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5USE OF CANONICAL CORRELATION ANALYSIS FOR DETERMINATION OF RELATIONSHIPS AMONG SEVERAL TRAITS IN EGG PLANT (*SOLANUM MELONGENA* L.) UNDER SALT STRESS

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Abstract

Morphological traits of the plants may depend on several biochemical traits. Thus, the determination of the relationships between morphological and biochemical traits of the plants may be important for plant scientists. The relationships between morphological (SL; Shoot length, RL; Root length, SW; Shoot weight, RW; Root weight and LW; Leaf weight) and biochemical traits (K, Na, Cl, Malondialdehyde and Chlorophyll) were studied by using canonical correlation analysis in egg plant grown under controlled climatic (salt-stress) conditions. Canonical correlation analysis was used to summarize the relationship between morphological and biochemical traits of the egg plant. This multivariate linear statistical analysis may be used in a wide range of disciplines to analyze the relationships between multiple independent and dependent variable sets.

As a result, the canonical correlation between the first canonical variates pair was found as 0.949 and the first canonical variate extracted 66.36 % of the variance in the morphological traits (Y set). It can be concluded that this multivariate analysis can be used to simplify the relationship between morphological and biochemical traits of the egg plant.

Introduction

Salinity is one of the major abiotic stress factors affecting plant growth and development. Growth stage of the plant is very important when considering salt tolerance of different crops (Ashraf & Harris, 2004). Many plants prove extremely sensitive to soil salt during germination or in the early seedling stage (Chartzoulakis & Loupassaki, 1997; Munss *et al.*, 2002; Qasim & Ashraf, 2006; Yasar, 2007). In most experiments salt tolerance have been evaluated only on mature plants (Luna *et al*., 2000; Shalata *et al*., 2001; Munss *et al*., 2002). Growth performance of crops may depend on several variables such as availability of nutrients, quality and quantity of physico-chemical properties of the soil as well as chemical and biological compartments of the system. Thus, crop growth performance may be adversely affected by salinity-induced nutritional disorders (Ashraf & Orooj, 2006). Separating salinity stress from other plant stresses is difficult because increased salt alters the ionic chemical balance in plants and affects water availability to plants. Therefore, salinity problems may contribute to other classes of plant stress.

There are several measures of correlation to express the relationship between two or more variables. For example, the standard correlation coefficient or Pearson product moment (*r*) measures the direction and amount (%) of the relationship between two variables. In addition, there are various nonparametric measures of relationships such as Kendall tau, Spearman rank correlation that are based on the similarity of ranks in two variables. Multiple regression allows assessing the relationship between a dependent variable and a set of independent variables. On the other hand, correspondence analysis is useful for determining the relationships between a set of categorical variables.

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Canonical correlation analysis is one of the most popular multivariate analysis techniques. The goal of canonical correlation is to determine simultaneous relationships between two sets of variable such as X and Y. In other words, canonical correlation analysis is a procedure to determine the strength of relationships among physiological, morphological or chemical traits of plants or animals. It may be useful to think of one set as independent variables and other set as dependent variables (Tabachnick & Fidell, 2001). In canonical correlation analysis, sets of variable (in general called X and Y sets) are combined to produce the highest correlation between sets. Because there are several variables in each set, several combination of the variable are possible. Thus, there are potentially as many ways to recombine the variables as there are variables in the smallest set (Tabachnick & Fidell, 2001).

Canonical correlation analysis can be used in wide fields like plant science, biology, chemistry, meteorology, demography, artificial intelligence, cognitive science, political science, sociology, psychometrics, educational research, economics and management science to analyze multidimensional relationships between multiple independent and dependent variable sets.

The main purpose of present study was to use canonical correlation analysis as a statistical approach to determine relationships between several morphological and biochemical traits of egg plant.

