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Evaluations morphological, physiological and biochemical of three tomato varieties (*Lycopersicum esculentum*) under salt stress at the University Jean Lorougnon Guede (Côte d'Ivoire)

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Abstract

Tomato, *Lycopersicom esculentum* Mill is a glycophyte plant, sensitive to salinity. However, the accentuated climate change causes abiotic (salinity) and biotic stress reducing plants production. Methodology: To better tame these plants, it is important to understand the mechanisms revealed during stress. Indeed, four concentrations of NaCl (0, 2, 4 and 6 g/L) were applied to seedlings of three tomatoes varieties grown in pots under greenhouse. Thus, morpho-physiological and biochemical behavior of varieties (Petomech, UC82B and Tropimech) were evaluated. Results: The results show that NaCl significantly reduces the morphological parameters. However, this reduction is pronounced in 6 g /L (NaCl). In physiological level, the NaCl significantly reduced water content of plant, chlorophyll a, b and total and the carotenoid. This reduction is independent of variety studied. Moreover, at biochemical level, proline increases with increasing salt concentration. Other side, catalase and ascorbate peroxidase, activity decreases significantly with increasing NaCl. After this work, all parameters studied were used to explain mechanisms involved in metabolism of tomato plants grown under salt stress.

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Introduction

Ivorian agricultural products account for 33% of gross domestic product (GDP) and 75% of export earnings. Agriculture also provides employment to 67% of the workforce. However, this agriculture is primarily based on industrial crops (wood, coffee, cocoa, cotton, rubber, oil palm, cashew, pineapple etc.) to the detriment of food crops which nevertheless are part of our daily regarding food. Of these crops, we can cite the tomato that plays a very important socio-economic role. The tomato, a widely cultivated plant in the world, is one of the most important vegetables in the diet after the potato. It is consumed fresh or processed (Derkaoui, 2011). It is cultivated in all latitudes with an area of about 3 million hectares (Abir *et al*., 2006). The global tomato production has continued to grow steadily in recent decades. It increased from 48 million tonnes in 1978 to 124 million in 2006 (Toufouti, 2013). The tomato is low in calories but rich in essential amino acids, vitamin B and C, iron and phosphorus where his interest in a balanced diet in preventing obesity (Chougar, 2011)

In Ivory Coast the annual production fluctuates between 22,000 and 35,000 tonnes (Sangare *et al*., 2009). Low productivity creates a structural deficit in the supply of tomato products. Indeed, the need for tomato estimated at over 100,000 tons are covered only 2/3 by local production (Soro *et al*., 2008). The country therefore imports a large quantity of tomatoes to meet demand.

However, despite the increase in world production of tomatoes, culture is facing many difficulties. These problems are due not only to climate change and the misuse of pesticides and especially the use of poor quality irrigation water (acid rain) that lead to soil salinity. Salt reduces crop yields and mainly vegetable crops which includes tomato (Belfakih, 2013). According to FAO, the most recent estimates show that salinity already affects at least 400 million hectares and threatens equivalent surfaces. Thus, the search for plants adapted to high salinity levels and understanding the mechanisms becomes imperative for agricultural production.

This study fits into the general context of food security and aims to evaluate the effect of salt stress on three tomato varieties grown in Cote d'Ivoire precisely in the department of Daloa.

Material and methods

Study site

The study was conducted in the University Jean Lorougnon Guede located in the town of Daloa in the west central Cote d'Ivoire. The geographical position is given by the following coordinates 6 ° 53 'North 6 ° 27'Ouest. It is located in a forest area with temperatures from 21-31 ° C. It is located 141 km from the political capital Yamoussoukro and 383 km from Abidjan the economic capital. The climate of the region is a four season climate. A great rainy season complicated by shoulder seasons and marked by storms (April to mid-July), the short dry season (mid-July to mid-September), a small rainy season (mid-September to November) and large dry season (December to March).

