CORRELATION AND SEQUENTIAL PATH ANALYSIS BETWEEN YIELD AND RELATED CHARACTERS OF WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES IN NON-STRESSED AND SALINITY-STRESSED CONDITIONS

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ABSTRACT

Study of interrelationships between grain yield and its contributing components can improve the efficiency of breeding programs through determining of appropriate selection criteria. The present research uses sequential path analysis to determine the interrelationships between grain yield and 16 related traits, by ordering the various variables in first, second and third order paths on the basis of their maximum directs model in discerning the actual effects of different predictor variables. Forty one genotypes of bread wheat were effects and minimal collinearity. The sequential path model has distinct advantages over the conventional path grown in two environments (non-stressed and salinity-stressed conditions) based on randomized complete block design with three replications. Electrical conductivity values of irrigation water were 2 and 10 dS.m^{-1} in non-stressed and salinity-stressed conditions, respectively. Correlation coefficient analysis showed that grain yield was positively correlated with all the traits except flag leaf length, chlorophyll content and days to heading in non-stressed environment. Grain yield was significantly correlated with all the traits except number of grain per spike, number of fertile tiller, number of spikelet per spike, inter-node weight and spike length in the salinity-stressed environment. Sequential path analysis in this research indicated that BY and HI traits as the first order variables in both environments and PH, NFT, NSP, SL, DH, CHL, SPS traits as second order variables can be used as selection criterion for improving of grain yield in wheat breeding programs.

Key words: correlation, collinearity, sequential path analysis.

Abbreviations: CPA (conventional path analysis), BY (biological yield), CHL (chlorophyll content), DH (days to heading), DM (days to maturity), FL (flag leaf length), GY (grain yield), HI (harvest index), NFT (number of fertile tiller), NSP (number of grain per spike), NSPS (number of spikelet per spike), PH (plant height), PL (peduncle length), IW (inter-node weight), SL (spike length), SPA (sequential path analysis), SPS (seed weight per spike), SW (spike weight), TSW (the 100-grain weight), VIF (variance inflation factor).

INTRODUCTION

S oil salinity is one of the most important factors that limit crop production in arid and semi-arid regions (Houshmand et al., 2005). Over 800 million hectares of worldwide land are affected salinity (Munns, 2005). Wheat is a moderately salt-tolerant crop (Mass and Hoffman, 1977) and is as the important grain and a stable food for more than one third of the word population, including Iran. On other hand, Iran is one of the countries that suffer from severe salinity problems. For example 44 million hectares of total land area in Iran has become salt affected to varying degrees. Wheat is the most

important and widely adapted food cereal in Iran. Therefore, it is necessary to increase wheat production Iran by raising the wheat grain yield. Obviously, the most efficient way to increase wheat yield is to improve the salt tolerance of wheat genotypes because salinity management through reclamation or improved irrigation techniques is often prohibitively expensive and provides only short-term solutions to overcome salinity (Ashraf and Wu, 1994; Shannon, 1997). Salt tolerance of crops may vary with their growth stage. However, a difference in the salt tolerance between genotypes may also occur at different growth stages. Therefore, the wheat genotypes must be evaluated at different growth stages regarding

to salt tolerance. Most of the experiments are carried out under controlled condition. This is in spite of the fact that genotypic differences observed controlled conditions may not correspond to those observed at the adult stages in the field conditions (Houshmand et al., 2005) because in controlled conditions the plants are not exposed with the conditions such as spatial and temporal heterogeneity of soil chemical and physical properties that prevail in salt affected field conditions (Munns and James, 2003). Therefore, in order to evaluate the efficiently of screening methods for improving salt tolerance in crops, reassessment should be carried out under field conditions and at different growth stages (Richards et al., 1987). Improving the salt tolerance of crop and species requires access to new genetic diversity and different techniques for identifying salt-tolerance (Munns and James, 2003). A degree of tolerance to salinity stress has been found in the Iranian landraces and attempts have been made to use landraces as parents in breeding programs intended to produce tolerant cultivars of bread wheat. Screening is an essential part of the breeding programs (Houshmand et al., 2005). The lack of reliable large-scale field screening techniques seems to be the biggest problem in genetic improvement of salt and drought tolerance of crop plants (Munns and James, 2003). Despite wide recognition of the key traits in the salt tolerant genotypes breeding, major gaps exist in knowledge needed to develop salty tolerance genotypes (Dehghani et al., 2012a).

Grain yield is a quantitative trait and highly affected by environmental factors and hence has a low heritability, so choose based on lower yield in order to improve it may not be very effective hence (Poormohammad-Kiani et al., 2009). Morphological traits and related characters with grain yield due to easy measurement and usually have a high heritability can be used as criteria for distinguish salinity tolerant plant and for improvement of grain yield in wheat genotypes especially under salt stress conditions. Plant breeders, commonly prefer yield components that indirectly increase grain yield (Yasin and sing, 2010). Therefore, there is the need to examine the relationships between various traits, especially between grain yield and other traits (Dehghani et al., 2012b).

