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# *In Vitro* Characterization of Indigenous Coconut Varieties (*Cocos nucifera* L.) of Sri Lanka for Water Stress Tolerance

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**Abstract**

Coconuts show a remarkable yield drop due to long dry periods caused by global climatic changes. Thus, breeding for drought tolerance has become a priority in coconut breeding in Sri Lanka. Five coconut varieties indigenous to Sri Lanka, namely Ran thembili, Gon thembili, Porapol, Bodiri and Red dwarf were screened for their drought tolerance potential *in vitro*. Mature zygotic embryos collected from self pollinated nuts of each variety were germinated and developed into plants with one photosynthetic leaf in $Y_3$ medium. Water stress condition was induced by application of Polyethylene Glycol (PEG) into the culture medium. The level of PEG in culture medium was gradually increased from 2% to 7% until the plants showed water stress symptoms in leaves; yellowing and necrosis. More than 75% of zygotic embryos of all tested coconut varieties successfully germinated *in vitro* and developed into plants. Plants of all tested coconut varieties survived water stress caused by 4% to 6% PEG and the percentages of plant survival at different levels of PEG varied among different coconut varieties. Variety Ran thembili showed the highest survival rate (27%) at 6% PEG and overall best plant growth performances at 4% PEG among the five coconut varieties tested. Red dwarf showed the lowest survival rate at 4% to 6% PEG indicating its susceptibility to water stress. Among the coconut varieties tested, zygotic embryos of Ran thembili showed the highest drought tolerance potential.

**Keywords:** drought tolerance; *in vitro* screening; PEG; polyethylene glycol; zygotic embryo

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## Introduction

The coconut palm, *Cocos nucifera* L. is a major plantation crop in the wet tropics and is the most extensively grown and used nut in the world, playing a significant role in the economic and social life of people in over 80 coconut growing countries. Coconut is also the most valued plantation crop in Sri Lanka as it provides food for 20 million people and direct livelihood for more than 50,000 people in the country while substantially contributes to foreign exchange earnings. The coconut palm remains an important crop especially for small farmers almost everywhere it grows and at least 96% of total world coconut production coming from small-holdings (Perera *et al*., 2009). Coconut is considered an orphan crop, because it is primarily grown in developing countries including Sri Lanka. Because of these reasons coconut can be considered as a potential tree crop for alleviation of poverty of the peoples in the developing countries such as Sri Lanka.

Due to the global climatic changes over the last two decades, the weather patterns in Sri Lanka as in many other coconut growing countries has changed drastically giving rise to continuous long dry periods of over 3-5 months. As a result, coconuts show a remarkable yield drop, such as over 700 million nuts in 2002 compared to 2000 in Sri Lanka due to the lagged effect of the drought that prevailed during 2001, resulting in the average rental prize of a nut increased by 36% (Anon, 2002). Hence breeding for drought tolerant in coconut has become the priority in the coconut breeding programs of Sri Lanka.

Indigenous coconut germplasm of Sri Lanka have been classified into different varieties and forms (Liyanage *et al*., 1988) and these coconut varieties and forms show very distinct morphological differences indicating higher level of possible genetic diversity for many traits of importance. These materials have never been evaluated for their drought tolerance potential and thus there is an urgent need to do this, as drought stands as a decisive constraint in coconut production in the country. Application of conventional methods to screen drought tolerance in coconut germplasm is limited due to the perennial nature, requirement of large extent of land for evaluation trials and because of the need of special control environment condition. Furthermore, conventional breeding efforts are time consuming and cost and labour intensive. *In vitro* culture of plant tissues on a medium containing selective agents offers a rapid and a reliable alternative to screen a large number of individual genotypes in a short period of time as a preliminary screening procedure. Polyethylene glycol (PEG) of high molecular weights and Mannitol have been used in culture media to simulate drought stress in different plants as non-penetrating osmotic agents lowering the water potential in a way similar to soil drying (Larher *et al*., 1993; Matheka *et al*., 2008; Rai *et al*., 2011). A positive correlation between germination of Alfalfa on PEG supplemented media and the whole plant behavior under drought stress in the field has also been identified (Petkova *et al*., 1995). Reports of attempts also exist for screening of coconut germplasm *in vitro* for drought tolerance using NaCl (Karunaratne *et al*., 1991) and PEG (Weerakoon, 2000). Those studies have revealed that survival of zygotic embryos of putative drought tolerant coconut cultivars under *in vitro* stress conditions was higher than that of known drought susceptible cultivars.

