African Journal of Biotechnology Vol. 12(13), pp. 1490-1494, 27 March, 2013
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.2793
ISSN 1684–5315 ©2013 Academic Journals

*Full Length Research Paper*

# Effects of salinity on growth and organic solutes accumulation of *Tabebuia aurea* Manso (Benth and Hook)

**Antonio Lucineudo de Oliveira Freire* and Artur Diego Vieira Gomes**

Unidade Acadêmica de Engenharia Florestal, Universidade Federal de Campina Grande, Patos, PB, Brazil, Av. Universitária, s/n, 58708-110.

Accepted 6 February, 2013

**Salinity is a problem that has increased annually and among the viable alternatives for the reintegration of saline areas to the productive system is the cultivation of tree species. This study aimed to evaluate the growth, dry matter distribution and organic solutes accumulation in *Tabebuia aurea* Manso (Benth and Hook) under salinity. The plants were grown in Hoagland solution with 0, 50, 100, 200 and 400 mM NaCl during 30 days distributed in a completely randomized design, with six replicates. The salinity affects growth and dry matter accumulation of *Tabebuia aurea* and promotes soluble sugar accumulation on the leaves and stem, and free amino acids in the roots, leaves and stems. Plants showed a sensibility to the salt levels used during the time they were exposed.**

**Key words:** Salt tolerance, osmotic adjustment, salt stress.

## INTRODUCTION

Some areas of arid and semiarid regions have problems with soil salinity, which has increased due to excessive fertilization and irrigation with saline water, preventing their exploitation for agriculture. It is estimated that about 25% of irrigated areas of the globe are affected by salinity (FAO, 2000), which is one of the main causes of the reduction in plant growth and yield.

The salts effects in plants has been studied, and its must be of osmotic, toxic or nutritional nature (Munns, 2002; Flowers, 2004). However, the mechanisms of salt tolerance are still not well understood (Hasegawa et al., 2000). To adapt and survive in these adverse conditions, the plants develop several mechanisms such as selectivity of the process of absorption by the cells of the roots, loading xylem preferably with $K^+$, than with $Na^+$, and removal of salt in the xylem top of the roots, stem, petioles or leaf sheaths (Munns et al., 2002), and osmotic adjustment, which plays an important role. This mechanism is based on the accumulation of compatible organic solutes (Meloni et al., 2004), which act as osmoprotectors and accumulate in the cytoplasm to balance the extremely low osmotic potential of the vacuole (Rontein et al., 2002). It also acts as protectors of proteins and membranes against denaturation caused by the high salinity (Munns, 2002). Among the main organic solutes that accumulate under salinity conditions are proline, glycinebetaine and polyols (Munns, 2002; Rontein et al., 2002).

In the semi-arid region of the Brazilian Northeastern, many areas are being abandoned because of salinization, and an alternative to the reintegration of these areas is the cultivation of trees, in view of the three main objectives, which are the revegetation of the area, the biomass production aimed at the recovery of fertility and physical attributes of these soils, and provide alternatives for producing firewood, decreasing its pressure on the native vegetation, already so heavily devastated. The use of wood in the State of Paraiba, Brazil, for energy purposes is intense and important contribution, bearing in mind that firewood and charcoal are among the main sour-

*Corresponding author. E-mail: lofreire@cstr.ufcg.edu.br.

ces of energy for domestic and industrial use.

Much of this wood comes from areas of Caatinga (dry forest), which contributes increasingly to the reduction of native vegetation. Thus, there is an urgent need to make the regeneration of this environment with the aim of restoring and conserving biodiversity.

The *Tabebuia aurea* Manso (Benth and Hook), Bignoniaceae, is a tree of 10 to 12 m tall, usually well branched; features trunk with 30 to 50 cm in diameter at breast height (dbh); canopy generally well branched, rounded, wide open pale gray bark, alternate leaves composed of three to five pairs of pinnae, each pinna with alternating small leaflets to large (1 to 20 cm), glabrous, oblong, bright green; fruit type follicle, seed with wings, dispersed by wind; prefers Brazilian Northeast riverbanks temporary; presents average growth may be recommended for reforestation, especially in riparian areas in regions of low rainfall (Lorenzi, 2000).

