1995). The variability of selected independent variables can be explained by $TV=1-R_i^2$ (R_i^2 is the coefficient of determination for the prediction of variable I by the predictor variables). On the other hand, VIF ($VIF=1/(1-R_i^2)$) demonstrates the extent of the effects of other independent variables on the variance of the selected independent variable. A high degree of multicollinearity can be explained by values greater than 10 for VIF and values smaller than 0.1 for TV (Wang *et al.*, 1999; Mohammadi *et al.*, 2003).

Results

Here, for the sake of simplicity the mid and sever stress treatments were combined and the average trait measurements were considered as the drought condition.

Simple correlation analyses

Simple correlation coefficients are presented in Tables 1 and 2 for both normal and drought-stressed conditions. In normal condition, a positive correlation was observed between WSY and SC (0.8) and negative significant correlations were observed with PDW and sodium. However, a non-significant negative correlation was seen with WSY (Table 1).

In stressed condition, SY and RY had the highest positive correlation with WSY, 0.95 and 0.76 respectively. RD also had a significant positive correlation with WSY (Table 2). WUE in stressed condition had lower effect on WSY compared to the normal condition (0.14 against 0.90).

Stepwise regression and sequential path analysis in normal condition

White sugar yield was considered as the dependent variable against the rest of the traits and stepwise regression was performed (Table 1 and Figure 1). Root diameter, sugar yield, molasses content and sugar content were kept in the model (R^2=0.98) and path analysis was followed (Table 1 and Figure 1). SY had the highest direct effect on WSY, while RD and MC had minor direct effect. Although SC had a high and positive correlation with WSY, its direct effect was much lower compared to SY (019). Meanwhile the SC's indirect positive effect via SY was high, suggesting its contribution to WSY.

In the second order, the above traits (RD, SY, MC and SC) were considered as dependent traits and stepwise regression was performed for each separately (Table 1 and Figure 1). Similarly, traits with the highest interrelationships with the above traits remained in the model. For RD, only root length remained in the model with the same correlation coefficient as RD (0.67, Table 1 and Figure 1). Water use efficiency, RY, sodium, and crown dry weight were present in the model for SY (Table 1). The first three traits had the highest direct effect on SY (0.47, 0.46, and -0.44, respectively). Although CDW had a negative correlation with SY (-0.45, Table 1), it had a positive direct effect on this trait (0.18, Figure 1). It appears the indirect negative effects through WUE and RY contributes to this negative correlation (Table 1). In case of MC, RY, sodium, potassium, α-amino-N were the remaining traits in the model (Table 1 and Figure 1). The highest direct effects belonged to Na^+ and K^+ (0.62 and 0.8, respectively). The indirect effects had minor values, suggesting that the major contribution of these traits were through their direct effects on MC. When SC was considered as dependent trait, the only trait appeared in the model was sodium content with a high negative effect (-0.88, Table 1 and Figure 1).

In the third order, traits appeared in the second step as independent

Table 1. Correlation coefficients between traits measured in three lines of sugar beet in normal condition. Correlation coefficients more than 0.32 and 0.43 are significant at the 0.05 and 0.01 probability levels, respectively.

