

## IN VITRO SELECTION AND CHARACTERIZATION OF SALVIA OFFICINALIS SALT-TOLERANT CLONES

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Scientific J. Flowers &  
Ornamental Plants,  
8(1):123-134 (2021).

Received:

18/1/2021

Accepted:

20/2/2021

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**ABSTRACT:** *Salvia officinalis* L. is a widest-spread attractive plant in different pharmacopoeias throughout the world. Shoot clumps were regenerated from leaf explants on MS medium enriched with 1 mg/l IAA + 1.5 mg/l TDZ. A 12-week old shoot clumps were subcultured on the same medium supplemented with 100 mM of NaCl. After 12 weeks 4 shoots were recovered from 60 cultured shoot clumps. These shoots were elongated and individually cloned on MS medium enriched with 1 mg/l IAA. Single nodes of the recovered clone, as well as, the control plants were cultured using a single node explants on MS medium enriched with 1 mg/l IAA and 0 or 100 mM NaCl. After a month of growth on this medium statistical analysis showed significant difference among the selected clones and the control plants on the base of the ability to develop new growth, plant height, shoot and root fresh and dry weights, as well as, chlorophyll content. Moreover, there were significant differences among the selected colons on proline and total soluble protein content. Also, some regenerated clone showed higher activity of the free radical scavenging enzymes; catalase, peroxidase, and ascorbate peroxidase under salt stress. Therefore, somaclonal variation was used to select a drought tolerant *S. officinalis* clone. Moreover, some physiological mechanisms that are crucial for salt stress tolerance were underlined.

**Key words:** abiotic stress, low-molecular-weight osmolytes, sage, scavenging enzymes, somaclonal variations.

## INTRODUCTION

*Salvia officinalis* L. (sage or salvia) which belongs to the Labiate family is a widest-spread attractive plant. It features clearly in different pharmacopoeias throughout the world. *Salvia* plants have been used in numerous ways, e.g. essential oils, the leaves, the flowers, the seed oil, and the roots (Dweck, 2000). The traditional use of this herb in the domestic medicine is endless; as a carminative, bactericidal, spasmolytic, mouthwash against its inflammation, a wound-healing agent, in skin and hair treatment; and against rheumatism and sexual debility in treating mental and nervous conditions as well as an

insecticidal (Dweck, 2000 and Baricevic and Bartol, 2000).

Salinity and drought are expected to cause thoughtful salinization of more than half of world land by 2050. The growth and productivity of numerous crops is badly affected by these stresses causing enormous economic losses in affected areas (Rao and Sandhya, 2019). About 20% of world irrigated land is significantly affected by salinity, decreasing crop yields. Plants are affected by salt stress in two chief ways: osmotic stress and ionic toxicity. These stresses affect all major plant processes, including photosynthesis, cellular metabolism, and plant nutrition (Gupta and Huang, 2014).

Low-molecular-weight osmolytes, comprising proline, as well as biosynthesis of free radical scavenging enzymes, such as catalase, peroxidase, and ascorbate peroxidase play important roles in sustaining cellular functions under abiotic stress (Brenes *et. al.*, 2020 and de Castro *et. al.*, 2020). The *in vitro* adjustment of proline accumulation was extensively estimated in sodium-chloride- shock (Alhasnawi, 2019) moreover, this review article defined a tissue culture technique for the assessment and enhancement of salt tolerance in many plants.

Keunen *et al.* (2013) emphasized the role of total soluble sugars (TSS) on salt tolerance due to promoting osmotic adjustment. They confirmed the extending concept of antioxidants functioning during abiotic stress, with special focus on the key role of sugars as true ROS scavengers. During the onset and development of abiotic stress, all the primary processes such as photosynthesis, protein synthesis and lipid metabolisms are affected. A higher content of total soluble proteins (TSP) have been observed in abiotic tolerant cultivars of many plant species however, on other species TSP increase at low salinity and decreases at high salinity stress (Parvaiz and Satyawati, 2008).

Effective strategy for the *in vitro* selection of desire traits is a vital step in plant breeding programs. There are some considerations to select plant cells and regenerated colons for a new trait using somaclonal variations. The most frequently used approach for selection of stress tolerant genotypes involves culturing explants on medium supplemented with, mannitol, NaCl, and PEG (Mohamed *et. al.*, 2000; Al-Amier and Craker, 2007; Ghaheri *et. al.*, 2017; Jolayemi *et. al.*, 2018; Singh *et. al.*, 2020 and De Castro *et. al.*, 2020).

