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Sensitivity of hybrid *Cymbidium* (Orchidaceae) to salt stress ($MgSO_4$, $CaCl_2$ and KNO_3)

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ABSTRACT
Hybrid *Cymbidium* Twilight Moon 'Day Light' is highly sensitive to salt (NaCl). This is not surprising given the natural growth locations of tropical and subtropical orchids, which usually receive clean, and unsalted water. However, to expand the potential culture of orchids using diluted salt water, or brackish water, there is interest in testing the response of orchids to different salts that occur in sea water or brackish water. In this study, the response of protocorm-like bodies (PLBs) to three salts ($MgSO_4$, $CaCl_2$ and KNO_3) was assessed. Unlike NaCl, *neo*-PLBs could form at higher concentrations of Mg^{2+}, Ca^{2+} and K^+ ions, with survival occurring at 50 mM. Although development was not optimum at this concentration of these salts, the ability to use diluted salt water or brackish water to induce orchid PLBs or to grow orchid plants would be an expansion of the possible culture conditions into developing countries or countries lined by the sea where access to clean or fresh water may be limited.

Keywords: $CaCl_2$, KNO_3, $MgSO_4$, PLB, polyamines, Teixeira *Cymbidium* (TC) medium

Abbreviations: NAA, α-naphthaleneacetic acid; PLB, protocorm-like body; PGR, plant growth regulator

Sensibilidad del *Cymbidium* híbrido (Orchidaceae) al estrés salino ($MgSO_4$, $CaCl_2$ and KNO_3)

RESUMEN
Cymbidium híbrido Twilight Moon 'Day Light' es altamente sensible a la sal (NaCl). Esto no es sorprendente teniendo en cuenta los lugares de crecimiento naturales de orquídeas tropicales y subtropicales, que por lo general reciben agua limpia y sin sal. Sin embargo, para ampliar el cultivo potencial de orquídeas con el uso de agua salada diluida o agua salobre, hay interés en probar su respuesta a diferentes sales que contiene el agua de mar o agua salobre. En este estudio, se evaluó la respuesta de cuerpos tipo protocormos (PLBs) a tres sales ($MgSO_4$, $CaCl_2$ y KNO_3). A diferencia de NaCl, los *neo*-PLBs se podrían formar en presencia de altas concentraciones de iones Mg^{2+}, Ca^{2+} y K^+, con supervivencia a 50 mM. Aunque el crecimiento no fue óptimo a esta concentración de sales, la capacidad de utilizar el agua salada diluida o agua salobre para inducir PLBs o para cultivar plantas de orquídeas sería una ampliación de las posibles condiciones de cultivo en los países en desarrollo o países situados junto al mar donde el acceso a agua limpia o fresca puede ser limitada.

Palabras clave: $CaCl_2$, KNO_3, $MgSO_4$, PLB, poliaminas, medio de Teixeira *Cymbidium* (TC)

INTRODUCTION

Water resources need to be better managed as fresh water is becoming more limited, and more wasted (Taft, 2015). The use of salt water or brackish water (the mixture of fresh water and sea water) in agriculture, greenhouse production or *in vitro* systems has always been a fascinating topic, but practical application has always been limited by stress that is often experienced by plants in response to salt. Filed trials have already taken place (Sánchez *et al.*, 2015) while only a single report has shown the potential application of salty water for ornamental plant production (Cassaniti *et al.*, 2013).

Unlike halophytic species, which grow well under saline conditions (Muscolo, 2011), such as *Conocarpus lancifolius* (Al-Kandari *et al.*, 2009), regular plants experience salt stress, affecting growth, physiology and yield (Ates and Tekeli, 2007) due to a disruption of metabolic processes caused by the accumulation of harmful oxygen radials (Cheeseman, 2007).

Teixeira da Silva (2015) showed that hybrid *Cymbidium*, an orchid, shows significant sensitivity to NaCl, the main constituent of sea water and that exists in ionic form, i.e., as Na^+ and Cl^- (as much as 0.5-30‰; Lin and Brown, 1993). After chlorine and sodium, the next most abundant salts or ions are magnesium, sulphate, calcium and potassium, followed by other constituents, but several fold lower. This response to NaCl would not be unusual given that orchids, whether terrestrial or epiphytic, would usually receive relatively clean rainwater or fresh water without salts. This study thus aimed to expose the same *Cymbidium* hybrid to three other compounds carrying these main salts/ions of salt water (except for NaCl) to assess the developmental response, fulfilling thus a gap in the objectives of orchid biotechnology (Hossain *et al.*, 2013).