The knowledge about tree tolerance to salinity is limited when compared with crops and pastures. In relation to research with *T. aurea*, this information is lacking. Such information can make allowances for the possibility of its use in the recovery of areas with soil salinity problems, becoming also an alternative to produce wood in plantings by decreasing the pressure on native vegetation.

This study aimed to assess the growth, dry matter distribution and organic solutes accumulation in plants kept under salinity.

## MATERIALS AND METHODS

The experiment was conducted in protected environment in forest nursery of Universidade Federal de Campina Grande (UFCG), Patos, Paraíba, Brazil (7°03'34" S - 37°16'29" W). The plants were kept in pots of ' Leonard ' filled with 1.5 kg of sand, in accordance to Vincent (1970), containing Hoagland and Arnon (1950) nutrient solution (½ strength) with the salinity treatments (0, 50, 100, 200 and 400 mM NaCl). The saline treatments started 15 days after the emergency, and adding 50 mM NaCl daily, until it reached the desired concentration. The solutions were changed every four days in order to keep the proper level of salinity and nutrient concentration of nutrient solution.

At 30 days after the beginning of saline treatments, the plants were evaluated for height, leaf number and specific leaf area of plants collected and the shoot and roots dry mass were obtained after drying at 65°C for 72 h. The determination of leaf area was made using foliar disks with a 1 $cm^2$ of diameter, placed to dry and then weighed. The leaf area was calculated based on the dry mass and in the area of the disks and the dry mass of leaves.

After drying, the material was submitted for weighing to get the dry mass determination of these components. In another group of plants, at the end of the experiment, plants analysis of soluble sugars, total amino acids and proteins contents were collected. The extractions were performed with methanol, chloroform and water (MCW) in 12:5:3 ratio (v/v/v) (Bieleski and Turner, 1966). The extract was prepared using 200 mg of fresh mass of leaves stem and roots, with 5 mL of MCW. After this, the samples were centrifuged three times to 3,000 rpm, for 3 min. The supernatant was placed in separating funnel on the 1 mL of chloroform and 1.5 mL of distilled water was added to each 4 mL of supernatant. After separation, the upper stage of the extract was kept in test tube in the water bath at 30°C for 2 h to evaporate the chloroform.

For the extraction of proteins, the above mentioned extraction precipitate was used, resuspending it three times with NaOH 0.1 mol/L, using 5 mL per gram of fresh mass, by centrifuging the extract to 3,000 rpm for three minutes. Soluble sugars, amino acids and protein contents were determined according to Yemm and Willis (1954), Yemm and Coccking (1955) and Bradford (1976), respectively.

The experiment was set up in complete randomized design (CRD) including 5 NaCl concentrations with five replicates. Data were analyzed according to Ferreira (2000) and analysis of variance and simple linear regression of the results were carried out.

## RESULTS AND DISCUSSION

Salinity significantly reduced all parameters evaluated ($P \leq 0.05$). Plant height, leaf number and leaf area decreased linearly with increasing salinity (Figure 1), and decreased 47% in plant height, 82% in leaf number and 65.5% in leaf area compared to 400 mM with 0 mM NaCl.

The salinity reduced the dry mass of shoot (82.48%), roots (80.26%) and total dry mass (81.74%) compared 0 to 400 mM NaCl treatments (Figure 2). The most severe reduction of these parameters was between 0 and 200 mM NaCl showing the sensibility of this species to high salinity levels. Analyzing Figure 2, the shoots were more affected by salinity than roots. Hsiao and Xu (2000) reported that in salinity conditions, the shoot growth is in most of the cases, more adversely affected than the root growth and according to Munns (2002), this is probably due to factors associated with water stress and not to the specific effect of salt. The reduction in leaf area should be influenced by reduced number of leaves (Figure 1). According to Lacerda et al. (2001), the reduction in leaf area in the presence of NaCl can be attributed to a lower division and/or cell expansion through the reduction in pressure turgidity, as in cell wall extensibility which is impaired due to saline stress, showing an important adjustment mechanism of plants to environments affected by such elements. Reduction in growth and dry mass of plants caused by salinity has been observed by several researchers, in many cultures, as *Vigna* and *Phaseolus* (Beckmann-Cavalcante et al., 2008), castor bean (Lima et al., 2007) and sorghum (Lacerda et al., 2001). This may be a reflection of the decline in water consumption (Beckmann-Cavalcante et al., 2008), causing water stress, affecting the photosynthetic capacity of plants (Flexas et al., 2004, Chartzoulakis, 2005; Stępień and Kłobus, 2006), due to the effects of salinity on stomatal conductance, reduction in specific metabolic processes in the carbon absorption, or a combination of both factors (Zhang et al., 2009). These changes in photosynthesis can also be non-stomatal as stated by Santos et al. (2009) and Huang et al. (2011).