Therefore, the aim of the current study was to induce somaclonal variations in regenerated *S. officinalis* plants in order to select NaCl-tolerant clones using a rapid *in vitro* regeneration protocol (Mohamed *et. al.*, 2019). Furthermore, some physiological

mechanisms that are crucial for drought stress tolerance were investigated.

## MATERIALS AND METHODS

### Plant materials and culture conditions:

*Salvia officinalis* single-node explants were collected from field-grown plants and cultured *in vitro* using a routine protocol previously described by Mohamed *et. al.* (2019). Apical shoots from developing plantlets were excised and individually cultured on MS medium (Murashige and Skoog, 1962) fortified with 30 g/l sucrose, and indole 3-acetic acid (IAA) at 1.0 mg/l (shoot growth medium; SGM). Developing plantlets were used as a plant material for the next experiments.

For all culture media pH was adjusted to 5.8 and gelled with 8 g/l agar then autoclaved for 20 min at 121 °C and 1.06 kg cm<sup>-2</sup>. The vessels (jam jar; 250 ml) containing the cultures were incubated in a culture room at 23±1 °C and 16 h photoperiod provided by Phillips TLD 50 W/ 84 HF fluorescent tubes giving a photon flux of approximately 40-50 µmol m<sup>-2</sup> s<sup>-1</sup>.

### Optimizing NaCl concentration for salt stress:

A preliminary experiment was carried out to optimize the NaCl concentration which could be used as a selective agent. Single nodes were cultured on SGM enriched with 0, 25, 50, 75, 100, 125, 150 mM of NaCl. For each treatment there were 6 jars each with 5 explants. Explants were transferred onto a fresh medium every 4 weeks. After 8 weeks, the ability of explants to show new growth, rooting %, and the fresh and dry (after drying at 70 °C) weights of both shoots and roots were measured.

### Shoot regeneration on medium containing mannitol:

Mohamed *et. al.* (2019) previously stated that leaf explants of *S. officinalis* plants cultured on MS medium with 1.5 mg/l thidiazuron (TDZ) + 0.1 mg/l IAA and 3% sucrose (regeneration medium; RM) had the highest significant shoot number (7.2

shoots/explant) after developing callus as compared with other investigated treatments. Therefore, this RM was used for *in vitro* selection of NaCl-tolerant clones.

Leaf explants as well as, the regenerated shoot clumps from leaf explants which cultured on RM for 4 or 12 weeks were cultured on RM supplemented with 100 mM NaCl for 12 weeks. The cultured explants were subcultured onto a fresh medium every 4 weeks. There were 30 explants for each treatment equally cultured on 6 jars.

#### **Shoot elongation:**

A number of 60 shoot clumps which developed from leaf explants on free-NaCl RM for 8 weeks before subculturing on the same medium but fortified with 100 mM NaCl for 12 weeks were recovered. These recovered shoot clumps were cultured on SGM supplemented with 100 mM NaCl. After 12 weeks 4 shoots were recovered and individually multiplied as clones by a single node on free-NaCl SGM till having 30 plants from each clone.

#### **Evaluation the selected clones for NaCl stress:**

Plantlets of the 4 selected clones were divided into single nodes and equally cultured on SGM with 0 or 100 mM NaCl. Explants were subcultured onto a fresh medium every 4 weeks. After 8 weeks shoot length, shoot and root fresh weight were assessed. Moreover, the following chemical and physiological parameters were measured.

#### **Chlorophyll content as SPAD unit:**

Total chlorophyll content was determined using a portable Minolta chlorophyll device SPAD-502 which has a 0.71 cm<sup>2</sup> measurement area and based on absorbance measurements at 660 and 940 nm (Spectrum Technologies, Inc., Plainfield, IL, USA). Thirty separate measurements were made using the leaves from 30 plants in each treatment (Uddling *et al.*, 2007).

#### **Proline, TSS and TSP determination:**

Fresh leaves from the same clone were mixed together then 3 randomized samples each of 0.1 g were used to assess the content of proline (Bates *et al.*, 1973), TSS, (Jermyn, 1975), and TSP (Lowry *et al.*, 1950).