Different statistical techniques have been used in modelling crops yield, including correlation, regression, path analysis, factor analysis, principal analysis and cluster analysis (Leilah and Al-Khateeb, 2005). Correlation coefficient is an important statistical procedure to evaluate breeding programs for indirect selection of genotypes for higher grain yield (Mohamed, 1999). Simple correlation analysis may not provide a complete understanding about the importance of each component in determining grain yield (Dewey and Lu, 1959). Partitioning the correlation coefficient into direct and indirect effects can be done through path analysis technique, so the contribution of each trait, to grain yield, can be estimated (Dewey and Lu, 1959). Path coefficient analysis have been widely used in plant breeding programs to determine the nature of the relationships yield and yield components that are useful as selection criteria to improve crop yield (Moghaddam et al., 1998). In most studies involving path analysis, researchers considered the predictor characters as first-order variables to analysis their effects over a dependent or response variable such as yield (Bhagowati and Saikia, 2003; Tuncturk and Çiftçi, 2005). This conventional approach might result in multicollinearity for variables, especially when correlations among some of the traits are high (Hair et al., 1995).

Also, there may be difficulties in interpretation of the actual contribution of each variable, as the effects are mixed or confounded because of collinearity (Pb Samonte et al., 1998). Recently the sequential path coefficient analysis has been used by many researchers for determining relationships between yield and related traits in different crops by organizing and analyzing various predictor variables in first, second and third order paths (Pb Samonte et al., 1998; Mohammadi et al., 2003; Asghari-Zakaria et al., 2007; Feyzian et al., 2009; Sabaghnia et al., 2010). The sequential path model has distinct advantages over the conventional path model in discerning actual effects of different predictor variables and can provided a better fit for various datasets (Mohammadi et al., 2003).

39

AMIR GHOLIZADEH AND HAMID DEHGHANI: CORRELATION AND SEQUENTIAL PATH ANALYSIS
BETWEEN YIELD AND RELATED CHARACTERS OF WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES
IN NON-STRESSED AND SALINITY-STRESSED CONDITIONS

The main objectives of this research were to analyze the correlation between grain yield and related traits in bread wheat by applying sequential path analysis and identifying traits, of genotypes, which may be useful in breeding higher-yielding genotypes in non-stressed and salinity-stressed conditions.

MATERIAL AND METHODS

The experiments were carried out during 2011-2012 growing seasons in two non-stress and stress location at the research field of the National Salinity Research Center (NSRC) at Yazd, Iran (31°86′N, 53°95′E, 1609 mail). Before starting the experiments was conducted sampling in the different layers of soli (0-30, 30-60, 60-90 cm depth) in both conditions. Relevant soil characteristics of the experimental sites are given in Table 1. Forty one genotypes of bread wheat (*Triticum aestivum* L.) were used in this study. We used almost all the commercial wheat genotypes cultivated in different parts of Iran. All wheat genotypes were selected from the germplasm collection at the Iranian Institute of Grain and

Plant Improvement Institute (SPII), Karaj, Iran. The genotypes cultivated in two non-stressed and salinity-stressed conditions with three replications in from of randomized complete block design.

The salinity of water used in irrigation in saline and non-saline conditions was 10 and 2 $dS.m^{-1}$ respectively. Each plot consisted two rows 20 cm apart and 2 m in length. Nitrogen, P and K fertilizers were applied based on the soil test and the Iranian Soil and Water Institute (ISWI) recommendation model (Milani et al., 1998). A total of 150 kg N ha^{-1} each time at sowing, top-dressed at tillering stage and at the start of heading. Other fertilizers were applied before plowing at the recommended rates of 115 kg P_2O_5 ha^{-1} and 80 kg K_2SO_4 ha^{-1}, for the purpose of determining the salinity of soil during growth season, sampling have been done from the depth of 0-30, 30-60, 60-90. The average of salinity rate during growth season in saline and non-saline condition was 9.5 and 2.7 $dS.m^{-1}$ respectively. Weeds were controlled by hand as needed.

Table 1. Soil properties of the two experimental sites

Location	Depth (cm)	pH	EC ($dS.m^{-1}$)	meq/L				P ($mg.kg^{-1}$)	K ($mg.kg^{-1}$)
				K^+	Na^+	Mg^+	Ca^+		
Normal	0-30	7.8	2.9	0.6	37.5	11	10	30.2	114
	30-60	7.5	2.3	0.7	30.8	7.5	9.6	17.45	121
	60-90	7.5	1.8	0.2	26.4	3.3	6.7	3.1	84
Stress	0-30	7.6	9.80	1.7	175.8	52.6	52.3	25.9	209
	30-60	7.6	7.89	0.9	149.8	41.2	38.2	5.00	177
	60-90	7.3	9.58	0.9	219.7	61.7	48.3	3.7	201

Data were collected on the following 17 characters in all replications on five randomly selected plants from each plot. The traits were plant height (PH), spike weight (SW), seed weight per spike (SPS), number of grain per spike (NSP), number of spikelet per spike (NSPS), inter-node weight (IW), spike length (SL), peduncle length (PL), days to heading (DH), days to maturity (DM), number of fertile tiller (NFT), flag leaf length (FL), grain yield (GY), biological yield (BY) were recorded. The harvest index (HI) was calculated as the ratio between grain yield and biological yield. The 100-grain weight (TSW)

was measured on a sub-sample of grain harvested from each plot. The chlorophyll content (CHL) of base, middle and tip (Munns and James, 2003) of the third leaves was measured using a SPAD-502 chlorophyll meter (Minolta, Japan) which provides rapid and non-destructive measurements of leaf chlorophyll content.

The datasets were first tested for normality by the Anderson and Darling normality test using MINITAB version 14 statistical software. Data from each trait were subjected to analysis of variance (ANOVA) using SAS version 9.1 statistical software. Phenotypic correlation

coefficients between various pairs of characters were calculated using the Pearson correlation coefficient. A primary analysis was carried out by means of conventional path model in which all traits were considered as first-order predictor variables with grain yield (GY) as response variable.

