

**EFFECT OF DIFFERENT SOIL WATER CONTENT EFFECT ON GENOTYPE
EXPRESSION IN PHOTOSYNTHETIC EFFICIENCY AND LEAF TEMPERATURE
IN SUNFLOWER**

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Sunflower (*Helianthus annuus* L.) has high needs for water but can tolerate drought very well because, under stress conditions, its well developed root system can supply water and mineral nutrients from deeper soil layers. Reduced water content in soil affects plant growth and development, photosynthetic rate and causes rapid leaf senescence. In this study, we measured maximum quantum yield of photosystem II (F_v/F_m), photosynthetic performance index (PI_{ABS}) and leaf temperature (LT) on 13 sunflower genotypes at different soil water contents. By calculating stress tolerance indices (STI) of F_v/F_m and PI_{ABS} parameters we evaluated drought tolerance for every tested sunflower genotype at given soil water contents. The experiment was set up in vegetation pots in two treatments with different soil water contents (60% and 80% of field water capacity) in three replications. Based on the obtained results for F_v/F_m and PI_{ABS} and STI values of F_v/F_m and PI_{ABS} parameters, we concluded that genotypes 5 and 12 had higher tolerance at both treatments, as opposed to genotypes 2 and 13 which were less tolerant. These analyses will help breeders to select genotypes adapted to different farming areas which is, along with the use of recommended production practices, the background for profitable sunflower production.

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INTRODUCTION

Sunflower (*Helianthus annuus* L.) has high needs for water as a key factor for plant growth and development, but can tolerate drought very well because, under stress conditions, its well-developed root system can supply water and mineral nutrients from deeper soil layers. During sunflower vegetation, along with the total amount of rainfall, water distribution is also very important, especially in the critical stages of growth i.e. from butonisation to flowering (GADŽO *et al.*, 2011). Lack of water, but also surplus of water, can cause weaker plant development. Apart from affecting the plants growth and development (HAO *et al.*, 2013), reduced soil water content affects photosynthetic rate (WARREN and ADAMS, 2006) and causes rapid leaf senescence (RIVERO *et al.*, 2009). Photosynthetic efficiency at different water contents was studied in many plant species such as barley, maize, soybean, winter wheat, etc. (OUKARROUM *et al.*, 2009; HAO *et al.*, 2013; KOVAČEVIĆ *et al.*, 2013; ŠIMIĆ *et al.*, 2014). Common method for investigation of photosynthetic efficiency is measurement of chlorophyll *a* fluorescence which is used for identifying differences in plant photosynthetic performance in rapid and nondestructive way (ŽIVČÁK *et al.*, 2014). ZHANG *et al.* (2010) stated that analysis of chlorophyll *a* fluorescence is widely and mostly used technique among plant ecophysicologists and physiologists. After chlorophyll *a* fluorescence measurement OJIP-test follows. OJIP-test gives information about the functioning of photosynthetic apparatus through parameters describing the absorption of photons, trapping of excitons, electron transport and energy dissipation (STRASSER *et al.*, 2004).

In this research, tolerance to different soil water contents of sunflower genotypes was evaluated by the maximum quantum yield of photosystem II (F_v/F_m), photosynthetic performance index (PI_{ABS}) and measured leaf temperature values. F_v/F_m provides information about the proportion of the light absorbed by chlorophyll in photosystem II and PI_{ABS} gives information on overall energy flow through photosystem II (ŠIMIĆ *et al.*, 2014).

Plant's photosynthetic apparatus and successively its photosynthetic efficiency can be damaged by high leaf temperatures (ZHANG *et al.*, 2010). Leaf temperature is affected by several simultaneous environmental factors, such as solar radiation, heating, evaporation and heat transfer (VON BERKUM, 2008). Generally, during transpiration leaf needs a considerable amount of energy for each molecule of water to be converted from liquid to vapour, i.e. process which is used for leaf cooling purpose (JONES *et al.*, 2009).

To determine the tolerance of genotypes to different soil water contents, one of the parameters which can be used is stress tolerance index (STI), proposed by FERNANDEZ (1992). STI helps plant breeders in further experiments and selection of tolerant genotypes in different environmental conditions.

