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REPEATED MEASURES ANALYSIS OF CHANGES IN PHOTOSYNTHETIC EFFICIENCY IN SOUR CHERRY DURING WATER DEFICIT

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SUMMARY

The objective of this study was to investigate changes in photosynthetic efficiency applying repeated measures ANOVA using the photosynthetic performance index (PI_{ABS}) of the JIP-test as a vitality parameter in seven genotypes of sour cherry (Prunus cerasus, L.) during 10 days of continuous water deficit. Both univariate and multivariate ANOVA repeated measures revealed highly significant time effect (Days) and its subsequent interactions with genotype and water deficit. However, the multivariate Pillai's trace test detected the interaction Time × Genotype × Water deficit as not significant. According to the Tukey's Studentized Range (HSD) test, differences between the control and genotypes exposed to water stress became significant on the fourth day of the experiment, indicating that the plants on the average, began to lose their photosynthetic efficiency four days after being exposed to water shortage. It corroborates previous findings in other species that PI_{ABS} is very sensitive tool for detecting drought stress.

Key-words: water deficit, JIP test, repeated measures ANOVA, sour cherry

INTRODUCTION

In the experimental studies, responses to treatments are measured frequently two or more times repeatedly over a period of time. This is particularly the case in the physiological studies when dynamics of a process is to be examined on the same subjects. Using a standard analysis of variance (ANOVA) to compare group means is not appropriate in this kind of research, as it does not consider dependencies between observations within subjects in the analysis. In this case, repeated measures ANOVA (Winer, 1971) should be used where strict analytical assumptions are to be met and specific analytical procedures followed.

We have recently used repeated measures ANOVA to demonstrate changes in photosynthetic efficiency during maturation in Norway maple (*Acer platanoides*, L.) (Lepeduš et al., 2011a) by measuring the time course of the chlorophyll *a* fluorescence, called the fast chlorophyll *a* fluorescence (OJIP) transient (Strasser and Govindjee, 1992a,b). The chlorophyll fluorescence parameters are strongly correlated with reducing plant vitality in response to environmental stresses including drought (Percival and Sheriffs, 2002) and are reliable early indicators of stress (Krause and Weiss, 1991; Schreiber et al., 1994). The procedure developed for quantification of OJIP transients is called JIP-test (Strasser and Strasser, 1995). The JIP-test is being used extensively in stress physiology in a range of plant species under controlled conditions. It is also adaptable to field conditions (Reddy and Strasser, 2000), which is particularly important for agricultural and horticultural research. Among other species, JIP-test parameters were investigated in grapevine (*Vitis vinifera* L.) for early detection of biotic and abiotic stresses *in situ* (Christen et al., 2007). However, no such studies were made in sour cherry (*Prunus cerasus* L.) specifically not in young *in vitro* propagated plants particularly susceptible to water stress. The objective of this study was to investigate changes in photosynthetic efficiency applying repeated measures ANOVA using herewith the

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parameter of photosynthetic performance index (PI_{ABS}) of the JIP-test in seven genotypes of sour cherry during 10 days of continuous water deficit in order to evaluate their water stress tolerance.