	LDW	RDW	PDW	CDW	TDW	LAI	SLW	LWR	LAR	RD	RL	K	Na	N	ALC	MC	WU	WUE	SC	SY	RY
LDW																					
RDW	-0.49																				
PDW	0.87	-0.77																			
CDW	0.87	-0.46	0.67																		
TDW	-0.1	0.90	-0.46	-0.12																	
LAI	0.98	-0.47	0.90	0.72	-0.08																
SLW	-0.82	0.34	-0.81	-0.58	-0.01	-0.8															
LWR	0.79	-0.86	0.87	0.52	-0.64	0.75	-0.60														
LAR	0.85	-0.82	0.93	0.60	-0.56	0.84	-0.73	0.97													
RD	-0.43	0.33	-0.30	-0.42	0.18	-0.3	0.15	-0.4	-0.41												
RL	-0.28	0.19	-0.22	-0.21	0.1	-0.3	0.11	-0.3	-0.29	0.67											
K	-0.50	0.39	-0.49	-0.43	0.21	-0.5	0.41	-0.4	-0.48	0.47	0.35										
Na	0.41	-0.51	0.57	0.65	-0.32	0.48	-0.45	0.42	0.52	-0.06	-0.06	-0.08									
N	-0.21	-0.20	0.04	0.01	-0.28	0.12	0.06	-0.1	-0.03	0.12	0.24	0.09	-0.02								
ALC	0.30	0.08	0.05	0.14	0.20	0.24	-0.02	0.12	0.10	-0.12	-0.22	-0.06	0.04	-0.90							
MC	-0.19	-0.03	-0.03	0.05	-0.07	-0.1	0.06	-0.1	-0.07	0.36	0.28	0.77	0.54	0.24	-0.18						
WU	0.62	-0.30	0.61	0.24	-0.08	0.62	-0.56	0.48	0.52	-0.21	-0.14	-0.56	-0.05	-0.09	0.12	-0.50					
WUE	-0.69	0.52	-0.78	-0.52	0.26	-0.7	0.73	-0.6	-0.70	0.14	0.30	0.43	-0.55	0.19	-0.18	0.03	-0.65				
SC	-0.36	0.49	-0.57	-0.50	0.33	-0.4	0.47	-0.4	-0.51	-0.02	0.14	0.03	-0.88	0.06	-0.01	-0.51	-0.17	0.66			
SY	-0.59	0.45	-0.67	-0.45	0.22	-0.6	0.63	-0.5	-0.60	0.12	0.34	0.36	-0.61	0.27	-0.28	-0.04	-0.47	0.92	0.67		
RY	-0.50	0.17	-0.42	-0.19	-0.05	-0.5	0.45	-0.3	-0.37	0.17	0.33	0.46	-0.07	0.36	-0.38	0.38	-0.48	0.67	0.05	0.76	
WSY	-0.50	0.48	-0.69	-0.49	0.26	-0.6	0.62	-0.4	-0.60	0.07	0.29	0.23	-0.73	0.20	-0.22	-0.22	-0.39	0.90	0.80	0.97	0.62

WSY, white sugar yield; RY, root yield; SY, sugar yield; SC, sugar content; WUE, water use efficiency; WU, water use; MC, molasses content; ALC, alkaline level content; N, α-amino N; Na, sodium; K, potassium; RL, root length; RD, root diameter; LAR, leaf area ratio; LWR, leaf weight ratio; SLW, specific leaf weight; LAI, leaf area index; TDW, total dry weight; CDW, crown dry weight; PDW, petiole dry weight; RDW, root dry weight; LDW, leaf dry weight.