Objective of this study was to determine differences in maximum quantum yield of photosystem II, photosynthetic performance index and leaf temperature at different soil water contents for 13 sunflower genotypes in order to assess their stress tolerance in these conditions. Also, using stress tolerance indices of F_v/F_m and PI_{ABS} parameters we were able to confirm genotype tolerance at different soil water contents along with average values of photosynthetic efficiency parameters. Our assumption was that tested genotypes would have different

photosynthetic efficiency and leaf temperature values i.e. they would react differently to different water contents in soil.

MATERIALS AND METHODS

Plant material and experiment conditions

Thirteen sunflower hybrids were chosen for this study. Ten genotypes were developed within the sunflower breeding program at the Agricultural Institute Osijek and three genotypes were introductions. All genotypes were developed from different source populations and as a result they differ in agronomic traits (plant height, head diameter, position of the head, the genetic potential for yield). Experiment was conducted at the Agricultural Institute Osijek (Osijek, Republic of Croatia) during the growing season of 2012. Each genotype was sown in six 12 liter pots, at three cm depth, eight seeds per pot (two seeds per hole). Sunflower were sown on 25th May, planted by hand and thinned at stage V4 (SCHNEIDER and MILLER, 1981) to two plants per pot at 10 cm distance. In order to provide optimal and uniform development conditions during the growing season, the plants were grown in a controlled greenhouse environment until July 13, to the R2 stage of sunflower development (SCHNEIDER and MILLER, 1981). After that vegetation pots were moved to the open field. Soil analysis was conducted at the Institute of Soil and Land Conservation, Osijek (Croatia), which determined that the soil texture was silty clay loam – SiCL (FAO, 2014). The same soil was in the each pot. Results of chemical analysis and mechanical composition of the soil on sodium pyrophosphate are shown in Tables 1 and 2.

Table 1. Chemical analysis of the soil

Components	pH KCl	pH H ₂ O	Humus %	P ₂ O ₅ mg 100 g ⁻¹	N %	K ₂ O mg 100 g ⁻¹	CaCO ₃ %	Al (mobile) mg 100 g ⁻¹
Results	7.3	7.9	2.18	>41	0.16	>40	0.9	0.26

Table 2. Mechanical composition of the soil on sodium pyrophosphate

Percentage content of particles / diametre mm	Sand		Powder		Clay
	2.000–0.200	0.200–0.063	0.063–0.020	0.020–0.002	<0.002
Content in the soil	0.30	1.25	37.99	31.44	29.02

The experiment was set up according to the randomized complete block design and it consisted of two treatments in three replications where each treatment had 78 plants. Plants in one treatment were maintained at about 60% of field water capacity (FWC) because wilting point according to the soil analysis data was 16% volume water content which represents 60% FWC, while plants in the other treatment were maintained at about 80% FWC according to JOSIPOVIĆ *et al.*, (2013) who proposed adding water as soon as the water content reduced to 75 - 80% FWC. According to the soil analysis data, soil water capacity was 28% volume water content which represents 100% FWC. FWC was determined using the gravimetric method. Soil water content in pots was monitored and maintained by weighing pots to determine water loss, and watering plants when soil water content lowered under desired level of 60% FWC for stress

treatment and 80% FWC for control treatment during the experiment. From phase V1 to R2 (SCHNEIDER and MILLER, 1981) all plants were maintained at 80% FWC (for both treatments). In the R3/R4 reproductive stage of sunflower, when FWC fell to 60% which caused a mild water stress in plants, photosynthetic efficiency measurements were made because sunflower in that phase was the most sensitive to water deficit (GADŽO *et al.*, 2011).