MATERIAL AND METHODS

Two-year old *in vitro* propagated plants of seven sour cherry genotypes were challenged to water deficit in the greenhouse of the Agricultural Institute Osijek during June, 2011. Water deficit is one of the restrictive factors in acclimatization of *in vitro* plants. The investigated genotypes (cultivars Kelleris 16, Maraska, Cigančica and Oblačinska which is represented with genotypes OS, 18, D6 and BOR) differ in their pomological characteristics (Puškar, 2005). Ten plants in pots of each of seven genotypes were placed on two benches. Plants allocated on one bench represented control plants watered every other day according to optimized propagation process. Plants on another bench were not watered during the experiment. Photosynthetic efficiency was measured as the photosynthetic performance index (PI_{ABS}) determined by measuring of the chlorophyll *a* fluorescence transient induced by pulse of saturating light (JIP-test) (Strasser and Govindjee, 1992a, b; Strasser and Strasser, 1995). The chlorophyll *a* fluorescence transient was measured by a Plant Efficiency Analyser (PEA, Hansatech, UK) on leaves between second and fifth nodium at one attached leaf per plant (ten leaves per genotype) at an ambient temperature every day in the morning (8:30-9:30 a.m.) during 10 days. Details about JIP-test and derivation of PI_{ABS} were given by Lepeduš et al. (2011b). PI_{ABS} represents the "vitality" index, a biophysical expression derived from the energy flux theory and calculated with the JIP-test (Christen et al., 2007). Relative water content (RWC) of leaves has been used as an indicator of water stress in leaves (Clavel et al., 2006) since it is immediately decreased in stress challenged plants. Relative water content (RWC) was determined on four leaves of each genotype and calculated as (fresh weight - dry weight)/ (turgid weight - dry weight) $\times$ 100. Averaged over seven genotypes, RWC mean was 61.53% at the beginning of the experiment. It was steadily reduced to 40.78% measured at the last tenth day, indicating water stress.

ANOVA repeated measures (Winer, 1971) were applied for the statistical analysis performed by the GLM procedure in the SAS program (SAS Institute, 2004) including univariate and multivariate ANOVA. The effects of interest were 1) between-subject effects (water deficit treatment), 2) within-subject effects (Time (Day)), and 3) interactions between the two types of effects. Multivariate ANOVA was applied since the sphericity test using Mauchly's criterion (Mauchly, 1940) was significant, thereby not validating univariate ANOVA. The multivariate tests provided for within-subjects effects and interactions involving these effects were Wilks'Lambda, Pillai's Trace, Hotelling-Lawley Trace, and Roy's largest root (Cole and Grizzle, 1966). Since the between-subject effect of water deficit was highly significant, means were subsequently compared by the *post-hoc* Tukey's Studentized Range (HSD) test.

RESULTS AND DISCUSSION

The repeated ANOVA measures testing between-subject effects showed highly significant effects of genotype and water deficit treatment (Table 1). It indicates that PI in the sour cherry genotypes was different in the control (watering) compared to plants exposed to water deficiency. The interaction genotype $\times$water deficit was also highly significant suggesting that genotypes responded differently on water deficit having different tolerance to water stress.

Table 1. F-statistics and the probability levels in the repeated ANOVA measures testing between-subject effects for index of photosynthetic efficiency ($PI_{ABS)}$ in sour cherry leaves

Tablica 1. Vrijednosti F testa i razine vjerojatnosti u analizi varijance ponovljenih mjerenja koje testiraju učinke između subjekata za indeks fotosintetske učinkovitosti ($PI_{ABS)}$ u listovima višnje

Source *Izvor*	Degrees of freedom *Stupnjevi slobode*	F value *F vrijednost*	Probability>F *Vjerojatnost>F*
Genotype – *Genotip*	6	13.03	<0.0001
Water deficit – *Vodni deficit*	1	479.44	<0.0001
Genotype $\times$Water deficit *Genotip $\times$ Vodni deficit*	6	19.23	<0.0001

The univariate repeated measures ANOVA revealed that the within-subject effect Time (Days) and its subsequent interactions are all highly significant (Table 2). As their validity depends on whether or not the covariance structure satisfies the sphericity condition, we applied the test based on Mauchly's Criterion to check for this condition. P values for the effect Time (Day) and its interactions are 0.000 meaning that the sphericity condition is not met. Thus, the test results of the significance of Time (Day) and the interaction on Table 2 are not completely valid and it justifies use of the multivariate repeated ANOVA measures.

