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Physiological parameters of tomato plants subjected to salinity and treated with brassinosteroid

Abstract - The objective of this work was to evaluate the effects of brassinosteroid on chlorophyll a fluorescence parameters, the SPAD index, and leaf succulence in young tomato (Lycopersicon esculentum) plants under salt stress. The experimental design was a randomized complete block, in a 5×2 factorial arrangement – five levels of water salinity (0.5, 1.5, 3.0, 4.5, and 6.0 dS m⁻¹) \times two rates of 24-epibrassinolide (0 and 10⁻⁶ mol L⁻¹ EBL). At 23 days, the treatment without EBL showed that the increase of water salinity reduced the maximum, primary, and effective photochemical efficiency of PSII, as well as the electron transport rate, the SPAD index, leaf succulence, total dry mass, and the stress tolerance index, but increased initial fluorescence and relative excess energy in PSII. With the increase of water salinity, in the EBL treatments, there was an increase in the effective photochemical efficiency of PSII, the electron transport rate, the SPAD index, leaf succulence, total dry mass, and the stress tolerance index. EBL promotes beneficial effects on PSII integrity, the chlorophyll index, and leaf succulence in young tomato plants subjected to salinity levels of up to 6.0 dS m⁻¹.

Index terms: *Lycopersicon esculentum*, 24-epibrassinolide, leaf succulence, NaCl, photosystem II.

Parâmetros fisiológicos em plantas de tomate submetidas à salinidade e tratadas com brassinosteroide

Resumo - O objetivo deste trabalho foi avaliar os efeitos de brassinosteroide sobre os parâmetros de fluorescência da clorofila a, o índice SPAD e a suculência foliar em plantas jovens de tomate (Lycopersicon esculentum) sob estresse salino. O delineamento experimental utilizado foi o em blocos ao acaso, em arranjo fatorial 5×2 – cinco níveis de salinidade da água (0,5, 1,5, 3,0, 4,5 e 6,0 dS m⁻¹) × duas doses de 24-epibrassinolídeo (0 e 10^{-6} mol L⁻¹ de EBL). Aos 23 dias, o tratamento sem EBL mostrou que o aumento da salinidade da água reduziu a eficiência fotoquímica máxima, primária e efetiva do PSII, assim como a taxa de transporte de elétrons, o índice SPAD, a suculência foliar, a massa de matéria seca total e o índice de tolerância ao estresse, mas aumentou a fluorescência inicial e o excesso relativo de energia no PSII. Com o aumento da salinidade, nos tratamentos com EBL, houve aumento da eficiência efetiva do PSII, da taxa de transporte de elétrons, do índice SPAD, da suculência foliar, da matéria seca total e do índice de tolerância ao estresse. O epibrassinolídeo promove efeitos benéficos sobre a integridade do PSII, o índice de clorofila e a suculência foliar em plantas jovens de tomate submetidas a níveis de até salinidade 6,0 dS m⁻¹.

Termos para indexação: *Lycopersicon esculentum*, 24-epibrassinolídeo, suculência foliar, NaCl, fotossistema II.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most consumed vegetables in the world, standing out among the most important ones as it represents an excellent source of antioxidants for human nutrition, such as lycopene and vitamin C (Toor et al., 2006; Filgueira, 2008). In Brazil, climatic conditions are favorable to the cultivation of this vegetable, but its performance is significantly hampered, especially in the semiarid regions, due to the presence of salt in the water used for irrigation, since the crop is moderately salt sensitive (Doorenbos & Kassam, 1994).

Salinity caused by excess Na^+ is one of the main factors of abiotic stress that limits plant development and production because it interrupts ion homeostasis, which results in the accumulation of toxic Na^+ in the cytoplasm with a concomitant deficiency of K^+ that induces osmotic, ionic, and oxidative stresses in tissues, besides nutritional imbalance, reducing nutrient absorption and transport (Lima et al., 2016; Cruz et al., 2018; Poór et al., 2019).