Materials and Methods

This research was conducted at Yuzuncu Yil University in Turkey. Long purple egg plant (*Solanum melongena* L.) was used. Plants were grown under controlled climatic conditions at 26/22 ^{0}C day/night temperatures, light/dark regimes of 16/8 h, light intensity of 280 µmol m^{-2} s^{-1} and 70% relative humidity. Seeds were germinated in vermiculite moistened with distilled water. After two weeks, seedlings were transferred to plastic vessels filled with 4-l half-strength Hoagland solution. The solution in the vessels was replaced every week. After 14 days of 150 mM NaCl treatment, plants were divided into separate leaf and root fractions. The fresh weights of shoots, roots and leaves were weighted and shoot and root length was measured. Lipid peroxidation was measured as the amount of Malondialdehyde (MDA) determined by the thiobarbituric acid (TBA) reaction (Heath & Packer 1968). Oxidative damage was evaluated by chlorophyll retention. Leaf segments (200 mg), either fresh or frozen at -40^{o}C were placed in 5ml 80% ethanol and heated in a water bath at 80 ^{0}C for 20 min. Chlorophyll was evaluated in the alcohol extracts from absorbance readings, taking into account the extinction coefficient for each one. Chlorophyll content was calculated as 1000 x A_{654}/(39.8 x sample fresh weight), according to Tetley & Thimann (1974). For ions determination, fresh leaf samples were extracted in concentrated 0.1 N Nitric acid. Na^{+}, K^{+} and Cl^{-} contents were determined by flame photometry in samples from leaves (Taleisnik *et al.,* 1997). For chloride determination, fresh leaves were extracted with 0.1 N HNO3 in 10% (v:v) Acetic acid and Cl^{-} was determined by the silver ion-titration method with a automatic chloridometer (Buckhler – Cotlove chloridometer) according to Bozcuk (1970). For the canonical correlation analysis; shoot length (SL), root length (RL), shoot weight (SW), root weight (RW), leaf weight (LW), Potassium (K), Sodium (Na), Chloride (Cl), Malondialdehyde (MDA) and Chlorophyll (Chl) of 144 plants were measured. From these traits; SL, RL, SW, RW and LW were considered as Y variable set. This set is also called as dependent variable set. Other traits (K, Na, Cl, MDA and Chl) were considered as X variable set or independent variable set. We looked for maximum correlations between the two sets of variables by considering linear combinations **U** = **a'X** and **V**= **b'Y** of the **X**'s and **Y**'s, respectively. We then have that $\sigma_U^2 = \mathbf{a}'\Sigma_{XX}\mathbf{a}$, $\sigma_V^2 = \mathbf{b}'\Sigma_{YY}\mathbf{b}$ and $\sigma_{UV} = \mathbf{a}'\Sigma_{XY}\mathbf{b}$.

$$\text{Hence, } C_{UV} = \frac{\mathbf{a}'\Sigma_{XY}\mathbf{b}}{\sqrt{\mathbf{a}'\Sigma_{XX}\mathbf{a}\ \mathbf{b}'\Sigma_{XY}\mathbf{b}}}$$

The estimation of a and b vectors were calculated according to Tabachnick & Fidell (2001). F test was used for testing of canonical correlation (Jobson, 1992; Tabachnick & Fidell, 2001). NCSS statistical package was used for all of the calculations (Hintze, 2001).

Results and Discussion

Descriptive statistics and Pearson correlation coefficients (r) for all variables were given Table 1 and Table 2 respectively.

Table 1. Descriptive statistics of the variables.