This study assessed the response of hybrid *Cymbidium* protocorm-like bodies (PLBs) on Teixeira *Cymbidium* (TC) medium (Teixeira da Silva, 2012) to $CaCl_2$, KNO_3, and $MgSO_4$.

MATERIALS AND METHODS

Plant material

PLBs of hybrid *Cymbidium* Twilight Moon 'Day Light' (Bio-U, Tokushima, Japan). Stock PLBs of hybrid *Cymbidium* Twilight Moon 'Day Light' were established and maintained as explained in detail in Teixeira da Silva (2012, 2014).

The application of the three compounds follow the protocol described for Teixeira da Silva (2015).

Neo-PLBs were kept in 100-mm diameter plastic Petri dishes (AsOne, Osaka, Japan) at 25°C, under a 16-h photoperiod with a light intensity of 45 µmol m^{-2} s^{-1} provided by plant growth fluorescent lamps (Homo Lux, Matsushita Electric Industrial Co., Japan).

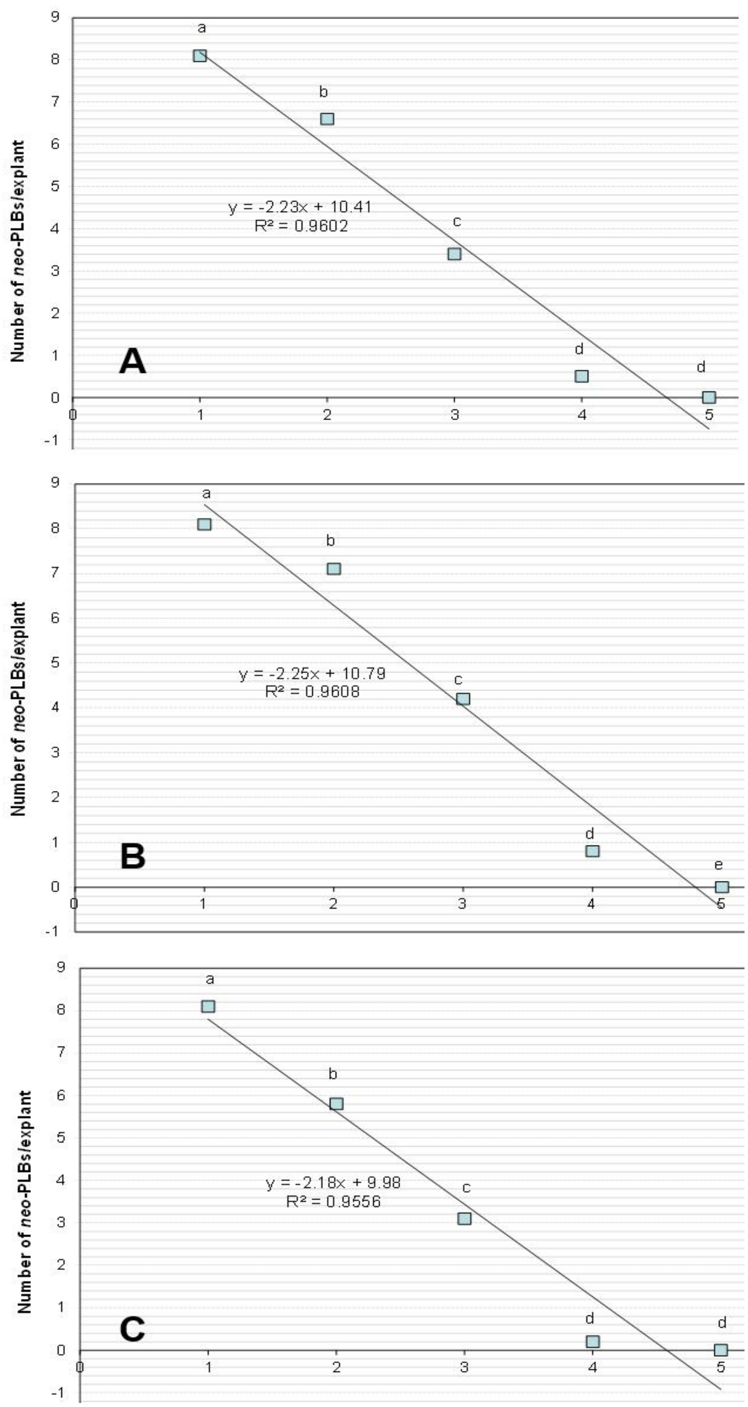
To test the effect of $CaCl_2$ (anhydrous), KNO_3 (anhydrous), and $MgSO_4$ (heptahydrate, i.e., $MgSO_4 \cdot 7H_2O$) on the development of *neo*-PLB (i.e., new PLBs), PLBs were placed on TC medium containing 0, 10, 50, 100 and 150 mM of each compound. The number of *neo*-PLBs/explant and percentage survival were determined for *neo*-PLBs after 30 days of culture.