Analysis of chlorophyll a fluorescence and leaf temperature

Chlorophyll *a* fluorescence and leaf temperature (LT) were measured in the R3/R4 reproductive stage on all genotypes included in this experiment (SCHNEIDER and MILLER, 1981). Thirty minutes after leaves were adapted to darkness, chlorophyll *a* fluorescence was measured on nine leaves per genotype in the pot (three leaves per repetition, i.e. 18 measurements per genotype in both treatments). Changes in fluorescence were measured in the morning (7:00-9:00 a.m.) using Plant Efficiency Analyser (PEA, Hansatech, UK). Obtained data were analysed by the OJIP test (STRASSER *et al.*, 2004) in order to calculate parameters of photosystem II functioning such as maximum quantum yield of photosystem II (F_v/F_m) and photosynthetic performance index (PI_{ABS}). Leaf temperature was measured at 10:00 a.m. on one developed apical leaf per genotype (six measurements per genotype in both treatments) using Dual Focus Infrared Thermometer (B+B Thermo-Technik GmbH, Germany). Device was adjusted to emissivity for sunflower tissue at 0.979 (COLL *et al.*, 2001). Republic of Croatia's Meteorological and Hydrological Service data on weather conditions during the measurements of fluorescence and leaf temperature are shown in Table 3. Photosynthetic efficiency was measured in the morning because at that period the weather conditions are optimal for normal photosynthetic apparatus function. Leaf temperature was measured after measuring photosynthetic efficiency, when the air temperature was 27.8 °C and 33% RH.

Table 3. Weather conditions in Osijek (measuring station Osijek-Klisa airport) during the measurements of fluorescence and leaf temperature ()*

Time a.m.	Temperature °C	Relative humidity %	Cloud cover Okta	Wind m s ⁻¹	Pressure Pa
7	19.2	77	0	4	100570
8	23.0	50	0	5	100580
9	26.0	38	0	5	100560
10*	27.8	33	0	6	100540

* – time when leaf temperature was measured

Stress tolerance indices of F_v/F_m and PI_{ABS}

We used stress tolerance index (STI) proposed by FERNANDEZ (1992) to evaluate genotype tolerance at different soil water contents. Stress tolerance indices based on maximum quantum yield of photosystem II and photosynthetic performance index were calculated by the following formula:

$$STI = \frac{Y_s \times Y_p}{\bar{Y}_p^2}$$

were Y_s and Y_p are values of the investigated traits for each genotype under stress (60% FWC) and nonstress (80% FWC) conditions and \bar{Y}_p is mean trait values of all genotypes evaluated in nonstress conditions.

Statistical analysis

Analysis of variance and correlation analysis were calculated using software SAS 9.3 for Windows (SAS Institute Inc., USA) with a level of significance threshold set at $\alpha = 0.05$ for analysis of variance and $\alpha = 0.01$ and $\alpha = 0.05$ for correlation analysis. In Figure 1 the letters indicate significant differences in mean values calculated with least significant difference (LSD) test at the level of 0.05. LSD test included data for 13 sunflower genotypes and two treatments.

RESULTS AND DISCUSSION

The effect of treatment and genotype as sources of variability for F_v/F_m and PI_{ABS} values of 13 tested sunflower genotypes were significant, while the effect of interaction between treatment and genotypes ($T \times G$) was not. At the same time, the analysis of variance confirmed no significant difference in LT between genotypes, treatments and their interactions (Table 4).

Table 4. Analysis of variance for maximum quantum yield of photosystem II (F_v/F_m), photosynthetic performance index (PI_{ABS}) and leaf temperature (LT) for 13 sunflower genotypes in two different treatments

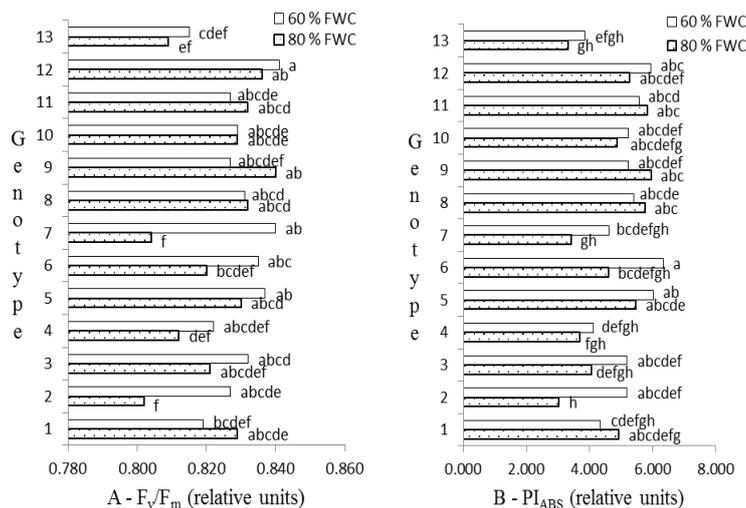
Source	Degree of freedom	Mean square			F value		
		F_v/F_m	PI_{ABS}	LT	F_v/F_m	PI_{ABS}	LT
Replication	2	0.01	34.48	3.84	37.54	34.91	1.65
Treatment (T)	1	0.00	5.46	0.42	4.89*	5.53*	0.18 ns
Genotype (G)	12	0.00	3.74	3.66	2.30*	3.79**	1.58 ns
Interaction $T \times G$	12	0.00	1.16	1.85	1.67 ns	1.17 ns	0.80 ns
Error	50	0.00	0.99	2.32	–	–	–
Total	77	–	–	–	–	–	–

