

Screening of Some Syrian Potato Lines Based on the Morphological Responses to Water Stress

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ABSTRACT

In order to screen 21 *in vitro* local lines of potato for water stress, the morphological responses of aerial and radical parts were studied. Water stress was mediated by adding 2, 4, 6, 8 and 10% (w/v) of sorbitol to Murashige and Skoog (MS) medium against 0% for the control. Plant length and diameter, leaf area, root number, length and diameter, as well as plant fresh and dry weight and plant water content, were measured. Water stress induced a decrease in several growth parameters. Using cluster analysis, based on the sum of relative values of water stress responses, three groups could be distinguished: (1) a tolerant group consisting of six lines (SY-C.09 > SY-C.32 > SY-C.42 > SY-C.49 > SY-C.48 > SY-C.08), (2) a moderately tolerant group consisting of eight lines (SY-C.10 > SY-C.51 > SY-C.16 > SY-C.06 > SY-C.30 > SY-C.41 > SY-C.50) and (3) a sensitive group consisting of seven lines (SY-C.36 > SY-C.44 > SY-C.33 > SY-C.45 > SY-C.40 > SY-C.05 > SY-C.04).

Keywords: potato, screening, water stress

INTRODUCTION

Water is essential for plant growth, since all physiological processes depend on it. Potato (Solanum tuberosum L.) is very sensitive to water stress because of its shallower root system (Iwama and Yamaguchi 2006). For developing drought-tolerant genotypes, improvement in root traits is considered to be important (Rossouw and Waghmarae 1995; Iwama and Yamaguchi 2006). However, the root traits in field-grown plants are exhausting and time-con-suming (Erusha et al. 2002). Thus, an in vitro method could be a possible alternative to overcome the problems associated with field evaluation of potato. The effectiveness of this technique has been studied to facilitate screening plants for diseases (Platt 1992) and drought resistance (Gopal and Iwama 2007). On the other hand, this technique is used in Syria to produce local potato varieties with high yield, improved quality, storage and processing characteristics, and stress resistance. In fact, potato producers in Syria tend to use imported seeds or tubers which increase the economic costs of the producers' budget, not only because of high costs of imported seeds and tubers, but also the magnitude of yield losses as a result of biotic and abiotic stresses.

Losses in agricultural yield due to water stress probably exceed the losses induced by all other causes combined (Harris *et al.* 2002). Under water stress, leaf turgor is lost and, consequently, cell elongation is inhibited (Taiz and Zeiger 2006). Likewise, water stress reduces photo-assimilation and metabolites required for cell division. As a consequence, impaired mitosis, cell elongation and expansion result in reduced plant height, leaf area and crop growth under water stress (Nonami 1998; Kaya *et al.* 2006; Hussain *et al.* 2008). Moreover, the effects of water stress on leaf number and area and stem length should reduce the number, growth and yield of tubers (Schittenhelma *et al.* 2006).

Although potato cultivars have a lower tolerance for water stress than other crops, it is generally accepted that there are differences in susceptibility to water stress among cultivars (Susnoschi and Shimshi 1985; Jefferies and MacKerron 1987). Screening for drought tolerance is complicated by the fact that the yield reduction cannot be traced back to one or a few major morphological, physiological or biological components (Schapendonk *et al.* 1989), but to their interaction with the environment. Thus, the objective of this study was to assemble and assess a morphological classification of 21 local lines of potatoes, and then to screen them for water stress tolerance. This study permits the establishment of genetic resources for breeding programs to produce potato seeds locally by classical breeding programs or new biotechnologies.