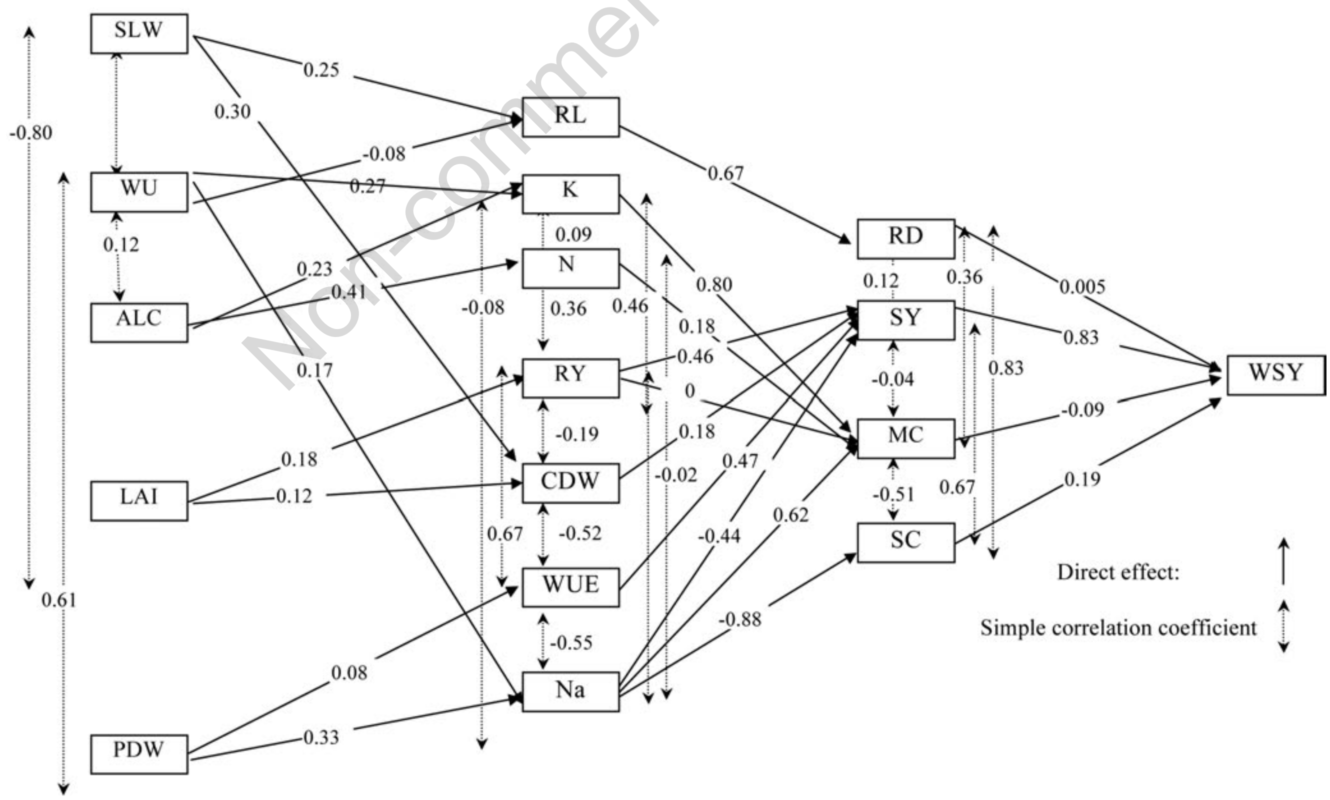

Figure 1. Sequential path model indicating interrelationships between white sugar yield and related traits in sugar beet in normal conditions.

Table 2. Correlation coefficients between traits measured in three lines of sugar beet in drought stress condition. Correlation coefficients more than 0.35 and 0.47 are significant at the 0.05 and 0.01 probability levels, respectively.

	LDW	RDW	PDW	CDW	TDW	LAI	SLW	LWR	LAR	RD	RL	K	Na	N	ALC	MC	WU	WUE	SC	SY	RY
LDW																					
RDW	-0.02																				
PDW	0.28	0.38																			
CDW	-0.29	0.25	0.49																		
TDW	0.8	0.98	0.49	0.30																	
LAI	0.94	0.02	0.40	-0.12	0.14																
SLW	-0.04	-0.09	-0.49	-0.42	-0.15	-0.34															
LWR	0.70	-0.58	-0.12	-0.59	-0.52	0.57	0.13														
LAR	0.71	-0.58	0.01	-0.45	-0.50	0.69	-0.15	0.94													
RD	-0.04	0.26	0.08	0.09	0.25	0.07	-0.18	-0.31	-0.20												
RL	0.02	-0.26	0.34	0.24	-0.21	0.14	-0.41	0.07	0.22	0.07											
