ISSN 1110-0419

Annals Of Agric. Sc., Moshtohor,
Vol. 46(1): Ho. 49-55, (2008).

IN VITRO SELECTION OF SALT - OLERANT OF *PASPALUM VAGINATUM* PLANTS.

BY

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ABSTRACT

The effects of salt on the growth, ion and proline accumulation were investigated *in vitro* in *Paspalum vaginatum*. Rhizome explant-drived calli obtained from *Paspalum vaginatum* were exposed to five concentrations of NaCl (0, 2, 4, 6, 8 gm/L). Relative growth rate, ion concentrations and proline accumulation were quantified. Relative growth rate influenced by increasing NaCl. In response to salinity, Na^+, Cl^- and proline concentrations increased significantly in calli with increasing NaCl concentrations while K^+ concentration decreased. These results suggested an implication of Cl^- and K^+ in salt resistance at cellular level in these genotypes and that proline is a symptom of injury in stressed *Paspalum vaginatum* calli rather than an indicator of resistance.

INTRODUCTION

Salt stress research benefits agriculture because soil salinity significantly limits plant productivity on agricultural lands. Plants need essential mineral nutrients to grow and develop. However, excessive soluble salts in the soil are harmful to most plants. In fact, no toxic substance restricts plant growth more than does salt on a world scale. It is estimated that salinity affects at least 20% of world's arable land and more than 40% of irrigated land to various degrees (Rhoades and Loveday, 1990). In costal areas periodic invasions of seawater directly add salts to the soil. Salinity is a major factor limiting the crop productivity in the arid and semi-arid areas of the world. Plant tissue culture techniques provide a promising approach to develop salt-tolerant plants. *In vitro* selection of salt tolerant cell lines and regenerated plants has been reported in several species such as Potato (Sabbah and Tal, 1990), rice (Lutts *et al*, 1999); Hordeum (Sibi and Fakiri, 2000), wheat (Barakat and Abdel-Latif, 1996) and sunflower (Alvarez *et al*., 2003). This suggests that tissue culture selection can be used to improve salt tolerance of plants.

Paspalum vaginatum is a warm season turfgrass. It has been around, but has yet to gain it's place in the turfgrass industry. *Paspalum vaginatum* is also referred to as Seashore Paspalum, Siltgrass, Sheathed Paspalum, Salt Jointgrass, Seaside Millet, Sand Knotgrass, and Saltwater Couch. It is native to East Central South America. Paspalum is being maintained on golf courses in Asia, South Africa, South America. Seashore Paspalum does not produce highly viable seed, and therefore must be propagated vegetatively. Paspalum is great for wet boggy areas where drainage is a problem. It has been known to survive being underwater for several days and still survive. Siltgrass can be watered with ocean water. It has the highest salt tolerance of all turfgrasses. There are golf courses throughout the world that use salt-water as their source of irrigation. Although it is beneficial to be able to water with fresh-water from time to time in order to alleviate the buildup of salts in the soil. As fresh water is becoming more scarce, increasing use of brackish water sources and sewage effluent for irrigation has resulted in an increasing need for salt tolerant turfgrasses. Though there are broad differences in salt tolerance among turfgrasses, little is known about how grasses adapt to salinity. Also, there are some alternative grasses which may hold promise for use as turfgrasses in extreme environments (e.g. high salinity, extreme drought) Geungjoo Lee *et al*, (2002). The aim of this study is to invistegate the effect of different concentration of NaCl on callus induction and to determine the relative salinity tolerances of a broad range of warm season grass (*Paspalum vaginatum*) to introduce it in the North-West Coastal region of Alexandria where irrigation with high- quality water is a problem.

MATERIALS AND METHODS

The present work was carried out at the Central Laboratory for Biotechnology Molecular Biology of the Faculty of Agriculture, Alexandria University, during 2006 and 2007. The present investigation consisted of two experiments. The first experiment was conducted on three different Turf grasses cultivars (*in vitro* culture technique) to identify the optimum conditions for plant regeneration. Results published in the 1st Part (El-Mokadem and El-Naggar. 2008).