Sequential stepwise multiple regression was performed to organize the predictor variables into first, second and third order paths on the basis of their respective contributions to total variation in grain yield and minimal collinearity using SPSS version 19 statistical software. The level of multicollinearity in each component path was measured from two common measures, namely, the "tolerance" value and the "Variance Inflation Factor" (VIF) as suggested by Hair et al. (1995). The tolerance value is the amount of variability of the selected independent variable not explained by other variables (1-R^2), where R^2 is the coefficient of determination for the prediction variable by the predictor variables. The variance inflation factor (VIF) indicates the extent of effects of other independent variables on the variance of the selected independent variable [VIF = 1/(1/R2)]. Therefore, very small tolerance values (much below 0.1) or high variance factor inflation (above 10) indicate high collinearity (Hair et al., 1995; Mohammadi et al., 2003). The partial coefficients of determination (analogous to R^2 of linear regression) were computed from the path coefficients for all predictor variables. To estimate the standard error of path coefficient, bootstrap analysis (Efron and Tibshirani, 1993) was performed using Amos version 19 (SPSS Inc.) statistical software. This was followed by the standard t test to verify the significance of path coefficients.

RESULTS AND DISCUSSION

The analysis of variance results indicated highly significant differences in bread wheat genotypes for all traits under study (data not shown). To determine in the most accurate procedure the relationship of direct and indirect grain yield components simple correlations were established and path coefficient analysis performed.

Simple correlation coefficients in the non-stressed environment (Table 2) showed that there were high positive correlations between grain yield and more of the measured traits except for FL, CHL and DH. As shown in Table 2, more traits were positively, and significantly, correlated with BY, except for FL, TSW, SPS, CHL, DH and HI.

Table 2. Pairwise correlation coefficients between 17 traits of 41 bread wheat genotypes
measured in a non-stressed environment

Trait[a]	NSP	NFT	SNPS	FL	TSW	SPS	IW	SW	PH	CHL	DH	DM	SL	PL	HI	GY
NFT	0.05b															
SNPS	0.65	0.16														
FL	-0.04	0.04	-0.21													
TSW	-0.23	0.44	-0.21	0.19												
SPS	0.71	0.27	0.57	-0.06	0.39											
IW	0.21	0.36	0.32	0.02	0.40	0.34										
SW	0.75	0.25	0.60	-0.02	0.33	0.07	0.54									
PH	0.08	0.59	0.30	0.12	0.38	0.17	0.55	0.33								
CHL	0.19	0.08	0.38	-0.26	-0.21	0.39	0.32	0.11	-0.06							
DH	0.14	-0.11	0.57	-0.35	-0.25	0.33	0.17	0.14	0.02	0.41						
DM	0.16	0.28	0.50	-0.26	0.18	0.17	0.53	0.37	0.33	0.38	0.70					
SL	0.29	0.32	0.45	0.21	-0.00	0.42	0.35	0.38	0.52	0.04	0.16	0.25				
PL	0.05	0.38	-0.05	0.29	0.37	0.46	0.54	0.20	0.75	-0.19	-0.36	0.05	0.31			
HI	0.33	0.10	-0.04	-0.08	0.27	0.38	0.03	0.37	-0.09	-0.15	-0.19	0.09	-0.25	0.13		
GY	0.31	0.53	0.30	0.13	0.30	0.34	0.40	0.44	0.54	0.03	0.05	0.43	0.30	0.48	0.39	
BY	0.30	0.62	0.37	0.12	0.23	0.07	0.48	0.40	0.63	0.15	0.05	0.39	0.34	0.52	0.13	0.91

[a] For trait abbreviations refer to text. [b] Critical values of correlation p< 0.05 and p< 0.01 (df 39) are 0.30 and 0.39, respectively.

41

AMIR GHOLIZADEH AND HAMID DEHGHANI: CORRELATION AND SEQUENTIAL PATH ANALYSIS
BETWEEN YIELD AND RELATED CHARACTERS OF WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES
IN NON-STRESSED AND SALINITY-STRESSED CONDITIONS

There was a statistically significant and positive correlation between HI and other bread wheat characters for NSP, SPS, SW, and GY (Table 2). The plant height (PH) had significant positive correlations with more other measured traits except for NSP, FL, SPS, HI, DH and CHL (Table 2).

In the salinity-stressed environment, all measured traits except for NSP, NFT, SNPS, IW and SL showed significant correlation with grain yield (Table 3). But, grain yield had a significant positive correlation with CHL while this trait did not had a significant correlation in the non-stressed environment. Also, grain yield was significantly positively correlated in NSP, NFT, SNPS, IW and SL in non-stressed environment while these traits did not have a significant correlation in the salinity-stressed environment. The HI was significantly correlated with SPS, IW CHL, DH, DM, SL and grain yield in the salinity-stressed environment. The highest correlation was between BY and grain yield in non-stressed and salinity-stressed environment (r=0.91 and r=0.88, respectively).

To find the relative significance of the traits the data were subjected to conventional path analysis. Path coefficient analysis is a statistical technique of partitioning the correlation coefficients into its direct and indirect effects, so that the contribution of each character to yield could be estimated.