Experimental design

Three local varieties of tomatoes (*Lycopersicom esculentum*) were used for this experiment. These are varieties Petomech, UC82 and B Tropimech. The seeds were purchased in the market Daloa. These varieties are fixed with a specific growth and high production. Three ridges of 2m length, height 20 cm, width 1m were used for the realization of the nursery for the production of tomato plants. These logs were treated with two nematicide and fungicide. The transplanting was done in 4-liter pots containing the treated soil. The pots were perforated at the base and a thin layer of gravel was deposited before filling with soil. Pots, under glass, are deposited on a support. Some days (21) after seed germination, the more vigorous plants were planted in pots. This experiment was conducted in a greenhouse 3m height. Salt solutions (2, 4 and 6 g.l-1) NaCl were used to water tomato selected plans. The witness watered by tap water. NaCl solutions and water were applied four days after planting tomato plants to the capture morphological data.

Data collection

During this experience, some morphological, physiological and biochemical parameters were evaluated after two months of treatment plants with different NaCl solutions and water. The vegetative parameters such as the diameter of the rod, the height and size of the plant, number of roots and finally sheet by plant leaf area were evaluated. Regarding the physiological parameters, variables such as the water content of plants, relative water content of the sheets (ERR) and the determination of chlorophyll pigments and carotenoids were determined. Extraction and assay of proline, catalase (cat) and ascorbate peroxidase were performed as biochemical parameters (Table 1).

Statistical analysis of the data

The one-way analysis of variance (ANOVA 1) was used to evaluate the effect of the concentration, variety and interaction through the comparison of the mean for each parameter. When a significant difference was observed ($P < 0.05$) between the different factors studied for a given parameter, multiple comparisons were performed using the test Least Significant Difference (LSD). This test identified the factor that significantly induces this difference.

Results

NaCl effect on germination

The depressive effect of NaCl was tested on the germination capacity of grain three tomato varieties to determine the concentrations that allow good germination of these seeds.

Table 1. Method of measurement of yield components of tomato varieties.

Yield and yield components	Measurement approach and sample size per plot
Morphological parametersperformed on three plants per treatment (concentration**)**	
Stem diameter (Cm)	Measuring the circumference of the rods, made on three plants per treatment (concentration)
Plant height (Cm)	Measuring the distance separating the outermost sheet from the surface, carried out on three plants per treatment (concentration)
Scale plants (Cm)	Measuring the distance separating the two most extreme sheets, carried out on three plants per treatment (concentration)
Number of sheets	Effective of all the leaves on three plants per treatment (concentration)
Foliar area (Cm^2)	Measuring the surface of the sheet, carried out on three plants per treatment (concentration)
Number of roots	Effective of all roots on three plants per treatment (concentration)
Root depth (Cm)	Measuring the length of the roots, carried on three plants per treatment (concentration)
Physiological parameters performed on three plants per treatment (concentration)	
Relative water content of leaves (%)	Difference between the fresh weight and dry weight of the leaves multiplied by 100
Water content of the plant TEP (ml)	Difference between the fresh weight and dry weight of the whole plant
Chlorophyll a (µg/mL)	Extraction and determination of chlorophyll pigments(a)
Chlorophyll b (µg/mL)	Extraction and determination of chlorophyll pigments(b)
Chlorophyll Total (µg/mL)	Extraction and determination of total chlorophyll pigments(t)
Carotenoide (µg/mL)	Extraction and determination of carotenoids
Biochemical parameters performed on three plants per treatment (concentration)	
Proline content (µg/g MF)	Extraction and determination of proline
Catalase (mol/mn/g MF)	Extraction and determination of catalase
Ascorbate Peroxydase (mol/mn/g MF)	Extraction and determination of catalase and ascorbate peroxidase

The results for the germination rate have shown that NaCl greatly reduces the germination of grains of three varieties. Thus, the lower the germination rate (2%) is obtained with the concentration 8 g / l. And beyond this concentration, no seed has germinated. These results allowed to wear our choices on levels 2, 4 and 6 g / l NaCl for evaluation of morphological parameters, physiological and biochemical (Figure 1).

Influence of the salt concentration and variety of the parameters studied three tomato varieties

Table 2 shows that all analyzed variables were significantly affected by the concentration factor. For cons, the variety and interaction effect had no influence on morphological, physiological and biochemical parameters analyzed giving all identical values. Consequently, only the results of the effect of NaCl concentration will be presented.

