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EFFECT OF SALT STRESS ON THE BEHAVIOR OF OLIVE VARIETIES UNGRAFTED AND GRAFTED ON THE OLEASTER (*Olea europea* VAR. *sylvestris*)

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Abstract

The objective of this work is to determine the effect of salinity on the morpho-physiological behavior of young olive plants in cuttings and grafted plants, including two local varieties (Chemlal and Sigoise) and two introduced varieties (Manzanilla and Arbiquina). The experiment started with the irrigation of the control with a nutrient solution, while the stressed plants received 100 mM NaCl with four replicates in a greenhouse. The analysis involved changes in the relative content (RWC) of water in plant leaves and their leaf area, stomatal density, stomatal size and wax level on the upper leaf epidermis. According to the results, the RWC leaf area and stomatal density of the treated plants decreased as compared to the control. On the other hand, the wax level increased in the case of salt stress in comparison with the control, both for the grafted plants and for the plants resulting from cuttings.

Key words: introduced varieties; local varieties; olive-tree; RWC; salt stress; wax rate

INTRODUCTION

The olive tree, a Mediterranean species, is renowned for its very long life and its resistance to cold. Its culture has a positive impact on the environment and the conservation of landscapes through the fight against soil erosion. It provides an area of refuge for certain animal species and contributes to the maintenance of biodiversity. According **to** Touzani (1984), the olive tree constitutes an economic and food source for the autochthonous inhabitants. The development of olive growing in Algeria was the subject of an extensive program to plant one million hectares of land in olive groves. Thus, new decisions are taken to improve the management of olive growing by extending it to land where intensification of production is possible. However, olive growing in some areas, such as the Tiaret region, is compromised by salinization of soil and irrigation water (Rozema and Flowers, 2008; Latef, 2010). This salinization is mainly anthropogenic (excessive use of chemical inputs and repeated irrigation with brackish water without drainage) (Pasternak and Malach, 1994; Villa-Castorena *et al*., 2003). It is noteworthy that in the ecosystem, marked by severe and frequent droughts, the soil salinization is one of the main factors limiting the development of plants. According to Kinet *et al.* (1998) in the West and the Middle East, about 15 million hectares of agricultural land are subject to increasing salinization. The aim of this work was to determine the effect of salinity on the leaf morpho-physiological behavior of four varieties of olive trees derived from cuttings or grafted, including two local varieties (Chemlal and Sigoise) and two introduced varieties (Manzanilla and Arbiquina).

MATERIALS AND METHODS

The experimental device: The experiment is carried out under a semi-automated greenhouse at the Ibn-Khaldoun University in Tiaret, Algeria, under semi-controlled environment. For the best results of this experiment, four blocks (four replicates) were set up to study the different parameters that demonstrate the physiological effects of salt water on the olive plants tested. The experiment is carried out on four varieties, two of which are for the production of table olives (Sigoise and Manzanilla) and two for olive oil production (Chemlal and Arbiquina).

The cuttings of the local varieties (Sigoise and Chemlal) came from a nebulization greenhouse (private greenhouse, Relizanewilaya and greenhouse of the ITAFV station in Birtouta, Algiers). Those of the cuttings of the introduced plants (Manzanilla and Arbiquina) came from Spain imported by SARL Presto "Agricultural Services" Saoula- Algiers. Finally, the plants grafted on Oleastre (Oleaeuropaeasylvestris) came from the nursery of the tree plants of the Wilaya of Tiaret.

The stressed plants of the four varieties were irrigated every other day with salt water at a concentration of 100 mM, while the controls were irrigated at the same frequency with a nutrient solution (Hoagland and Arnon, 1938).

Analyzes and measures: A series of morpho-physiological parameters analyses, after six (06) months of saline stress were carried out.

The relative water content (RWC): The method of Ladiges (1975) in relation to that of Clarke and McCaig (1982) and **Rascioa** et al. (1988) was used to determine the RWC. The last leaf was excised at its base and immediately weighed to determine the initial fresh weight (PFi); then it was deceived in a test tube containing distilled water and was then placed in the dark at a temperature of 4 °C. for 12 hours. The leaves were again weighed in full turgor (Ppt). The dry weight (PS) was obtained by passing in an oven for 48 hours at a temperature of 80 °C. The relative water content of leaves was estimated by the equation:

RWC (%) = [PFi - Ps / Ppt -Ps] x 100

Pfi = initial fresh weight; Ppt = weight in full turgor; PS = dry weight

The leaf area: The area of the excised leaves (SF) is measured using an LICOR-3000A electronic planimeter that determines the geometry of the sheets (surface, length, width) with a resolution of 1 mm^2.