Table 3. Pairwise correlation coefficients between 17 traits of 41 bread wheat genotypes measured in a salinity-stressed environment

Trait	NSP	NFT	SNPS	FL	TSW	SPS	IW	SW	PH	CHL	DH	DM	SL	PL	HI	GY
NFT	0.22a															
SNPS	0.61	0.05														
FL	-0.28	0.22	-0.25													
TSW	-0.55	0.44	-0.35	0.62												
SPS	0.54	0.19	0.33	0.25	0.28											
IW	0.29	0.22	0.31	0.04	-0.00	0.26										
SW	0.59	0.11	0.35	0.21	0.12	0.86	0.12									
PH	-0.20	0.44	0.05	0.29	0.38	0.26	0.30	0.15								
CHL	0.33	0.21	0.40	-0.13	-0.28	0.22	0.45	0.24	0.17							
DH	0.16	-0.02	0.46	-0.39	-0.35	0.03	0.30	0.05	0.01	0.60						
DM	0.08	0.28	0.40	-0.12	-0.10	0.10	0.46	0.01	0.35	0.40	0.79					
SL	-0.13	0.30	-0.01	0.12	0.03	0.00	0.39	0.07	0.62	0.25	0.18	0.40				
PL	-0.19	0.34	-0.17	0.44	0.39	0.27	0.16	0.19	0.82	0.31	-0.30	0.02	0.32			
HI	0.12	-0.15	-0.24	0.22	0.21	0.31	-0.37	0.21	-0.16	-0.40	-0.65	-0.45	-0.47	0.16		
GY	-0.02	0.27	-0.08	0.39	0.31	0.38	0.04	0.32	0.61	0.39	-0.31	0.30	0.24	0.59	0.31	
BY	-0.03	0.40	0.06	0.31	0.21	0.26	0.20	0.26	0.66	0.40	0.13	0.42	0.38	0.54	-0.14	0.88

[a] Critical values of correlation p< 0.05 and p< 0.01 (df 39) are 0.30 and 0.39, respectively.

Estimation of direct effects by conventional path analysis, where the yield-related traits were considered as the first-order variables with grain yield as the response variable, and analysis of multicollinearity (Tolerance and Variance Inflation Factor) conventional path analysis are given in Table 4. According to the conventional path analysis and multicollinearity analysis, there are conflicting relations among the variables. Results from this analysis in the non-stressed environment, where all traits were considered as the first-order variables (Model I) with grain yield as the response variable, indicated high multicollinearity for some traits such as number of grain per spike (NSP) (VIF=11.8), seed weight per spike (SPS) (VIF= 60.9) and spike weight (SW) (VI=52.7). These traits were removed as first-order variables from the analysis in the non-stressed environment.

In the salinity-stressed environment (Table 4), the traits number of grain per spike (NSP) (VIF=13.6), 100-grain weight (TSW) (VIF=11.4), seed weight per spike (SPS)

(VIF=10.8), plant height (PH) (VIF=20.8), chlorophyll content (CHL) (VIF=20.9) and peduncle length (PL) (VIF=13.5) showed multicollinearity when all traits were considered as first-order variables with grain yield as the response variable. So these traits were removed as first-order variables from the

analysis in the salinity-stressed environment. This method for the evaluation of different traits correlations and path analysis has been studied by Pb Samonte et al. (1998) in rice, Mohammadi et al. (2003) in maize, Asghari-Zakaria et al. (2007) in potato, and Sabaghnia et al. (2010) in canola (*Brassica napus* L.).

Table 4. Direct effects of first-order predictor variables on the grain yield of 41 bread wheat genotypes in a non-stressed and salinity stressed environments and two common measures of collinearity in conventional path analysis

Trait	Non-stressed environment			Salinity stressed environment		
	Direct effect	Tolerance	VIF[a]	Direct effect	Tolerance	VIF
NSP	-0.110	0.085	11.831	0.031	0.073	13.615
NFT	-0.135	0.334	2.992	-0.065	0.500	1.999
SNPS	-0.087	0.203	4.923	-0.129	0.292	3.428
FL	0.075	0.594	1.682	0.035	0.342	2.924
TSW	-0.093	0.114	8.759	-0.027	0.088	11.407
SPS	0.765	0.016	60.936	0.305	0.092	10.839
IW	-0.001	0.226	4.421	-0.056	0.283	3.530
SW	-0.649	0.019	52.715	-0.193	0.134	7.453
PH	-0.018	0.118	8.507	0.188	0.048	20.872
CHL	-0.201	0.483	2.068	-0.030	0.048	20.945
DH	-0.017	0.146	6.867	0.064	0.154	6.474
DM	0.111	0.189	5.294	0.153	0.163	6.133
SL	0.096	0.404	2.472	-0.024	0.213	4.701
PL	0.003	0.117	8.546	-0.096	0.074	13.509
HI	0.271	0.297	3.372	0.181	0.253	3.949
BY	0.934	0.347	2.881	0.912	0.380	2.628

[a] VIF: variance inflation factor.

In contrast to the above results, sequential path provided a better understanding of the interrelationships between the various traits and their relative contribution to grain yield. Estimation of direct effects by sequential path analysis (Table 5), were considered where yield-related traits, as grouped into first, second, and third-order variables, with grain yield (Model II). The results of tolerance and VIF values for predictor variables in non-stressed and salinity-stressed environments indicated a considerable decrease of VIF values in Model II in contrast with Model I.

Sequential stepwise multiple regressions was performed to organize the predictor variables into first, second, third-order paths on the basis of their relative contributions to the total variation of grain yield and minimal collinearity measures (tolerance and VIF) of all variables. So, very small tolerance values (much lower than 0.1) or high VIF values

(>10) indicate high collinearity (Hair et al., 1995; Mohammadi et al., 2003). The benefit of sequential path method compared with conventional path analysis in minimizing collinearity problems and identifying factual contributions of each component in different path components. This method was used in other crop (rice: Pb Samonte et al., 1998; maize: Mohammadi et al., 2003; potato: Asghari-Zakaria et al., 2007; and canola (*Brassica napus* L.): Sabaghnia et al., 2010) that show it is very effective in achieving the favourable results.

