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An *in vitro* assay for drought-tolerant coconut germplasm

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Summary

The feasibility of developing an *in vitro* technique for screening drought-tolerant coconut germplasm has been investigated. Embryos excised from mature nuts of Sri Lanka-tall coconut were cultured as described previously. Water-stress in the culture system was progressively increased with each passage, by incorporating polyethylene glycol (PEG-6000), mannitol and sodium chloride into the culture medium. PEG and mannitol were observed to be growth inhibitory in action even at low concentrations and these two compounds were abandoned. In NaCl-stressed media, about 21% of randomly selected Sri Lanka-tall embryos died before reaching the 170 mM NaCl. About 78% survived 170 mM NaCl and only 12.6% were able to resist 320 mM NaCl. When zygotic embryos derived from two known drought-susceptible cultivars of coconut, CRIC-65 and Dwarf (from *pumila*) were tested using the same technique, 29% and 73% of embryos respectively died due to stress damage caused by 170 mM NaCl and none of either cultivar survived a salt concentration above 230 mM.

However, embryos originated from two putative drought-tolerant cultivars showed a higher survival rate when subjected to salt stress. At 170 mM NaCl, all the embryos had developed into seedlings. In fact, percent germination of embryos was somewhat higher in 170 mM NaCl than in the control, that was devoid of NaCl. However, percent survivors gradually dropped with increase in salt concentration and about 18% survived the 330 mM NaCl. The technique seems to have great potential in screening drought-tolerant coconut germplasm.

Introduction

A great majority of coconut plantations in Asian countries were stricken by drought recently. In Sri Lanka alone, a biometrical survey showed that 15% and 35% of the standings in the North-Western Province and the Tangalle area, respectively, were damaged beyond recovery (D. Mathes, personal communication). Being a perennial crop producing for more than 40 years and attaining bearing age in 3–7 years, depending on the variety, the loss of palms has severe economic consequences.

Coconut generally withstands moderate drought to some extent, yet severe drought causes the frequent loss of existing plantations. Traditional measures to combat drought have laid emphasis on more efficient irrigation and moisture conservation in coconut lands. Although there is a much felt need to breed specifically drought-tolerant coconut germplasm, practical steps have been neglected

because of the technical problems particular to the coconut. Thus, selection methods adopted for other species such as withholding irrigation, germination in mannitol (Ashrof & Abu-Shakra, 1978) or survival through desiccation (Sullivan & Ross, 1979) cannot be applied to the coconut because of seed-nut and seedling size which discourage greenhouse experimentation. Field experimentation is, besides, hindered by the low planting density (about 200 palms/ha) and the difficulty of controlling the physical environment and nutritional status of plants grown in the open. The tissue-culture approach, whereby tolerance to a variety of stresses may be selected for among calluses and cells, will have to await a reliable technique for the consistent plant regeneration of coconut *in vitro*.

However, the germination of zygotic embryos of the coconut *in vitro* (Karunaratne et al., 1985) has the advantages of laboratory manipulation and ensures at the same time the subsequent establishment of individuals in the field. We report here on the possibility of screening the germplasm of plantations for stress tolerance by progressively increasing the stress with each passage of zygotic embryo culture and appraising the survivors. Although the osmotic stress caused by polyethylene glycol is commonly applied to simulate drought, we have preferred the more indirect physiological stress associated with sodium chloride in this study.

Materials and methods

Mature seednuts of *Cocos nucifera* L var typica were harvested from randomly selected palms unless otherwise stated in the text. Their embryos were dissected and cultured as described previously (Karunaratne et al., 1985) in a medium (Eeuwens, 1978) where the potassium chloride was replaced by 40 mM sodium chloride and sodium dihydrogen phosphate was replaced by 4.0 mM potassium dihydrogen phosphate. Concentration of magnesium sulphate, calcium chloride and ammonium chloride has been doubled. At three-weekly intervals, the germinating embryos were transferred to fresh media containing progressively higher levels of NaCl, at the rate of a 20 mM increase per passage for the first 3–4 months in culture and then by 30 mM per passage, at four-weekly intervals until the majority of plants showed severe symptoms of water stress as judged by the cessation of growth or even actual necrosis of the tissues. Control embryos were cultured on the original medium, that is, devoid of sodium chloride.

Survivors of water stress were expressed as percentages of the total number of embryos cultured and as percentages of the controls as well. Survivors referred to in the text are percentages of the respective controls, unless otherwise stated.

Results

Choice of a suitable drought-mimicking compound

The putative drought mimicry of polyethylene glycol was investigated using the form of the compound with an average molecular weight of 6000 (PEG-6000). However, after three attempts the results were found to be erratic and difficult to interpret. PEG-6000 generally inhibited plant development even at low concentrations of 1–5%, w/v. Similar results were obtained when mannitol was incorporated into the culture medium. These two compounds were therefore abandoned and subsequent investigations confined to the use of NaCl.

Unlike PEG which simulates water stress without penetrating the cell, NaCl is absorbed into cells readily. It can have the deleterious effects of ion toxicity. However, salt also functions as an osmotic agent since its presence in water lowers the osmotic potential and, in addition to salt stress, exposes the plant to a secondary osmotic stress, which has also been called a physiological drought stress (Levitt, 1980). Thus, NaCl may be used not only for salt screening but drought screening as well. Cell lines have been described growing in high salt (Ben-Hayyim and Kochba, 1983; Croughan et al., 1982; Nabors et al., 1980) and an increased tolerance to NaCl has been reported to confer increased tolerance to osmotic stress (Heyser & Nabors, 1981).