The second experiment included the *in vitro* selection among cells under different levels of salt as a mean for selecting salt tolerance.

Only one cultivar was used for *in vitro* selection, *Paspalum vaginatum* which was chosen as it has the highest capacity to regenerate plants from rhizomes cultures.

The plants were grown at the Floriculture and Ornamental Horticulture Research Garden, El Shatby. Rhizomes explants were excised and then surface sterilized by immersing them in 95% ethanol for 1 minute followed by 0.1% mercuric chloride for 15 min and washed with six changes of sterile distilled water.

Explants were cultured on modified MS medium supplemented with 1 mg/L BA, 5 mg/L 2,4-D, 30 g/L sucrose and 10 g /L agar. The pH was adjusted to 5.8 using 1.0 M HCl or 1.0 NaOH prior to autoclaving at 1kg/cm^2, 121 °C for 15 minutes.

Explants were aseptically excised and placed in 9-cm Petri dishes containing 20 ml of culture medium and each dish contained 5 explants under 16 hours illumination (2,000 Lux, daylight fluorescent tubes) and 25±1 °C for 30 days.

In vitro salt treatments

For this work the selection strategy was made by using direct selection methods. After two subcultures, the growing calli were transferred to callus culture medium containing 0, 2, 4, 6, 8 g/L NaCl. Three successive subcultures (4 weeks each) were performed at each NaCl concentration. Petri dishes with the explants were arranged in a totally randomized design with a complete factorial distribution for 5 treatments. Six replicates were taken into regeneration media of each NaCl concentration. To determine the growth from the exposed calluses to the different concentrations of NaCl, the difference in fresh weight between initial and 30, 60, and 90 days was measured. The following *in vitro* traits were recorded:

Callus induction response

1- Callus induction (%).
2- Calus weight (mg).
3- Embryogenic callus (%).

Callus necrosis percentage was determined visually as percentage of necrotic callus and relative fresh weight growth of non-necrotic calli.

Estimation of K^+ and Na^+ were determined as described by Gulati and Jaiwal (1993). chloride was determined according to Gilliam (1971). Proline content was estimated according to Bates *et al.* (1973).

Morphogenetic response:

Embryogenic cultures from all generations were placed on regeneration media in jars containing 25 ml of sterile media without NaCl for shoot elongation. The regeneration medium had the same components as callus induction medium, except for the omission of the auxin source.

Regenerated plants were acclimated off by removing culture Jars caps and exposing the succulent plant tissue to the atmosphere. Acclimated plants were washed by tap water to remove the agar then established in plastic pots filled with sterilized peat moss. The pots were incubated under moist conditions for adaptation establishment

Statistical analysis:

Data were statistically analyzed as a completely randomized design (CRD) with six replicates. All *in vitro* traits data, except for the callus weight were subjected to arcsine transformation, before statistical analysis. Comparisons among means were made by using the L.S.D. test of significance. Analysis was carried out using SAS program.

RESULTS AND DISCUSSION

Selection for Tolerance to NaCl:

Data presented in Table 1 indicated that the percentage of callus induction, embryogenic callus and callus weight (mg), were significantly influenced by NaCl concentrations. It is clear that the relative growth of callus was markedly decreased, in response to increasing NaCl concentration as compared to the control (Figure1). These results may be attributed to the toxic effects of Na^+ and Cl^- ions accumulated in the cytoplasm causing reduction in cell division and elongation (Khan *et al.* 2000). Similar effects of high concentrations of sodium chloride on the callus weight have been reported. Smith and McComb (1981) reported that the exposure of callus to a saline environment may lead to water stress and specific ionic imbalances perhaps resulting in ion toxicity. Furthermore, cells grown under stress may have to spend more metabolic energy than those grown in the absence of stress (Croughan *et al.*, 1981). They also reported that this extra energy most probably is used up in regulating osmotic adjustment. Reddy and Vaidyanath (1986) reported that the callus growth of rice decreased markedly with increasing NaCl concentration in the medium. They also suggested that the observed decline in fresh weight in the NaCl environment may be due to the diversion of some quantum of energy from growth and metabolism. Lupotto *et al.* (1989) reported that the effect of salt on the established embryogenic cultures derived from immature embryos of maize resulted in a rapid depression of the general callus growth, with necrosis and degeneration of the tissue at concentrations above 80-90 mM NaCl (5 g/L NaCl). Explant necrosis was observed at high NaCl concentrations this result was in agreement with Jain *et al.*(1991) on *Brassica junica* and Sandra and Jaime (2006) on *Melia azedarach L.* The presence of a high concentration of salt in the culture medium produces a loss in regeneration potential. This result may be due to the high osmotic potential of the saline medium, a higher osmotic potential affects water and nutrient uptake, which may in turn inhibits the metabolic activities necessary for bud initiation and growth (Vijayan *et al.* 2003).

Table (1): Means of callus induction %, embryogenic callus and callus weight (mg) of *Paspalum vaginatum* as influenced by NaCl concentrations.

Concentrations of NaCl (gm/L)	Callus induction %	Callus weight (mg)	Embryogenic callus %
0	71.5	91.5	49.1
2	60.7	60.7	44.6
4	48.4	21.9	40.4
6	33.1	15.4	33.9
8	25.4	7.1	11.9
L.S.D at 0.05	3.7	2.8	2.1

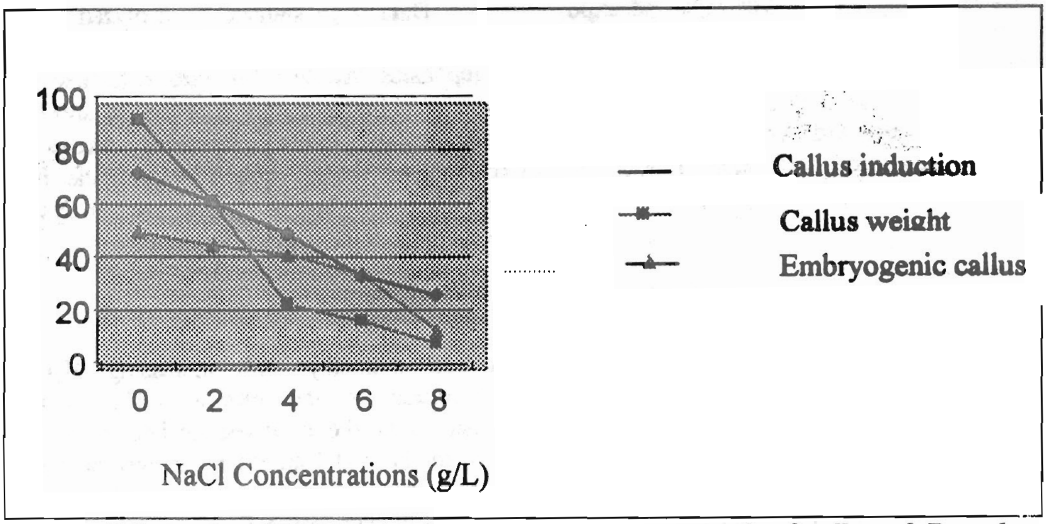

Fig (1): Effect of NaCl concentrations on the relative growth of callus of *Paspalum vaginatum*

