CHAPTER ONE

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is an important sugar crop for producing the sweetener and sugar. Commercial production of sugar from sugarcane began in India and China approximately 2500 years ago and spread to Western Europe in the eighteenth century **(James, 2004)**. Today, sugarcane is used as raw materials for fuel production, chemicals, bio-fertilizers, paper and pulp (**Arruda, 2011**).

Sugarcane is an important agro-industrial sugar crop, contributing about 70% world sugar production. Globally, it occupies more than 23.98 million hectares of land worldwide, generating 1.71 billion tons of harvested cane in 2010 **(FAO, 2012)**.

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 the root zone of sugarcane decreases sucrose yield through its effect on both biomass and juice quality (**Lingle and Weigand, 1996)**. A large acreage of land is affected with abiotic stress i.e., world's 20% cultivated land and nearly half of all irrigated land is affected by salinity (**Rhoades and Loveday, 1990**) and 93 Mha of cultivable land is rain fed.

Tissue culture system is useful for the evaluation of tolerance to environmental stresses because the stress conditions can be easily controlled *in vitro*. Moreover, *in vitro* culture provides a uniform population of synchronously developing plant cells without involving regulatory mechanisms that naturally repaired at the whole plant level (**Tal, 1983**).

In vitro tissue culture constitutes an important tool to study the physiological and biochemical mechanisms that operate in response to stress conditions at the cellular level (**Lerner, 1985**). Furthermore, the plant tissue culture allows the control of stress homogeneity and the characterization of cell behavior under stress conditions, independently of regulatory systems that take place at the whole plant level (**Lutts *et al.* 2004).**

Abiotic stresses especially salinity and drought limit crop productivity (**Akhtar *et al.* 2003**). Sugarcane is extensively cultivated in irrigated lands worldwide. Being a typical glycophyte, it exhibits stunted or no growth under salinity, with its yield falling to more than 50% as compared to its true potential

(**Wiedenfeld, 2008**) due to alterations in water relations, ionic and metabolic perturbations, and tissue damage (**Patade *et al.* 2011**).

In tolerant plants, there are many defense mechanisms such as osmoregulation, ion homeostasis, antioxidant and hormonal systems, helping plants to stay alive and development prior to their reproductive stages **(Hasegawa *et al*. 2000; Wang *et al*. 2003; Reddy *et al*. 2004; Sairam and Tyagi, 2004; Mahajan and Tuteja, 2005; Ashraf, 2010).**

Stress reduced the growth of cultured sugarcane cells significantly over the control. This growth limitation is possibly due to abiotic stress may presumably be due to the osmotic effect of salt earlier phase or the ionic stress which, impacts growth much later (**Munns and Tester, 2008**).

Excess of Na^+ and Cl^- ions may lead to conformational changes in protein structures, while the osmotic stress leads to turgor loss and cell volume change (**Chinnusamy and Zhu, 2003**). However, the precise mechanisms underlying these effects are not fully understood because the resistance to salt stress is a multi-genic trait (**Parida and Das, 2005**).

To achieve salt tolerance, plant cells evolve several biochemical and physiological pathways. These processes are thought to operate additively to ensure plants and cells survival and they include the exclusion of Na^+ ions and their compartmentation into vacuoles as well as the accumulation of compatible solutes such as proline, glycinebetaine and polyols (**Hasegawa *et al.* 2000; Chinnusamy and Zhu, 2003; Parida and Das, 2005)**.

Actually, the stress-resistant in genotypes accumulated proline at lesser extent than the stress-sensitive one. This finding let us suggest that proline accumulation among sugarcane cultivars is merely a symptom of injury rather than a stress resistance trait. Identical statements were reported in several other species (**Cano *et al.* 1996; Garcia *et al*. 1997; Tonon *et al*. 2004**).

Furthermore, the contribution of proline to osmotic adjustment, from a quantitative point of view, in sugarcane cultivars seemed to be insignificant as was reported previously in other species under mannitol and NaCl- induced stress (**Mohamed *et al.* 2000; Benlloch-Gonza` lez *et al.* 2005**).

Therefore, the present investigation was undertaken to fill in some of lacunae with the following objectives:

1- To evaluate three sugarcane (*Saccharum officinarum* L.) genotypes for their capabilities for callus induction, day of callus initiation and embryogenic callus,

2- To study the effect of drought stress using different concentration of mannitol on callus to determine the tolerance of each genotypes,

3- To study effect of relative growth rate (RGR), water content (WC), accumulation of Na^+ and K^+ ions and determine the proline content at different concentration of mannitol on callus,

4- To identify the genetic variation among sugarcane genotypes via RAPD-PCR and,

5- To study the effect of drought stress on shoot and root formation.