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EFFECT OF SUCROSE INDUCED OSMOTIC STRESS ON CALLUS GROWTH AND BIOCHEMICAL ASPECTS OF TWO WHEAT GENOTYPES

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Abstract

The research work was carried out to study the effect of sucrose induced osmotic stress on callus growth and biochemical aspects of two wheat genotypes (S-24 and MH-97). The seeds were cultured on Linsmaier and Skoog medium containing 30g sucrose, 8g agar, 5mg L^{-1} thiamine HCl and 3mg L^{-1} 2, 4-Dichlorophenoxyacetic acid. One month old calli were subcultured for 15 d in liquid LS-medium supplemented with same concentration of thiamine HCl and 2,4-D. and different concentrations of sucrose [control, (3%), 4%, 6% 5% and 8%]. After 15 d of sucrose induced osmotic stress the results revealed that relative growth rate (fresh), macro cations (K^+, Ca^{2+}, Mg^{2+})and micro cations (Mn^{2+}, Fe^{2+}) significantly decreased, while dry weight, free proline, total soluble carbohydrates contents and water relation parameters significantly increased (more negative) as concentration of sucrose increased in the culture medium. The effects of sucrose induced osmotic stress was greater on MH-97 than S-24.It is concluded that increasing sucrose concentrations in the medium above control caused osmotic stress and it also been found that accumulation of free proline and total soluble carbohydrates accumulated in greater amount responsible for turgor maintenance and increase in callus dry weights.

Introduction

Plants resort to many adaptive strategies in response to abiotic environmental stresses such as high salt, dehydration, cold, heat and osmotic stress which affect plant growth (Epstein *et al.*, 1980; Shu *et al*., 2004). It has been reported that osmotic stress effects callus growth, colony formation, shoot regeneration, somatic embryogenesis, adjustment in ion transport i.e extrusion or uptake of ions, metabolic changes e.g carbon metabolism, the synthesis of compatible solutes such as proline has been suggested as one of the possible means for overcoming osmotic stress (Shankhadhar *et al*., 2000; Al-Khayri & Al-Bahrany, 2002) and the metabolism of spesific compounds (Huang & Liu, 2002) and plants exhibit a wide range of responses at the moleculer, cellular and whole plant levels (Hasegawa *et al*., 2000).

Osmotic agents such as sucrose, mannitol and sorbitol not only acts a common source of carbon in the cell culture media of cereal (Al-Khayri & Al-Bahrany, 2002) and energy but also as an osmotica during organogenesis (Huang & Liu, 2002) and accumulated in many plant tissues in response to environmental stress, including water deficit (Ramos *et al.*, 1999) for playing a role in osmoregulation and cryoprotection. It has also been reported that sucrose in lower concentration (2% and 4%), is necessary for optimal growth and multiplication (George & Sherington 1984, Hazarika, 2003), decreases the fresh weight (Plas & Wagner, 1984), increases the dry weight (Kishore & Dange, 1990) but at high concentration reduces the growth rate (Shibli *et al*., 1992),

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chlorophyll formation to induce embryogenic callus formation (Tremblay & Tremblay, 1991; George, 1993) and water and osmotic potential i.e. more negative (Huang & Liu, 2002) that reduces the optimal turgor pressure (Seijo, 2000).

As there is no information on link between osmotic stress and callus growth of wheat, so this study was used to clarify the relationship between osmotic stress, and morpho-biochemical aspects in wheat callus tissues.

Materials and Methods:

The experiment was conducted in the Tissue Culture Laboratory, Department of Botany, University of Agriculture, Faisalabad, to study *In Vitro* assessment of sucrose induced osmotic stress in callus tissues of two bread wheat (*Triticum aestivum* L) genotypes, obtained from IRRI and Botany Department, University of Agriculture, Faisalabad. The mature embryos (grains) of both wheat (*Triticum aestimvum* L.) genotypes i.e. MH-97 and S-24 were used as a source of explants. The medium used in this study was LS (Linsmaier & Skoog, 1965) plus 8g agar, 5mg thiamine HCl and 3mg L^{-1} 2,4-dichlorophenoxy acetic acid (2,4-D). The pH 5.8 was adjusted before autoclave. The grains were washed first with detergent and rewashed with running tap water followed by thrice with distilled water. For surface sterilization seeds were first dipped in 90% ethyl alcohol for 10 seconds followed by 20% (v/v) sodium hypochlorite solution for 25 minutes and finally by 0.01% mercuric chloride for 4 minutes. The grains were then rinsed with autoclaved distilled water aseptically in laminar flow cabinet, till to ensure complete removal of mercuric chloride.