#### **Estimation of some antioxidant enzymes activity:**

A Sample of 0.5 g of fresh leaves was frozen in liquid N and finely ground by pestle in a chilled motor as described by Mukherjee and Choudhuri (1983). The activity of CAT enzyme was estimated by the decrease of absorbance at 240 nm as a consequence of H<sub>2</sub>O<sub>2</sub> consumption and was expressed according to Havar and Mellate (1987). The POX activity was assessed following Maehly and Chance (1954). The increase in absorbance due to formation of tetraguaiacol was observed at 470 nm (Klapheck *et al.*, 1990). The APX activity was determined from the decrease in absorbance ascorbic at 290 nm as ascorbic acid oxidized (Asada, 1992).

## **RESULTS AND DISCUSSION**

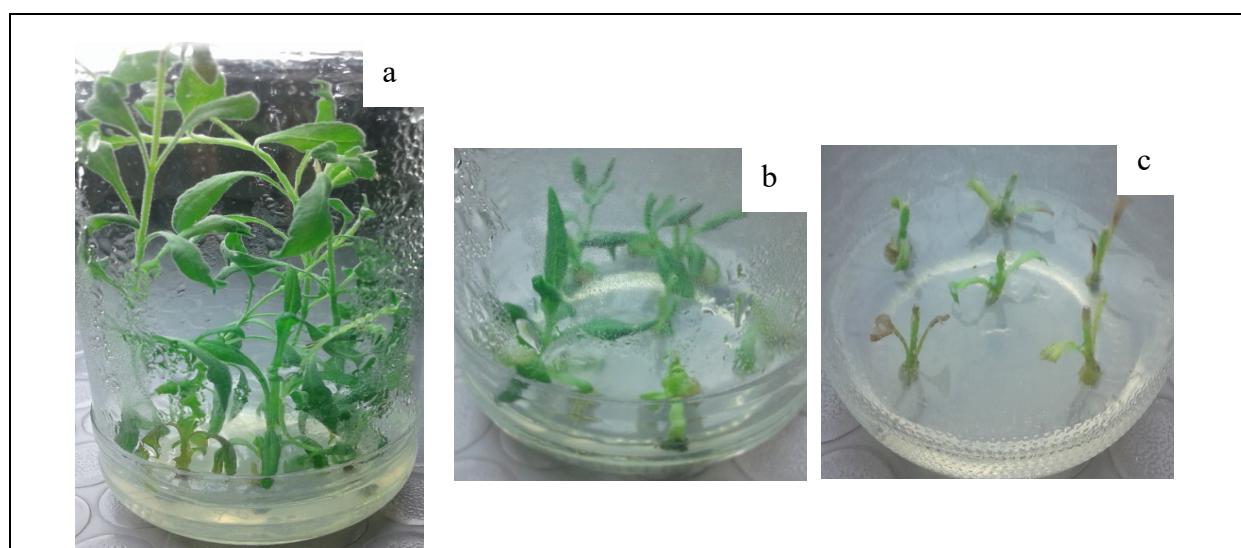
#### **Optimizing NaCl concentration for water stress:**

Table (1) shows that all estimated traits were significantly reduced by the addition of NaCl to the growth medium even at 25 mM. The ability of cultured nodes to show new growth was significantly reduced from 100% to 43% by increasing the concentration of NaCl from 0 to 100 mM. However, there was no difference among 25, 50 and 75 mM but, at 125 mM all explants died. Shoot length and fresh weights (FW) and dry weights (DW) were decreased ( $p \leq 0.5$ ) due to NaCl treatments. Plant heights on 100 mM NaCl were significantly reduced to 1.6 cm (compared with the control treatment; 8.2 cm) (Fig. 1). Also, shoot dry weights under this concentration were reduced by more than 50% compared with the control treatment (1.80 g/plant). Similar trend for the effect of NaCl on shoot DW were observed (Table, 1). The effects of NaCl on root FW

**Table 1. Effect of NaCl on growth of *Salvia officinalis* single node explants after 8 weeks of culture on MS medium.**

NaCl (mM)	Shoot				Root		
	Growth (%)	Length (cm)	Fresh weight (g)	Dry weight (mg)	Rooting (%)	Fresh weight (mg)	Dry weight (mg)
0	100.0a	8.2a	1.80a	186a	93.2a	216a	24.0a
25	66.2b	4.8b	1.24b	140b	72.8b	152b	10.6b
50	59.8b	3.0c	1.26b	130b	72.8b	84c	7.4c
75	59.6b	2.2d	1.02b	88c	59.8b	58c	4.6d
100	43.2c	1.6d	0.96c	82c	43.2c	52c	1.6d

Means in the same column followed by the same letter(s) are not significantly different according to Dancan's multiple range test ( $P \leq 0.05$ ).