In relation to the accumulation of organic solutes, significant effects of NaCl levels were observed only on soluble sugars and total amino acids. Analyzing the soluble sugars contents (Figure 3), an increase was observed

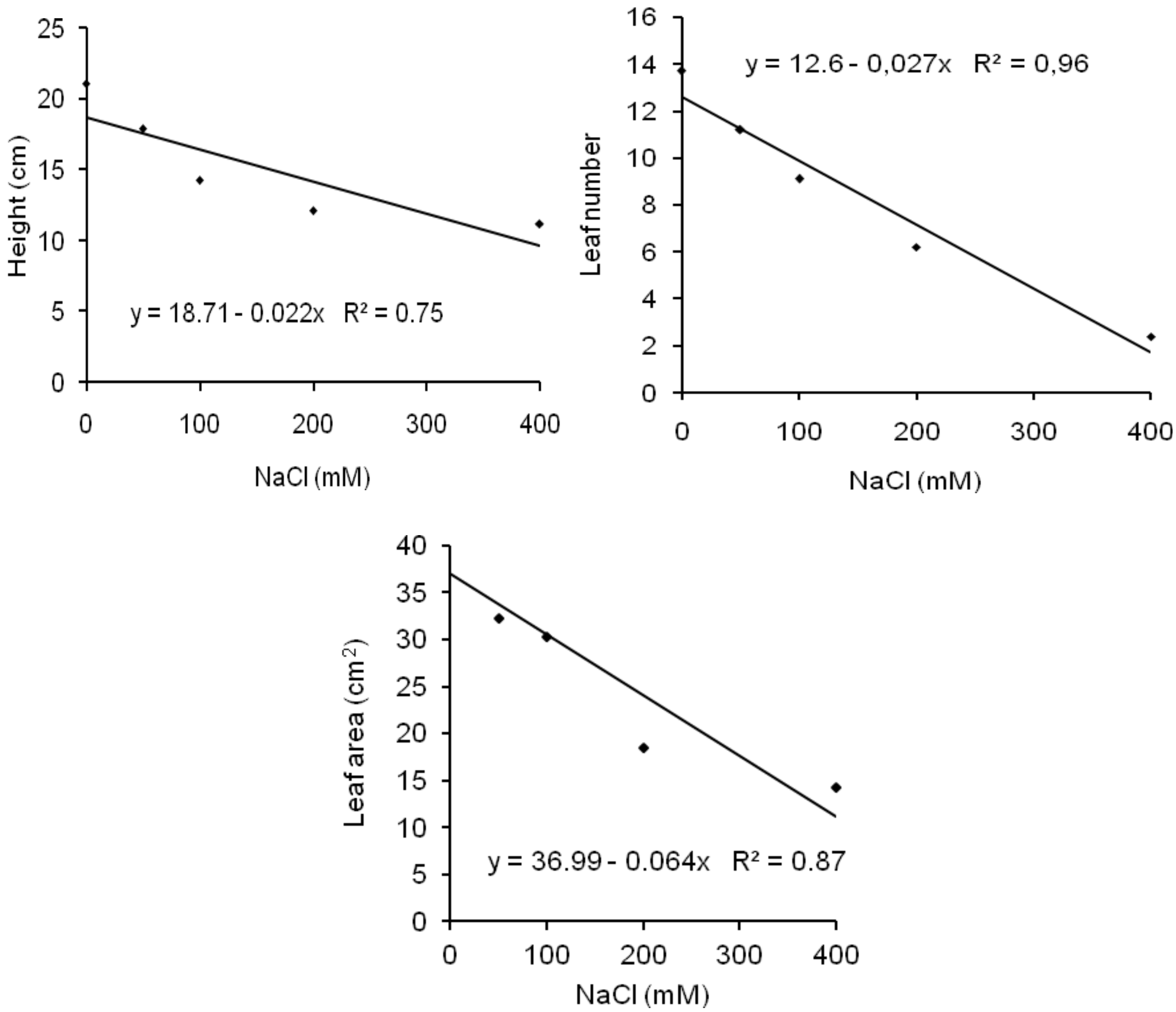


**Figure 1.** Effect of NaCl on height, number of leaves and leaf area of *Tabebuia aurea* plants.

with the elevation on salinity level, this behavior being more pronounced in leaves.

Comparing non-saline treatment with 50 mM NaCl, the soluble sugar contents increased from 14.8 to 17.68 µg $g^{-1}$fwt, representing elevation of 19.6%. In roots, the increases were 18 and 53%, respectively compared to the control treatment with 50 and 400 mM NaCl. On the stem, quadratic reduction in soluble sugar concentrations reached the lowest value (3.63 µg $g^{-1}$ fwt) in 185 mM NaCl, according to the regression equation (Figure 3). When 400 mM NaCl was used, there was a reduction of only 6.96% in relation to the non-saline treatment. Oliveira et al. (2006) and Lacerda et al. (2001) observed significant elevations of soluble carbohydrates in sorghum in the presence of NaCl. According to these authors, carbohydrates were the main contributor to the osmotic adjustment.

There was gradual increase in amino acids content as there was rise in the level of NaCl (Figure 4) in nutrient solution and, in the same way as in soluble sugar (Figure 3), this behavior was more pronounced in the leaves. Comparing non-saline treatment with 200 mM NaCl, it can be verified that there was an increase (838%) from 0.59 to 5.54 µg $g^{-1}$ fwt which increased to 8.41 µg $g^{-1}$ fwt in 400 mM NaCl (1334%) in the leaves. Both on the leaves as in stem, the salt effects became apparent from 200 mM NaCl, while in the roots, it was from 100 mM NaCl. Evidences of elevation of amino acids with salinity were also presented by Costa et al. (2003), Lacerda et al. (2003), and Oliveira et al. (2006). Lacerda et al. (2003) reported that amino acids were the solutes that mostly contributed to the osmotic potential in roots of sorghum genotypes tolerant to salt, followed by the soluble carbohydrates and proline.

According to Saneoka et al. (1995), the total free amino acids and soluble carbohydrates are considered the main organic solutes in osmotic adjustment of plants subjected to water stress and saline conditions. According to Munns