Callus initiation: The surface sterilized grains were placed in the culture tubes containing L.S medium supplemented with $3mgL^{-1}$ of 2,4-D (2, 4-dichlorophenoxy acetic acid) to initiate calli. After culturing the explants, the cultured tubes were sealed with cotton plugs and incubated in the growth room under 2000 Lux light at $25\pm2^{o}C$ for 30 days.

Sucrose treatments: There were five sucrose (osmotic stress) treatments including control [control (3%), 4%, 5%, 6%, and 8%] and each treatment was replicated thrice.

Application of sucrose (osmotic stress) treatments: Thirty days old calli were harvested and subjected to sucrose treatments. About 2g (fresh weight) calli were cultured in Erlen-meyer flasks (100mL Pyrex) containing 40ml of LS liquid medium, supplemented with above mentioned sucrose concentrations and 3mg L^{-1} of 2,4-D. The flasks were placed on orbital shaker at Completely Randomized Design, under above mentioned environment conditions. After 15 days of stress treatment, calli were harvested, and various morpho-biochemical parameters were studied.

Relative growth rate (fresh) and dry weight of callus: Calli after 15 d of treatments harvested from liquid medium, were first washed with deionized distilled water for 2 min. with continuous agitation. After dried with tissue paper, fresh weights of calli were determined and RGR was calculated with the following formula.

$$= \ln(F.\ wt) - \ln(I.\ wt)$$

Callus dry weights were determined after calli dried in oven at 65°C for 74 hours.

Cations accumulation: K^+ and Ca^{2+} were determined with flame photometer (Model PFP-7. Jenway, UK), while Mg^{2+}, Fe^{2+} and Mn^{2+} were determined with atomic absorption spectrophotometer. About 0.04g oven dried material was digested with acid (5ml HNO_3) and finally volume was made up to 50 ml.

Water relations of callus tissues: The calli were first freezed at -70°C in ultra freezer (SANYO MDF-382, ultra low). After 7 days, the calli were defrosted and extract the solutes. 10 µL of the extract was used to determine the osmotic potential of callus with the help of osmometer (Vapor-Vapour pressure osmometer 5520). To determine the water potential of callus tissues, osmotic potential of the respective medium of each replicate from which the callus harvested was determined to assume that osmotic potential of the medium equal to the water potential of the callus tissues. The turgor potential was calculated as the difference between water potential and osmotic potential.

Free proline and total soluble carbohydrates contents: Extraction and estimation of free praline and total soluble carbohydrates was conducted according to the procedure described by Bates *et al*., (1973) and Yemm & Willis (1954).

Statistical analysis: A two way analysis of variance of data for all the parameters was computed, using the COSTAT computer package (Cohort software Berkeley, California). The least significant differences between means were calculated.

Results

The results obtained after 15 days of sucrose induced stress with regard to callus tissues relative growth rate (fresh) revealed that increasing concentration of sucrose in culture medium showed a significant decline, while dry weights increased significantly in of both the genotypes (Fig.1). S-24 gave more dry weight and less reduction in relative fresh weight than MH-97.

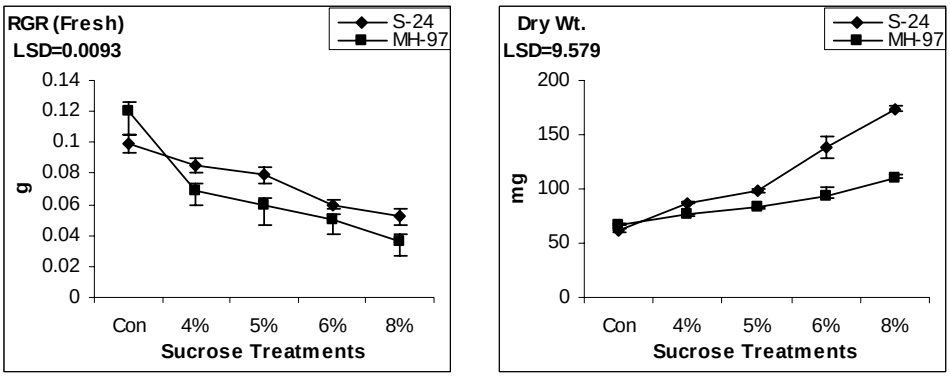

Fig.1. RGR (Fresh) and dry weights of callus tissues of two wheat genotypes after 15 d treatments with various concentrations of sucrose. SE and LSD are shown.

