Available online at www.elixirpublishers.com (Elixir International Journal)

Agriculture

Elixir Agriculture 97 (2016) 42189-42191

ISSN: 2229-712X

CP73-21 Sugarcane Regeneration at Salinity Stress by Emphasis on Variety of Somaclonal

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ARTICLE INFO

Article history:
Received: 30 July 2016;
Received in revised form:
10 August 2016;
Accepted: 15 August 2016;

Keywords
Proline,
Regeneration,
Sugarcane.

ABSTRACT

For CP73-21 sugarcane regeneration at salinity stress an experiment was conducted in 2014-2015 year, in tissue culture laboratory of Islamic Azad University of Ahvaz. According to past results, 3 mg/l of 2-4D treatment was applied as callus induction treatment. Then calluses were transferred to regeneration medium. The effect of treatments was significant at 1% on indirect regeneration. In between treatments the highest of mean was related to 1 BAP + 0.2 NAA treatments, also the effect of salinity on regeneration was significant at 1%. The highest regeneration value was obtained in control and 33 mM treatments. Effect of Salinity on proline was significant at 1% and to increase the level of stress, increased proline content. In both laboratory and greenhouse conditions the investigated of traits were decreased with increasing salinity levels.

Introduction

The over salinity of the soil is one of the main factors that limits the spread of plants in their natural habitats. It is an ever-increasing problem in arid and semi-arid regions(Shannon, 1996). Fisher and Turner (1978) estimate that arid and semi-arid lands represent around 40% of the earth's area. The property of salinity tolerance is not a simple attribute, but it is an outcome of various features that depend on different physiological interactions, which are difficult to determine(Fischer and Turner, 1978). The morphological appearance presented by the plant in response to salinity, may not be enough to determine its effect, so it is important to recognize other physiological and biochemical factors, including proline. Plants are classified as glycophytes or halophytes according to their capacity to grow on high salt medium. Most plants are glycophytes and cannot tolerate salt-stress. High salt concentrations decrease the osmotic potential of soil solution creating a water stress in plants. Secondly, they cause severe ion toxicity, since Na+ is not readily sequestered into vacuoles as in halophytes. Finally, the interactions of salts with mineral nutrition may result in nutrient imbalances and deficiencies. The consequence of all these can ultimately lead to plant death as a result of growth arrest and molecular damage (McCue and Hanson, 1992). Sugarcane (*Saccharum officinarum* L.) is an important agro-industrial sugar crop, contributing about 70% of the world sugar production. Globally, it occupies about 20 Mha of land, a little about 2% of total cropped area, producing 1350 million MT of cane (FAO, 2004). Sugarcane is cultivated as a commercial crop in nearly 60 countries spread over the world. However, being a typical glycophyte, it exhibits stunted growth or no growth under salinity, with its yield falling to 50% or even more of its true potential (Subbarao and Shaw, 1985). Besides this, salinity in root zone of sugarcane decreases sucrose yield through its effect on both biomass and juice quality (Lingle and Wiegand, 1996). At this study we evaluated CP73-21 sugarcane regeneration at salinity stress by emphasis on variety of somaclonal.

Material and methods

Experiment was conducted in 2014-2015 year, in tissue culture laboratory of Islamic Azad University of Ahvaz. According to past results (Mohammadnejad et al., 2016) 3 mg/l 2-4-D was applied as callus induction treatment, so sodium chloride was used for to determine the salinity tolerance of viable callus. For this purpose, 5 treatments were prepared 0, 33, 66, 99 and 132 mM. MS medium was used for regeneration medium and treatments included 0.5mg/l of BAP +0.5mg/l Kinetin, 1.0 mg/l of BAP +1.0 mg/l Kinetin, 1.0 mg/l of BAP +0.2 mg/l NAA, 2.0 mg/l of BAP +0.1 mg/l NAA. The genesis of root was obtained by 0.1 mg/l IBA, 0.1 mg/l NAA, 0.03 mg/l IBA, 0.03 mg/l NAA treatments. To study the variety of regenerated plantlet, plantlets were transferred to green house and after hardening process, Proline content was evaluated to determination of tolerant plant. Analysis of variance and Duncan mean comparison were performed by using SAS statistical software, also, Probit analysis was carried out by using the software Minitab version 16 and graphs were drawn by using Excel software.

Result and discussion

Indirect regeneration

Between treatments, highest regenerations were obtained by application of 1BAP + 0.2 NAA and 2BAP + 0.1 NAA, respectively. Also, it was founded that other treatments did not show any regeneration. So, 1BAP + 0.2 NAA was selected as best treatment. These data confirm the results reported in Primula ssp. (Schween and Schwenkel, 2003) Oryza sativa (Hoque and Mansfield, 2004) , and Triticosecale (Birsin and Ozgen, 2004).

Indirect regeneration at salinity stress

According to previews results, 1BAP + 0.2 NAA was selected as best treatment for indirect regeneration at salinity stress. Means comparisons showed that the highest regeneration was observed in control and 33 mM On the other hand no regeneration was observed in 132 mM. The success of in vitro culture depends mainly on the growth conditions of the source material (Caswell et al., 2000, Delporte et al., 2001)

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,medium composition and culture conditions(Saharan et al., 2004) and on the genotypes of donor plants. Among those factors, the genotype appears to be important factor influencing the efficiency of in vitro culture.