Under salt stress, one of the first negative events that occur in plants is the degradation of the photosynthetic apparatus, with negative interferences in the electron transport rate and photochemical efficiency of the photosystem II (Wani et al., 2019). Thus, chlorophyll fluorescence is a fast, accurate, and reliable tool in the evaluation of the effects of environmental stresses, such as salinity (Khalaji et al., 2016). Another problem caused by this stress is related to leaf chlorosis, because it reduces chlorophyll synthesis and, consequently, the green intensity indicated by the low SPAD index reading (Wani et al., 2019), besides the lower water absorption in stressed plants, as shown by the reduction of leaf succulence (Cruz et al., 2018). However, alternatives to increase agricultural production under salinity conditions, through exogenous supplementation of phytohormones such as brassinosteroid have already been evaluated (Ding et al., 2012; Tanveer et al., 2018).

The exogenous use of brassinosteroid through foliar spraying can mitigate salt stress, as reported by Wei et al. (2015), who observed in *Cucumis sativus* L. that 1.0 μ mol L⁻¹ 24-epibrassinolide (EBL) increased the maximum quantum yield of the photosystem II under salt stress condition. In the evaluation of three EBL applications at 10⁻⁸ mol L⁻¹ in [*Brassica juncea* (L.) Czern. & Coss], the authors also observed that the phytohormone maintained the maximum efficiency of

the photosystem II and SPAD index in plants under salt stress (Wani et al., 2019). The EBL application at 0.1 mg L⁻¹ to *Pisum sativum* L. increased the electron transport rate and effective quantum yield of the photosystem II in the absence of stress, according to Dobrikova et al. (2014).

Brassinosteroid is likely to improve salt tolerance in tomato by maintaining the integrity of the photosynthetic apparatus and the absorption of water, ultimately attenuating the damage caused by salinity. However, studies on the induction of salt tolerance in tomato plants by brassinosteroid are scarce.

The objective of this work was to evaluate the effects of brassinosteroid on chlorophyll a fluorescence parameters, the SPAD index, and leaf succulence in young tomato plants under salt stress.

Materials and Methods

The study was carried out in a protected environment in the academic unit of agricultural engineering, at the Centro de Tecnologia e Recursos Naturais, of the Universidade Federal de Campina Grande, in the municipality of Campina Grande, in the state of Paraíba, Brazil (7°15'18"S, 35°52'28"W, at 550 m altitude), from June to September 2019.

Seed of the IPA 6 tomato cultivar were placed to germinate in expanded polystyrene trays with 128 cells. When the seedlings had four to five true leaves (at 25 days after sowing), they transferred to 20 L pots, filled with 17 kg of soil. One seedling was planted per pot and fertilized according to Novais et al. (1991).

The experimental design was a randomized complete block, in a 5×2 factorial arrangement – five levels of water salinity (0.5, 1.5, 3.0, 4.5, and 6.0 dS m⁻¹) × two rates of EBL (0 and 10⁻⁶ mol L⁻¹ EBL) obtained from Sigma-Aldrich (St., MO, USA), totaling ten treatments, with six replicates.

At 48 days after sowing, the plants were divided into two groups subjected to foliar spraying: one with 10^{-6} mol L⁻¹ EBL, for three consecutive days; and the other, with deionized water. Simultaneously to the last EBL application, the treatments with concentrations of saline water (0.5, 1.5, 3.0, 4.5, and 6.0 dS m⁻¹) began to be applied and continued until the end of the experiment. From transplantation to the beginning of the treatments, plants were irrigated with publicsupply water (0.5 dS m⁻¹; control). The electrical conductivity of the sodium chloride solutions was obtained according to Richards (1954).

The irrigations were performed in the late afternoon (17:00 h), following a two-day interval, in which each treatment received a specific volume of irrigation, corrected in each irrigation event, based on the water consumption by plants in the previous irrigation. Thus, soil moisture was restored to field capacity, obtaining a leaching fraction (LF) of approximately 0.1 for all treatments, according to the equation: Vi = Va - Vd/1 - LF, in which: Vi is the water volume (mL) to be applied in the irrigation; Va is the water volume (mL) applied in the previous irrigation; and Vd is the water volume (mL) drained in the previous irrigation. Drained water was collected in the morning of the day after irrigation, and the leached volume was measured using the collector installed in each pot.

Plants were arranged in six rows of ten plants spaced at 1.0 m between plants and 0.5 m between rows. Along each row, a wooden bar was installed at 1.10 m height to assist in conducting and training the plants.