** – F test significant at $P < 0.01$, * – F test significant at $P < 0.05$, ns – not significant ($P > 0.05$)

Average values of F_v/F_m for investigated samples (Fig. 1), in the treatment which had 60% FWC, ranged from 0.815 (genotype 13) to 0.841 (genotype 12), while for the treatment with 80% FWC the lowest value of F_v/F_m was 0.802 (genotype 2) and the highest value was 0.840 (genotype 9). In the treatment with 60% FWC almost all genotypes had higher values of F_v/F_m compared to the treatment with 80% FWC which indicates that the plants had experienced mild water stress. Although the difference between treatments for F_v/F_m existed, it was significant only for genotypes 2 and 7. Similar results were obtained by JANSEN *et al.* (2009) on *Arabidopsis thaliana*. In their experiment drought stressed plants showed significantly higher values of F_v/F_m compared to well-watered plants. Another investigation of water stress influence (LI *et al.*, 2008) showed that in cucumber seedlings values of F_v/F_m were significantly decreased

in severe drought stress. The first step in plants defence from water stress is reducing its stomatal aperture but this does not affect the photoinhibition and values of F_v/F_m remain almost unaffected while water stress leads to chronic photoinhibition and causes changes in F_v/F_m values (JANSEN *et al.*, 2009). According to KALAJI and GUO (2008), maximum F_v/F_m value of healthy samples can reach approximately 0.85, while the limit value at which photosystem II functions normally is 0.75 (BOLHÁR NORDENKAMPF *et al.*, 1989). LSD_{0.05} value for F_v/F_m was 0.021 while for PI_{ABS} it was 1.630.

The most sensitive parameter of the OJIP-test is PI_{ABS} which shows plant vitality and can quickly detect stress even before the appearance of visible symptoms on leaves (STRASSER *et al.*, 2004). In this experiment average values of PI_{ABS} ranged from 3.031 to 6.358 (Fig. 1B). Genotype 13 had the lowest average value and genotype 6 had the highest average value in the treatment with 60% FWC. Genotype 2 had the lowest average PI_{ABS} value in the treatment with 80% FWC while genotype 9 which had the highest average value. Majority of genotypes in the treatment with 60% FWC had more expressed photosynthetic performance index than genotypes in the treatment with 80% FWC, which also suggests that plants had experienced mild water stress, but the difference between treatments for PI_{ABS} was significant only for genotypes 2 and 6. Similar results were reported by KOVAČEVIĆ *et al.* (2013) while investigating PI_{ABS} in drought conditions on wheat and soybean (unpublished data).



Notes. The letters indicate significant differences in the mean values calculated with LSD test at $P < 0.05$. Means with the same letter are not significantly different.

Figure 1. Maximum quantum yield of photosystem II (F_v/F_m) (A) and photosynthetic performance index (PI_{ABS}) (B) average values for 13 sunflower genotypes in two treatments (60% and 80% of field water capacity, FWC)

Measurement of chlorophyll *a* fluorescence can be used to find differences in the response of plants to environmental conditions and thus for screening for tolerance to environmental stress (OUKARROUM *et al.*, 2009). In this research the most unstable genotypes according F_v/F_m and PI_{ABS} values were genotypes 2, 6 and 7.