**Fig. 1. *Salvia officinalis* single node explants grown on medium enriched with 0, 100 and 125 mM NaCl (a, b and c, respectively).**

and DW were more pronounced than that of shoots (Table, 1). At 25 and 50 mM NaCl the percentage of explants which developed roots were higher than that of which showed shoot growth. Results showed that 100 mM NaCl reduced the ability of explants to develop new growth and rooting of *S. officinalis* explants to 43%. Additionally, plantlet growth in terms of shoot and root FW and DW were significantly reduced for more than 50%. But, it should be noticed that all traits except rooting percentage did not varied ( $p \leq 0.5$ ) between 75 and 100 mM of NaCl treatments. Therefore, 100 mM NaCl was considered as a selective agent for the next experiments.

Reducing survivability of *S. officinalis* explants due to high salt stress (125 mM NaCl) and inhibition the percentage of explants which showed growth under 75 and 100 mM were observed on this investigation. The reduction on root fresh and dry weight under salt stress is a known phenomenon under salt stress. Reduction of plant growth and dry matter accumulation under salt stress conditions has been reported in several medicinal plants (Mohamed *et al.*, 2000 and Muhammad and Hussain, 2010). Glenn *et al.* (1999) stated that excessive salt above what plants tolerate limits plant growth and productivity and could lead to plant death. Siler *et al.* (2007) demonstrated that roots are

the first organs affected and most sensitive ones by salt stress. This retardation could be attributed to the inhibition of cell elongation (Bandeoglu *et al.*, 2004)

#### ***In vitro* selection NaCl-tolerant clone:**

All leaf explants as well as 4-month old shoot clumps cultured on RM enriched with 100 mM NaCl showed necrosis during the 1<sup>st</sup> culture. Thereafter, these cultures get brown and died after subculturing. On contrast to that, shoot clumps which originally cultured on RM for 12 weeks showed different levels of necrosis (Fig., 2). Nevertheless, green survived clumps were recovered after the 3<sup>rd</sup> subcultures. The recovered shoot clumps were subcultured onto SGM where 4 shoots were elongated. These shoots were individually elongated on free-NaCl medium and called clone 1, 2, 3 and 4. The elongated shoots were individually cloned by single node explants on free-NaCl SGM. Following the theoretical base of somaclonal variations *in vitro* culture techniques was applied to produce variants which were more resistant to different environmental abiotic stress assuming that there is a parallel between cellular and *in vivo* plant responses (Mohamed *et al.*, 2000; Rai *et al.*, 2011; Krishna *et al.*, 2016; Rao and Sandhya, 2019). These investigators showed similar responses to our study of different plant species to *in vitro* stress agent such as NaCl, mannitol and PEG.