Table 2. F-statistics and the probability levels in the univariate repeated ANOVA measures testing within-subject effects for index of photosynthetic efficiency (PI_{ABS}) in sour cherry leaves

Tablica 2. Vrijednosti F testa i razine vjerojatnosti u univarijatnoj analizi varijance ponovljenih mjerenja koje testiraju učinke unutar subjekata za indeks fotosintetske učinkovitosti (PI_{ABS}) u listovima višnje

Source *Izvor*	Degrees of freedom *Stupnjevi slobode*	F value *F vrijednost*	Probability>F *Vjerojatnost>F*
Time (Days) – *Vrijeme (dani)*	9	108.99	<0.0001
Time × Genotype *Vrijeme × Genotip*	54	5.18	<0.0001
Time × Water deficit *Vrijeme × Vodni deficit*	9	133.28	<0.0001
Time × Genotype × Water deficit *Vrijeme × Genotip ×Vodni deficit*	54	4.62	<0.0001

The multivariate repeated measures ANOVA (Table 3) testing within-subject effects showed that all four multivariate tests provided similar statistics. However, according to Pillai's trace statistics, the interaction Time × Genotype × Water deficit was not significant indicating that Pillai's trace (Pillai, 1960) was most conservative test with respect to violations of the assumptions of multivariate ANOVA. It is particularly useful when sample sizes are small or unequal, or covariances are not homogeneous, offering the greatest protection against Type I errors with small sample sizes.

Table 3. Probability levels of exact F values according to four statistics in the multivariate repeated ANOVA measures testing within-subject effects for index of photosynthetic efficiency (PI_{ABS}) in sour cherry leaves

Tablica 3. Razine vjerojatnosti egzaktnih F vrijednosti prema četiri statistička parametra u multivarijatnoj analizi varijance ponovljenih mjerenja koje testiraju učinke unutar subjekata za indeks fotosintetske učinkovitosti (PI_{ABS}) u listovima višnje

Source *Izvor*	Degrees of freedom *Stupnjevi slobode*	Probability>F - *Vjerojatnost >F*			
		Wilks' lambda	Pillai's trace	Hotelling-Lawley trace	Roy's greatest root
Time (Days) – *Vrijeme (dani)*	9	<0.0001	<0.0001	<0.0001	<0.0001
Time × Genotype – *Vrijeme × Genotip*	54	<0.0001	0.0003	<0.0001	<0.0001
Time × Water deficit – *Vrijeme × Vodni deficit*	9	<0.0001	<0.0001	<0.0001	<0.0001
Time × Genotype × Water deficit – *Vrijeme × Genotip ×Vodni deficit*	54	0.0003	0.0538	<0.0001	<0.0001

Figure 1 gives log transformed values of the photosynthetic performance index (PI_{ABS}) averaged over seven genotypes of sour cherry under conditions of continuous no watering (water deficit) compared to the control during 10 days. According to the Tukey's Studentized Range (HSD) test, differences between the control and plants exposed to water deficiency became significant on the fourth day of the experiment. It suggests that the plants of seven sour cherry genotypes, on the average, began to show the symptoms of water stress with respect to the fluorescence of chlorophyll *a* after four days of no watering. The considerable decrease of PI occurred in plants exposed to water stress on the sixth and tenth day of the experiment. This is in accordance with other reports (Živčak et al., 2008; Christen et al., 2007; Oukarroum et al., 2007) where the PI_{ABS} parameter was shown to be very sensitive tool for detecting plant tolerance to drought stress and strongly correlated with RWC (Živčak et al., 2008).