Variables	Mean ± S. Error	Minimum	Maximum
SL (cm)	10.378 ± 0.252	6.00	17.4
RL (cm)	12.363 ± 0.319	8.00	23.6
SW (g)	1.357 ± 0.051	0.46	2.51
RW (g)	1.317 ± 0.039	0.66	2.20
LW (g)	1.741 ± 0.066	0.55	3.99
K (µ g /mg F.W.)	1.746 ± 0.045	1.01	2.68
Na (µ g /mg F.W.)	16.051 ± 0.392	8.00	26.5
Cl (µ g /mg F.W.)	13.691 ± 0.382	6.80	22.4
MDA (µ mol/g F.W.)	12.601 ± 0.297	7.90	21.0
Chl (µ mol/g F.W.)	0.135 ± 0.003	0.07	0.23

SL: Shoot length, RL: Root length, SW: Shoot weight, RW: Root weight, LW: Leaf weight, K: Potassium, Na: Sodium, Cl: Chlorine, MDA: Malondialdehyde Chl: Chlorophyll.

Table 2. Pearson correlation coefficients among all variables.

	SL	RL	SW	RW	LW	K	Na	Cl	MDA
RL	0.858**								
SW	0.866**	0.859**							
RW	0.831**	0.807**	0.893**						
LW	0.507**	0.472**	0.433**	0.371**					
K	0.878**	0.830**	0.828**	0.796**	0.366**				
Na	-0.820**	-0.801**	-0.836**	-0.762**	-0.409**	-0.829**			
Cl	-0.838**	-0.763**	-0.822**	-0.778**	-0.397**	-0.861**	0.856**		
MDA	-0.605**	-0.633**	-0.759**	-0.722**	-0.335**	-0.547**	0.696**	0.656**	
Chl	0.632**	0.552**	0.692**	0.653**	0.249**	0.493**	-0.572**	-0.519**	-0.651**

*:p<0.05, **:p<0.01, SW: Shoot length, RL: Root length, SW: Shoot weight, RW: Root weight, LW: Leaf weight, K: Potassium, Na: Sodium, Cl: Chlorine, MDA: Malondialdehyde, Chl: Chlorophyll

Table 3. Canonical correlations between canonical variates

Canonical variates	Canonical correlations	F values	P Values	Wilks' lambda
U_1V_1	0.949	$16.81_{(25.\ 388)}$	0.000	0.065
U_2V_2	0.481	$2.92_{(16.\ 321)}$	0.000	0.660
U_3V_3	0.313	$1.84_{(9.\ 258)}$	0.061	0.859
U_4V_4	0.194	$1.30_{(4.\ 214)}$	0.271	0.953
U_5V_5	0.097	$1.03_{(1.108)}$	0.311	0.990

Table 4. Canonical coefficients and canonical loadings between first canonical variates and original variables.

X variable set			Y variable set		
Variables	Canonical loadings	Standardized canonical weights	Variables	Canonical loadings	Standardized canonical weights
K	-0.937	-0.541	SL	-0.957	-0.457
Na	0.902	0.112	RL	-0.901	-0.088
Cl	0.904	0.129	SW	-0.966	-0.424
MDA	0.750	0.137	RW	-0.917	-0.116
Chl	-0.724	-0.237	LW	-0.422	0.078

SW: Shoot length, RL: Root length, SW: Shoot weight, RW: Root weight, LW: Leaf weight, K: Potassium, Na: Sodium, Cl: Chlorine, MDA: Malondialdehyde, Chl: Chlorophyll.

As seen in Table 2, all Pearson correlation coefficients ranged from -0.836 to 0.878 and were statistically significant ($p<0.01$). Thus, growth traits (morphological traits) may be influenced by biochemical traits.

In this study, **X** and **Y** variable sets had p = 5 and q = 5 variables, respectively. Thus five canonical variables (U_iV_i) (usually called as canonical variates) were formed and canonical correlations between them are presented in Table 3.

As shown in Table 3, first (0.949) and second (0.481) canonical correlations between first (U_1V_1) and second (U_2V_2) canonical variates were significant ($P<0.01$) however, other correlations were non-significant. Although the first two canonical correlations were found statistically significant, the second canonical correlation is quite low. Thus, only the first canonical correlation was interpreted. The first canonical correlation is 0.949 representing 90% overlapping variance of first pair of canonical variate.