#### **Evaluation of the selected clones for NaCl stress:**

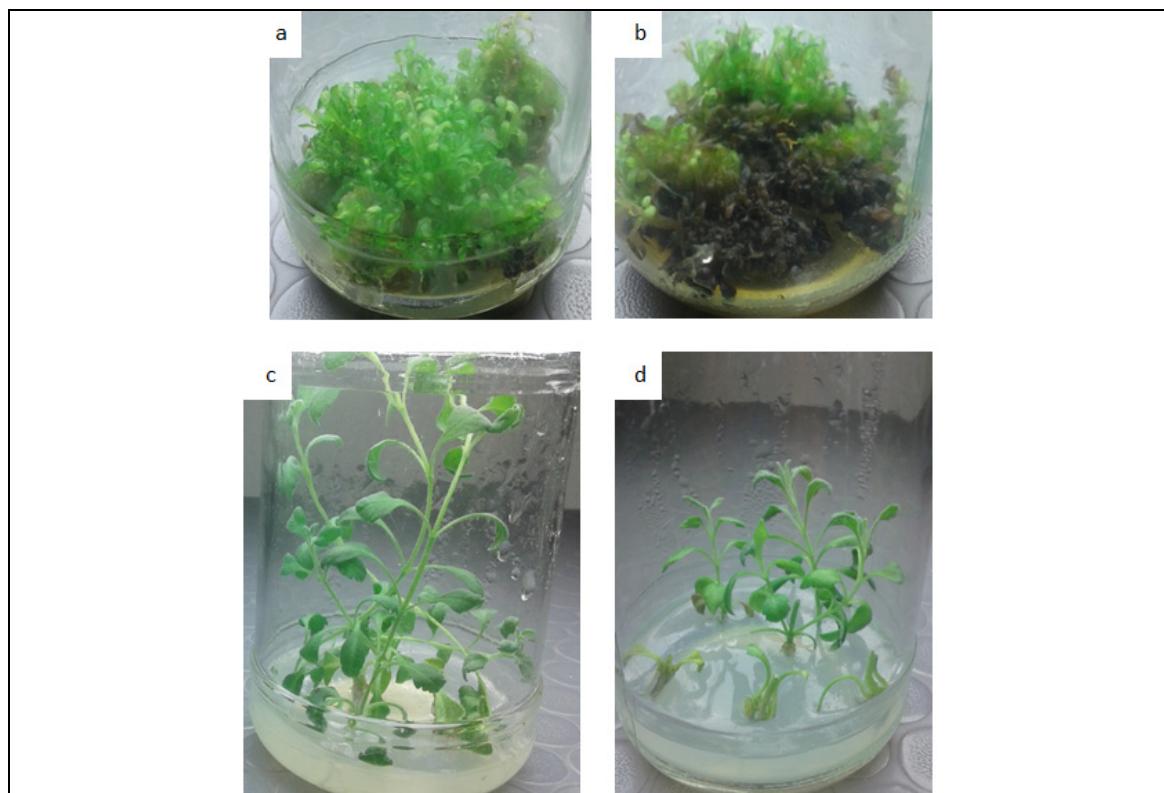
##### **1. Explant growth:**

The number of control explants of *S. officinalis* that showed new growth on stressed medium was reduced ( $p \leq 0.5$ ) to 60% nevertheless, this reduction was similar to all clones. The percentage of selected clone-explants which showed further shoot growth on NaCl enriched medium was ranged from 50 to 72%. Salt enriched medium clearly reduced shoot lengths of the selected clone (Fig., 2). The control plantlets and clone 1 cultured on free-NaCl medium had the highest shoot length (10.7 and 11.3

cm. respectively;  $p \geq 0.5$ ) which reduced to 1.3 and 3.6 cm respectively, under stress conditions (Table, 2). Under this condition clone 1 and 3 had higher shoot length than the control and other clones. Overall, the minimum reduction (63%) on shoot FW due to salt stress was for clone 3 however, the maximum reduction (88%) was for control plant.

Control plantlet grown on free-NaCl medium had the highest shoot FW (330 mg) whereas, this value was significantly reduced to 208, 196, 141, and 135 mg/plantlet for the four selected clones, respectively (Table, 2). Overall, clone had the highest shoot FW (159 mg/plantlet) whereas control plantlets grown on stress conditions had significantly the lowest shoot FW (90 mg/plantlet). Stress condition significantly reduced the FW for control and clone 2 however, the FW of the other clones did not reduce due to NaCl. Moreover, these plantlets had significantly lower shoot DW under stress conditions. Shoot DW were significantly affected with and varied among the clones. Under non-stress conditions all clones had shoot DW lower than that of the control treatment however, the variance among the clones on this condition was not significant (Table, 2).

All explants developed roots on NaCl-free medium while on NaCl-enriched medium the percentage of explants which developed roots were significantly reduced to 60-72% with no significant difference among them. However, for all tested plants NaCl significantly reduced the percentage of rooting. Clone 1 had significantly the highest root FW (92.7 mg/plant) whereas the control plant had 65.3 mg/plantlets. Salt stress significantly reduced the root FW of control and clone 2 and 4 plantlets (Table, 2). The reduction on root FW of these plantlets compared with these on NaCl-free medium were 75, 48 and 84% respectively. Root DW did not represent as it was difficult to measure it for many plantlets. The effect of NaCl on growth of *S. officinalis* nodal-explant was discussed above. Whereas, the variation among *in vitro* regenerated plantlets



**Fig. 2.** *Salvia officinalis* shoot clumps grown on MS medium with 1.5 ml TDZ and 1 mg/l IAA (a), shoot clumps grown on same medium included 100 mM NaCl (b), single node explants of clone 1 grown on free-NaCl medium (c) and on medium with 100 mM NaCl.