In this present study, five of the coconut varieties indigenous to Sri Lanka were assessed for their response to *in vitro* growth and induced moisture stress using PEG 6000 with the objective of screening and selecting candidate coconut varieties for future coconut breeding programmes for enhanced drought tolerance.

## Materials and Methods

Self pollinated and 12 months maturity nuts of coconut varieties; Ran thembili, Gon thembili, Porapol, Bodiri and Red dwarf which were planted at the e*x-situ* gene-bank of the Coconut Research Institute of Sri Lanka, harvested and collected in four batches were used for this study. The zygotic embryos of the first batch were excised and cultured in the modified Eeuwens $Y_3$ (Eeuwens, 1978) liquid medium. At 5 weekly intervals, cultures were transferred to

the fresh media. Due to the poor performance of some embryos in the liquid medium, the embryo culture protocol in subsequent batches was modified and the embryos were thereafter cultured in solid Eeuwens $Y_3$ for two passages and then transferred to liquid medium. Once the embryos germinated and produced the first photosynthetic leaf (Fig. 1), water stress conditions were induced by incorporating 2% (w/v) polyethylene glycol (PEG) into the culture medium. Then the concentration of PEG was increased step-wise for 4%, 5%, 6% and up to maximum of 7% until the plants showed symptoms of water stress; yellowing and necrosis of leaves (Fig. 2). Cultures were maintained in each PEG level for 5 weeks. The plants showing stress symptoms were acclimatized as described previously (Vidhanaarachchi *et al*., 1998)

Twenty to twenty five self pollinated zygotic embryos per variety per batch, depending on the availability were used in this study. Number of embryos germinated and numbers of embryos developed into plants in each variety were counted and the percentages were calculated. The plant height (cm) and average root length of plants (cm), before and after the induction of the stress were also measured at different stress levels in each variety. The number of plants that were tolerated and survived at 6% PEG was also counted for each variety and percentages were calculated.

The data was analyzed using the General Liner Model (GLM) procedure using the statistical data analysis package; the SAS (Version 9.1.3 portable) according to a Completely Randomized Design (CRD). The mean separation was done by LSD procedure. Percentage data were transformed to Arc sign values before the analysis to convert the data distribution to normal. The plant height and root length recorded before the introduction of the stress were used as a covariate to analyze the among variety difference for response to different levels of PEG.

## Results and Discussion

General: Performance of *in vitro* cultured zygotic embryos

*In vitro* culture of coconut zygotic embryos has been done in several laboratories worldwide. Each laboratory worked on different genotypes of coconut that are locally available and thus the success depended on the genotype and the culture conditions (Batugal and Engelmann, 1998). Attempts have been made to achieve higher embryo germination (>90%) and plant development (>85%) rates by changing culture conditions such as use of semi solid medium (Pech y Ake *et al*. 2004) and application of Gibberellic acid (Pech y Ake *et al*. 2007).

The zygotic embryos of tested coconut varieties varied in their response to germination in liquid Eeuwens $Y_3$ medium (the medium routinely used in the laboratory), recording 70% of zygotic embryos for Bodiri and Ran thembili and only 20% to 30% of zygotic embryos for Gon thembili, Red dwarf and Porapol. Application of modified protocol; the culturing of embryos in solid Eeuwens $Y_3$ medium for two passages and then transferring to liquid medium, enhanced successful germination of embryos over 75% for all different varieties and as a result enhanced development of embryos into plants which was used for subsequent *in vitro* embryo screening for drought tolerance.