Resampling techniques, such as the bootstrab, provide estimates of the standard error, confidence interval, and the distribution of any statistic. To use these procedures, the mean direct effects estimated from a set of 200 bootstrap samples were in close agreement with the observed direct effects of various traits (Table 5). The low standard

43

AMIR GHOLIZADEH AND HAMID DEHGHANI: CORRELATION AND SEQUENTIAL PATH ANALYSIS
BETWEEN YIELD AND RELATED CHARACTERS OF WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES
IN NON-STRESSED AND SALINITY-STRESSED CONDITIONS

error for all of the direct effects and low bias also indicated the robustness of the sequential path model. The direct and indirect effects for response variables are shown in Table 5 for the non-stressed and salinity-stressed conditions. The T-test of significance, using standard error values, obtained through bootstrap resampling, indicated that all the direct effects were significant (data not shown). The direct and indirect effects in the non-stressed and salinity-stressed environment for the predictor variables in sequential path analysis (grouped into first, second and third order variables) are given in Tables 6 and 7.

Table 5. Measures of collinearity values (tolerance and variance inflation factor, VIF) for predictor variables in conventional path analysis (CPA, all predictor variables as first-order variables) and sequential path analysis (SPA, predictors grouped into first-, second-, and third-order variables) and Estimation of standard error values of path coefficients using bootstrap analysis

Predictor variable	Response variable	Tolerance		VIF		Adj. R^2	Direct effect	Bootstrap		
		CPA	SPA	CPA	SPA			Bias	Mean	SE
Non-stressed environment										
BY	GY	0.34	0.98	2.88	1.01	0.90	0.86	0.00	0.33	0.01
HI		0.29	0.98	3.37	1.01		0.28	0.00	0.02	0.00
PH	BY	0.11	0.63	0.11	1.57	0.53	0.36	0.00	0.02	0.00
NFT		0.33	0.63	2.99	1.56		0.42	0.01	0.25	0.06
NSP		0.08	0.97	11.83	1.02		0.24	0.00	0.02	0.00
SPS	HI	0.01	0.88	60.93	1.12	0.33	0.55	0.00	0.02	0.00
SL		0.40	0.88	2.47	1.12		-0.46	0.00	-0.02	0.00
PL	PH	0.11	0.85	8.54	1.17	0.72	0.70	-0.01	1.20	0.14
SNPS		0.20	0.44	4.92	2.27		0.56	0.20	1.62	0.70
TSW		0.11	0.57	8.75	1.75		0.31	0.36	1.72	0.14
SW		0.01	0.40	52.71	2.46		-0.26	-0.25	-1.52	0.90
TSW	NFT	0.11	1.00	8.75	1.00	0.17	0.44	0.03	1.87	0.67
SW	NSP	0.01	0.82	52.71	1.20	0.85	0.99	-0.02	1.59	0.41
TSW		0.11	0.79	8.75	1.25		-0.59	0.03	-0.63	0.93
DH		0.14	0.87	6.86	1.14		-0.15	-0.00	-0.21	0.09
SW	SPS	0.01	0.67	52.71	1.48	0.97	0.98	-0.00	0.73	0.02
TSW		0.11	0.70	8.75	1.41		0.13	-0.01	0.32	0.08
DH		0.14	0.61	6.86	1.62		0.08	0.00	0.01	0.00
IW		0.22	0.83	4.42	1.20		-0.11	-0.00	-0.57	0.19
PL	SL	0.11	0.99	8.54	1.00	0.27	0.33	-0.00	0.06	0.03
SNPS		0.20	0.99	4.92	1.00		0.46	0.01	0.26	0.06
Salinity-stressed environment										
BY	GY	0.38	0.97	2.62	1.02	0.85	0.90	0.00	0.34	0.01
HI		0.25	0.97	3.94	1.02		0.41	0.00	0.07	0.02
PH	BY	0.04	1.00	20.87	1.00	0.43	0.66	0.01	0.20	0.14
DH	HI	0.15	0.96	6.47	1.03	0.50	-0.47	0.00	-0.00	0.00
CHL		0.21	0.96	4.70	1.03		-0.37	0.00	-0.02	0.00
SPS		0.09	0.99	10.83	1.00		0.28	0.00	0.02	0.00
PL	PH	0.07	0.99	13.50	1.00	0.78	0.81	-0.03	1.49	0.15
DM		0.16	0.99	6.13	1.00		0.32	0.00	0.61	0.12
PL	DH	0.07	0.79	13.50	1.25	0.74	-0.23	-0.00	-0.33	0.15
DM		0.16	0.97	6.13	1.02		0.76	-0.01	1.01	0.14
FL		0.34	0.78	2.92	1.27		-0.18	0.01	-0.82	0.44
PL	CHL	0.07	0.99	13.50	1.00	0.22	0.31	0.00	0.06	0.03
DM		0.16	0.99	6.13	1.00		0.39	0.00	0.08	0.03
DM	SPS	0.16	0.98	6.13	1.01	0.86	0.12	-0.00	0.01	0.01
SW		0.13	0.35	7.45	2.86		0.46	0.01	0.36	0.07
TSW		0.08	0.33	11.40	2.65		0.53	-0.00	0.98	0.19
NSP		0.07	0.24	13.61	4.05		0.53	-0.00	0.06	0.01