**Table 2.** Effect of NaCl on growth of *Salvia officinalis* single node explants excised from in vitro NaCl-tolerant selected clone.

Treatment Explant	NaCl (mM)	Shoot				Chlorophyll (SPAD)	Root	
		Growth (%)	Length (cm)	Fresh weight (mg)	Dry weight (mg)		Rooting (%)	Fresh weight (mg)
<b>Control</b>	<b>0</b>	100a	10.7a	330a	32.3a	45.2a	100.0a	65.3a
	<b>100</b>	60c	1.3e	90e	6.7e	30.8b-d	60.7c	16.0cde
<b>Clone 1</b>	<b>0</b>	89b	11.3a	208b	23.0bc	36.1b	88.7ab	92.7bc
	<b>100</b>	72c	3.6d	159b-d	15.7cd	24.3d	61.0c	48.3b
<b>Clone 2</b>	<b>0</b>	100a	8.4b	196bc	25.7ab	25.8cd	100a	60.3a
	<b>100</b>	72c	2.2de	116d	9.3d	33.1bc	60.0c	31.0bc
<b>Clone 3</b>	<b>0</b>	100a	9.3b	141b-d	16.3b-d	38.8ab	100a	26.3b-d
	<b>100</b>	66c	3.4d	117d	17.0b-d	22.3bc	72bc	14.0de
<b>Clone 4</b>	<b>0</b>	83c	6.4c	135cd	17.0b-d	36.3b	100a	68.7a
	<b>100</b>	50c	1.5e	103d	7.2d	23.8d	66bc	11.2e

Means in the same column followed by the same letter(s) are not significantly different according to Dancan's multiple range test ( $P \leq 0.05$ ).

under stress condition which can be due to somaclonal variation was discussed above.

## **2. Chlorophyll content:**

The chlorophyll content measured as SPAD unit was significantly varied among tested plantlets, as well as, the concentration of NaCl. Moreover, there was a significant interaction among the type of plantlets and NaCl concentrations (Table, 2). Under non-stress conditions the analysis of variance for SPAD measurements showed differences ( $p \leq 0.5$ ) among the control plantlets (45.2) and the selected clones except clone 3 which had (38.8). Under stress conditions the lowest SPAD value (23.8) was being for clone 4 plantlets whereas, the highest value being for these of clone 3. Only, clone 2 had higher SPAD under stress conditions than non-stress ones (25.8 and 33.1, respectively). Our results regarding the reduction of chlorophyll content due to NaCl stress is in agreement with previous results of Taibi *et al.* (2016) and Turan *et al.* (2007). The decrease in chlorophyll content in salt-stressed plants has been considered as a classic symptom of oxidative stress which inhibits its biosynthesis, together with the activation of its degradation by the enzyme chlorophyllase (Santos, 2004).

## **3. Proline content:**

Proline content (Table, 3) was affected by NaCl and varied ( $p \leq 0.05$ ) among clones and the control plants. Control and clone 1 plantlets and had significantly the lowest proline content (4.48 and 5.05  $\mu\text{g/g}$  FW, respectively) with no significant difference between them. Under stress conditions clone 2 had overall, the highest proline content (29.26  $\mu\text{g/g}$  FW) however, clone 1 had the lowest content (9.43  $\mu\text{g/g}$  FW) which was similar to that of control plantlets (8.42  $\mu\text{g/g}$  FW). The highest increment (about 3.5-fold) of proline due to NaCl stress was assessed for clone 2. The positive correlation between plant salt tolerance and proline accumulation has been provide in different plant species (Xue *et al.*, 2009 and Shahbaz *et al.*, 2011, Brenes *et al.*, 2020). Proline could be

directly involved in the mechanisms of salt tolerance, so that higher contents mean higher tolerance (Al Hassan *et al.*, 2016). Our results showed a significant variation among the selected clone in proline content which confirmed with *in vitro* results obtained by Mohamed *et al.* (2000) and Androw *et al.* (2005) due to somaclonal variations. They stated that selected clones of different plant species with high proline content could withstand *in vivo* abiotic stress.