Experiments were organized according to a randomized complete block design with three blocks of 10 replicates per treatment. All experiments were repeated in triplicate. Percentage values were arcsine transformed prior to analysis. Data was subjected to analysis of variance (ANOVA) with mean separation ($P \leq 0.05$) by Duncan's Multiple Range test (DMRT) using SAS® vers. 6.12 (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

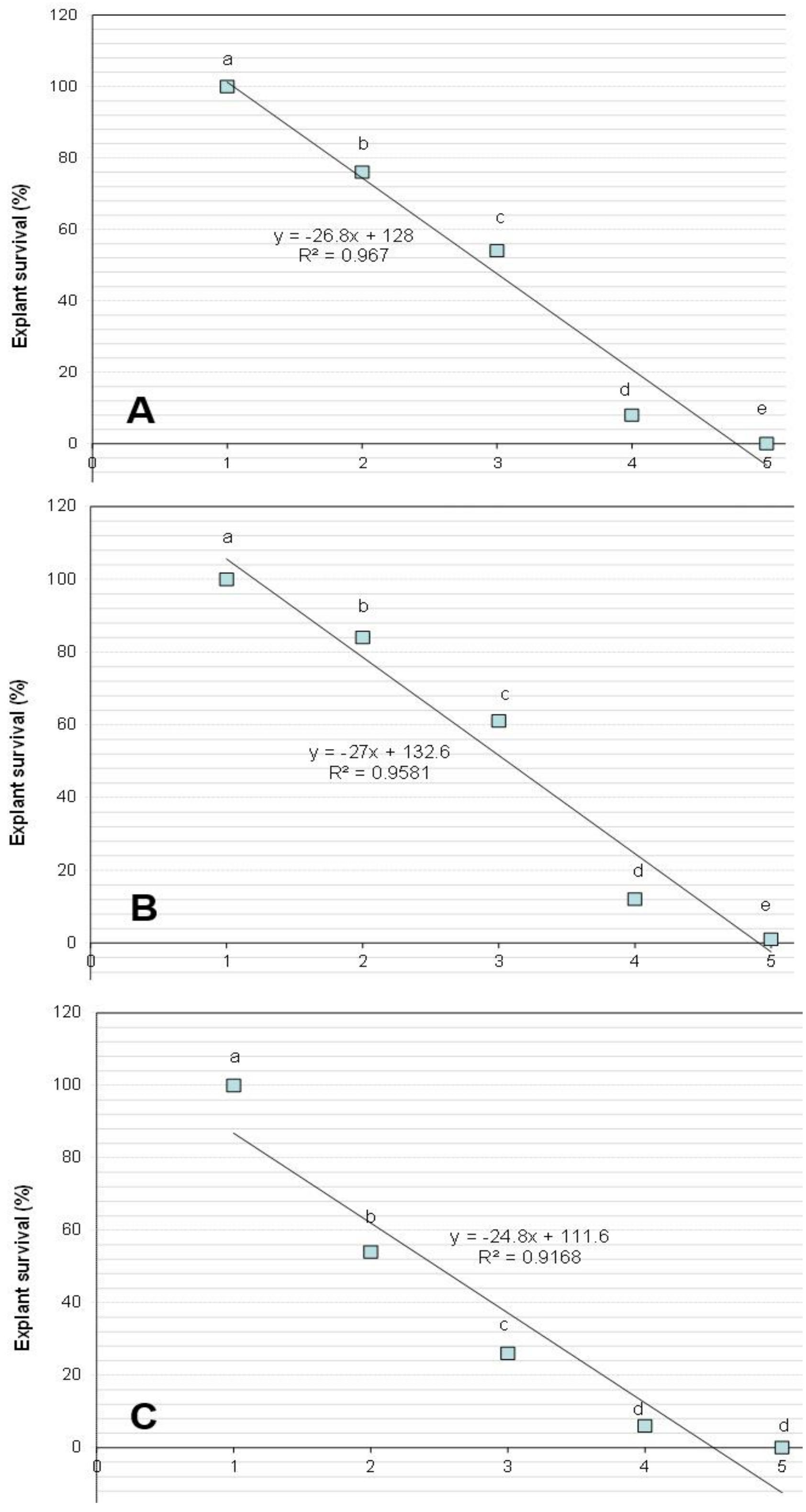
Theoretically, one would not expect to find orchids to be tolerant to salt stress caused by growth after irrigation with salt water or brackish water. These ornamentals tend to be highly sensitive to extreme abiotic conditions, and exposure to NaCl *in vitro* at as little as 20 mM can significantly reduce the formation of *neo*-PLBs in hybrid *Cymbidium* (Teixeira da Silva, 2015). However, sea or brackish water contains other ions, primarily Mg^{2+}, S^{2-}, Ca^{2+} and K^+, and thus there is interest in knowing the response to such ions. It should be noted that most artificial *in vitro* basal media for *Cymbidium* culture are made up of a wealth of macro- and micronutrients, and high or low ion media can significantly influence the formation of *neo*-PLBs (Teixeira da Silva *et al.*, 2005; Teixeira da Silva, 2013).