Determination of the soluble sugars: The dosage of the soluble sugars was carried out according to the method of Schields et Burnett (1960) utilisée par REKIKA (1997).

Determination of proline content: The proline assay was performed according to the method of Troll and Lindsley (1955) improved by Lahrer and Magnecité by LEPORT, (1992).

Statistical analysis: The results obtained were processed through Statistical Software (Stat32.exe; Version 8.0) by analysis of the three-factor variance and the correlation matrix.

The values obtained were the statistical average of four repetitions with a confidence interval calculated at the 5% threshold.

RESULTS

The relative water content (TRE): The relative water content of the leaves provides information on the relative turgescence of the tissues and is one of the criteria for assessing stress tolerance.

The results of the analysis of the saline treatment effect and the various interactions at the 5% error threshold are shown in Table-1.

Table-1. Analysis of the variance of the Relative Water Content of stressed and un-stressed olive trees

Variable	Effet variété(F1)	Effet plant(F2)	Effet stress(F3)	F1*F2	F2*F3	F1*F3	F1*F2*F3
	P	P	P	P	P	P	P
TRE	0,026	0,22	0,63	0,57	0,85	0,76	0,09

Our statistical results are significant for the varietal effect on the relative water content and not significant for the effect of the plant type. Indeed, Fig. 2 shows that after six months of treatment with 100 mM.l-1 of NaCl, the majority of the genotypes studied did not show a significant decrease in the relative water content. The reduction of the TRE of all the tested plants fluctuates between 1% and 2% compared to the control. Thus, the varieties of olive trees studied (local and introduced) have retained a high TRE in the presence of salt stress, indicating that the olive tree is of the "stay green" type. The olive tree retains green leaves and photosynthetically active allowing having reasonable yields even in the presence of abiotic stresses. However, a high content can be found in local varieties of olive trees compared to introduced varieties. For the local plants treated with 100 mMNaCl and in absence of salinity, the sheet exhibited a TRE of 70% and 60% respectively for Chemlal and Sigoise, and 50% on average for the introduced varieties.

The leaf area: The same effect (Table-2) is observed when analyzing the variance of the leaf area. Statistical calculations appear to be significant for the varietal effect on the leaf area and not significant for the effect of the plant type under the saline treatment effect and the various interactions at the 5% error threshold. Saline stress affects the leaf area slightly (p = 7.4%). Indeed, the foliar area recorded in the control plants was 49.34 cm^2 and then it dropped to 27.19 cm^2 for the leaves of the plants treated with 100 mM L^{-1} NaCl, (Fig. 3).