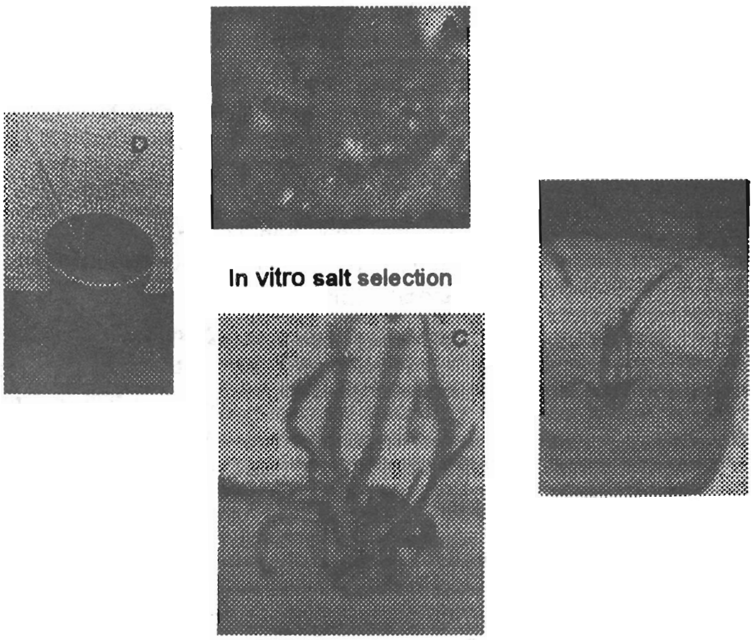

Fig. (2): *In vitro* salt tolerance selection of *Paspalum vaginatum*. Regeneration of callus in culture media (A), organogenic callus (B,C). Plants obtained from *in vitro* NaCl selection (D).

The obtained results in the present investigation agree with previous studies (Croughan *et al.*,1978, Watad *et al.*, 1983, Winicov, 1991). Croughan *et al.* (1978) observed that the concentrations of Na^+ and Cl^- in alfalfa cells cultured on media containning NaCl increased with increasing NaCl level while the concentration of K^+ was decreased. Watad *et al.* (1983) reported that Na^+ content rose steadily in Nicotiana cell line as external NaCl concentration increasing. The K^+ content decreased with increasing NaCl levels in the medium. Winico (1991) reported that Na^+ content in the regenerated shoots of alfalfa increased with increasing NaCl concentration while K^+ content in the regenerated shoots was decreased.

Table (2): Mean effect of NaCl concentrations on ion and proline contents (mg/gram dry weight) in *Paspalum vaginatum*.

NaCl (g/L)	Ion contents (mg/gm D.W)			Proline content (mg/gm D.W)
	Na^+	K^+	Cl^-	
0	5.2	17.15	7.42	5.05
2	25.14	15.21	35.63	7.03
4	33.51	15.52	55.51	10.22
6	39.65	16.03	65.40	12.03
8	44.89	13.22	85.63	17.25
L.S.D	3.8	2.2	3.7	2.1

Proline content:

Proline content in stressed calli was different from non-stressed (control). It increased with the increasing in salt stress (Table 2). The highest proline content was obtained at (8g/L NaCl). Proline has been shown to accumulate in a number of plant tissues in response to salt stress (Greenway and Munns, 1980; Chandler *et al.*, 1986. Chandler and Thorpe, 1987). Greenway and Munns (1980) suggested an adaptive role for proline accumulation in relation to survival, rather than maintained growth, since it only accumulates when growth inhibition is already pronounced. It has been found that the accumulated proline mediates tolerance by serving as a source of cytoplasmic osmoticum (Stewart and Lee, 1974; Watad *et al.*, 1983; Daines and Gould, 1985) and protecting cytoplasmic enzymes and cellular structures (Le Rudulier *et al.*, 1984). It has also been argued that proline counteracts the inhibitory effect of NaCl (Pandey and Ganapathy, 1985).

Plant regeneration:

The growth of callus on the medium containing 8 g/l NaCl was very poor and became necrotic after two subcultures (Figure 3). The regeneration of plants from some calli occurred after two subcultures and the regeneration was successful (Figure 4).