There was no significant differences in both embryo germination and plant development efficiencies among five varieties tested (Table 1) with the modified protocol. The positive impact of modified embryo culture procedure might be due to the culturing of embryos in solid medium with the plumule end upward. This orientation results in good aeration conditions for the cultured embryo. In liquid medium, embryos are submerged and poorly aerated. The beneficial effect of well aerated embryos on embryo germination has shown by Ake *et al*. (2002). In their study, germination and conversion of Malayan Green Dwarf embryos cultured in liquid medium was poorer than embryos cultured in solid medium with plumule end upward or horizontal. Furthermore, when embryos were

**Figure 1. Embryos produced with the first photosynthetic leaf (Ran thembili varieties)**

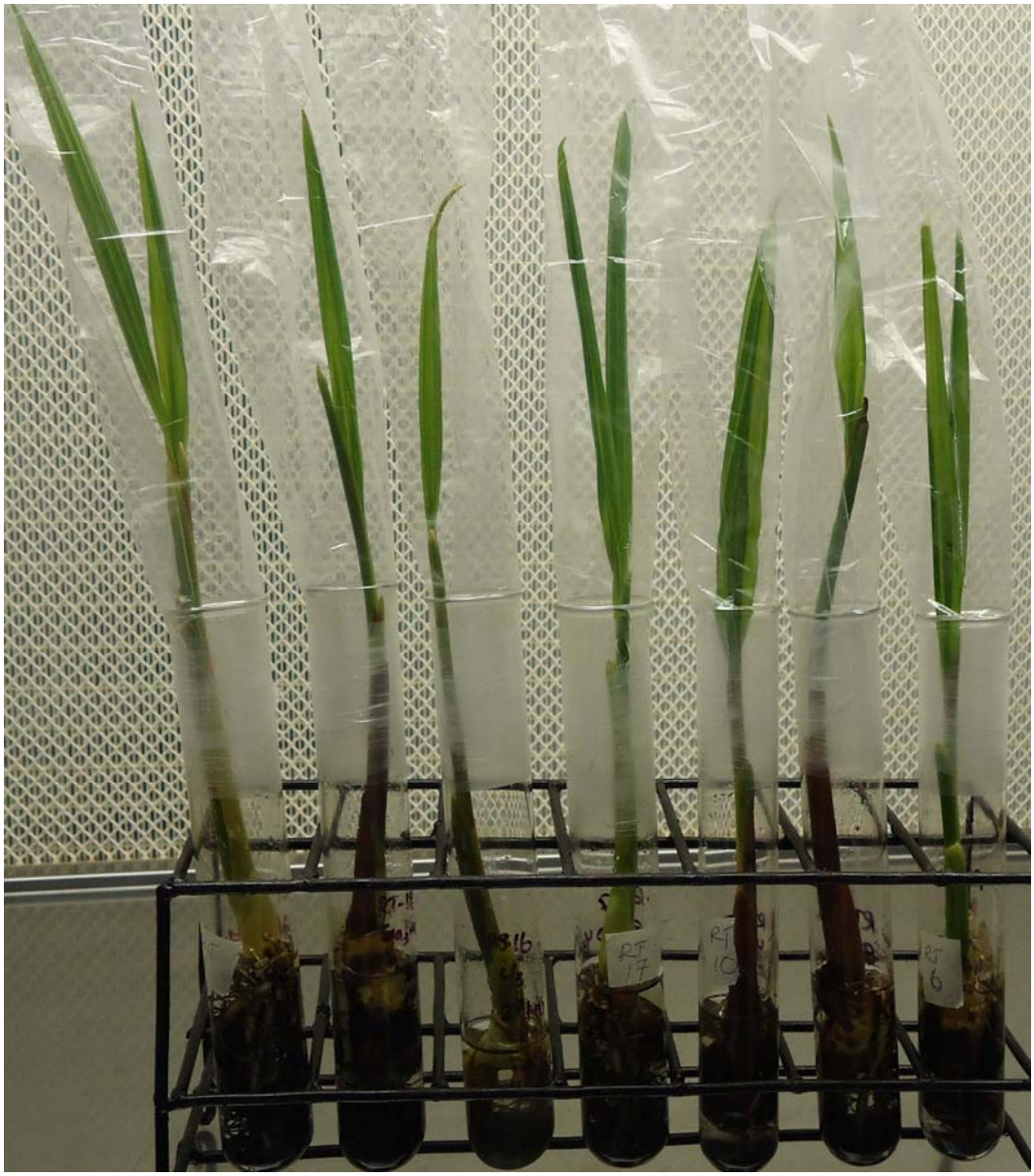


**Figure 2. *In vitro* symptoms of water stress: yellowing and necrosis of leaves**

**Table 1. The percentage of germination and embryos development into plants (plant development) of cultured zygotic embryos of different coconut varieties *in vitro***