Table 2. Results of statistical tests assessing the influence of the salt concentration, variety and interaction concentration-varieties of the parameters studied three varieties of tomato.

Variables	Concentrations		Varieties		Concentrations-varietiess	
	F	P	F	P	F	P
Morphological parameters performed on three plants per treatment (concentration)						
Stem diameter(Cm)	14.714	< 0,001	1,255	0,303	0,152	0,986
Plant height(Cm)	4,140	0,016	3,735	0,038	0,668	0,676
Scale plants(Cm)	15,565	< 0,001	17,819	0,10	1,655	0,175
Number of sheets	4,244	0,015	4,272	0,25	0,259	0,950
Foliar area(Cm²)	11,228	< 0,001	0,585	0,564	0,288	0,936
Number of roots	6,726	< 0,001	10,616	0,15	0,279	0,941
Root depth(Cm)	4,642	< 0,001	0,170	0,844	0,898	0,512
Physiological parameters performed on three plants per treatment (concentration)						
Relative water content of leaves(%)	36,160	< 0,001	2,180	0,134	0,790	0,585
Water content of the plantTEP (ml)	8,833	< 0,001	2,543	0,099	1,078	0,403
Chlorophyll a (µg/mL)	15,565	< 0,001	1,449	0,306	0,175	1,655
Chlorophyll b (µg/mL)	9,840	< 0,001	0,174	0,843	0,432	0,849
Chlorophyll Total (µg/mL)	40,517	< 0,001	0,914	0,414	0,732	0,628
Carotenoide (µg/mL)	30,885	< 0,001	1,167	0,328	0,526	0,782
Biochemical parameters performed on three plants per treatment (concentration						
Proline content(µg/g MF)	169,85	< 0,001	2,152	0,138	5,166	< 0,001
Catalase (mol/mn/g MF)	11,429	< 0,001	2,882	0,075	1,679	0,169
Ascorbate Peroxydase (mol/mn/g MF)	13,135	< 0,001	0,195	0,823	1,365	0,267

Effect of different NaCl concentrations on the morphological parameters of three tomato varieties

Salinity has a significant influence on the development of leaves, number of roots and scale of the studied tomato plants (Table 3).

These parameters decrease with increasing NaCl concentration in the medium. Thus, lower values for these parameters are obtained at concentrations of 4 and 6 g / L NaCl. For cons, the highest values are observed in the control and the lowest concentration (2 g / L).

The results showed a complete difference in the depths of the roots for the four concentrations studied.

Thus, when the NaCl concentration increases, the depth of the roots increases. The greatest value of the depth of the roots is obtained at the highest concentration (6 g / L). In the parameters such as the diameter of the rod, the plant height and leaf area, a partial difference has been observed. This difference shows that these parameters decrease with increasing concentration of the medium. And the larger values are obtained with low concentrations of NaCl.

Table 3. Effect of different NaCl concentrations on morphological parameters.

	stem diameter(Cm)	plant (Cm)	height Scale (Cm)	plants Number sheets	of Foliar (Cm²)	area Number of roots	Root depth (Cm)
Witness	0,4± 0,05 ^{a}	32,8± 4,09^{a}	34,7± 3,3 ^{a}	8,51± 0,80 ^{a}	48,1± 5,04 ^{a}	175,6± 22,59 ^{a}	10,7 ±1,63^{d}
2 g/L	0,3± 0,4 ^{b}	31,2± 2,06^{ab}	32,3±3,1^{ab}	7,6± 0,91 ^{ab}	44,14±2,7 ^{ab}	150,4±25,7^{ab}	12,54±3,11^{c}
4 g/L	0,3±0,1 ^{bc}	29,3± 2,53^{ab}	30,0±3,2^{b}	7,37± 0,63 ^{b}	41,41± 5,6^{b}	143,7± 23,18 ^{b}	13,2± 1,33^{b}
6 g/L	0,3± 0,02 ^{c}	28,6± 2,70 ^{b}	28,7±2,4^{b}	7,19± 1,11 ^{b}	35,4± 3,46 ^{c}	139,81±18,18^{b}	14,3±1,37 ^{a}
P	< 0,001	0,016	< 0,001	0,015	< 0,001	0,001	0,010
F	14,717	4,140	15,565	4,244	11,228	6,726	4,642

Mean values within column by parameter followed by the same superscriptedletter were not significantly different at p = 0.05 level, on the basis of the least significant difference test.

