ESTABLISHMENT OF *in vitro* GERMINATION AND GROWTH CONDITIONS OF *Distichlis spicata*.

Castelán M.L., Villalobos M.A., Arroyo A.
Centro de Investigación en Biotecnología Aplicada-IPN Tlaxcala, Ex-Hacienda San Juan Molino, Tepetitla Tlaxcala, México.
E-mail castelanmarialuisa@hotmail.com; mvillalobosl@ipn.mx; alarroyo@ipn.mx

ABSTRACT

Highly tolerant plant species, such as the native grass *Distichlis spicata* var. stricta (L) Greene shows great potential for use as a model system to study salinity and water stress tolerance in plants; and as forage during drought or flooding periods, or in saline/alkaline and infertile soils. However, *D. spicata* is known to have an extremely low germination rate (3%) in field. To strictly and consistently study this system, it is essential to establish *in vitro* germination and growth conditions, as in field multiple factors affect results during experimentation. In order to establish *in vitro* germination and growth under controlled laboratory conditions as well as to enhance germination rates, we tested the effects on contamination and germination rates of several antiseptic agents and mechanical scarification and stratification seed treatments. Results from seed treatments that included mechanical scarification, stratification and treatment with sodium dicloroisocyanurate (NaDCC) 20% (w/v) proved to be the best procedure to significantly enhance disinfection and germination up to 80% in only 6 days. After germination tests, ten days after seeded, plantlets were successfully established in pots in growth chamber and green house conditions. Thus, we have successfully established *D. spicata in vitro* germination and growth conditions which allow the study of this grass under controlled conditions, and increase its potential for propagation in poor soil conditions.

INTRODUCTION

Currently, climate change and increasing agricultural irrigation has generated world-wide salinity and drought concerns. Highly tolerant plant species, like the native grass *Distichlis spicata* var. stricta (L.) Greene has great potential for use as a model system to study salinity and water stress tolerance in plants. Nevertheless, this perennial halophyte (O'leary and Glenn, 1994) commonly known as saltgrass, has been proposed as potential forage because it is generally green during drought or flooding periods, or in saline/alkaline and infertile soils, where other pasture grasses are not available and is tolerant to wear. Markedly it is well known that it has an extremely low germination rate (3%) likely because seed coat in the wild need to be decayed or to be broken by freezing and thawing conditions (Qian *et al*., 2006, 2007; Shahba *et al*, 2008). Saltgrass is native to America and is widely distributed in México, from Coahuila to Yucatán (Rzedowski and Rzedowski, 2001). *Distichlis spicata* belongs to the family *Poaceae*, it is a low perennial with wiry culms and creeping or deeply running rhizomes 2-3 dm tall, often prostrate with a strong tendency to form stolons; blades erect, 1-2 dm long, the upper exceeding the female panicle and often equaling the male; saltgrass flowering period is in April-July (USDA PLANTS Database).

In vitro contamination by fungi or bacteria is one of the most serious problems of commercial and plant tissue culture research laboratories (Leifert *et al*., 1994); especially when plant material comes directly from field. The establishment of *in vitro* germination of *D. spicata* is of primary importance, because no standard protocol exists to get high germination percentages under strictly controlled conditions. Thus the aim of this work was to establish *D. spicata in vitro* germination and growth conditions that will allow the study of this grass at the molecular level to get a better understanding of molecular basis of water deficit stress, and increase its potential for propagation in poor soil conditions.

MATERIALS AND METHODS

Biological Material

D. spicata (Saltgrass) seedheads were collected on September 2007 from "Llano Salado" halofit turfgrass, NW El Carmen (19º 17` 36.8" N, 97º 40` 00" W, 2350 meters above sea level); then seeds were separated from hulls and other debris using tweezers. Seeds were immediately stored at 4 °C.