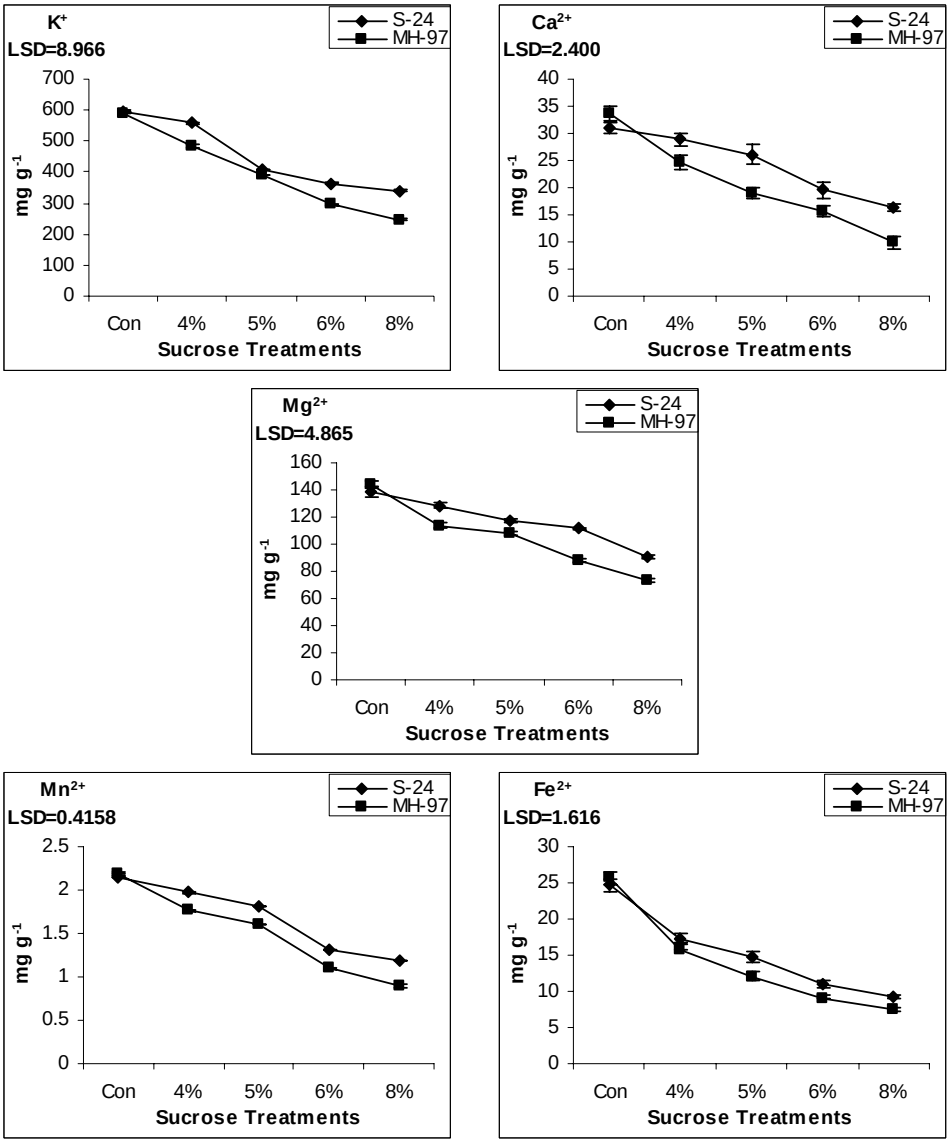

Fig.2. Cations (macro and micro) accumulation in callus tissues of two wheat genotypes after 15 d treatments with various concentrations of sucrose. SE and LSD are shown.

Accumulation of macro cations **(**K^+, Ca^{2+}, and Mg^{2+}) in callus tissues of both the genotypes affected significantly. Data revealed decline with increasing sucrose concentrations of culture medium. In S-24, K^+ accumulation was more effected followed by Ca and Mg.MH-97 showed the same pattern of reduction but extent of reduction was greater than S-24. Among the macro cations Mg content showed more reduction than K and Ca in both the genotypes. Micro cations (Fe and Mn) also showed significant

reduction but the extent was greater than macro cations in callus tissues of both the genotypes (Fig.2).