NaCl's effect on physiological parameters of varieties of Tomato

The statistical analysis of these parameters showed a significant effect on physiological parameters for the different concentrations of NaCl used (Table 4). The results show that in the total chlorophyll and carotenoid, the highest values were obtained at the low concentration (2 g / L). Beyond 2 g / L, it was observed that concentrations of 4 g / L and 6 g / L have the same values at these two parameters. At the water content and chlorophyll (a), a decrease of the values was observed with the increase in salt concentration.

Table 4. Results of statistical tests assessing the influence of salt concentration on the relative water content of the leaves, the water content and the contents of chlorophyll pigment tomato plants.

	TRE	TEP	(Chl a)	(Chl b)	(ChlT)	Caro
Witness	66,58 ± 7^{a}	10,1 ± 0,8^{a}	11,2±2,2 ^{a}	6,2 ± 2,3 ^{a}	17,77± 4,3 ^{a}	9,923 ± 2,6 ^{a}
2 g/L	60,1 ± 3,3 ^{a}	9,23 ± 1,4 ^{a}	9,4± 2,1 ^{a}	5,1± 2,2 ^{a}	13,6 ± 2,4 ^{b}	7,00 ± 1,7 ^{b}
4 g/L	47,2 ± 5,8 ^{b}	8,37 ± 0,5 ^{ab}	5,8± 2,1 ^{b}	2,7± 1,0 ^{b}	7,20 ± 2,1 ^{c}	3,79± 1,4 ^{c}
6 g/L	40,5 ± 7,1^{b}	7,75 ± 1,1^{b}	2,9± 0,9 ^{c}	1,8± 1,3 ^{b}	4,48 ± 1,2 ^{c}	2,23± 0,7 ^{c}
P	< 0,001	< 0,001	< 0,001	< 0,001	< 0,001	< 0,001
F	36,162	8,830	15,565	9,894	40,517	30,885

Mean values within column by parameter followed by the same superscriptedletter were not significantly different at p = 0.05 level, on the basis of the least significant difference testTRE : teneur relative en eau des feuilles; TEP : Teneur en eau de la plante; Chl a: Chlorophylle a; Chl b: Chlorophylle b; Chl t: Chlorophylle Totale ; Caro : Caroténoïde.

NaCl's effect on biochemical parameters varieties Tomato

The analysis of variance showed a significant effect of salt concentration on the biochemical parameters tested (Table 5). Indeed, under the effect of salt stress, proline is accumulated in the leaves. This accumulation is either a function of the intensity of stress or salt concentration. Thus, accumulation highest proline is observed in concentrations 4 and 6 g / L NaCl. By cons, Under the effect of the same salt stress, the activity of catalase and ascorbate peroxidase decreases relative to controls in three tomato varieties studied. The lower activity in both enzymes was observed in the highest concentration of NaCl (6g / L). With a reduction rate compared to the control of 85% for each of the enzymes studied.

Discussion

Morphological parameters

The results of this study revealed decrease morphological and physiological parameters of the three varieties according to the increase of NaCl concentration.

Similar results were obtained by Hassani (2008) on some chili varieties. Indeed this author in a study on pepper varieties showed that high salt concentrations have a negative influence on the growth of the pepper varieties. The decrease in the growth of the vegetative observed in tomato plants can be explained by the fact that the NaCl acts by increasing the osmotic pressure of the medium.

This prevents the absorption of water by the root system. This lack of water absorption leads to the reduction in growth is a result of a decrease in the number of cell divisions (Benmahioul *et al.* 2009). The reduction of the growth may result from the increase in abscisic acid concentration in the aerial part or cytokinin concentration reduction.

Table 5. Results of statistical tests assessing the influence of salt concentration on the biochemical parameters of the leaves of three varieties of tomato.