## **4. Total soluble sugars content:**

The analysis of variance showed TSS was significantly varied among tested plants and due to salt stress moreover, there were a significant interaction between the plant types and NaCl treatment. The control plantlets grown on salt-stress and non-stressed medium had 35.26 and 36.92 mg/g FW, respectively; ( $p \leq 0.05$ ). On the non-stressed medium plantlets of clone 1 and 3 had significantly the highest concentration (38.33 and 37.74 mg/g FW, respectively). This value significantly reduced to 36.71 mg/g FW. Although TSS play a vital role in osmoregulation under stress conditions in some plant species for clone 1 however, the reduction under stress condition 36.78 mg/g FW for clone 3 was not significant. Therefore, these results could suggest that TSS contents do not associate with the degree of salt tolerance, and probably do not play any significant role in the responses to salt stress of *S. officinalis*. Gil *et al.* (2013) referred that TSS play a vital role in osmoregulation under stress conditions however this depending on plant species. Recently, Brenes *et al.* (2020) had similar results however these results were in contrast with Mohamed *et al.* (2000).

## **5. Total soluble protein content:**

Total soluble proteins of *S. officinalis* plants were significantly varied among the selected clones and the control plants which had 26.1 mg/g FW on NaCl-free medium. There was no significant increment on TSP

**Table 3. Effect of NaCl on proline and total soluble sugars and protein content as well as the activity of CAT, APX and POX of *Salvia officinalis* plantlets.**

Treatment		Proline ( $\mu\text{g/g FW}$ )	TSS ( $\text{mg/g FW}$ )	TSP ( $\text{mg/g FW}$ )	POX ( $\text{mmol/min/mg FW}$ )	CAT ( $\text{mmol/min/mg FW}$ )	APX ( $\text{mmol/min/mg FW}$ )
Plant	NaCl (mM)						
Control	<b>0</b>	4.48g	36.92bc	26.10e	3.25de	1.25c	1.50b
	<b>100</b>	8.42f	35.26e	25.65e	4.50de	1.12c	1.56b
Clone 1	<b>0</b>	5.05g	38.33a	25.29e	5.50d	0.75c	1.50b
	<b>100</b>	9.43ef	36.71bcd	17.01f	4.50de	0.75c	1.75b
Clone 2	<b>0</b>	8.46f	34.14f	23.85e	2.50e	0.75c	1.50b
	<b>100</b>	29.26a	37.38ab	38.88d	5.00de	1.50c	1.75b
Clone 3	<b>0</b>	10.38e	37.74ab	53.91c	20.00b	1.00c	1.75b
	<b>100</b>	12.64d	36.78bcd	128.6a	58.00a	18.08a	5.50a
Clone 4	<b>0</b>	18.03c	35.86de	24.21e	2.25e	1.25c	1.75b
	<b>100</b>	21.15b	36.21cde	60.57b	15.5c	8.75b	1.50b

Means in the same column followed by the same letter(s) are not significantly different according to Dancan's multiple range test ( $P \leq 0.05$ ).

for control plantlets due to salt stress however, all other clones had significantly higher TSP under salt stress. The content of TSP for clone 1 which was the lowest value (17.0 mg/g FW) was significantly decreased under salt stress compared with non-stress condition (Table, 3). However, all other clones had significantly higher TSP under stress conditions. Clone 3 and 4 plantlets which grow on NaCl-enriched medium had more than 2-fold of TSP than that of plantlets on non-stressed medium. Overall, under stress conditions clone 3 had significantly the highest TSP (128.1 mg/g FW) which is about 5-fold than that of control plants on non-stressed medium and about 2.5-fold than that assessed on the same clone under non stress condition. The obtained results are in agreement with those obtained by Ayala-Astorga and Alcaraz-Meléndez (2010). To survive under stress, plants accumulate organic solutes included proteins that protect cells from stress damage. Timasheff and Arakawa (1989) presumed that the accumulation of organic solutes under salt stress was involved in some protection mechanisms such as the restoration of turgor and cell volume, the decrease exogenous nitrate of the cell damage prompted by free radicals, the

protection of enzymes and membrane structures.