Osmotic and water potential of the callus tissues of both the genotypes increased (more –ve), while turgor potential increased with the increasing concentration of sucrose in the growth medium (Fig.3). Genotype S-24 showed more reduction in osmotic and water potentials, while less in turgor than MH-97.

Free proline and total soluble carbohydrates significantly increased in both genotypes callus tissues as the sucrose concentration increased of the culture medium (Fig. 4). The increase was many fold and extents of both contents were more in S-24 than MH-97 callus tissues

Discussion

Sucrose is the mist commonly used carbohydrate in plant tissues culture. This is due to wide spread of this particular disaccharide as a transporter molecule, suppose to its high solubility in water. Majority of in vitro studies have calculated that sucrose supports near optimum rates of growth (Swedlund & Locy, 1993). In spite of this, some others (Carrier *et al.*, 1997; Johnson *et al.*, 1997) reported that sucrose might play multiple roles including the prevision of carbon and energy, causing an osmotic effect. In vitro addition of sucrose in the growth medium acts as osmotic agent that may introduce osmotic stress

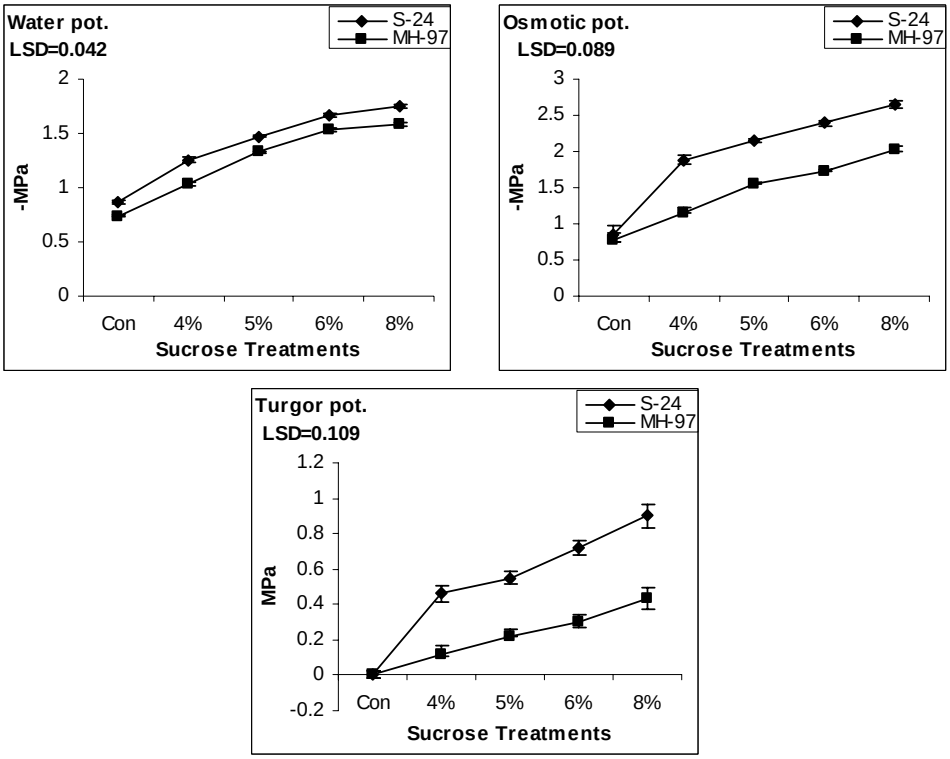

Fig.3. Water, osmotic and turgor potentials of callus tissues of two wheat genotypes after 15 d treatments with various concentrations of sucrose. SE and LSD are shown

above certain concentrations lead to decrease in growth (Kishore & Dange, 1990; Mehta *et al.,* 2000; Kim & Kim, 2002), while dry weights increased under increasing sucrose osmotic stress (Kishore & Dange, 1990; Juhasz *et al.,* 1997). The present study revealed that relative growth rate decline constantly in both the genotypes, while dry weights of callus tissues of both the wheat genotypes increased consistently and extent was more in S-24 than MH-97. The increase in dry weight of callus tissue was due the more accumulation of proline and total soluble carbohydrates in the callus tissues.

Cations accumulation under the drought and salt stress conditions decreased at whole plant level as well as at cellular level (Javed, 2002a; Ahmad *et al.*, 2007), same pattern reveled by the present study that callus tissues of both the genotypes showed reduction in cations accumulated and effect was more as the concentration of sucrose in the culture media. Although cations accumulation was decreased, the reduction was more in MH-97 than S-24.