During the experimental period, the temperature ranged from 21 to 32°C, with average 27.5°C and 11°C thermal amplitude, while the relative humidity ranged from 46 to 87%. Evaluations were performed at 23 days after the treatments began.

Chlorophyll a fluorescence was measured in leaves pre-adapted to the dark using leaf clips for 30 min between 10:00 and 11:00 h, in the last leaflet of the third expanded leaf from the apex, using a OS5p pulse-modulated fluorometer (Opti Sciences, Hudson, NH, USA). In this measurement, the following fluorescence parameters were obtained: initial fluorescence, maximum fluorescence, variable fluorescence, maximum photochemical efficiency of PSII (Fv/Fm), and primary photochemical efficiency of PSII (Fv/Fo) (Maxwell & Johnson, 2000). Besides, using the fluorometer, the evaluations were performed under light conditions, through the yield protocol. The readings were obtained by applying an actinic light source with multi-flash saturating pulse, coupled to a clip for determining photosynthetically active radiation (PAR-clip) in order to estimate the electron transport rate (ETR) and the effective quantum efficiency of the photosystem II (Y). The relative excess energy in PSII (EXC) was calculated by the formula EXC = (Fv/ Fm) - (Y / (Fv/Fm), according to Pereira et al. (2019).

The SPAD index was determined from five readings in the third expanded leaf from the apex using the SPAD-502, and the results were expressed in the reading units of the device (SPAD units).

Leaf succulence was determined according to the methodology proposed by Mantovani (1999), using the equation LS = (LFM - LDM)/LA, in which: LS is the leaf succulence (mg H₂O cm⁻²); LFM is the leaf fresh mass (mg); LDM is the leaf dry mass (mg); and LA, the leaf area (cm²).

At the end of the crop cycle, the plants were collected and dried in an oven at 65°C until constant weight was attained, to obtain the total plant dry mass. From the total dry mass, the salinity tolerance index was calculated according to the method of Fageria et al. (2010), based on the classification for four levels of dry mass reduction: tolerant (T), 0–20%; moderately tolerant (MT), 21–40%; moderately sensitive (MS), 41–60%; and sensitive (S), >60%; calculated as STI = TDMs/TDMc×100, in which STI is the stress tolerance index, TDMs is the total dry mass under stress, and TDMc is the total dry mass in the control.

The data were subjected to the analysis variance and, when significant, salinity levels were subjected to polynomial regression, and brassinosteroid treatments were compared by the F test at 5% probability level.

Results and Discussion

There were significant effects of salinity on the parameters initial fluorescence, maximum photochemical efficiency of PSII (Fv/Fm), and primary efficiency of PSII (Fv/Fo), as well as effects of the interaction between salinity and EBL on the effective photochemical efficiency of PSII (Y), electron transport rate (ETR), excess energy in PSII (EXC), SPAD index (SPAD), leaf succulence (LS), total dry mass (TDM), and stress tolerance index (STI), while maximum fluorescence and variable fluorescence were not affected by any of the treatments (Table 1, Figures 1 and 2).

The initial fluorescence was affected by irrigation water salinity, and it increased linearly as salinity increased by 10% from 0.5 to 6.0 dS m⁻¹ (Figure 1). Similarly, the maximum photochemical efficiency (Fv/Fm) and the primary photochemical efficiency of PSII (Fv/Fo) were affected only by the salinity levels. However, the increase in salinity levels from 0.5 to

6.0 dS m⁻¹ reduced maximum photochemical efficiency and primary photochemical efficiency of PSII by 2.0% and 7.8%, respectively.

Because of the use of saline water to irrigate crops, plants first experience osmotic stress as a consequence of the reduced osmotic potential of the soil solution. Subsequently, an accumulation of salts occurs in the plant tissues resulting in ionic stress, which may eventually increase up to toxic concentrations and cause ionic toxicity, thereby reducing the nutrient acquisition or causing nutritional imbalance (Munns & Tester, 2008).