K	-0.59	0.51	0.05	0.33	0.44	-0.54	-0.01	-0.67	-0.68	0.24	-0.11										
Na	-0.22	0.38	0.17	0.36	0.37	-0.11	-0.30	-0.32	-0.25	-0.06	-0.03	0.53									
N	0.23	0.16	0.24	0.02	0.19	0.35	-0.42	0.06	0.18	0.37	0.59	0.01	-0.15								
ALC	-0.41	0.06	-0.31	0.12	-0.05	-0.53	0.55	-0.32	-0.45	-0.10	-0.50	0.29	0.19	-0.84							
MC	-0.51	0.54	0.11	0.38	0.49	-0.42	-0.15	0.63	-0.60	0.22	-0.03	0.96	0.71	0.07	0.19						
WU	-0.24	0.31	0.52	0.60	0.34	-0.02	-0.59	-0.55	-0.36	0.59	0.18	0.24	0.10	0.26	-0.16	0.26					
WUE	0.14	-0.43	-0.58	-0.47	-0.46	-0.04	0.65	0.37	0.22	-0.02	-0.23	-0.11	-0.51	-0.15	0.28	-0.26	-0.51				
SC	-0.02	-0.29	-0.27	-0.24	-0.31	-0.21	0.57	0.17	-0.03	-0.04	-0.05	-0.27	-0.82	0.09	0.02	-0.46	-0.14	0.61			
SY	-0.36	0.04	0.10	0.52	0.04	-0.21	-0.28	-0.51	-0.37	0.68	0.12	0.36	-0.01	0.16	0.11	0.31	0.77	0.04	0.09		
RY	-0.34	0.17	0.22	0.59	-0.17	-0.10	-0.53	-0.55	-0.34	0.65	0.13	0.48	0.35	0.12	0.08	0.51	0.78	-0.23	-0.36	0.89	
WSY	-0.26	-0.10	0.05	0.43	-0.09	-0.14	-0.20	-0.36	-0.24	0.65	0.13	0.12	-0.25	0.16	0.07	0.04	0.73	0.14	0.29	0.95	0.76