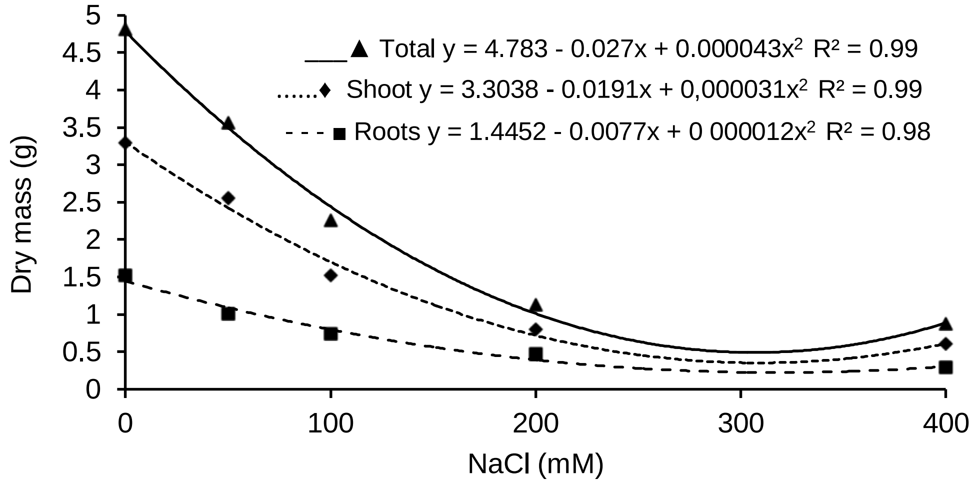


**Figure 2.** Effect of NaCl on dry mass of *Tabebuia aurea* plants.

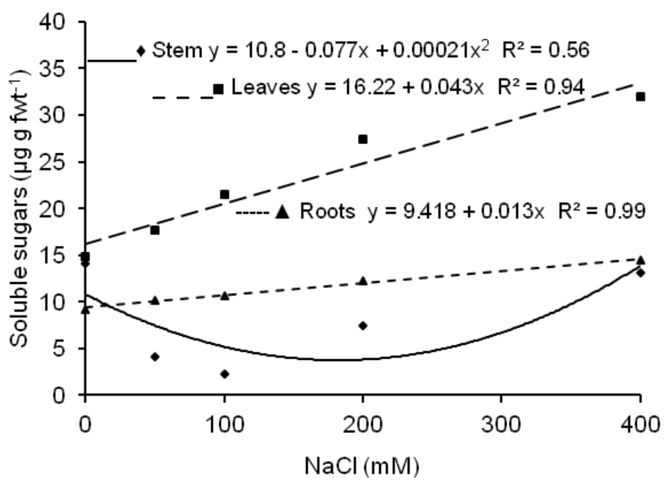


**Figure 3.** Effect of NaCl on soluble sugar content in *Tabebuia aurea* plants.

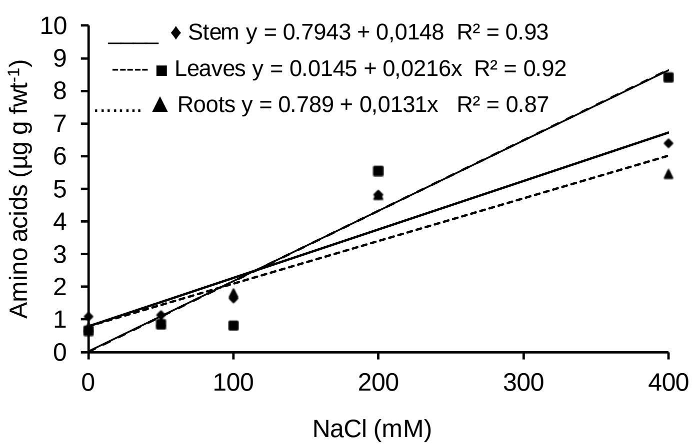


**Figure 4.** Effect of NaCl on amino acids content in *Tabebuia aurea* plants.

(2002), the increase in the concentrations of organic and inorganic solutes in the cytoplasm and vacuole is a mechanism of plant tolerance to survive under water stress conditions and saline. This author also stated that the accumulation of these solutes in leaves can be so high that it is not confined to specific cytoplasmic compartments, and its accumulation level highlights the osmotic effect of salts (it is not specific effect). In this experiment, the older leaves showed chlorosis and necrosis at the onset, showing the toxicity levels of salt employed.

The results demonstrate that salinity affects growth and dry matter accumulation of *Tabebuia aurea*, and the increase of salinity on substrate promotes soluble sugar accumulation on the leaves and stem, and free amino acids in the roots, leaves and stems.

In conclusion, we suggest that experiments be conducted by subjecting the plants for a longer period of time, so that the time at which the salt begins to affect the plants can be determined.

## ACKNOWLEDGEMENTS

The authors are thankful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Universidade Federal de Campina Grande (UFCG) for financial support and scholarship.

### REFERENCES

Beckmann-Cavalcante MZ, Cavalcante IHL, Cavalcante LF, Sousa GB, Santos JB, Souza MSM (2008). Growth and water consumption of two bean species under irrigation with saline water. Sci. Agraria 9: 349-355.

Bieleski RI, Turner NA (1966). Separation and estimation of aminoacids in crude plants extracts by thin-layer electrophoresis and chromatography. Anal. Bioch. 17: 278-293.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein binding. Anal. Bioch. 72: 248-254.

Chartzoulakis KS (2005). Salinity and olive: growth, salt tolerance, photosynthesis and yield. Agric. Water Manag. 78: 108-121.

Costa PHA, Silva JV, Bezerra MA, Enéas Filho J, Prisco JT, Gomes Filho E (2003). Crescimento e níveis de solutos orgânicos e inorgânicos em cultivares de *Vigna unguiculata* submetidos à salinidade. Rev. Bras. Bot. 26: 289-297.