In conclusion, turf-type *Paspalum Vaginatum* cultivars with the best callus induction and regeneration rates have been identified. Tissue culture conditions of turf-type *Paspalum Vaginatum* have been optimized. Also we are determind the relative salinity tolerances of a broad range of warm season grass (*Paspalum vaginatum*) to introduce it in the North-West Coastal region of Alexandria where irrigation with high-quality water is a problem.

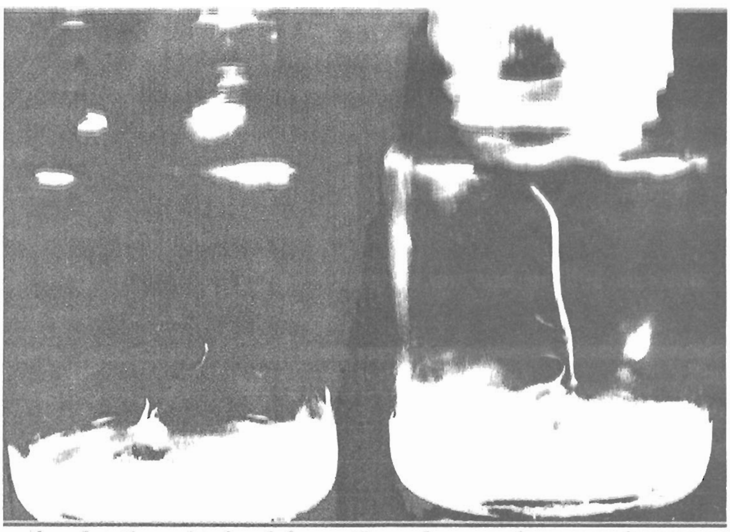

Fig. (3): Necrosis of callus obtained from *Paspalum vaginatum* after two subculture as affected by NaCl concentrations.

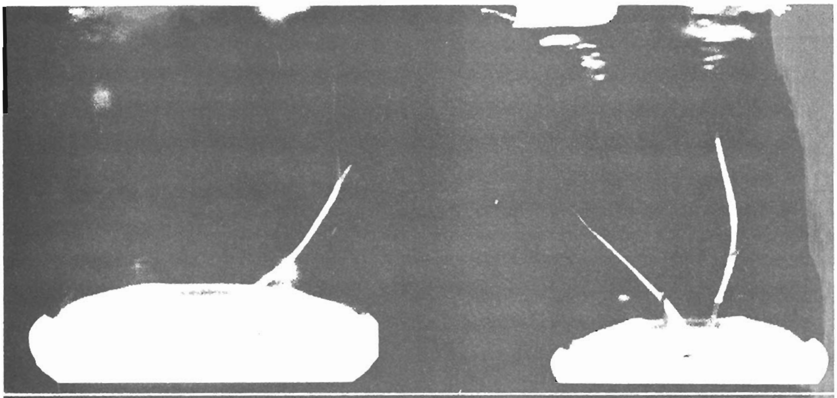

Fig. (4): Regeneration of plants of *Paspalum Vaginatum*

REFERENCES

Alvarez I.; Tomaro, LM and Benavides, PM (2003): Changes in polyamines, proline and ethylene in sunflower calluses treated with NaCl. Plant Cell, Tissue and Organ Culture 00:1-9.

Barakat, M.N. and Abdel-Latif, TH. (1996): *In vitro* selection of wheat callus tolerant to high levels of salt and plant regeneration. Euphytica 91:127-140.

Bates, L.S.; Waldrem, R.P. and Teare, I.D. (1973): Rapid determination of free proline for water stress studies. Plant and Soil 39: 205-208.

Chandler, S.F. Mandal, B.B. and Thoorpe, T.A. (1986): Effect of sodium sulfate on tissue cultures of Brassica napus L. cv. Westar and Brassica compestris cv. Tobin.J. Plant Physiol. 126: 105-117.

Chandler, S.F. and Thorpe, T.A. (1987): Proline accumulation and sodium sulfate tolerance in callus of Brassica napus L. cv. Westar. Plant Cell Rep. 6: 176-179.