MATERIALS AND METHODS

Plant material and culture conditions

The experiment was carried out in the laboratories of the National Commission for Biotechnology (NCBT, Damascus, Syria). In total, 21 potato local lines were obtained in form of in vitro shoot tips from Plant Genetic Resources Conservation Unit, National Commission for Biotechnology and were studied: SY-C.4, SY-C.5, SY-C.6, SY-C.8, SY-C.9, SY-C.10, SY-C.16, SY-C.30, SY-C.32, SY-C.33, SY-C.36, SY-C.37, SY-C.40, SY-C.41, SY-C.42, SY-C.44, SY-C.45, SY-C.48, SY-C.49, SY-C.50 and SY-C.51. The sprouted healthy tubers of local lines were planted in 50×80 cm slabs divided into holes containing steamy disinfected compost. In order to be used as primary explants, the stems of grown plants were cut into nodal parts consisting of a single node and leaf. Nodal parts were disinfected in a solution of 0.5% (v/v) sodium hypochlorite (Clorox: water, 1: 9) for 5 min. Then they were rinsed with distilled water three times and were transferred on 15 ml of Murashige and Skoog (MS) medium (Murashige and Skoog 1962) supplemented with 20 g.l⁻¹ sucrose and 7 g.l⁻¹ agar. Cultures were then maintained under a 16-h photoperiod with 150 µmol.m⁻¹.s⁻¹ natural light intensity supplemented with sodium vapour pressure lamps at 25±1°C. In vitro grown plants were propagated with at a 4-week interval. Water stress was assessed by transferring single nodes to MS medium containing 0, 2, 4, 6, 8 or 10% (w/v) sorbitol

with eight replicates per treatment. In order to screen the local lines for water stress tolerance, the plants were subjected to stress for a sufficient period to stimulate long-term effects (6 weeks). *In vitro* grown plants were harvested after 6 weeks (all plants were at the tuber initiation stage of development, which is the most drought sensitive development stage in potatoes) for measuring the morphological parameters.

Measurements

Eight plants per line were rinsed in distilled water and separated into leaves, stems and roots. Leaves and roots number were recorded. Roots length and diameter, as well as stem length and diameter were carried out using digital caliper (500-181U Mitutoyo, precision 1/100th). Leaf area was measured using a Li-Cor 3100 area meter (Li-Cor, Lincoln, NE, USA). The plant fresh and dry weights were determined (oven-dried at 70°C for at least 72 h) (Schafleitner *et al.* 2007). Plant water content (PWC %) was estimated according to the equation of Guo *et al.* (2008):

PWC (%) = [(Fresh weight – Dry weight)/Fresh weight] \times 100

Experimental design and statistical analysis

The experiment was designed as a Completely Random Design. Each local line had eight replicates for each treatment. Significant differences between all lines were assessed according to the LSD test at the 5% level (Little and Hills 1968) using R-version 2.5.3 statistical software (The R Project for Statistical Computing, http://www.r-project.org/). To examine the degree of association between parameters studied, correlation coefficients and cluster analysis, based on the sum of relative values of the differences between the control and stressed plants for the ten morphological parameters, were assessed using Systat software.

RESULTS

Growth parameters

Potato growth parameters decreased under water stress mediated by the variation of sorbitol concentration in MS medium. While severe leaf damage was shown at $\geq 6\%$ sorbitol, similar responses to the control were observed at 2% sorbitol (data not shown). Significant differences were only appeared at 4% sorbitol. Therefore, in order to screen the potato local lines for water stress tolerance, only the results of 4% sorbitol, as a critical threshold, are presented.

The water stress significantly ($P \leq 0.05$) affected the stem length and diameter. The stem length has decreased under water stress according to the line. The decrease generally varied between 2- and 3-fold relative to the control (Fig. 1). A relative reduction of stem diameter, depending on the line, was shown (Fig. 2). Unlike SY-C.8, SY-C.9, SY-C.10, SY-C.41, SY-C.42, SY-C.44 and SY-C.48, the remaining lines showed a significant decrease in stem diameter with water stress compared to the control. A distinctive decrease (70, 59, 50 and 46%) was observed in the stem diameter of SY-C.36, SY-C.4, SY-C.5, SY-C.37, respectively. Assuming the plant stem to be a cylinder, the volume (V) can be estimated as a proportional to the square of the stem diameter and length $(\pi^* r^{2*}L)$. Significant differences were observed in the stem volume, not only between lines but although between water stress treatments (data not shown).

The fresh weight decreased significantly under water stress (**Table 1**). Such a decrease reached 82, 72, 67 and 66% in SY-C.5, SY-C.50, SY-C.33 and SY-C.36, respectively. Dry weight under water stress (**Table 1**) increased by 1-2-fold in the majority of lines, with the exception of SY-C.4, where it decreased. The PWC varied depending on the line and water stress treatment (**Table 1**). PWC decreased significantly under water stress.