The increase of initial fluorescence and the reduction of maximum photochemical efficiency (Fv/Fm) and primary photochemical efficiency of PSII (Fv/Fo), with the increase of salinity levels indicate that plants undergone damage in the PSII reaction centers because of salt stress. The increase of Fo indicates that the PSII reaction center was compromised, or the transfer of excitation energy from antenna to the reaction centers was hampered (Maxwell & Johnson, 2000), which can be confirmed by the reductions of maximum photochemical efficiency of PSII (Fv/Fm) and primary photochemical efficiency of PSII (Fv/Fo). Reductions in these ratios caused by salinity have also been found in Brassica juncea (Gupta et al., 2017; Wani et al., 2019), Cucumis sativus (Wei et al., 2015), and in different cultivars of Solanum melongena (Hanachi et al., 2014). These reductions are probably due to the accumulation of toxic ions of Na⁺ and Cl⁻ and decrease of K⁺ in the leaves, as occurred in *Lycopersicon esculentum* (Santos et al., 2017) and *Solanum melongena* (Ding et al., 2012). However, the application of EBL can mitigate these effects through the induction of lower ionic toxicity and improvement of the homeostasis of the K⁺/Na⁺ ratio, as reported for *Eucalyptus* under salinity at 250 mmol L⁻¹ NaCl and application of 50 nmol L⁻¹ EBL (Oliveira et al., 2019).

The effective photochemical efficiency of PSII and the electron transport rate decreased linearly with the increase of salinity levels in both EBL treatments (Figure 3). However, the foliar application of EBL promoted higher values of these variables, and their lower reduction with increased salt stress. Salinity in plants without EBL application reduced the effective photochemical efficiency of PSII and the electron transport rate by 22.7 and 44.6%, respectively, while with the EBL application, the reduction was of 20.8 and 35.5%.

However, the relative excess energy in PSII increased with the increment of salinity levels, and it was superior in the treatment without EBL application; under this condition, the increase of relative excess energy in PSII was 130%, between 0.5 and 6.0 dS m⁻¹, while under EBL application the increase was 112% (Figure 3). Thus, this fact suggests that EBL acted on

Source of variation	Degree of	F test					
		Fo	Fm	Fv	Fv/Fm	Fv/Fo	Y
Salinity	4	*	ns	ns	*	*	**
EBL	1	ns	ns	ns	ns	ns	**
Salinity×EBL	4	ns	ns	ns	ns	ns	*
Error	45				-		
CV (%)		8.43	7.39	7.87	1.60	6.69	8.47
		ETR	EXC	SPAD	LS	TDM	STI
Salinity	4	**	**	**	*	**	**
EBL	1	**	**	**	**	**	**
Salinity×EBL	4	*	*	**	**	**	*
Error	45						
CV (%)		14.20	12.50	4.19	5.33	4.86	5.78

Table 1. Analysis of variance for the physiological parameters of the IPA 6 tomato (*Lycopersicon esculentum*) cultivar subjected to some salinity levels and epibrassinolide (EBL) application.

Physiological arameters: Fo, initial fluorescence; Fm, maximum fluorescence; Fv, variable fluorescence; Fv/Fm, maximum photochemical efficiency of PSII; Fv/Fo, primary photochemical efficiency of PSII; Y, effective photochemical efficiency of PSII; ETR, electron transport rate; EXC, relative excess of energy in PSII;SPAD, SPAD index; LS, leaf succulence;TDM, total dry mass; and STI, stress tolerance index. CV: coefficient of variation. ^{ns} Nonsignificant. *, ** Significant at 5% and 1% probability, respectively.

effective photochemical efficiency of PSII, electron transport rate, and relative excess energy in PSII in tomato plants.

Effective photochemical efficiency of PSII expresses the proportion of light that is absorbed and used in the photochemical phase in PSII (Maxwell & Johnson, 2000). Thus, better efficiency of absorbed light was found in tomato plants treated with EBL at all salinity levels, similarly to what occurred with the electron transport rate and relative excess energy in PSII, although salt stress interrupts the transport of electrons from the reaction centers to plastoquinone (Kalaji



Figure 1. Fluorescence parameters of the IPA 6 tomato (*Lycopersicon esculentum*) cultivar subjected to salinity levels: A, initial fluorescence (Fo); B, maximum fluorescence (Fm); C, variable fluorescence (Fv); D, maximum photochemical efficiency of PSII (Fv/Fm); and E, primary photochemical efficiency of PSII (Fv/Fo).