Croughan, T.P.; Stavarek, S.J. and Rains, D.W. (1981): *In vitro* development of salt resistant plants. Environ. Exp. 21: 317-324.

Croughan, T.P.; Stavarek, S.J. and Rains, D.W. (1978): Selection of NaCl tolerant lines of cultured alfalfa cells. Crop Sci. 18: 959-963.

Daines, R.J. and Gould, A.R. (1985): The cellular basis of salt tolerance studies with tissue cultures of the halophytic grass *Distichlis spicata*. J. Plant physiol. 119: 269- 280.

El-Mokadem H.E. and El-Naggar H.(2008): *In vitro* Somatic embryogenesis and plant regeneration from rhizomes of some turfgrasses. Alex. J. Agric. Res. 53:123- 130.

Geungjoo Lee, Ronny.R. Duncan, and Robert. N. Carrow (2002): Initial selection of salt-tolerant Seashore Paspalum Ecotypes. Turfgrass and Environmental Research Online. Vol.1, No. 11.

Gilliam, J. W. (1971): Rapid measurement of chloride in plant material. Proceedings of the American Society of Soil of Science 35: 512-513.

Greenway, H. and Munns, R. (1980): Mechanisms of salt tolerance in non-halophytes. Annu. Rev. Plant physiol. 31: 149-190.

Gulati, A. and Jaiwal, P.K. (1993): Selection and characterization of mannitol-tolerant callus lines of *Vigna radiata* (L.) Wilczek. Plant Cell, Tissue and Organ Culture, 34: 35-41.

Jain, RK; Jain, S and Chodhury, J. (1991): *in vitro* selection for salt tolerance in *Brassica junicea* L. using cotyledon explants, callus and cell suspension cultures. Annals of Botany 67, 517-519.

Khan, A.; Unger, A.and Showalter, M. (2000): The effect of salinity on the growth, water status and ion content of a leaf succulent perennial halophyte Suaeda fruticosa. J. Arid Envir., 45: 73-84.

Le Rudulier, D.: Strom, A.R; Dandekar, A.M.; Smith, L.T. and Valentine, R.C. (1984): Molecular biology of osmo regulation. Science 224:1064-1068.

Lupotto, E.; Lusardi, M.C. and Mongodi, M. (1989): *In vitro* selection of maize (*Zea mays* L.) salt tolerant somaclones and plant regeneration.J. Genet.& Breed 43: 215-222.

Lutts S, Bouharmont J and Kint JM (1999): Physiological characterization of salt-resistant rice (*Oryza sativa*) somaclones. Aust. J. Bot. 47:835-849.

Pandy, R. and Ganapathy, P.S. (1985): The proline enigma: NaCl-tolerant and NaCl-sensitive callus lines of *Cicer arietinum*. Plant Sci. Lett. 40: 13-17.

Reddy, P.J. and Vaidyanath, K. (1986): *In vitro* characterization of salt stress effects and the selection of salt tolerant plants in rice (*Oryza sativa* L.). 71: 757-760.

Rhodes, J. D., and Loveday, J. (1990): Salinity in irrigated agriculture. In American Society of Civil Engineers, Irrigation of Agricultural Crops (Steward, B.A. and Nielsen, D.R., eds), Am.Soc. Agronomists, Monograph 30 1089-1142.

Sabbah, S and Tal, M (1990): Development of callus and suspension cultures of potato resistant to NaCl and mannitol and their response to stress. Plant Cell, Tissue and Organ Culture 21:119-128.

Sandra E.S, and Jaime A.T. (2006): Effective organogenesis, somatic embryogenesis and salt tolerance induction *in vitro* in the Persian Lilac tree (*Melia azedarach* L.). Floriculture, Ornamental and Plant Bio-technology. Vol. 2: 317-324.