The three ionic compound tested ($CaCl_2$, KNO_3, and $MgSO_4$) showed a similar trend and response, with a significant negative effect on *neo*-PLB development and percentage survival of explants, necrosing at 100 and 150 mM in TC medium, and with an equally high correlation between compound ionic concentration and parameter (Fig. 1 y 2). Unlike NaCl, in which *neo*-PLB formation was negatively affected with as little as 20 mM NaCl (Teixeira da Silva, 2015), in this study, the negative impact of other salt ions was felt only at fold-higher concentrations. This indicates that hybrid *Cymbidium* is much more sensitive to NaCl (or Na^+ and Cl^- ions) that to other ions, or ionic compounds. Sea water or brackish water, if sufficiently diluted to 20-40 mM NaCl or maximum 50 mM of $CaCl_2$, KNO_3, and $MgSO_4$, could in principle be used to induce *Cymbidium* PLBs *in vitro* or grow plants in the greenhouse where fresh water resources are not available, or are scant.



Different letters between treatments indicate significant differences according to DMRT (P≤0.05).

Figure 1. Response (number of *neo*-PLBs/explant) of *Cymbidium* Twilight Moon 'Day Light' PLBs 30 days after culture initiation to: A-$CaCl_2$, B- KNO_3, C- $MgSO_4$, n = 30 per treatment. X-axis ($CaCl_2$, KNO_3, $MgSO_4$ concentration): 1 = control (no $CaCl_2$, KNO_3, $MgSO_4$); 2 = 10 mM; 3 = 50 mM; 4 = 100 mM; 5 = 150 mM.



Different letters between treatments indicate significant differences according to DMRT (P<0.05).

Figure 2. Response of *Cymbidium* Twilight (% survival of PLB explants) Moon 'Day Light' PLBs 30 days after culture initiation to: A-$CaCl^2$, B- KNO_3, C- $MgSO_4$, n = 30 per treatment. X-axis ($CaCl_2$, KNO_3, $MgSO_4$ concentration): 1 = control (no $CaCl_2$, KNO_3, $MgSO_4$); 2 = 10 mM; 3 = 50 mM; 4 = 100 mM; 5 = 150 mM.

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