Leaf number differed depending on the line (**Table 2**). While it reached 13 and 14 leaves in SY-C.44 and SY-C.5, respectively, it was < 12 leaves/plant for the remaining lines.

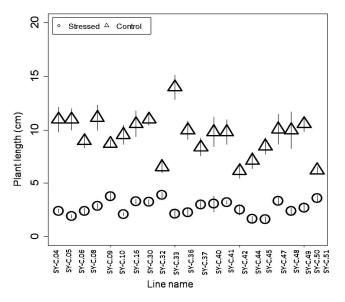


Fig. 1 Stem length according to the lines and water stress treatment. Control: control plants, Stressed: plants subjected to water stress mediated by 4% sorbitol. Values are means \pm standard errors (n=8).

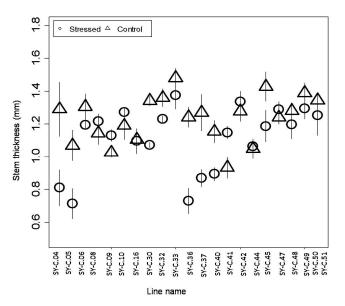


Fig. 2 Stem diameter according to the lines and water stress treatment. Control: control plants, Stressed: plants subjected to water stress mediated by 4% sorbitol. Values are means \pm standard errors (n=8).

Leaf number decreased under water stress. For example, SY-C.5, SY-C.45, SY-C.40 and SY-C.33 showed the highest decrease in leaf number compared to the control (i.e. 72, 38, 37 and 36%, respectively). Total leaf area varied, depending on the line, from 400 to 600 mm² (**Table 2**). This important morphological criterion decreased significantly ($P \le 0.05$) under water stress, regardless of the line. This decrease ranged between 2- and 17-fold compared to the control, in SY-C.32 and SY-C.4, respectively.

Fig. 3 represents the variations in roots number, length and diameter under water stress. Root number varied depending on the line (**Fig. 3A**). SY-C.10 had fewer roots (i.e. 3 roots/plants) while SY-C.8 and SY-C.9 had more roots (i.e. 9 roots/plant). Root number varied under water stress. Several lines showed an increase in root number under water stress, such as SY-C.10, SY-C.30, SY-C.33, SY-C.41, SY-C.42, SY-C.45, SY-C.50, SY-C.6, SY-C.8 and SY-C.9. However, the remaining lines showed a reduction in this trait (**Fig. 3A**). Root length ranged between 5 cm for SY-C.05 and 11 cm for SY-C.44, SY-C.45 and SY-C.49 (**Fig. 3B**). Water stress significantly decreased root length in most lines, greatest in SY-C.4 with a 70% decrease compared to

Table 1 Variations of fresh weight (FW, g), dry weight (DW, g) and plant water content (PWC,%) under water stress according to the line. Control:
control plants, Stressed: plants subjected to water stress mediated by 4% sorbitol. The values are means \pm standard errors (n=8).