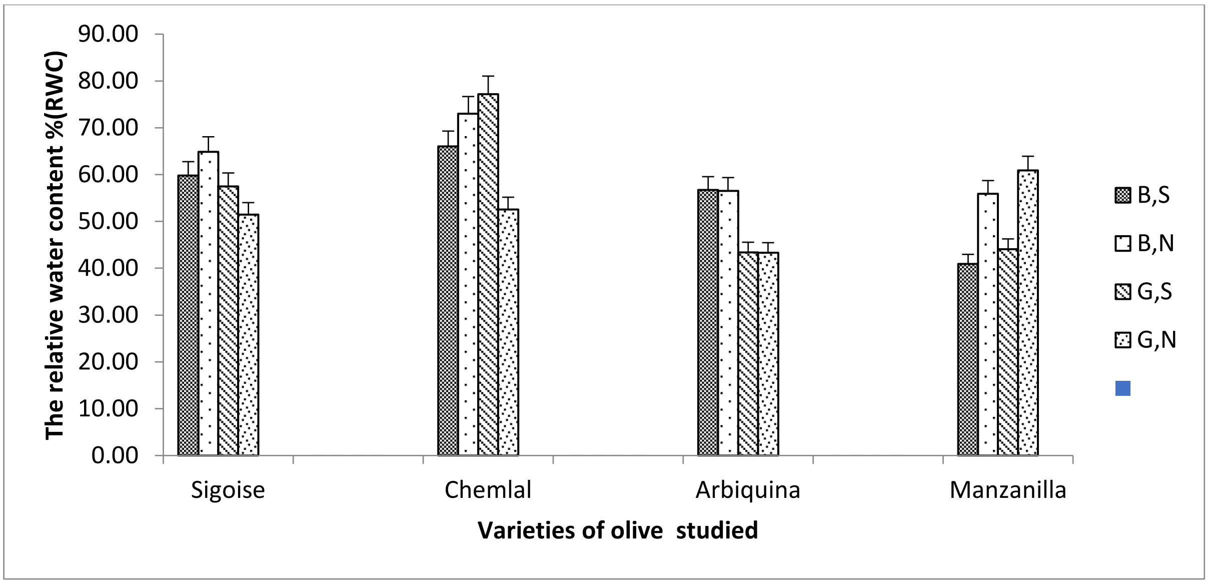

Figure 2. Effect of saline stress on RWC of olive trees from herbaceous cutting and grafted to oleaster (BS: Stressed Cut, BN: Cutting Control, GS: Stressed Graft, GN: Grafted Control)

Table-2. Analysis of the variance of the leaf area of stressed olive trees and unstressed

Variable	variétal effect (F1)	Plant type effect (F2)	Stress effect (F3)	F1*F2	F2*F3	F1*F3	F1*F2*F3
	P	P	P	P	P	P	P
Foliar area	0,0069	0,0745	0,4628	0,1329	0,7261	0,9426	0,5881

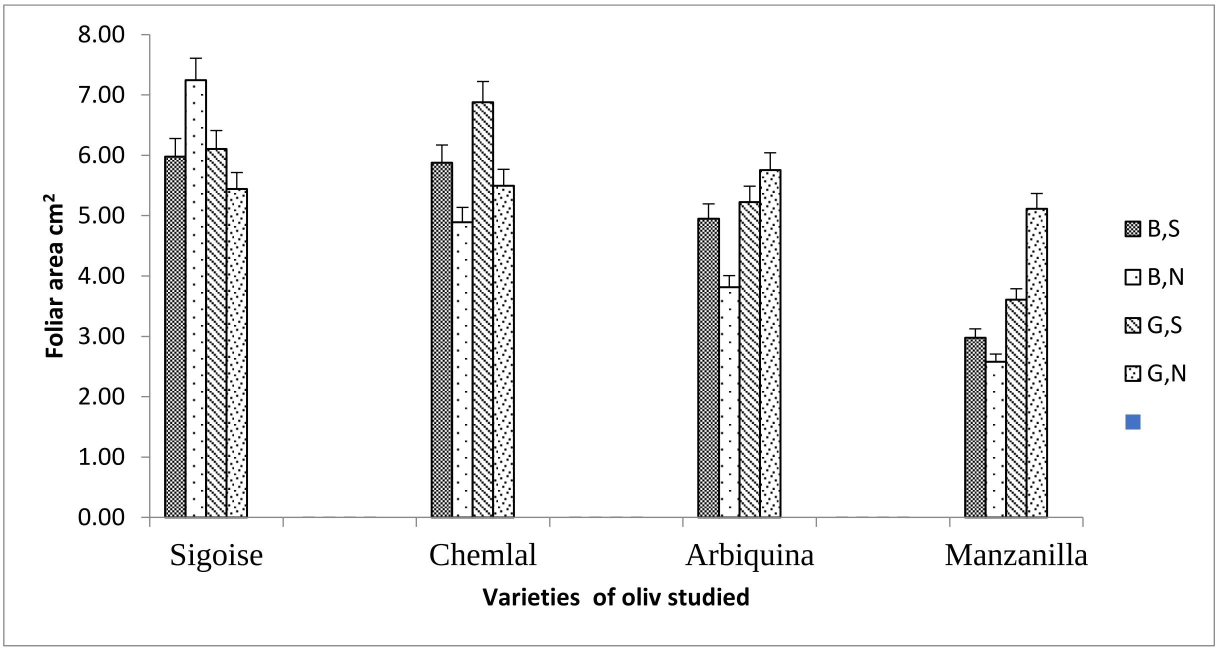

Fig. 3: Histogram showing the effect of saline stress on the leaf area of olive trees grown from herbaceous cuttings and grafted on oleaster (BS = Stressed Cut, BN: Cutting Control, GS: Grafted Stress, GN: Grafted Control)