## 6. Antioxidant enzymes activity

Significant differences were found among the selected clones and control plants in terms of CAT, POX, and APX activity (Table, 3). The highest activities of these enzymes (58.00, 18.08, and 5.50  $\mu\text{mol/min/mg FW}$ ) occurred in clone 3 under stress condition. Whereas, the activities of these enzymes on the control plants which grow in NaCl-free medium were 3.25, 1.25, and 1.5  $\mu\text{mol/min/mg FW}$  respectively. On NaCl-enriched medium plantlets of clone 3 and 4 had higher ( $p \leq 0.5$ ) activity of CAT than the control plantlets. Whereas, there was a higher significant activity on APX which grow on salt-stressed medium. The highest increase of CAT due to salt stress was assessed for clone 3 where it increased from 20.0 to 58.0  $\mu\text{mol/min/mg FW}$ . Compared with the control plant grown on non-stressed medium (1.25  $\mu\text{mol/min/mg FW}$ ) the activity of POX. Only plantlets of clone 3 and 4 had higher activity of POX (18.08 and 8.75  $\mu\text{mol/min/mg FW}$ ) under stress condition compared with other

treatments. However, there was a significant difference between these values.

Our results regarding the antioxidant enzymes activity of some was similar to these obtained by Çelik, and Atak (2012) and extensively revived by Joseph and Jini (2010) and in plant some enzymatic processes contribute in the detoxification of ROS which could cause oxidative injury to many cellular components such as membrane lipids, proteins and nucleic acids (Guo *et al.*, 2019). Several anti-oxidant molecules/enzymes are involved in this detoxification of ROS in plants. In our study, a significant varietal dissimilarity had been found regarding the antioxidant enzyme activities of selected clone. The enzyme activities (CAT, APX and POX) were found constitutively higher in some clone under salt-tolerant as compared to the non-stressed conditions (de Castro *et al.*, 2020).

Obtained results from this study were in concurrence with the previous obtained results of many studies showed a using somaclonal variation as an efficient tool for *in vitro* selection a stress-tolerant clones of many plant species. A negative correlation between increased salt stress and plant growth was well documented. However, there were positive correlation between accumulation of some organic molecules such as proline and TSP as well as antioxidant activity of some enzymes and salt tolerance. From this it is possible to concluded that *in vitro* culture of *S. officinalis* can be easily used in screening and selecting for the salt tolerance plant with other physiological criteria.

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### الانتخاب المعملي وصفات السلالات المنتسبة لنباتات المريمية

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تعد نباتات المريمية من النباتات الجذابة واسعة الانتشار في العديد من دساتير الأدوية العالمية. تم الحصول على كل من الاشطاء عمرها ١٢ أسبوع تكشفت من زراعة اجزاء ورقية في بيئة MS مضاد إليها ١,٥ ملجم/لتر من منظم النمو TDZ و ١ ملجم/لتر من IAA. زرعت هذه الكتل من الاشطاء في نفس البيئة السابقة بعد اضافة ١٥٠ مليمول من كلوريد الصوديوم إليها. وبعد ١٢ أسبوع من الزراعة تم الحصول على عدد ٤ اشطاء ناتجة من زراعة ٦٠ كتلة من الاشطاء. حفزت هذه الاشطاء بصورة فردية للاستطاله حيث اعتبر كل منها سلاله مستقلة في بيئة MS المضاد إليها ١ ملجم/لتر من IAA حتى تم الحصول على ٣٠ نبات من كل سلاله. عندها زرعت العقد الساقية في بيئة MS المضاد إليها ١ ملجم/لتر من IAA المضاد إليها صفر أو ١٥٠ مليمول من كلوريد الصوديوم. بينت النتائج وجود فروق معنوية بين السلالات المنتسبة ونبات المقارنة على اساس القدرة على نمو المستأصلات المتزرعة وطول النباتات والوزن الطازج والجاف للنمو الخضري والجذور تحت ظروف الاجهاد. وعلاوة على ذلك كان هناك فروق معنوية ايضاً في محتوى النباتات من الكلورو فيل مقدر على اساس وحدات SPAD والبرولين، والبروتين الذائية الكلية. بالإضافة إلى ذلك بينت بعض السلالات زيادة في نشاط بعض الانزيمات مثل الكتاليز والبيروكسيديز والاسكوربيت بيروكسيديز وهي المعروفة بدورها في الحماية من الاكسدة بفعل الشوارد الحرية تحت تأثير الاجهاد. وعلى هذا يمكن القول بأنه أمكن استخدام التباينات الجسدية في نباتات المريمية الناتجة معملياً في انتخاب نباتات مقاومة للإجهاد المائي. وقد تم دراسة بعض العوامل الفسيولوجية الحاسمة في مقاومة النباتات لـاجهاد الملوحة.