WSY, white sugar yield; RY, root yield; SY, sugar yield; SC, sugar content; WUE, water use efficiency; WU, water use; MC, molasses content; ALC, alkaline level content; N, α-amino-N; Na, sodium; K, potassium; RL, root length; RD, root diameter; LAR, leaf area ratio; LWR, leaf weight ratio; SLW, specific leaf weight; LAI, leaf area index; TDW, total dry weight; CDW, crown dry weight; PDW, petiole dry weight; RDW, root dry weight; LDW, leaf dry weight.

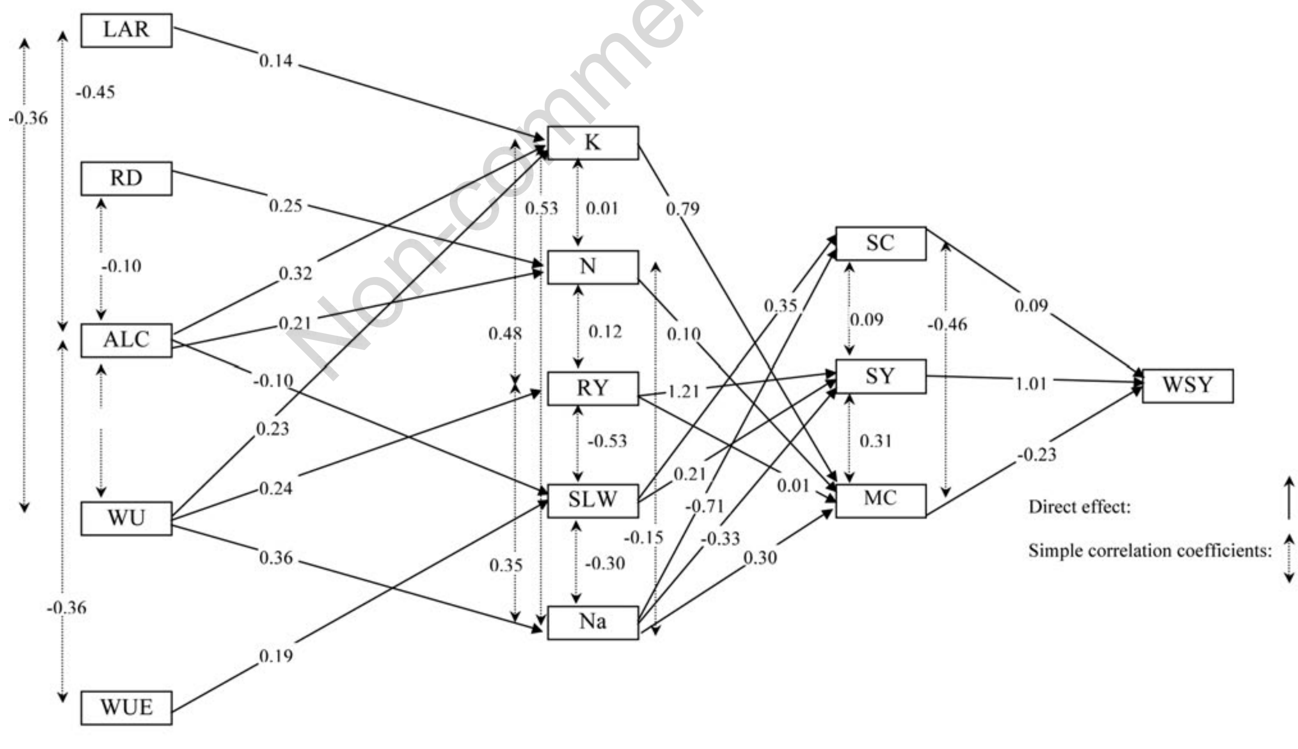

Figure 2. Sequential path model indicating interrelationships between white sugar yield and related traits in sugar beet in drought-stress conditions.

variables (RL, K, Na, N, RY, CDW, WUE) were considered dependent variables. The interrelationships of these traits with the remaining traits were evaluated. Since the coefficients of determination (R^2) of all models were very low, path analysis for the third order has not been discussed.

Stepwise regression and sequential path analysis in drought-stressed condition

Similar to normal condition, WSY was considered as the dependent variable and its interrelationship with other traits was evaluated. Three variables including sugar content, sugar yield and molasses content remained in the model following the first stepwise regression. Similar to the normal condition SY had the highest effect on WSY, while MC had a negative direct effect (Table 2 and Figure 2). In this model, RD was not kept and the direct effect of SC was minor.

For the second order path analysis, the above traits (SY, SC, and MC) were used as dependent variables in a stepwise regression and remaining traits were considered as independent traits. Root yield, specific leaf weight and sodium demonstrated a direct meaningful relation with sugar yield (Table 2 and Figure 2). Since the coefficient of determination was not as high as expected (R2=0.63), it may suggest that other traits might be involved which either at this point were not included into the model or they have not been measured. RY had the highest direct effect on SY. SLW had a negative simple correlation with RY, but it demonstrated positive direct effect on SY. However, the indirect effect of SLW via RY was significant and negative. Moreover, the root sodium content also demonstrated a negative direct effect on SY. When MC was considered as the dependent variable, four independent variables including K^+, Na^+, RY and α-amino-N were retained in the regression model. Among these variables, K+ and Na+ had the highest direct effect on MC with 0.79 and 0.3, respectively (Figure 2). In addition to the direct effect of Na+ on MC, it demonstrated a considerable amount of indirect effect mainly via K+. By considering the SC as the dependent variables, SLW and root Na+ were the only traits that entered into the model (Figure 2). The coefficient of determination was low (R^2=0.57), suggesting probable presence of other traits affecting SC that were not measured in this experiment.

In the third order of stepwise regression where the independent variables of second step considered being the dependent variables, the coefficients of determination were low and similar to the normal condition the path analysis was not conducted.

Comparative studies of single-step path analyses and sequential path analyses

Tolerance values (TV) and variance inflation rates (VIF) were calculated for both single-step path analyses and sequential path analyses (Tables 3 and 4). The values demonstrated elimination of multicollinearity in the latter approach.

Table 3. Comparison of multicollinearity in sequential path model and conventional path analysis by tolerance value xand variance inflation factor at normal condition.