Measuring leaf temperature enables us to determine when cooling or heating of leaves occurs (VON BERKUM, 2008). Leaf temperature depends on the energy leaves receive and return. Leaf temperature is affected by the ambient temperature and by the soil water content. In terms of water saturated atmosphere, when leaf and ambient temperatures are the same, transpiration and transpirational cooling are being discontinued. Leaf temperature is higher in water nonsaturated atmosphere than in the saturated atmosphere. Also, cooling and transpiration rate are usually different for each leaf (BUCHNER *et al.*, 2013), which requires more measurements in order to get reliable data. FALKENBERG *et al.* (2007) in their study established that lack of accessible water prevented plants from having proper transpiration and from heat release, which caused increase in canopy temperature and subsequent damage to cells. Almost nonexistent difference between environment (Table 3) and leaf temperatures (Fig. 2) confirms that there was no significant difference between genotypes which is in agreement with results of ANOVA (Table 4). Similar results were confirmed by FALKENBERG *et al.* (2007) in their research. They did not found significant temperature difference between 100% and 75% ET_c irrigation regimes stating that the amount of irrigation in the 75% regime was enough to maintain canopy temperature. The same was not true for plants which were irrigated in 50% irrigation regime where plants did not have enough accessible water which caused an increased canopy temperature.

Our next step in this research will be to test the leaf temperature in field conditions in order to determine whether measuring of leaf temperature can be used to find differences in drought tolerance between sunflower genotypes.

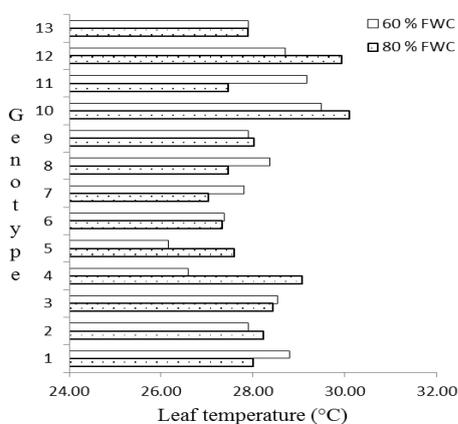


Figure 2. Leaf temperature (LT) average values for 13 sunflower genotypes in two treatments (60% and 80% of field water capacity, FWC)

Correlation analysis was used to determine the relationship between the investigated traits (Table 5). F_v/F_m and PI_{ABS} parameters showed a significant positive correlation in both treatments which means the values of both parameters changed in the same direction. Also, analysis did not show significant correlation between F_v/F_m , PI_{ABS} and LT which demonstrates their mutual independence. Positive correlation between F_v/F_m and PI_{ABS} parameters in both treatments (control and water stress) was determined by KOVAČEVIĆ *et al.* (2011) on winter barley and GHOBADI *et al.* (2013) on sunflower.

Table 5. The correlation coefficients of maximum quantum yield of photosystem II (F_v/F_m), photosynthetic performance index (PI_{ABS}) and leaf temperature (LT) for 13 sunflower genotypes in two treatments (60% and 80% of field water capacity)

Correlated variables	F_v/F_m^2	F_v/F_m^1	PI_{ABS}^2	PI_{ABS}^1	LT^2
F_v/F_m^1	0.180				
PI_{ABS}^2	0.954**	0.230			
PI_{ABS}^1	0.483	0.715**	0.555*		
LT^2	0.220	0.012	-0.006	-0.031	
LT^1	0.318	-0.090	0.236	-0.005	0.328

¹ – treatment with 60% of field water content, ² – treatment with 80% of field water content; ** – $r = 0.684$ – significant at $P < 0.01$, * – $r = 0.553$ – significant at $P < 0.05$

Stress tolerance index (STI) is useful for identification of genotypes more or less tolerant to different soil water contents. The tolerance of genotypes to different soil water contents will be higher when the value of STI is higher. PORCH (2006) noted that, in investigation of heat stress in field and greenhouse conditions, STI and GM (geometric mean) are good indices for genotype selection under stress and no stress conditions. In this research the STI value of F_v/F_m for genotype 13 (0.973) was lower than for other genotypes while genotype 12 (1.039) had the highest STI value (Fig. 3A). According to obtained STI of PI_{ABS} values, genotype 5 (1.536) showed more tolerance than genotype 13 (0.597) (Fig. 3B). STI values determined for F_v/F_m and PI_{ABS} values of the investigated genotypes indicated different levels of tolerance at different soil water contents. STI has been a common method used to compare genotype tolerance at different levels of water content in the soil for different plants such as wheat (ANWAR *et al.*, 2011), sunflower (ABDI *et al.*, 2013) and barley (KHOKHAR *et al.*, 2012). STI values for LT were not calculated because analysis of variance did not show any statistically significant differences.