Canonical coefficients and canonical loadings between first canonical variates and original variables were presented in Table 4. Loadings are considered as the correlation coefficients between original variables and canonical variates. As pointed out by Tabachnick & Fidell (2001), correlations between original variables and canonical variates (loadings) in excess of 0.300 are interpreted.

Thus, the first canonical variate (U_1) for the X variable set contains relatively large positive loadings of Na, Cl and MDA and relatively large negative loadings of K and Chl. The U_1 canonical variate measures a contrast between the variables Na, Cl and MDA and the variables K and Chl. From the canonical loadings, it was concluded that higher values of Na, Cl and MDA, and the lower values of K and Chl formed the greater value of U_1. The first canonical variate (V_1) for the Y variable set contains the negative loadings of all variables in the set. From the estimated canonical loadings, it was determined that V_1 canonical variate is high when all of own variables are lower.

The proportion of variances extracted from a set of variables by a canonical variety of the set is equal to the sum of squared of loadings divided by the number of variables in the set. Thus, the first canonical variate in the X and Y set was found as 0.7191 [$(-0.937^2 + \ldots + (-0.724^2) / 5 = 0.7191$] and 0.7360 respectively. The first canonical variate extracts 71.91% of the variance in the X set and 73.60% in the Y set. However, redundancy index in a canonical variate is the percentage of variance it extracts from its own set of variables. So, the first canonical variate extracts 66.36 % (0.7368×0.949^2) of the variance in the Y set.

If the aim of the study is production of score values, the coefficients of them can be available. Standardized canonical weights in the Table 4, can also be considered as regression coefficients of U_1 and V_1 canonical variates.

It clearly indicates that all variables (K, Na, Cl, MDA and Chl) in the X set have significant effects on Y variable set (SL, RL, SW, RW, and LW). In other words, Y variable set i.e., (growth traits) is influenced by all traits in the X variable set. Increases in K, Chl and decreases in Na, Cl, and MDA resulted in significant increase in SL, RL, SW, RW, and LW. As pointed out by Chartzoulakis & Loupassaki (1997), Munss *et al.* (2002) and Yasar (2007), available NaCl causes increasing of Na and Cl and decreasing of K concentrations in plant. This results in toxic levels of sodium as well as insufficient K concentration for enzymatic reaction and osmotic adjustment (Yasar, 2003; Zhu, 2003). A high Na and Cl content results in increased cell permeability (Levitt, 1980; Yasar, 2007). Ion disorders caused from salinity may also lead to changes in plant lipid metabolism (Kuiper, 1985). When examining the effects of environmental stresses on plant membranes, many studies have measured the products of lipid peroxidation, such as malondialdehyde (MDA) (Wilson & McDonald, 1986). MDA content, a product of lipid peroxidation, has been considered an indicator of oxidative damage from stress (Dhindsa & Matowe, 1981). Thus, cell membrane stability has widely been utilized to differentiate salt-tolerant and salt-sensitive cultivars (Luna *et al.*, 2000; Meloni *et al.*, 2003). As reported from Dhindsa & Matowe (1981) and Luna *et al*., (2000), our results show that salt stress elicits oxidative stress, measured as lipid peroxidation,

Conclusion

In the present study, the relationships between several morphological and biochemical traits of egg plant was studied by using canonical correlation analysis. High and significant canonical correlation (0.949) was found between morphological and biochemical traits of egg plant. The results of this study indicate that salinity is an important concern for egg plant and high K, Chl and low Na, Cl and MDA increase growth traits of egg plant. It was found that selected biochemical traits account for 66.36 % of the variation in the selected morphological traits.

Several univariate and multivariate correlation measurements can be used to determine relationships among the variables. However, univariate correlation coefficients may fail to determine complex relationships. Multivariate models can be appropriate for assessing the relationships between large numbers of variables. Canonical correlation analysis can be used to determine relationships between multivariate dependent and independent variables. Therefore, this analysis may be more successful for determining the complex relationships in Biological sciences.

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