Line name	FW		DW		PWC	
	Control	Stressed	Control	Stressed	Control	Stressed
SY-C.4	0.55 ± 0.13	0.13 ± 0.01	0.05 ± 0.008	0.05 ± 0.002	62.79 ± 1.30	90.61 ± 0.75
SY-C.5	0.36 ± 0.09	0.06 ± 0.02	0.03 ± 0.007	0.03 ± 0.010	64.33 ± 7.09	90.23 ± 0.43
SY-C.6	0.53 ± 0.05	0.22 ± 0.03	0.04 ± 0.003	0.09 ± 0.006	55.86 ± 8.47	92.22 ± 0.31
SY-C.8	0.64 ± 0.09	0.57 ± 0.08	0.05 ± 0.006	0.11 ± 0.017	77.38 ± 3.94	91.67 ± 0.23
SY-C.9	0.31 ± 0.03	0.27 ± 0.04	0.03 ± 0.003	0.09 ± 0.016	66.75 ± 2.11	90.53 ± 0.45
SY-C.10	0.46 ± 0.11	0.26 ± 0.03	0.04 ± 0.007	0.09 ± 0.01	64.28 ± 5.85	91.38 ± 0.37
SY-C.16	0.56 ± 0.07	0.38 ± 0.06	0.05 ± 0.006	0.07 ± 0.01	82.33 ± 2.13	90.64 ± 0.46
SY-C.30	0.63 ± 0.06	0.31 ± 0.05	0.04 ± 0.003	0.06 ± 0.006	80.12 ± 0.97	93.2 ± 0.35
SY-C.32	0.60 ± 0.10	0.45 ± 0.09	0.04 ± 0.005	0.08 ± 0.014	80.71 ± 1.30	92.41 ± 0.50
SY-C.33	0.94 ± 0.16	0.38 ± 0.08	0.07 ± 0.01	0.06 ± 0.011	82.97 ± 0.67	92.85 ± 0.36
SY-C.36	0.88 ± 0.18	0.30 ± 0.04	0.05 ± 0.01	0.08 ± 0.007	71.42 ± 2.87	93.99 ± 0.52
SY-C.37	0.71 ± 0.07	0.29 ± 0.04	0.06 ± 0.004	0.08 ± 0.008	69.81 ± 4.13	91.86 ± 0.47
SY-C.40	0.75 ± 0.16	0.26 ± 0.07	0.06 ± 0.009	0.06 ± 0.013	72.44 ± 2.08	90.87 ± 0.54
SY-C.41	0.94 ± 0.09	0.36 ± 0.03	0.07 ± 0.005	0.09 ± 0.006	75.38 ± 0.82	93.06 ± 0.29
SY-C.42	0.47 ± 0.08	0.32 ± 0.07	0.04 ± 0.006	0.08 ± 0.015	74.68 ± 2.68	91.74 ± 0.66
SY-C.44	1.10 ± 0.12	0.29 ± 0.04	0.05 ± 0.01	0.06 ± 0.005	76.50 ± 2.66	95.27 ± 0.75
SY-C.45	1.26 ± 0.32	0.34 ± 0.04	0.07 ± 0.012	0.06 ± 0.007	81.14 ± 0.49	88.80 ± 5.71
SY-C.48	0.61 ± 0.08	0.58 ± 0.13	0.06 ± 0.01	0.08 ± 0.01	80.84 ± 3.70	90.15 ± 2.27
SY-C.49	0.92 ± 0.23	0.60 ± 0.09	0.07 ± 0.013	0.13 ± 0.017	77.63 ± 1.61	92.47 ± 0.59
SY-C.50	0.94 ± 0.10	0.45 ± 0.07	0.06 ± 0.012	0.10 ± 0.011	78.48 ± 1.08	93.30 ± 0.45
SY-C.51	1.08 ± 0.15	0.52 ± 0.11	0.07 ± 0.010	0.12 ± 0.019	67.49 ± 6.79	93.78 ± 0.15

Table 2 variations of leaves number and leaves area (mm^2) under water stress according to the line. Control: control plants, Stressed: plants subjected to water stress mediated by 4% sorbitol. The values are means \pm standard errors (n=8).

Line name]	Leaves number	Lea	Leaves area (mm ²)		
	Control	Stressed	Control	Stressed		
SY-C.4	7.66 ± 0.66	2.40 ± 0.25	26.80 ± 4.03	475.33 ± 95.68		
SY-C.5	14.00 ± 1.43	3.83 ± 0.91	59.51 ± 11.47	573.17 ± 126.24		
SY-C.6	12.00 ± 1.54	6.40 ± 0.4	118.60 ± 5.41	460.67 ± 113.53		
SY-C.8	10.83 ± 0.74	6.42 ± 0.61	159.29 ± 19.40	575.33 ± 67.99		
SY-C.9	10.33 ± 0.76	6.55 ± 0.41	149.22 ± 30.83	432.83 ± 68.64		
SY-C.10	9.17 ± 0.47	4.89 ± 0.48	82.78 ± 8.60	413.33 ± 87.09		
SY-C.16	9.83 ± 0.95	8.50 ± 0.79	235.00 ± 48.79	607.17 ± 127.50		
SY-C.30	11.00 ± 0.31	6.14 ± 0.59	77.43 ± 9.25	407.00 ± 45.65		
SY-C.32	9.66 ± 0.33	7.77 ± 0.54	239.89 ± 43.09	491.17 ± 92.06		
SY-C.33	10.00 ± 0.25	4.87 ± 0.66	44.13 ± 8.27	535.83 ± 83.39		
SY-C.36	10.50 ± 0.76	9.22 ± 1.43	125.67 ± 23.39	488.50 ± 64.76		
SY-C.37	10.00 ± 0.81	9.55 ± 1.66	132.14 ± 36.35	495.00 ± 26.46		
SY-C.40	10.50 ± 0.84	5.28 ± 1.08	67.00 ± 18.82	467.16 ± 56.59		
SY-C.41	8.16 ± 0.54	4.44 ± 0.68	97.89 ± 17.01	422.83 ± 47.60		
SY-C.42	9.42 ± 0.52	8.75 ± 0.85	170 ± 34.89	506.57 ± 65.78		
SY-C.44	13.16 ± 0.65	9.12 ± 0.87	107.50 ± 8.90	422.33 ± 97.55		
SY-C.45	12.00 ± 1.03	11.71 ± 2.04	146.86 ± 26.30	514.50 ± 103.47		
SY-C.48	10.66 ± 0.55	10.5 ± 1.08	234.88 ± 36.19	520.00 ± 79.47		
SY-C.49	10.80 ± 1.24	8.87 ± 1.04	260.13 ± 58.92	608.21 ± 109.16		
SY-C.50	11.33 ± 0.66	7.00 ± 1.12	141.66 ± 32.89	571.67 ± 101.39		
SY-C.51	10.00 ± 1.00	8.11 ± 0.63	188.44 ± 49.36	582.80 ± 86.93		