Proline content

Results showed that the effects of the treatments were significant at 1% statistically probability and it founded that with increasing levels of stress, proline content increased. So, application of 33, 66 and 99 mM salinity stress increased proline 1.8, 2.6 and 3 fold in compare to control, respectively. In organisms ranging from bacteria to higher plants, there is a strong correlation between increased cellular proline levels and the capacity to survive both water deficit and the effects of high environmental salinity. It may also serve as an organic nitrogen reserve that can be utilized during recovery although proline can be synthesized from either glutamate or ornithine; glutamate is the primary precursor in osmotically stressed cells. The biosynthetic pathway consists of two important enzymes, viz. pyrroline carboxylic acid synthetase and pyrroline carboxylic acid reductase. Transcripts corresponding to both cDNAs accumulate in response to NaCl treatment. Both these regulatory steps are keys to developing strategies for overproducing proline in a selected plant species(Iyer and Caplan, 1998, Sairam and Tyagi, 2004). Totally, the effect of different levels of salinity 0, 33, 66, 99 and 132 mM were investigated to tolerance of callus in completely randomized design. After 8 weeks, the callus value reduction by 33, 66, 99 and 132 treatments in compare to control were obtained 31, 33, 22 and 26%, respectively. Calluses were transferred to regeneration medium. The effect of treatments was significant at 1% on indirect regeneration. In between treatments the highest of mean was related to 1 BAP + 0.2 NAA treatments, also the effect of salinity on regeneration was significant at 1%. The highest regeneration value was obtained in control and 33 mM treatments. Effect of Salinity on Proline was significant at 1% and to increase the level of stress, increased proline content. In both laboratory and greenhouse conditions the investigated of traits were decreased with increasing salinity levels.

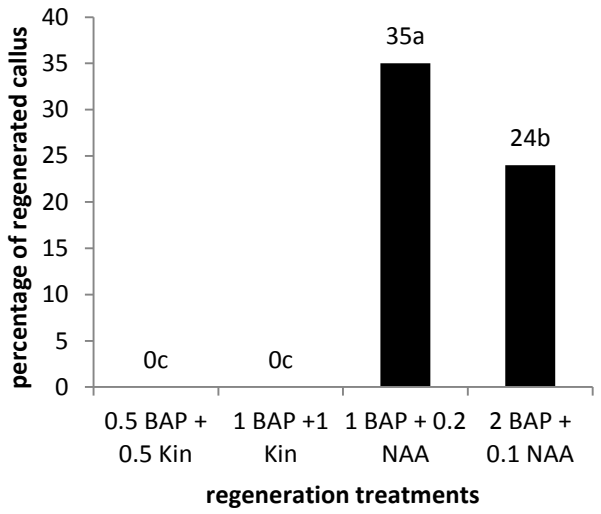

Figure 1. Effect of regeneration treatments of indirect regeneration

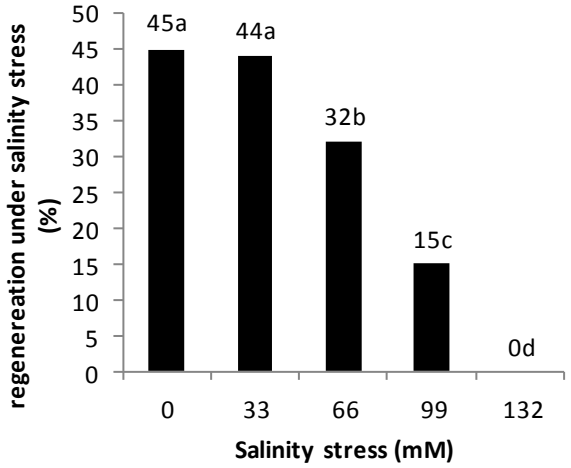

Figure 2. Effect of salinity stress on indirect regeneration

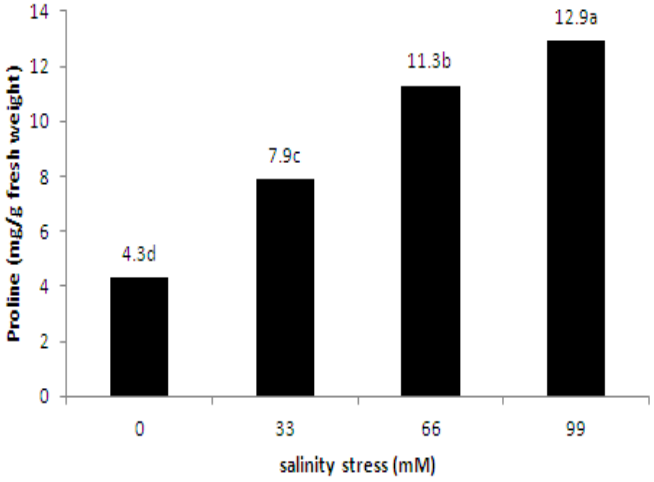

Figure 3. Effect of salinity stress on proline content

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