FAO (2000). Global network on integrated soil management for sustainable use of salt-affected soils. http://www.fao.org/ag/AGL/agll/spush/intro.htm.

Ferreira DF (2000). Sistemas de análise estatística para dados balanceados. UFLA/DEZ/SISVAR, Lavras.

Flexas J, Bota J, Loreto F, Cornic G, Sharkey TD (2004). Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. Plant Biol. 6: 269-279.

Flowers TJ (2004). Improving crop salt tolerance. J. Exp. Bot. 55: 307-319.

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000). Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51: 463–99.

Hoagland DR, Arnon DI (1950). The water culture method for growing plants without soil. California Agriculture Experimental Study, USA. (Circular, 347).

Hsiao TC, Xu LK (2000). Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relation to water transport. J. Exp. Bot. 51: 1595-1616.

Huang Y, Bie ZL, Liu ZX, Zhen A, Jiao XR (2011). Improving cucumber photosynthetic capacity under NaCl stress by grafting onto two salt-tolerant pumpkin rootstocks. Biol. Plant. 55: 285-290.

Lacerda CF, Cambraia J, Cano MAO, Ruiz HA (2001). Plant growth and solute accumulation and distribution in two sorghum genotypes, under salt stress. Rev. Bras. Fisiol. Veg. 13: 270-284.

Lacerda CF, Cambraia J, Cano MAO, Ruiz HA (2003). Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. Envir. Exp. Bot. 49: 107-120.

Lima CB, Santos Filho SV, Santos MA, Oliveira M (2007). Influência da água salina nas características físico-químicas do solo e no desenvolvimento da mamoneira cultivada em vasos. Rev. Caat. 20: 132-136.

Lorenzi H (2000). Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas do Brasil. 3.ed. Instituto Plantarum, Nova Odessa, Brasil.

Meloni DA, Gulotta MR, Martinéz CA, Oliva MA (2004). The effects of salt stress on growth, nitrate reduction and proline and glycinebetaine accumulation in *Prosopis alba*. Braz. J. Plant Physiol. 16: 39-46.

Munns R (2002). Comparative physiology of salt and water stress. Plant. Cell Envir. 25: 239-250.

MUNNS R, Husain S, Rivelli AR, James RA, Condon AG, Lindsay MP, Lagudah ES, Schachtman DP, Hare RA (2002) Avenues for increasing salt tolerance of crops, and the role of physiologically based selection traits. Plant and Soil, v. 247, p. 93-105, 2002.

Oliveira LAA, Barreto LP, Bezerra Neto E, Santos MVF, Costa JCA (2006). Solutos orgânicos em genótipos de sorgo forrageiro sob estresse salino. Pesq. Agrop. Bras. 41: 31-35.

Rontein D, Basset G, Hanson AD (2002). Metabolic engineering of osmoprotectants accumulation in plants. Metab. Eng. 4: 49-56.

Saneoka H, Nagasaka C, Hahn DT, Yang WJ, Premachandra GS, Joly RJ, Rhodes D (1995). Salt tolerance of glycinebetaine-deficient and -containing maize lines. Plant Physiol. 107: 631-638.

Santos MG, Ribeiro RV, Machado EC, Pimentel C (2009). Photosynthetic parameters and leaf water potential of five common bean genotypes under mild water deficit. Biol. Plantarum 53: 229-236.

Stepień P, Klobus G (2006). Water relations and photosynthesis in *Cucumis sativus* L. leaves under salt stress. Biol. Plantarum 50: 610-616.

Vincent JM (1970). A manual for the pratical study of root-nodule bacteria. Blackwell Scientific Publications, Oxford, USA. (IBP Hand book, 15).

Yemm EW, Coccking EC (1955). The determination of amino acid with ninhidrin. Analyst 80: 209-13.

Yemm EW, Willis AJ (1954). The estimation of carbohydrates in plant extracts by anthrone. Bioch. J. 57: 508-14.

Zhang RH, Li J, Guo SR, Tezuka T (2009). Effects of exogenous putrescine on gas-exchange characteristics and chlorophyll fluorescence of NaCl-stressed cucumber seedlings. Photos. Res. 100: 155-162.