Figure 2. Fluorescence parameters of the IPA 6 tomato (*Lycopersicon esculentum*) cultivar subjected to salinity levels and treated with epibrassinolide applications: A, initial fluorescence (Fo); B, maximum fluorescence (Fm); C, variable fluorescence (Fv); D, maximum photochemical efficiency of PSII (Fv/Fm); and E, primary photochemical efficiency of PSII (Fv/Fo).



Figure 3. Physiological parameter of the IPA 6 tomato (*Lycopersicon esculentum*) cultivar subjected to salinity levels and treated with epibrassinolide: A, effective photochemical efficiency of PSII (yield); B, electron transport rate (ETR); C, relative excess energy in PSII (EXC); D, SPAD index; and E, leaf succulence.

et al., 2016). In *Solanum melongena* under 90 mmol L⁻¹ salinity NaCl, the application of 100 nmol L⁻¹ EBL improved the effective efficiency of PSII, according to Wu et al. (2012). Similarly, in *Eucalyptus* plants stressed with 250 mmol L⁻¹ NaCl, the use of 50 nmol L⁻¹ EBL recovered the effective photochemical efficiency of PSII by 25% (Oliveira et al., 2019).

The improvement of effective photochemical efficiency of PSII is a consequence of the EBL action on the electron transport rate, since this phytohormone increases the rate of electron transport, improving the open reaction centers of PSII and the oxygen evolution kinetics, thus improving the overall efficiency of PSII (Dobrikova et al. 2014). In the present study, the electron transport rate was 25.6% higher in plants treated with EBL, despite its decrease with the increment of salt stress under both EBL applications (0 and 10⁻⁶ mol L⁻¹). Similar results were reported for Eucalyptus under 250 mmol L⁻¹ NaCl, in which the application of 50 nmol L⁻¹ EBL recovered the electron transport rate by 23.9%, in comparison with plants without EBL (Oliveira et al., 2019). Glycine max subjected to water stress also showed recovered electron transport rates at 35.2 and 31.0%, when treated with 50 and 100 nmol L⁻¹ EBL applications, respectively, according to Pereira et al. (2019).

The electron transport rate showed an inverse behavior to that of relative excess of energy in PSII, as observed in Glycine max plants under water stress and treated with EBL (Pereira et al., 2019). Therefore, the higher values of effective photochemical efficiency of PSII and electron transport rate found in tomato plants treated with EBL are likely related to the lower excess energy not used in the photochemical phase, due to the increased dissipation of excess energy in the form of heat, which preserved the integrity of the structures of chloroplasts. The effective efficiency of PSII was improved by 100 nmol L⁻¹ EBL application to Solanum melongena plants under salt stress, due to the dissipation of energy favored by the phytohormone, which protected the PSII from damage, according to Wu et al. (2012). In addition, the application of 0.1 mg L⁻¹ EBL was efficient to increase the effective photochemical yield of PSII and the electron transport rate in non-stressed plants of Pisum sativum (Dobrikova et al., 2014).

The SPAD index decreased linearly with the increment of salinity in both EBL treatments, but the

foliar application of EBL promoted higher SPAD index and lower reduction of this parameter under salt stress. The increase of salinity levels from 0.5 to 6.0 dS m⁻¹ reduced the SPAD index by 7.7% without EBL, while with the phytohormone the reduction was equal to 5.7% (Figure 3).

Although the SPAD chlorophyll index was reduced because of salinity, its increase or improvement was 26% higher in plants treated with EBL, which is probably associated with lower damage to chloroplasts induced by the phytohormone. These results are in agreement with those by Wu et al. (2012), who reported the improvement of the SPAD chlorophyll index with the application of 50 and 100 nmol L⁻¹ EBL to Solanum melongena plants under 90 mmol L⁻¹ NaCl salinity. Moreover, the chlorophyll content was reported as partially recovered in Capsicum annuum plants treated with 4 g L^{-1} NaCl + 0.5 mg EBL, in comparison with the control, according to Houimli et al. (2010). Also, the application of 10⁻⁸ mol L⁻¹ EBL to two Lycopersicon esculentum cultivars under cadmium stress was efficient to recover the SPAD index at the control level (Hasan et al., 2011). Brassinosteroids act by reducing the activity of chlorophyllase, an enzyme responsible for chlorophyll degradation under stress condition (Tanveer et al., 2018). Therefore, brassinosteroid applications can help with the recovery of the chlorophyll index in the leaves.