Table 03: Analysis of the variance of the stomatal density of stressed and unstressed olive trees

Variable	variétal effect F1	Type of plant effect F2	Stress effect F3	F1*F2	F2*F3	F1*F3	F1*F2*F3
	P	P	P	P	P	P	P
Stomatal Density	0,000	0,378	0,000	0,000	0,003	0,041	0,036

With the exception of the effect of the plant type, the impact of salinity is very highly significant on the stomatal density (p = 0%), as well as the varietal effect and the various interactions, particularly at leaf level of the plants receiving a salinity of 100 mM L^{-1} NaCl. (Table-3)
Figure 3 shows a significant variation in the number of stomata. In fact, Sigoise cuttings have 40 stomata / unstressed (control) versus 26 (stressed) stomata. The Chemlal presents 45 against 24, the Arbiquina 44 against 24 and the Manzanilla 30 against 23 stomata / unstressed (us).
For the genotypes grafted on oleaster the fall is more important. According to Fig. 5, the values are 49 (control) versus 14 for the Sigoise, 45 against 24 for Chemlal, 62 against 21 for Arbiquina and 48 against 21 stomata / us for the variety Manzanilla.

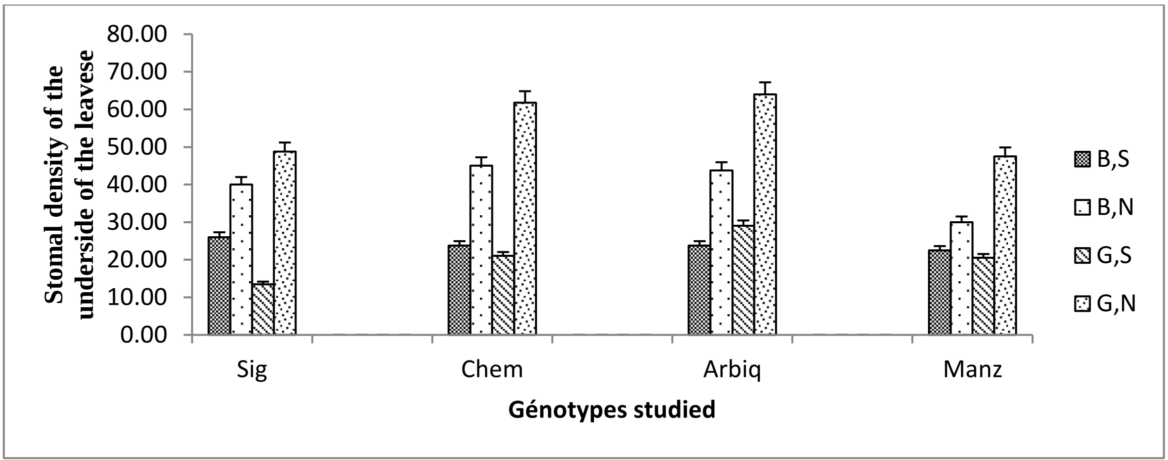

Fig. 5: Histogram representing the stomatal density (mm^{-2}) of the underside of the leaves of the genotypes studied (SS: stressed Sigoise, SN: Sigoise control, MS Manzanilla stressed, MN: Manzanilla control).

The length and width of the stomata: The impact of salinity on the length of stomata, particularly on the leaves of plants treated with 100 mM L^{-1} NaCl except for the plant type, is highly significant (p = 0%).

It is the same for the varietal effect and the different interactions. The width of the stomata had the same fate (Fig. 6).

Table-4: Analysis of the variance of the length and width of the stomata (µm) of the leaves of olive trees, stressed and unstressed.