The diagram of sequential path analysis for the non-stressed and salinity-stressed environments (Figures 1 and 2) are given a better understanding of the interrelationships between the various variables and their relative contribution to grain yield. In both environments, BY and HI as first-order variables were positively associated with grain yield and have positive direct effects on grain yield in both the non-stressed and salinity-stressed environments (Figures 1 and 2). The BY and HI as first-order variables, accounted for 90 and 85% (Table 5) of the variation in grain yield in the non-stressed and salinity-

stressed environments, respectively. In both the non-stressed and salinity-stressed environments between the BY and HI traits, the BY had the greater direct effect than HI on grain yield. The Indirect effects to the BY and HI in both the non-stressed and salinity-stressed environments were low but the indirect effect on the HI was relatively high via BY. These two traits have direct effects of a positive on grain yield and recommended that be used as selection criterion in selecting for increased for grain yield in bread wheat in both non-stressed and salinity-stressed conditions.
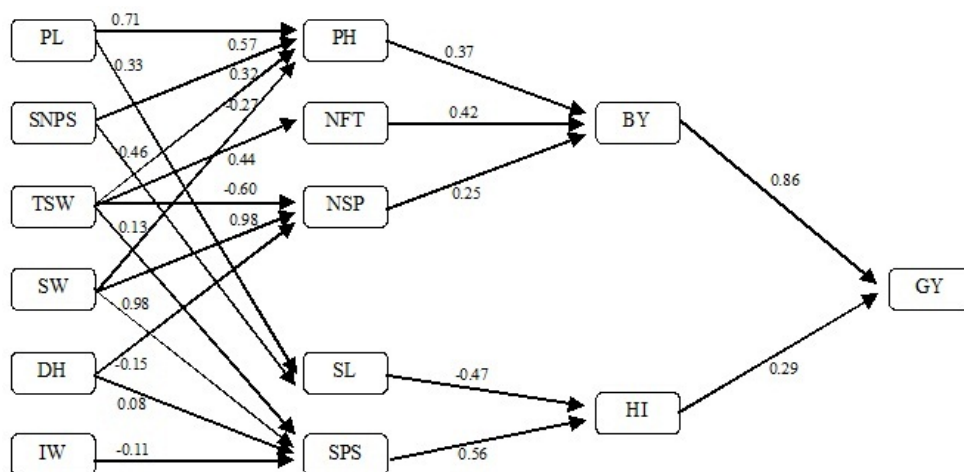

Figure 1. Sequential path analysis diagram illustrating the interrelationships between various traits contributing to grain yield in a non-stressed condition. For trait abbreviations refer to text
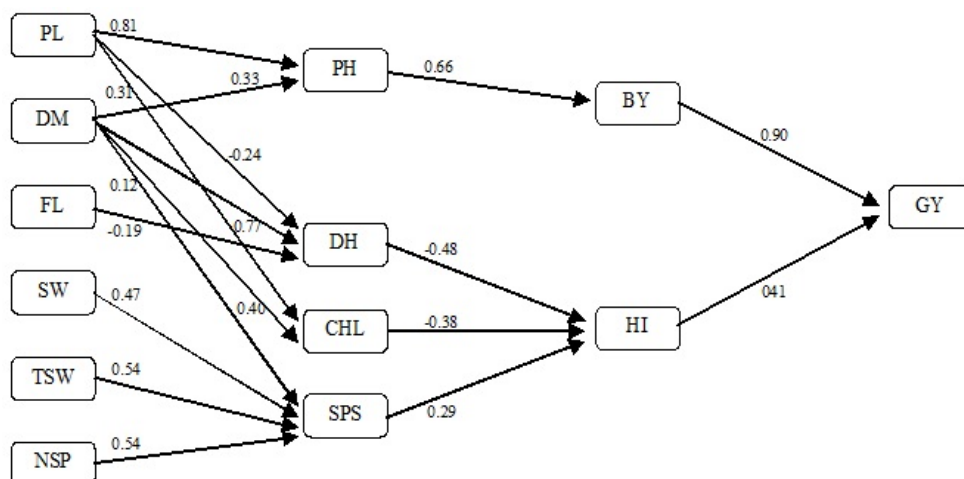

Figure 2. Sequential path analysis diagram illustrating the interrelationships between various traits contributing to grain yield in a salinity-stressed condition

The results of sequential path analysis, when the second-order variables were used as predictor, and the first-order variables as response variables, indicated that PH, NFT and NSP positively influenced on BY (Table 5) and accounted for 53% of the observed

variation in the non-stressed environment (Figure 1). The SPS positively and the SL negatively influenced on HI and accounted for 33% of the observed variation of HI in the non-stressed environment (Table 5).When the third-order variables were used as predictors

45

AMIR GHOLIZADEH AND HAMID DEHGHANI: CORRELATION AND SEQUENTIAL PATH ANALYSIS
BETWEEN YIELD AND RELATED CHARACTERS OF WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES
IN NON-STRESSED AND SALINITY-STRESSED CONDITIONS

and second-order variables as response variables the results indicated PL, SNPS and TSW positively and the SW negatively influenced on PH and accounted for 72% of the observed variation of PH. The TSW positively influenced on NFT and accounted for 17% of the observed variation of NFT while the TSW and DH negatively and the SW positively influenced of NSP and accounted for about 85% of the observed variation of NSP in the non-stressed environment (Table 5). Also PL and SNPS positively influenced on SL and accounted for about 27% of the observed variation while The IW negatively and SW, TSW and DH positively influenced on SPS and accounted for about 97% of the observed variation of SPS in the non-stressed environment (Table 5).