Single-step path analysis					Sequential path analysis				
R^2	TV	VIF	DV	IV	R^2	TV	VIF	DV	IV
0.99	0.001	980.75	WSY	RD	0.98	0.82	1.20	WSY	RD
	0.017	57.71		SY		0.66	1.51		SY
	0.01	104.78		MC		0.72	1.37		MC
	0.02	48.78		SC		0.86	1.15		SC
	0.03	31.63		RL	0.47	0.99	1.01	RD	RL
	0.003	314.57		RY	0.94	0.72	1.37	SY	RY
	0.001	671.83		CDW		0.86	1.16		CDW
	0.008	127.68		WUE		0.70	1.41		WUE
	0.003	310.68		Na		0.68	1.45		Na
	0.004	247.31		K	0.88	0.96	1.03	MC	K
	0.01	78.17		N		0.61	1.62		N
	0.14	7.02		RD		0.72	1.37		RY
	0.01	54.02		WU		0.24	4.04		Na
	0.006	173.23		LAI	0.56	0.31	3.16	SC	Na
	0.02	43.92		PDW	0.36	0.59	1.67	RL	SLW
						0.41	2.39		WU
					0.28	0.62	1.60	K	WU
						0.55	1.81		ALC
					0.21	0.66	1.49	N	ALC
					0.31	0.86	1.15	RY	LAI
					0.19	0.61	1.62	CDW	SLW
						0.71	1.40		LAI
					0.58	0.50	1.98	WUE	PDW
					0.24	0.55	1.81	Na	PDW
									WU

A high degree of multicollinearity can be explained by values greater than 10 for VIF (variance inflation factor) and values smaller than 0.1 for TV (tolerance value); DV, dependent variable; IV, independent variable; WSY, white sugar yield; RY, root yield; SY, sugar yield; SC, sugar content; WUE, water use efficiency; WU, water use; MC, molasses content; ALC, alkaline level content; N, -amino-N; Na, sodium; K, potassium; RL, root length; RD, root diameter; LAR, leaf area ratio; LWR, leaf weight ratio; SLW, specific leaf weight; LAI, leaf area index; TDW, total dry weight; CDW, crown dry weight; PDW, petiole dry weight; RDW, root dry weight; LDW, leaf dry weight.

Discussion

Although analysis of path coefficients has been around for some time, the reports indicating its use in sugar beet is very limited (Behl and Singh, 1977; Smith *et al.*, 1977; Er *et al.*, 2009). In the latest report a positive correlation (+0.630) was found between rainfall and monthly mean sugar content (Er *et al.*, 2009), emphasizing on the effect of negative effect of drought.

Because of current environmental changes, drought stress has become the major limitation factor on plant yield at global scale (Yordanov *et al.*, 2000). In sugar beet, water shortage greatly affects beet root quality (Pidgeon *et al.*, 2001; Bagatta *et al.*, 2004) and to a less degree affects root yield. Despite the drastic influence of drought on sugar beet shoot, the taproot yield mainly remains less affected since an effective osmotic adjustment mechanism is present (Clarke *et al.*, 1993; Abdollahian-Noghabi and Froud-Williams, 1998; Hsiao, 2000; Shaw *et al.*, 2002). However in an earlier report, a reduction of 16.1-51.6% in both root yield and sugar yield was noted upon water stress depending on the drought timing during the growth season (Choluj *et al.*, 2004). Meanwhile, water stress causes the accumulation of non-sugar compound impurities (glycine betaine, proline, potassium, sodium, α-amino-N) leading to a great reduction of taproot sugar yield (Harvey and Dutton, 1993).

An effective approach towards lessening the effects of drought on sugar beet is selection within cultivated and wild types for tolerant varieties and their use in future crossing programs. Taking these measures would not be successful, unless a comprehensive knowledge of interrelationship among traits would be available. Simple correlation studies might be informative in selection steps, but fails to produce a rather perfect picture about relative importance of each trait. Furthermore, it does not reveal how the other traits affect the target trait, usually yield; is it through direct effects? or considering that we are living at the era of systems biology, does it affect indirectly and via other biological networks or traits? To address these matters, a multivariate statistical analysis such as path analysis may unravel the interrelationships of these traits. In path analysis, the direct and indirect shares of each trait on yield are being revealed according to the relative magnitude of correlation coefficients (Board *et al.* 1997; Hobbs and Mahon, 1982). Our aim here was to develop selection indices for higher white sugar yield at normal and drought stress condition via both simple correlation analysis and sequential path analysis and further to demonstrate the advantage of this method over single-step path analysis.

Farmers usually withdraw irrigation some time before harvest to increase the sugar yield by taproot dehydration. Though, a balance needs to be established between dehydration and stressing sugar beet plants (Morrilo-Velarde and Ober, 2006). In this study a negative correlation was noticed between water used and both white sugar yield and root yield under normal condition (Table 1), emphasizing on better field management through managing the irrigation at late season.