the control. Likewise, root diameter changed under water stress depending on the line (**Fig. 3C**). Water stress significantly reduced this parameter in all lines with the exception of SY-C.09, SY-C.32 and SY-C.42, where it increased. The decline in root diameter varied between 31 and 78% compared to the control in SY-C.4 and SY-C.33, respectively.

Å correlation analysis of the morphological parameters (Fig. 4) showed a strong correlation between leaf area and fresh weight through a linear model (Fig. 4A), and plant length through a binominal model (Fig. 4B). These models were fitted using the datasets of both control and stress treatments. However, the parameters of linear models between fresh and dry weight were significantly different according to the water stress treatment, so they were illustrated as separated linear models (Fig. 5).

Cluster analyses

The lines tolerance for water stress were evaluated by clus-

ter analysis based on the sum of relative values of the differences between the control and stress treatments for the 10 morphological parameters (**Fig. 6**). Three distinct groups resulted. Firstly, the water-stress tolerant group consisting of six lines: SY-C.09 > SY-C.32 > SY-C.42 > SY-C.49 > SY-C.48 > SY-C.08. Secondly, the moderately tolerant group consisting of eight lines: SY-C.10 > SY-C.51 > SY-C.16 > SY-C.06 > SY-C.30 > SY-C.37 > SY-C.41 > SY-C.50. And thirdly, the sensitive group consisting of seven lines: SY-C.36 > SY-C.44 > SY-C.33 > SY-C.45 > SY-C.40 > SY-C.05 > SY-C.04.

DISCUSSION

Screening an enormous numbers of lines and varieties in the field for their responses to stress can be exhausting and expensive work. Thus, *in vitro* screening of lines for stress tolerance is an alternative approach (Zhang and Donnelly 1997; Aghaei *et al.* 2008). Various studies have revealed similar effects of *in vitro* water or salinity stress on plants to

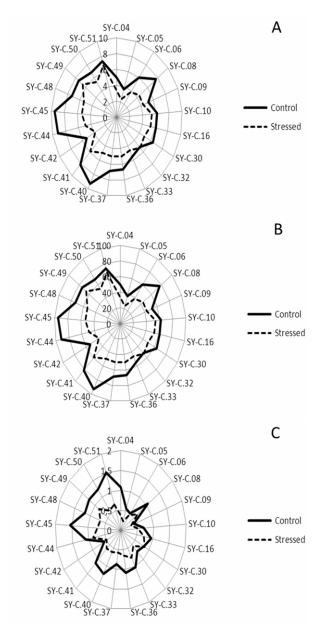


Fig. 3 Average of root number (A), length (B) and diameter (C) for 8 plants per treatment. Control: control plants, Stressed: plants subjected to water stress mediated by 4% sorbitol.

those observed in field (Zhang and Donnelly 1997; Gopal and Iwama 2007; Aghaei *et al.* 2008). Sorbitol is an alditol found in higher plants that is considered to be a nonmetabolite because it is metabolically more inert than other saccharides (Lambers *et al.* 1981). Limited information is available about the effects of sorbitol on plant growth. However, Liu and Lai (1991) found that sorbitol induced osmotic stress in rice callus. Sorbitol was also used to induce osmotic stress in suspension-cultured sweet potato cells (Wang *et al.* 1999), in cucumber callus (Abu-Romman 2010) and in cucumber microshoots (Abu-Romman and Suwwan 2011). Moreover, Gopal and Iwama (2007) reported that sorbitol decreased plant water potential, inducing water stress, so they used it as a method to screen potato genotypes for drought tolerance.