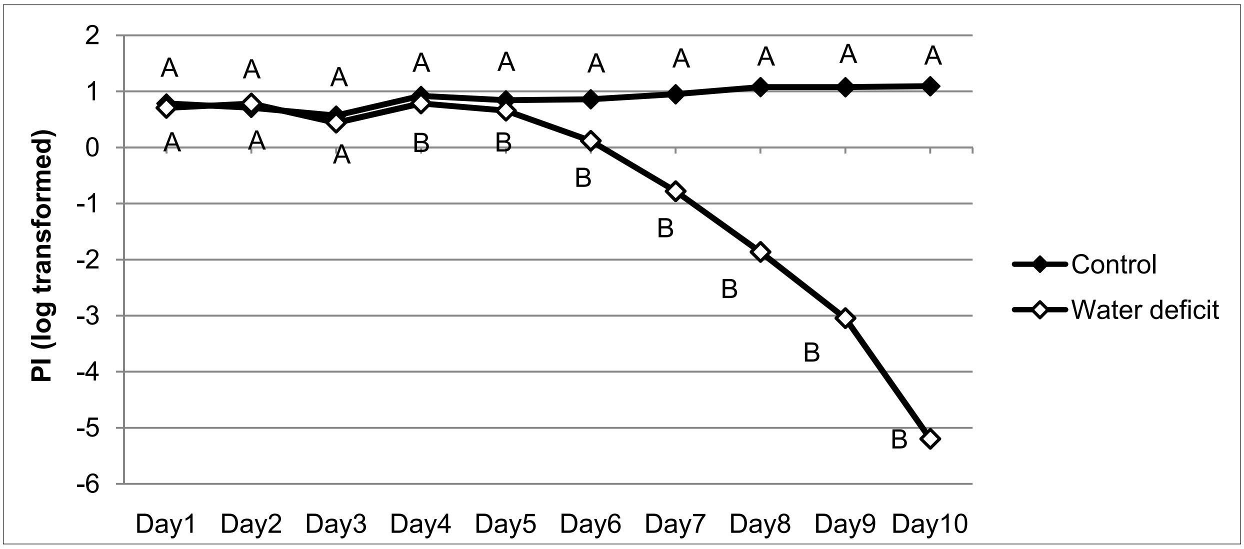

Figure 1. Changes in the photosynthetic efficiency index (PI_{ABS}) under conditions of continuous no watering (water deficit) during 10 days averaged over seven genotypes of sour cherry. Significant differences between the control and no watering-treated genotypes according to Tukey's Studentized Range (HSD) Test are designated by an upper-case letter (A and B)

Slika 1. Promjene u indeksu fotosintetske učinkovitosti (PI_{ABS}) u uvjetima kontinuiranoga nezalijevanja (vodnoga deficita) tijekom 10 dana prema prosječnim vrijednostima sedam genotipova višnje. Značajnost razlika između kontrole i tretmana–nezalijevanja izražene su prema Tukey's Studentized Range (HSD) testu velikim slovom (A i B)

CONCLUSION

Statistically sound repeated measures ANOVA (both univariate and multivariate analyses) revealed highly significant within-subject effect Time (Days) and its subsequent interactions with effects of genotype and water deficit in seven sour cherry genotypes during 10 days of no watering. According to the Tukey's Studentized Range (HSD) test, differences between the control and plants exposed to water deficiency became significant on the fourth day of the experiment indicating that the plants, on the average, began to show disturbances in photosynthetic processes already four days after the water was withheld. According to PI_{ABS} and RWC genotype OS was the most tolerant genotype to water deficit while genotype Kelleris 16 was the least tolerant all together with genotypes Maraska and Cigančica.

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ANALIZA PONOVLJENIH MJERENJA ZA PROMJENU FOTOSINTETSKE UČINKOVITOSTI KOD VIŠNJE TIJEKOM VODNOGA DEFICITA

SAŽETAK

Cilj je ovoga rada bilo istražiti promjene u fotosintetskoj učinkovitosti uporabljajući ANOVA-u ponovljenih mjerenja za indeks fotosintetske učinkovitosti (PI) dobivenoga JIP testom kao parametra vitalnosti biljaka sedam genotipova višnje (Prunus cerasus L.) tijekom 10 dana kontinuiranoga vodnoga deficita. Univarijatna i multivarijatna ANOVA ponovljenih mjerenja ukazale su na visoko značajni učinak Vrijeme (dan) i njegovih odnosnih interakcija s učincima Genotip i Vodni deficit. Međutim, multivarijatni Pillai's trace test nije detektirao značajnu interakciju Vrijeme× Genotip × Vodni deficit. Prema Tukey's Studentized Range (HSD) testu, razlike između biljaka u kontroli i tretmanu - nezalijevanju postale su statistički značajne četvrtoga dana u pokusu, što ukazuje da su biljke višnje u prosjeku počele gubiti vitalnost nakon četiri dana nezalijevanja. To potvrđuje prijašnja istraživanja na drugim biljnim vrstama, gdje se PI pokazao kao vrlo senzitivan pokazatelj stresa izazvanoga sušom.

Ključne riječi: vodni deficit, JIP test, ANOVA ponovljenih mjerenja, višnja

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