In both conditions sugar yield had the highest effect on WSY. Consecutively, root yield and sodium content had the major positive and negative effects on SY respectively. This demonstrates that sodium content inhibits the extraction of sugar and lowers both SY and WSY. The remaining traits with minor effects on WSY were molasses content (negative effect) and sugar content (positive effect) in both conditions, except that root diameter appeared only in normal condition.

Higher percentage of coefficient (close to 1.00) between a predictor

Table 4. Comparison of multicollinearity in sequential path model and conventional path analysis by tolerance value and variance inflation factor at drought-stressed condition.

Single-step path analysis					Sequential path analysis				
R^2	TV	VIF	DV	IV	R^2	TV	VIF	DV	IV
0.98	0.003	332.55	WSY	RD	0.93	0.32	3.07	WSY	SC
	0.01	78.17		SY		0.36	3.78		SY
	0.002	501.03		MC		0.56	1.77		MC
	0.005	195.36		SC	057	0.61	1.63	SC	SLW
	0.02	38.03		RL		0.76	1.30		Na
	0.14	6.74		RY	0.63	0.30	3.33	SY	RY
	0.15	6.27		CDW		0.54	1.84		SLW
	0.15	6.37		WUE		0.56	1.78		Na
	0.28	3.51		Na	0.74	0.50	1.96	MC	K
	0.003	396.57		K		0.52	1.91		N
	0.007	139.63		N		0.38	1.62		RY
	0.01	54.02		RD		0.45	2.02		Na
	0.006	173.23		WU	0.36	0.48	2.05	K	LAR
	0.02	38.03		LAI		0.38	2.57		ALC
	0.003	310.85		PDW		0.82	1.24		WU
					0.28	0.45	1.20	N	RD
						0.37	2.67		ALC
					0.31	0.55	1.79	RY	WU
					0.41	0.81	1.22	NSLW	ALC
						0.51	1.94		WUE
					0.26	0.21	4.60	Na	WU

A high degree of multicollinearity can be explained by values greater than 10 for VIF (variance inflation factor) and values smaller than 0.1 for TV (tolerance value); DV, dependent variable; IV, independent variable; WSY, white sugar yield; RY, root yield; SY, sugar yield; SC, sugar content; WUE, water use efficiency; WU, water use; MC, molasses content; ALC, alkaline level content; N, α-amino-N; Na, sodium; K, potassium; RL, root length; RD, root diameter; LAR, leaf area ratio; LWR, leaf weight ratio; SLW, specific leaf weight; LAI, leaf area index; TDW, total dry weight; CDW, crown dry weight; PDW, petiole dry weight; RDW, root dry weight; LDW, leaf dry weight.

variable and the response variable may mask the real effects of other predictor variables. For instance, sugar content appeared only to have a major indirect role on WSY via SY ($r_{WSY, SY}$ = + 0.83) in normal condition. In this case, if the SY is being removed from the model it can be noted that SC will strongly predict the WSY (*data not shown*). When SC was considered as the dependent trait, sodium content had the major negative effect in both conditions and eventually on WSY. Once molasses content with negative effect on WSY was considered as dependent variable, potassium, sodium and α-amino-N, non-sugar impurities, had the positive effect on MC. Nonetheless, in all steps the negative effect of Na on WSY is clear either by lowering sugar yield and content or by improving the molasses content. Furthermore, K and N had a major contribution on lowering the WSY via increasing the molasses content.

An important observation was that the specific leaf weight appeared to be meaningful and effective on SY, SC, and WSY in drought-stress condition. This means that selection for sugar beets with leaves that withstand the stress and continue cell expansion and division would be beneficial in breeding programs under stress conditions.

In general, it could be concluded that under normal and drought-stressed conditions the traits that have to be brought under scrutiny during selection procedures are; selection for higher sugar yield and content, and lower non-sugar compounds. Moreover in drought-stressed condition, the plants that withstand better upon water shortage are the varieties with better potential to accumulate more sugars in their taproots. Furthermore we have noticed that similar to previous studies, sequential path analysis provides a more reliable analysis in contrast to single-step path analysis.

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