Sibi ML and Fakiri M (2000): Androgènese et gynogenèse, sources de vitrovariation et de tolèrance à la salinite chez l'orge *Hordeum vulgare*? Sècheresse. 11(2): 125-132

Smith, M.K. and McCpmb, J.A. (1981): Effect of NaCl on the growth of whole callus cultures. Aust. J. Plant Physiol. 8: 267-275.

Stewart, G.R. and Lee, J.A. (1974): The role of proline accumulation in halophytes. Planta 120: 279-281

Vijayan, K, Chakraborti, S, and Ghosh, P (2003): *In vitro* screening of mulberry (*Morus spp.*) for salinity tolerance. Plant Cell Reports 22, 350-357.

Watad, A.E.A.; Reinhold, L. and Lerner, H.R. (1983): Comparison between a stable NaCl-selected Nicotiana cell line and the wild type. Plant physiol. 73: 624-629.

Winicov, I. (1991): Characterization of salt tolerant alfalfa (*Medicago sativa L.*) plans regenerated from salt tolerant cell lines. Plant Cell Reports 10: 651-564.

إنتخاب نباتات باسبالم *Paspalum Vaginatum* مقاومة للملوحة بواسطة زراعة الأنسجة النباتية

هدى السيد المقدم

قسم الزهور ونباتات الزينة وتنسيق الحدائق كلية الزراعة جامعة الأسكندرية

أجريت هذه الدراسة فى معمل التقنية الحيوية، المعمل المركزى، كلية الزراعة، بالشاطبى جامعة الإسكندرية خلال الفترة من عام ٢٠٠٦- ٢٠٠٧ أجرى البحث على مرحلتين:

المرحلة الأولى: تم عمل تقييم لثلاثة أنواع من المسطحات الخضراء لإختبار قدرتها على إنتاج الكالس من الريزومات ثم التكشف وإعطاء نموات خضرية وذلك على ثلاثة بيئات غذائية مختلفة لتحديد أحسن نوع يمكنه النمو وإنتاج الكالس ثم التكشف وإنتاج نباتات على أفضل بيئة غذائية. وتم التوصل الى أن النوع *Paspalum Vaginatum* قد تفوق على باقى الأنواع فى إنتاج كالس وتكوين أفضل نموات خضرية أمكن الحصول منها على نباتات.

المرحلة الثانية: تمت دراسة تأثير إضافة ملح كلوريد الصوديوم بتركيزات ٠، ٢، ٤، ٦، ٨جم/اللتر الى بيئة MSالمضاف اليها (2,4-D٥ ملجم/لتر) و (BA١ ملجم/لتر). على الخلايا الناتجة من زراعة الريزومات لنبات المسطح الأخضر *Paspalum Vaginatum*

وكانت النتائج المتحصل عليها كما يلى:

١- تأثر وزن الكالس معنويا بالتركيزات المختلفة من كلوريد الصوديوم فى البيئة الغذائية النامى عليها.

٢- أظهر التحليل الأحصائى لمحتوى الخلايا من الصوديوم والبوتاسيوم والكلوريد أنها كانت معنوية و أنه زاد محتوى الخلايا من الصوديوم والكلوريد فى الكالس بزيادة تركيزات ملح كلوريد الصوديوم فى البيئة بينما تناقص محتوى الخلايا من البوتاسيوم مع زيادة تركيزات ملح كلوريد الصوديوم فى البيئة.

٣- زاد محتوى الخلايا من البرولين نتيجة زيادة تركيزات ملح كلوريد الصوديوم فى البيئة الغذائية.

٤- وجد أن عدد النباتات المتحصل عليها ليس بالقدر الكافى ولكنه يفتح الطريق لعمل أبحاث مكملة والتوسع فى أعداد النباتات المستخدمة لكى يمكن الحصول على العدد الكافى اللازم للتطبيق العملى وبذلك يمكن استخدام هذا النبات كمسطح أخضر يمكن ريه بمياه البحر المالحة.