	Proline content	Catalase	Ascorbate Peroxidase
Witness	0,331 ± 0,09 ^{c}	0,2647 ± 0,17 ^{a}	0,00245 ± 0,001 ^{a}
2 g/L	0,500 ± 0,06 ^{c}	0,1153 ± 0,08 ^{b}	0,00102 ± 0,001 ^{b}
4 g/L	0,809 ± 0,12 ^{b}	0,0660 ± 0,04 ^{bc}	0,00073 ± 0,0004 ^{bc}
6 g/L	1,483 ± 0,28 ^{a}	0,0435 ± 0,03 ^{bc}	0,00036 ± 0,0002 ^{bc}
P	$<$ 0,001	$<$ 0,001	$<$ 0,001
F	169,850	11,429	13,135

Mean values within column by parameter followed by the same superscripted letter were not significantly different at p = 0.05 level, on the basis of the least significant difference test;

The reduction in growth is an adaptation strategy allowing the plant to reduce resource spending. These strategies, implemented to maintain homeostasis under conditions of stress, are consuming energy and resources they divert the expense of growth. According to our results, the salt stress causes a delay in plant growth. This results in a reduction in plant height. A decrease in leaf area that is accompanied by symptoms of stress such as foliar chlorosis and necrosis, leading to death of the leaves. This same symptom was observed by Chartzoulakis and Klapaki, (2000). This result is explained by a specific harmfulness of Cl ions accumulated at levels exceeding the compartmentation capacity. These toxicity symptoms reduce the active surface for the photosynthesis and markedly reduced growth. Our results are similar to those of Munns and Tester (2008) who observed that the vegetative growth, and particularly leaf expansion, is severely inhibited by salt stress. We also note that salinity further reduced the growth of the aerial parts of the tomato compared to that of roots, this agreeing well with the results of the work of Kara and Brinis (2012), which explain that the reduction of growth of parts air is an adaptive capacity necessary for the survival of plants exposed to abiotic stress.

Indeed, developmental delay allows the plant to accumulate energy and resources to combat stress before the imbalance between the inside and outside of the organism increases to a point where the damage is irreversible.

Physiological parameters

The physiological approach made in this study shows that chlorophyll a, b, and total carotenoid is negatively influenced by salt diet. Indeed, in our study, a decrease in the content of these pigments was observed with increasing salt concentration. This is explained by the fact that a plant under stress reduced all metabolic activities that may induce excessive energy expenditure. Thus, reduction of the leaf surface involves a reduction in CO_2 uptake and consequently a reduction of the photosynthetic activity. The similar results were observed by Baghizadeh explains that under saline regime, chlorophyll a, b, and total carotenoids were significantly reduced in two wheat varieties (Baghizadeh *et al.*, 2014). Other studies have also revealed similar results to ours. Indeed, Abbas *et al.* (2013) found that excessive amounts of toxic ions in leaf tissue of tomato cultivars can act as agent degrading chlorophyll pigments.

Achour *et al.* (2015) in their work on two varieties of okra (*Abelmoschus esculentus* L.) observed a decrease in the synthesis of chlorophyll and explains that this decrease in chlorophyll synthesis may be due, among other things, a decrease of 5-aminolevulinic acid. According Nunkaew *et al.* (2014) NaCl inhibits the synthesis of 5-aminolevulinic acid, a precursor of chlorophyll. In addition, the growing of plants in saline solutions is known to damage the photosynthetic enzymes (Youssef Awad, 2006).

The study also revealed a decrease in the relative water content of the leaves and the water content in the tomato varieties. Indeed, increasing the NaCl concentration results in a decrease of tissue hydration increasing the concentration inside and therefore prevents the water outlet of the plant to the outside. The intensification of salt treatment is accompanied by a decrease in the level of hydration which still remains tolerable. Maintaining a relative high content of water, under salt stress, is a remarkable form of resistance.