Leaf succulence increased by 7.5% when EBL was applied, even with the increase of salinity from 0.5 to 6 dS m^{-1} , while in the absence of this phytohormone, the leaf succulence decreased by 8.2% with the increment in of salinity (Figure 3).

EBL also improves the turgor of stressed plants, as confirmed by the increase of the leaf water potential of *Glycine max* under water stress (Pereira et al., 2019), and *Lycopersicon esculentum* under cadmium stress (Hasan et al., 2011). Under these conditions, the benefits promoted in the leaf water potential indicate that EBL improves the osmotic adjustment process (Shahid et al., 2014; Gupta et al., 2017). Thus, it is possible that these effects occurred on tomato plants under salt stress, since leaf succulence increased in stressed plants treated with EBL and decreased in those plants without the EBL treatment.

The increase of leaf succulence promoted by EBL is an indication of the plant capacity to adapt to salt stress, facilitating the absorption of water by the plant roots, which results in higher water content in the leaf tissues (Cruz et al., 2018) that are important to dilute the concentration of salts in the leaves, avoiding the toxic effect of salt accumulation in these organs (Leite et al., 2017). Studies report that the increase of salt stress decreases the leaf succulence in different plant species, indicating low osmotic adjustment (Acosta-Motos et al., 2017; Cruz et al., 2018), which possibly occurred in the tomato plants under salt stress without the EBL application.

The total dry mass decreased linearly with the increase of the salinity levels in both EBL treatments, but it was higher with the foliar EBL application, showing a lower reduction of this parameter with the salt stress. Salinity in treatments without EBL application reduced the total dry mass by 53%, while with the EBL, the reduction of this parameter was 46.6% (Figure 4).

The stress tolerance index also decreased with the increase of salinity levels in both EBL treatments; however, it was higher after the foliar application of EBL and reduced less under salt stress. Salinity in plants without the EBL application reduced the stress tolerance index by 52.8%, while with the application of EBL, the reduction was 46.5% (Figure 4). At salinity levels 0.5, 1.5, 3.0, 4.5, and 6.0, the estimated stress tolerance index corresponded to 97.9, 89.6, 77.2, 64.8,

and 52.4% in plants treated with EBL, and to 95.2, 86.0, 72.3, 58.6, and 44.9% in plants without the EBL application, respectively.

The 13.5% increase of the total dry mass of plants treated with EBL, compared to those without EBL, is probably due to the effects promoted by the phytohormone, which avoided damage to PSII, preserving the photosynthesis and, consequently, increasing the biomass production. Similar results were reported for Lycopersicon esculentum plants under water stress, for which the application of 0.01 and 1 µmol L⁻¹ EBL increased the production of shoot dry mass in stressed and control plants, respectively (Behnamnia et al., 2009), and for Solanum melongena plants under 90 mmol L-1 NaCl salt stress and treated with 0.1 mg EBL, which increased shoot dry mass by 20.3% in comparison to the control (Ding et al., 2012). Such increase of dry mass affects the tolerance index, which in the present study was slightly higher in plants treated with EBL. Thus, according to Fageria et al. (2010), plants treated with EBL were classified as tolerant at 0.5 and 1.5 dS m⁻¹ salinity levels, moderately tolerant at 3.0 and 4.5 dS m⁻¹, and as moderately sensitive at 6.0 dS m⁻¹, while plants without the EBL treatment had already reached moderate sensitivity at 4.5 dS m⁻¹. Therefore, based on the dry biomass

Figure 4. Total dry mass (A) and stress tolerance index (B) of the IPA 6 tomato (*Lycopersicon esculentum*) cultivar subjected to salinity levels and treated with epibrassinolide (EBL) application.



production, EBL increased the tolerance of tomato plants, especially up to salinity of 4.5 dS m⁻¹.

Conclusions

1. Epibrassinolide (EBL) promotes beneficial effects on PSII integrity and on the chlorophyll index in young tomato (*Lycopersicon esculentum*) plants subjected to salinity levels of up to 6.0 dS m⁻¹.

2. Leaf succulence is improved in tomato plants treated with EBL and subjected to a salinity level of 3.0 dS m^{-1} .

3. EBL has an attenuating effect on the photochemical apparatus of tomato plants under salt stress.

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