Genotypes 5 and 12 were the most tolerant at both soil water contents as opposed to genotypes 2 and 13 which were the least tolerant. This conclusion was based on average F_v/F_m and PI_{ABS} values and STI results.

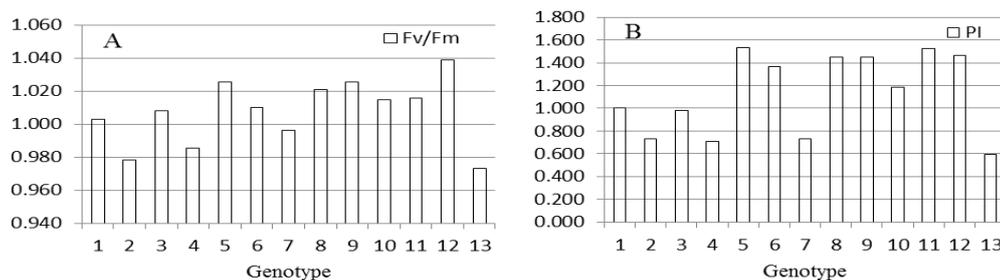


Figure 3. Stress tolerance indices values calculated for the maximum quantum yield of photosystem II (F_v/F_m) (A), and for photosynthetic performance index (PI_{ABS}) (B)

CONCLUSIONS

Based on average values of maximum quantum yield of photosystem II, photosynthetic performance index and stress tolerance index, we concluded that genotypes 5 and 12 had the highest tolerance at investigated conditions. We found no significant differences in leaf temperatures and assumed surrounding temperature was suitable for stomata to normally perform their functions so plants could defend themselves from heat. Analyses of photosynthetic efficiency parameters and stress tolerance index are very useful methods for detecting tolerant genotypes in different environmental conditions. These analyses will help breeders to select genotypes adapted to different farming areas. Genotype adaptability combined with the use of recommended production practices is the background for profitable sunflower production. Selection of genotypes that are tolerant to environmental conditions may reduce the negative impact on yield which will enable undisturbed production of healthy, functional and safe food.

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**EFEKAT RAZLIČITIH SADRŽAJA VODE U ZEMLJIŠTU NA EKSPRESIJU
GENOTIPA U FOTOSINTESKOJ EFIKASNOSTI I TEMPERATURA LISTA KOD
SUNCOKRETA**

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Izvod

Suncokret (*Helianthus annuus* L.) ima velike potrebe za vodom, ali i visoku toleranciju na sušu. U stresnim uslovima gajenja njegov koren crpi vodu i mineralna hranjiva iz dubljih slojeva zemljišta. Smanjeni sadržaj vode u zemljištu utiče na rast, razvoj i fotosintezu biljaka te izaziva ubrzanu senescenciju listova. U ovom istraživanju analizirali smo maksimalni kvantni prinos fotosastava II (F_v/F_m), indeks fotosintetske efikasnosti (PI_{ABS}) i temperaturu lista na 13 genotipova suncokreta gajanih na zemljištu s različitim sadržajem vode. Pomoću F_v/F_m i PI_{ABS} parametara izračunat je indeks tolerantnosti na stres (STI) s kojim smo procenili tolerantnost genotipa na sušu s obzirom na sadržaj vode u zemljištu. Ogljed je postavljen u vegetacijskim posudama u dva tretmana koji su predstavljali različit sadržaj vode u zemljištu (60 i 80 % poljskog vodnog kapaciteta) u tri ponavljanja. Na osnovu dobijenih rezultata za F_v/F_m i PI_{ABS} , zaključili smo da su genotipovi 5 i 12 imali veću toleranciju u oba tretmana, za razliku od genotipa 2 i 13 koji su bili manje tolerantni. Ove analize će pomoći oplemenjivačima kod izbora genotipova koji su prilagođeni različitim područjima gajenja, što je uz korišćenje preporučene proizvodne prakse, dobra podloga za profitabilnu proizvodnju suncokreta.

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