Variable	Variétal effect F1	Plant effect F2	Stress effect F3	F1*F2	F2*F3	F1*F3	F1*F2*F3
	P	P	P	P	P	P	P
Length of the stomata	0,000	0,481	0,001	0,000	0,000	0,020	0,037
Width of the stomata	0 ,000	0,239	0,000	0,007	0,024	0,000	0,000

The analysis of the variance of the two parameters studied reveals a significant effect on the varietal effect on the length and width of stomata as well as on the saline treatment effect and genotype-type plant interactions, genotype-stress, genotype-Treatment and genotype-type plant-treatment. However, this variance is not significant for the plant type effect at the 5% error threshold (Table-4).
The Fig. 6a and 6b showed that the length and width of stomata in the leaves of plants treated with 100 mM L^{-1} NaCl react differently. The two local varieties (Sigoise and Chemlal) in cuttings and grafted mark an increase in the length of the stomata with the exception of the grafted sigoise which records an average decrease of 4µm. The allochthonous varieties, however, show an average fall in the length of the stomata that varies between 0.4 and 4.5 µm. On the other hand, the width of the stomata of all the genotypes in their two forms (from cuttings and grafted) shows a remarkable drop which oscillates between 1.33 and 16.51m, with the exception of the grafted Manzanilla variety which marks an increase of The width of the stomata 0.75 µm relative to the control.

The analysis of wax variance shows a very highly significant influence for all factors tested at $p < 0.05$, in particular the varietal effect on stomatal density, "plant type" effect, Saline.

Table-6: Correlation at $p < 0.05$ between variety, plant type, stress and physio-biochemical parameters

	TRE	Foliar surface	Somatic density	Somatal length	Stomatal width	The wax rate
Variety	-0, 34*	-0, 38*	0,09	0,38*	0,25	-0 ,20
Type of plant	-0,15	0,21	0 ,04	0,05	-0,09	-0,27*
Stress	-0,06	-0,09	0,82***	0,23	-0,49*	-0,38*

DISCUSSION

Our study emphasizes the irrigation of olive trees of local origin and introduced or allochthonous (Spanish) with brackish water which is about 6 g L^{-1} of soluble salts (NaCl at a concentration of 100 mM L^{-1}), for 24 weeks. It enabled us to determine certain characteristics of the morpho-physiological behavior of this plant species in a situation of salt stress. The water state of the plant, expressed by the relative water content, was sensitive to the applied treatments (r = - 0.200 *). Indeed, salt stress causes a decrease in the values of the relative water content. This parameter, according to Monneveux and Bensalem (1992), is one of the criteria for evaluating abiotic stress tolerance because it indicates the state of turgescence of the plant tissues and its ability to maintain a level of hydration of the tissues to guarantee the continuity of its metabolism. According to Mehani *et al*. (2012), the water state of a plant can be expressed by its relative water content. The results obtained during our study demonstrate that the TRE of all stressed varieties varies with salt. However, the reduction is small compared to that of the control (between 1 and 2%). Local varieties retained a higher TRE than the introduced varieties. It is also noted that saline treatment did not cause a significant reduction in water content. The analysis of the relative water content allows describing in a global way the water status of the plant. On the other hand, salinity is a complex phenomenon that often leads to an osmotic stress due to the reduction of the quantities of water available in the rhizosphere, due to the reduction of the ability of plants to absorb water. The immediate response to salt stress is expressed by a reduction in leaf area as noted by Wang and Nil (2000). Munns and Termaat (1986) showed that decreased vegetative growth, expressed as leaf area reduction or leaf area, is generally the first response of glycophytes exposed to saline stress. As regards our results, the leaf area of the olive varieties studied seems to be a more or less stable parameter in saline conditions. It is observed that the decrease in the leaf area of all the genotypes studied shows only a slight drop which is between 1 and 2% compared to the control. This is confirmed by the results of the analysis of the correlation matrix between variety and stress (p = -0.38 *).

For cuttings and grafted plants, it was found that the local ecotypes grafted on oleaster and stressed showed an increase in the leaf area compared to their control and those from cuttings and stressed showed a decrease in leaf area by Compared to their control (less than 1.1 cm^2 compared to the control).

Thus, decreasing leaf area is considered as a form of adaptation to salt stress, by reducing water losses through perspiration, but it may also cause a decrease in yields due to the reduction of photosynthesis (Bidinger and Witcombe, 1989). The stomatal density of leaves of all stressed genotypes decreased compared to that of no salt (Fig. 4). Moreover, this decrease is greater in the ecotypes obtained from herbaceous cutting (i.e. 65% for Sigoise, 52.78% for Chemlal, 54.29% Arbiquina and 75% for Manzanilla) than those grafted on oleaster (69% for Sigoise, 34.01% for Chemlal, 45.31% for Arbiquina and 43.2% for Manzanilla).