Osmotic adjustment through the accumulation of cellular solutes, such as proline has been suggested as one of the possible means for overcoming osmotic stress caused by the loss of water (Al-Bahrany, 1994; Shankhadhar *et al.,* 2000). Free proline and total soluble carbohydrates accumulation increased many folds upon exposure to abiotic stresses (Geetha *et al.,* 1996; Al-Khayri & Al-Bahrany, 2002; Javed, 2002b; Ahmad *et al.*, 2007; Ahmad *et al.,* 2006). The present study revealed that sucrose induced osmotic stress increased free proline and total soluble carbohydrates contents in both wheat genotypes callus tissues (Al-Khayri & Al-Bahrany, 2002). The extent of proline and soluble carbohydrates accumulation were more in S-24 callus tissues than MH-97. Osmotic potential is one of the most important parameter often affected by abiotic stresses. Under drought stress the osmotic potential in tolerant plants is reduced (more –ve) with the increasing intensity of stress. This reduced osmotic potential helps the plants to uptake more water and maintain growth (Almansouri *et al.,* 2000). Osmotic potential of the calli also increased (more-ve) with increasing stress level of the culture medium (Bajji *et al.*, 2000; Huang & Liu, 2002; Javed, 2002c; Ahmad *et al.*, 2007), particularly by sucrose (Riham *et al.,* 2001). This increase (more-ve) in water potential and accumulation of osmotica like, total soluble carbohydrates and proline were to accelerate the water uptake and hence enhance growth (Javed, 2002c; Ahmad *et al.*, 2007), Our present study

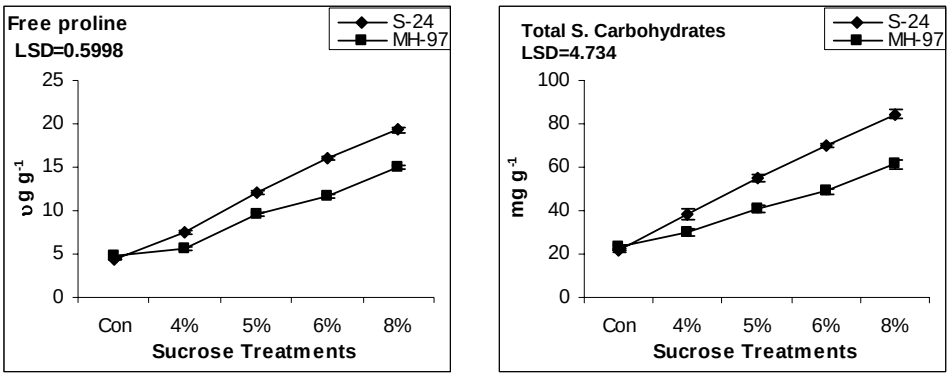

Fig.4. free proline and total soluble carbohydrates accumulation in callus tissues of two wheat genotypes after 15 d treatments with various concentrations of sucrose. SE and LSD are shown.

exhibited the similar results under sucrose induced osmotic stress, because proline and total soluble carbohydrates contents of both the wheat genotypes callus tissues increased (Fig.4). Callus tissue of S-24 exhibited more –ve water potential, since less reduction in cations accumulation and more accumulation of proline and total soluble carbohydrates than MH-97.

Abiotic stress does not affect the turgor potential (Gibbs *et al*., 1989; Ikeda *et al*., 2002) or in some cases increases it (Javed, 2002c). The maintenance of turgor is very important under stress condition in tolerant cultivars for enhanced growth (Ikeda *et al*., 2002). This maintenance is brought about by increase (more-ve) in osmotic and water potential of the tissues (Gupta *et al*., 1995). Those cultivars which can not maintain turgor are unable to grow and maintain growth with the increasing intensity of stress (Newton *et al.*, 1987). Present study revealed similar results under sucrose induced osmotic stress. Callus tissue of S-24 wheat genotypes showed more turgor than MH-97 at all the concentrations of sucrose.

In conclusion, sucrose induced osmotic stress has showed significant effect on callus growth as well as biochemical parameters in both the wheat genotypes. Reduction in growth and accumulation of free proline and total soluble carbohydrates in callus tissues of both the wheat genotypes, responsible for the increase (more –ve) in osmotic and water potentials while, decreased in turgor potential and collectively showed impact on the enhancement of callus dry weights. It has also been found that excessive production of free proline and total soluble carbohydrates were responsible for turgor maintenance in the callus tissues during sucrose induced osmotic stress.

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