Results of sequential path analysis in the salinity environment, when second-order variables were used as predictor variables and first-order variables as response variables, showed that PH positively influenced on BY (Table 5) and accounted for about 43% of the observed variation of BY (Figure 2). The PH trait had a positive direct effect of relatively high (0.66) on biological yield (BY) and recommended it to be used as selection criteria in selecting for increased for biological yield and finally grain yield in bread wheat genotypes in the salinity-stressed conditions. The DH and CHL negatively and SPS positively influenced on HI and accounted for 50% of the observed variation of HI. Results of sequential path analysis when the third-order variables were used as predictor variables, and second-order variables as response variables, showed that PL and DM positively influenced on PH and accounted for about 78% of the observed variation of PH in the salinity-stressed environment (Figure 2). Also, PL and FL negatively and DM positively influenced on DH and accounted for 74% of the observed variation of DH. The PL and DM positively influenced on CHL and accounted for 22% of the observed variation of CHL. Also DM, SW, TSW and NSP positively influenced on

SPS and accounted for about 86% of the observed variation of SPS in the salinity-stressed environment (Table 5). Yield component studies, in both environments, showed that BY and HI traits were positively associated with grain yield and have direct effects of a positive on grain yield in both the non-stressed and salinity-stressed environments.

The results of simple correlation analysis indicated that in both the non-stressed and salinity-stressed conditions, the highest correlation for grain yield was between BY and grain yield (r=0.91 and r=0.88 respectively in non-stressed and salinity-stressed conditions). High correlation between grain yield with BY shows that with increasing BY also was been increased grain yield. As regards the grain is the result of photosynthetic activity of organs such as stems and leaves, so not unexpected strong and high correlation between the two traits. This shows that for high yield in both the non-stressed and salinity-stressed conditions is required to plants with good growth and vigor. Also, in both the non-stressed and salinity-stressed conditions between HI and grain yield was a significant positive correlation. Harvest index is the ratio of grain yield to the biological yield. So with increasing the grain yield or both (grain yield and biological yield) can be increased HI.

The positive and significant correlation in the non-stressed and salinity-stressed conditions was observed between grain yield and days to maturity (DM), those observations leading that in breeding programs, the appropriate estimation of days to maturity and maturity period of the studied plant could help to select the choice quickly. Also, between days to maturity (DM) and biological yield was a significant positive correlation, which shows with increasing of the maturity period too increased total plant biomass.

The result of this study showed a significant negative correlation between grain yield and DH in the salinity-stressed environment, because with increasing vegetative growth is reduced the growth of economic organs and grain yield.

In the both non-stressed and salinity-stressed was a high, positive, significant, correlation between PH and BY. Also, correlation analysis show that plant height (PH) was correlated positively with SL, PL, IW, TSW and days to maturity (DM). The result of this study showed that with increasing the days to maturity (DM) and vegetative growth, other components such as PH, PL and finally will increase the biological yield.

Results revealed that grain yield was positively correlated with PH, TSW, SPS, HI and biological yield in both conditions. Our observations were consistent with Kumbhar et al. (1983), Mohamed (1999) and Leilah and Al-Khateeb (2005) investigations on wheat plant yield components. However, Moghaddam et al. (1998) showed a negative correlation between plant height and grain yield. They attributed that to the lower number of grain per spike with the tallest wheat plants. Also, the results of this study showed a significant positive correlation between grain yield and CHL in salinity-stressed environment. Salinity stress significantly reduces the total chlorophyll content and the rate of reduction in total chlorophyll depending on salt tolerance of plant species and salt concentrations. In salt-tolerant species, chlorophyll content increased, while in salt-sensitive species it was decreased (Ashraf and McNeilly, 1988). Plant physiologists have found chlorophyll content to be a valuable tool to monitor plant stress response. The chlorophyll content meter is useful for improving nitrogen and fertilizer management and is ideal for crop stress, leaf senescence, plant breeding, health determination and other studies. Determination of the relationships of the chlorophyll content, yield and yield components facilitates selection of high yielding varieties from breeding programs. The results of correlation coefficient analysis revealed that leaf chlorophyll content was positively and significantly correlated with the grain yield under salinity conditions. In contrast Araus et al. (1998), Boggs et al. (2003) and Bronson et al. (2003) also were found a positive correlation between leaf chlorophyll content and yield for wheat and other crops. This information indicated that increase in leaf chlorophyll content increases grain yield. Therefore, further investigation is required on the leaf chlorophyll content and physiological studies. In the present study, correlation analysis revealed that leaf chlorophyll content can be advised one of the indicators in the improvement of grain yield in bread wheat under salinity-stressed conditions.