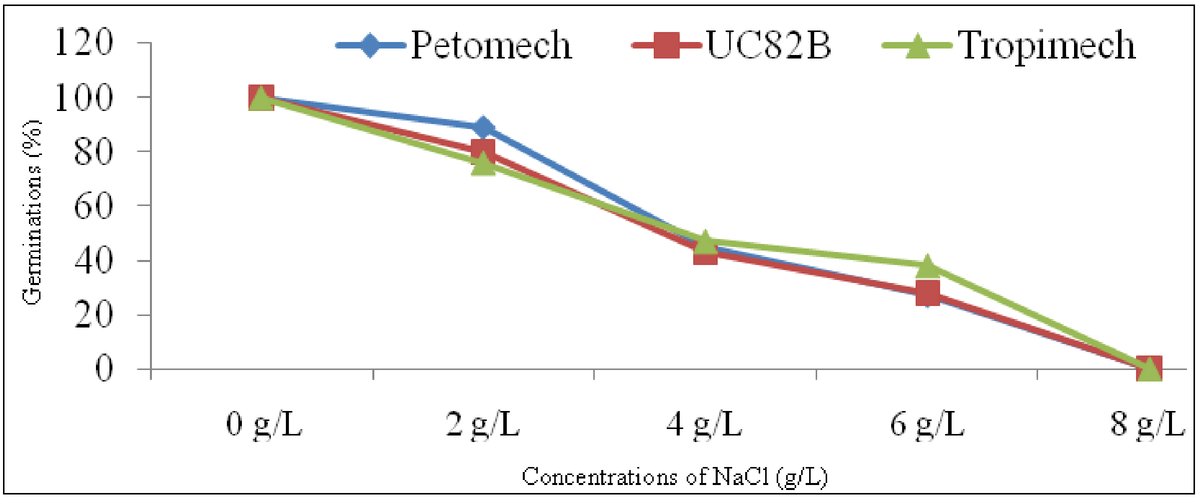

Fig. 1. NaCl effect on germination.

Biochemical parameters

The results of biochemical parameters showed significant differences in the activities of catalase and ascorbate peroxidase between the control plants and treaties. In fact, a negative correlation was observed upon application of different treatments for these two enzymes. This decrease of the activity of catalase and ascorbate peroxidase is due not only to the development stage of the plant but also to various NaCl concentrations because beyond a certain concentration (6g / L) increased the activity of these two enzymes was observed (Yang et Poovaiah, 2002) .These Similar results are observed by Naike *et al.* (2005). According to these authors, the increasing concentrations of NaCl, cause a decrease in the activity of catalase and ascorbate peroxidase. This indicates that the high salinity generally reduces the activity of catalase whatever the variety studied up to a certain concentration.

However, our results differ from those Rajaravindran and Natarajan (2014) by which their work has revealed that the activity of these enzymes increases with increasing salt concentration. According to them, the catalase is essential to maintain the redox balance during oxidative stress. It works well as a cell and allows the plant to stand in water or salt stress. A positive correlation between the intensity of the accumulation of free catalase and stress tolerance has been suggested as an indicator to determine the potential stress tolerance of the cultivars. The main reason for the increase in the concentration of catalase during salt stress is due to the continuous synthesis of this amino acid during stress (Tabatabaei, 2013). The results obtained in this study indicate that excess salt causes in plants proline accumulation.

Proline, usually low in tissues of plants grown on salt-free environment and therefore not binding on the water plan is accumulated dramatically in response to salt stress. Several authors whose Mohamdi *et al.* (2008), had mentioned that this amino acid is part of osmoticums synthesized by plants when exposed to water or salt stress. Its role is necessary for tonicity adjustment and to balance the osmotic potential of the plant.

Conclusion

Salt stress exerts in three varieties of tomatoes a depressive effect on all morphological parameters, physiological and biochemical. The degree of sensitivity or tolerance depends on the variety, and the intensity of stress. In fact, the contents of chlorophyll a; b total and are very sensitive parameters that are indicators of the degree of tolerance of the three varieties of tomato.

The action of salt on the three varieties has led proline contents that go along with the NaCl concentration. The selection of salt tolerant plant material depends on a thorough understanding of the physiological and biochemical mechanisms. Finally this study showed that there is some variability genotypic analyzed for metabolites, they may therefore represent potential criteria to characterize the salt tolerance in tomato.

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