According to Guyot (1998), the stomatal transpiration accounts for 90% of total sweating during 24 hours. The opening and closing of the stomata are controlled by the turgidity of their guard cells, which depend on soil and air moisture, sheet temperature, incident radiation, wind and concentration CO_2 in the air as well as in the chamber under stomatal conditions (Teare and Kanemasu, 1972). However, whenever the plant reduces its transpiration by closing the stomata, it causes the reduction of the production of dry matter following the reduction of the chlorophyll assimilation. Moreover, the results obtained showed a strong positive relationship between salt stress and stomatal density in the lower leaf epidermis. Indeed, the high and negative correlation obtained between salinity and stomatal density (r = 0.850 ***) showed that the presence of NaCl leads to an increase in the number of stomata, which is not logical when we know that they are small stomata in order to reduce water losses and increase the TRE. Indeed, according to Nemmar (1983), the plants that live in dry environments have many stomata with small sizes. Erchidi et al. (2000) confirmed that the presence of small and large stomata allows a much more effective regulation of sweating than that of large and small stomata.

Moreover, Monneveux (1989) believe that this stomatal resistance is manifested by the presence of numerous stomates of small sizes and quick closing. According to Slama (2002), the increase in the number of stomata per unit area could be one of the factors of resistance to water deficit if accompanied by a good physiological activity. The increase in stomatal density can increase the net assimilation of CO_2 and decrease the loss of water. In fact, a large number of stomata can cause small stomata and rapid closure (Slama *et al*., 2005). Heller *et al*. (2004) noted that plants undergoing salt stress close their stomata earlier than plants under normal conditions. This increases the stomatal resistance due to decreased water uptake. In addition, Nilson (1985) established the relationship between transpiration and stomatal resistance in *Arabidopsis thaliana* by increasing this resistance during salt stress, in order to minimize the water loss.

According to Hopkins (2003), sweating becomes more important in the case of thin cuticle leaves. According to our results, the stressed genotypes have cuticles thicker than those under normal conditions that can explain the resistance of the tested plants by keeping their leaves throughout the duration of the stress applied.

CONCLUSION

The morpho-physiological parameters of the leaf, retained in this study, would be closely involved in the regulation of the water state of the leaf tissues. Salt resistance is a polygenic character that can be controlled at different levels of organization, from the cell to the entire plant. However, the diversity of salt effects on plants offers a wide range of physiological and biochemical criteria that can be the basis for rapid testing for large-scale selection. Application of brackish water resulted in a lower TRE, but relatively high compared to other plants in the same situation. This characteristic can be attributed to the osmotic fit of the stressed plant. This control of the hydration reveals a good ability to adjust the osmotic potential in the olive tree in general and especially in the varieties studied. For this purpose, it can be deduced that the varieties of olive trees studied have retained a high ERR in the presence of saline stress, indicating that this plant is of the "stay green" type, which keeps synthetically active green and photo leaves, have reasonable yields even in the presence of abiotic stresses. Under arid climates and in conditions of ionic stress accompanied by osmotic stress, the plant must maintain a dynamic balance between the opening and closing of the stomata. This activity allows it to increase the fixation of the carbon and a better transpiration thus avoiding the heating of the plant. In Mediterranean regions, physiological drought (due to excess salinity) is often chronic, resulting in a decrease in photosynthesis. Our results showed that all the varieties studied, under the effect of salinity, developed a thick cuticle compared to those under normal conditions, thus decreasing leaf transpiration. This may explain the resistance of the olive tree in general and especially the genotypes studied allowing it to keep their leaves throughout the duration of the stress applied. One can also conclude that thanks to its leaves, the olive tree can survive in arid environment. The results obtained in this study showed that the olive tree is a salinity-resistant plant that is a characteristic of arid and semi-arid areas. Olivier, then, is a promising alternative to improve the productivity of marginalized lands. The genetic origin, the rusticity, the adaptation of the olive tree to the conditions of aridity and its specificity to the Mediterranean regions, give it the capacity to constitute a means of fight against the desertification.

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