Table 6. Direct and indirect effects in a non-stressed condition for predictor in sequential path analysis
(grouped into first, second and third order variables)

GY	BY	HI		PH	PL	SNPS	TSW	SW
BY	0.86	0.03		PL	0.70	-0.02	0.11	-0.05
HI	0.11	0.28		SNPS	-0.03	0.56	-0.06	-0.15
HI				TSW	0.25	-0.11	0.31	-0.08
	SL	SPS		SW	0.14	0.33	0.10	-0.26
SL	-0.46	0.23		SPS				
SPS	-0.19	0.55			TSW	SW	DH	IW
SL				TSW	0.13	0.32	-0.02	-0.04
	PL	SNPS		SW	0.04	0.98	0.01	-0.06
PL	0.33	-0.02		DH	-0.03	0.13	0.08	-0.01
SNPS	-0.01	0.46		IW	0.05	0.53	0.01	-0.11
BY				NSP				
	PH	NFT	NSP		TSW	SW	DH	
PH	0.36	0.24	0.01		TSW	-0.57	0.32	0.03
NFT	0.21	0.42	-0.01		SW	-0.19	0.99	-0.02
NSP	0.02	0.02	0.24		DH	0.14	0.13	-0.15

47

AMIR GHOLIZADEH AND HAMID DEHGHANI: CORRELATION AND SEQUENTIAL PATH ANALYSIS
BETWEEN YIELD AND RELATED CHARACTERS OF WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES
IN NON-STRESSED AND SALINITY-STRESSED CONDITIONS

The results of sequential path analysis in both the non-stressed and salinity-stressed environments showed that biological yield (BY) and harvest index (HI) have positive direct effects on grain yield. These two traits have direct effects of a positive on grain yield and recommended them to be used as selection criterion in selecting for grain yield in bread wheat in both non-stressed and salinity-stressed conditions (Table 6 and 7). Also, these characters are a yield component and are easier to determine than yield and generally have a high heritability. Donald et al. (1976), Mohammed. (1999) and Leiah and Al-Khateeb (2005) reported a high, positive, significant, correlation between BY and HI with grain yield. This study indicated the advantage of sequential path analysis over conventional path analysis in understanding the direct and indirect effects of different yield-related traits. The traits PL, TSW, SW, DH, PH, NSP, SPS, BY and HI were identified as the first, second and third order variables in both the non-stressed and salinity-stressed environments (Figures 1 and 2). Sequential path model in both the non-stressed and salinity-stressed conditions not only demonstrated that other traits, namely, plant height (PH), number of fertile tiller (NFT), number of grain per spike (NSP), spike length (SL), days to heading (DH), chlorophyll content (CHL) and seed weight per spike (SPS), exercise their influence as second-order variables but also provided a better understanding of their relative contributions to the first-order variables (Figures 1 and 2). However, the sequential path model clearly showed that the second-order traits (PH, NFT, NSP, SL, DH, CHL and SPS) have significant effects on grain yield through biological yield and harvest index (Figures 1 and 2). Therefore, these traits (second-order traits) can be used as selection criteria for increasing of biological yield (BY), harvest index (HI) and finally grain yield in wheat breeding programs.

Table 7. Direct and indirect effects in a salinity-stressed condition for predictor variables
in sequential path analysis (grouped into first, second and third order variables)

GY	BY	HI		HI	DH	CHL	SPS
BY	0.90	-0.04		DH	-0.47	-0.22	0.00
HI	-0.11	0.41		CHL	-0.28	-0.37	0.26
PH				SPS	0.09	-0.08	0.28
	PL	DM		DH			
PL	0.81	0.00			PL	DM	FL
DM	0.01	0.32		PL	-0.23	0.01	-0.08
CHL				DM	-0.00	0.76	0.02
	PL	DM		FL	-0.10	-0.0	-0.18
PL	0.31	0.00					
DM	0.00	0.39					
SPS							
	DM	SW	TSW	NSP			
DM	0.12	-0.00	-0.05	0.04			
SW	-0.00	0.46	0.06	0.31			
TSW	-0.01	0.05	0.53	-0.29			
NSP	0.00	0.27	-0.29	0.53			

According to simple correlation and sequential path analysis, BY and HI traits were positively associated with grain yield and have direct effects of a positive on grain yield in both the non-stressed and salinity-stressed environments. Studies showed that the increasing in biological yield will be effective while produced carbon of during photosynthesis process collected into economic organs or grains (Reynold et al.,

ROMANIAN AGRICULTURAL RESEARCH

2009). In other words, the wheat varieties that have high biological yield and harvest index, are likely have high grain yield. Donmez et al. (2001) and Reynolds et al. (2009) reported that the yield improvement in recent years has been due to increasing of plant dry matter. Increasing in the allocation of photosynthesis products into economic organs have been among the most important aspects of development and selection of crops (Hay, 1995). The investigations showed that a wide variety for harvest index was observed among wheat cultivars. The differences in plant high have been suggested as the most important cause of variation in harvest index. Hay (1995) reported that the reduction of plant high using dwarfing genes (Rht) was the most important cause of increasing in harvest index. Also, the grain filling period has effects on harvest index. In this regard, Hay (1995) reported that the higher harvest index in England, with humid and temperate climate, as compared with Australia, was due to the grain filling period being longer. According to the relationship between harvest index (HI) and grain yield, it seems that harvest index and finally grain yield could still be improved in Iranian cultivars.

CONCLUSIONS

In summary, we tried to use sequential path analysis as a predictive tool for analysis of interrelationships between yield-related characters in bread wheat under both non-stressed and salinity-stressed conditions. Generally, sequential path analysis in this research indicated that BY and HI traits, as the first order variables in both environments, and PH, NFT, NSP, SL, DH, CHL, SPS traits as second order variables, can be used as selection criterion for improving of grain yield in wheat breeding programs.

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49

AMIR GHOLIZADEH AND HAMID DEHGHANI: CORRELATION AND SEQUENTIAL PATH ANALYSIS
BETWEEN YIELD AND RELATED CHARACTERS OF WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES
IN NON-STRESSED AND SALINITY-STRESSED CONDITIONS

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