Earlier studies showed a decrease in potato plant length (Deblonde and Ledent 2001; Tourneux *et al.* 2003; Lahlou and Ledent 2005), leaf area (Jefferies and MacKerron 1987) and fresh and dry weight (Heuer and Nadler 1995) under stress conditions. Furthermore, the reduction in yield, including tuber number and dry matter accumulation, can be as a result of reduced plant growth under stress conditions (Jefferies 1993; Deblonde *et al.* 1999). In this study, the growth parameters (i.e. stem length and diameter; leaf num-

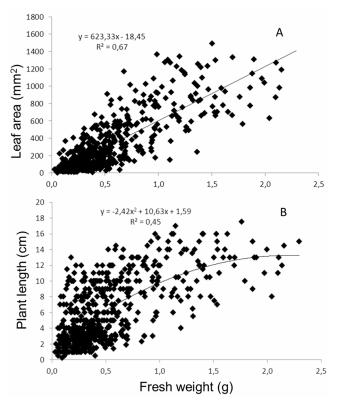


Fig. 4 Correlation between leaf area and fresh weight (A) and plant length (B) for all lines and treatments.

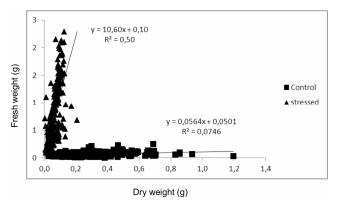


Fig. 5 Correlation between fresh and dry weight for all lines and treatments.

ber and area; number, length and diameter of roots; fresh and dry weight and PWC) decreased and differed under water stress depending on the line. Our results that showed a reduction in growth criteria under water stress are identical to those of others (Frensh 1997; Schittenhelma *et al.* 2006; Sánchez-Rodríguez *et al.* 2010).

Potato is considered to be highly susceptible to water stress (Frusciante et al. 1999; Iwama and Yamaguchi 2006; Hassanpanah et al. 2008), while several studies have indicated that such susceptibility to water stress differs depending on the line (Steckel and Gray 1979; Levy 1983, 1986; Susnoschi and Shimshi 1985). Screening of potato lines to water stress tolerance is an economic agricultural project in Syria. Taking into account that screening under field conditions is complicated, screening crops in vitro for stress tolerance has been used as an alternative approach. Frusciante et al. (1999) has suggested some morphological and physiological parameters like canopy expansion, chlorophyll fluorescence and leaf water content as characters for stress tolerance screening while others have used only morphological (Zhang and Donnelly 1997) or physiological (Ranalli et al. 1996; Rampino et al. 2006), or both morphological and molecular parameters (Aghaei et al. 2008). In

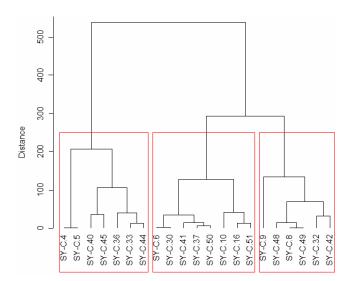


Fig. 6 Dendrogram based on the sum of relative values of ten morphological and physiological growth parameters of potato lines under drought. "Distance" is the distance between lines (unless unit). The dendrogram is cut at a certain level into several groups either by specifying the desired number of groups or the cut height.

this study, 10 growth plant parameters were used to screen 21 *in vitro* potato local lines for water stress tolerance. The screening results revealed three distinct groups: tolerant, moderately tolerant and sensitive lines (**Fig. 6**).

We recommend screening potato genotypes for water stress tolerance using the responses of plant growth under water stress mediated by 4% sorbitol. This method could be used to identify suitable parental lines with improved stress tolerance.

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