| Coconut varieties | % Embryo germination | % Plant development |
|---|---|---|
| Gon thembili | 80.7 | 75.4 |
| Red dwarf | 75.2 | 75.9 |
| Bodiri | 85.0 | 85.0 |
| Porapol | 81.6 | 81.6 |
| Ran thembili | 92.0 | 89.1 |
| Significance | NS | NS |

NS = Not significant

placed in solid medium with plumule end downward, the germination and conversion was lower than in embryos in liquid medium.

Initial growth response of varieties for *in vitro* culturing

Once the cultured embryos developed into plants with the first photosynthetic leaf, plants were selected for *in vitro* screening. As the PEG induced stress was applied batch-wise, at the time of commencing the experiments some plants had more than one photosynthetic leaf per plant. The initial growth performance of plants used for drought screening is summarized in the Table 2. The results clearly showed genotypic difference of plant growth *in vitro*. Among the coconut forms tested, Ran thembili recorded the significantly taller (20.7 cm) plant height and significantly increased number (2.9) of primary roots per plant while the Red dwarf had significantly higher number of leaves per plant (1.4) at the time of PEG application indicating their high response to *in vitro* culturing compared to other varieties tested.

*In vitro* screening for drought tolerance

In this study, the experimental material was subjected to physiological drought during growth. The level of drought tolerance of the coconut varieties was evaluated based on their % of survival to PEG induced stress condition. Plants of all five varieties survived the stress caused by 2% PEG and none survived that of 7% PEG. The percentage of plant survival at 4% to 6% PEG varied among different coconut forms showing the genotypic difference among them in response to PEG induces stress *in vitro*. Varieties; Ran thembili and Gon thembili showed highest percentages of survival at 4% and 5% PEG, but the difference was not statistically significant among varieties. The survival rate of all the varieties at 6% PEG was below 30%. In general the survival rate of all the varieties at 6% PEG is below 70% (Ran thembili; only 29% survived and 10% of for Bodiri and Gon thembili and nil for Porapol and Red dwarf). However, interestingly, the variety Ran thembili showed statistically significant high survival percentage (27%) at 6% PEG compared to 10% survival for varieties Bodiri and Gon thembili and zero survival for varieties Porapol and Red dwarf. This indicated that variety Ran thembili which showed the significantly improved initial growth of plants has the highest drought tolerant potential among the five coconut varieties tested in this study. Red dwarf showed the lowest survival percentage (49%) at 4% PEG indicating its high level of susceptibility to water stress (Figure 3). This is in agreement with a previous finding by Weerakoon (2000), where embryos of Dwarf green and Dwarf yellow had the lowest levels of tolerance to PEG induced drought stress condition *in vitro*.

Furthermore this present study disclosed "among genotype variation" within a variety for drought resistance, thus indicating the usefulness of further *in vitro* screening of individuals within a known drought tolerant variety for identification of highly drought tolerant individuals for the breeding program. This finding is attributed to fact that coconut is highly cross pollinating and therefore inherently highly heterozygous in nature except for dwarfs which are highly self-pollinated. This observation has been previously also noted by Karunaratne *et al*. (1991).

Growth performance of plants under PEG induced stress conditions

In this study, plant growth measurements were taken after stress caused by different levels of PEG. However, due to the nature of experimental conditions, reliable growth measurements could not be taken after the 4% PEG treatment. Therefore only the growth measurements of plants taken after stress caused by 4% PEG was used for evaluating the plant growth performance of different coconut varieties under stress conditions.

Based on the growth of Ran thembili that survived 4% PEG (> 98%), it can be said that overall plant growth of variety Ran thembili under stress conditions is significantly better than that of other four varieties of coconut. It is worthwhile to note here that variety Ran thembili had a significantly higher number of primary roots per plant than other coconut varieties (Table 3). The results are in agreements with the

**Table 2. Growth performance of different coconut varieties *in vitro* before imposing the PEG treatment**

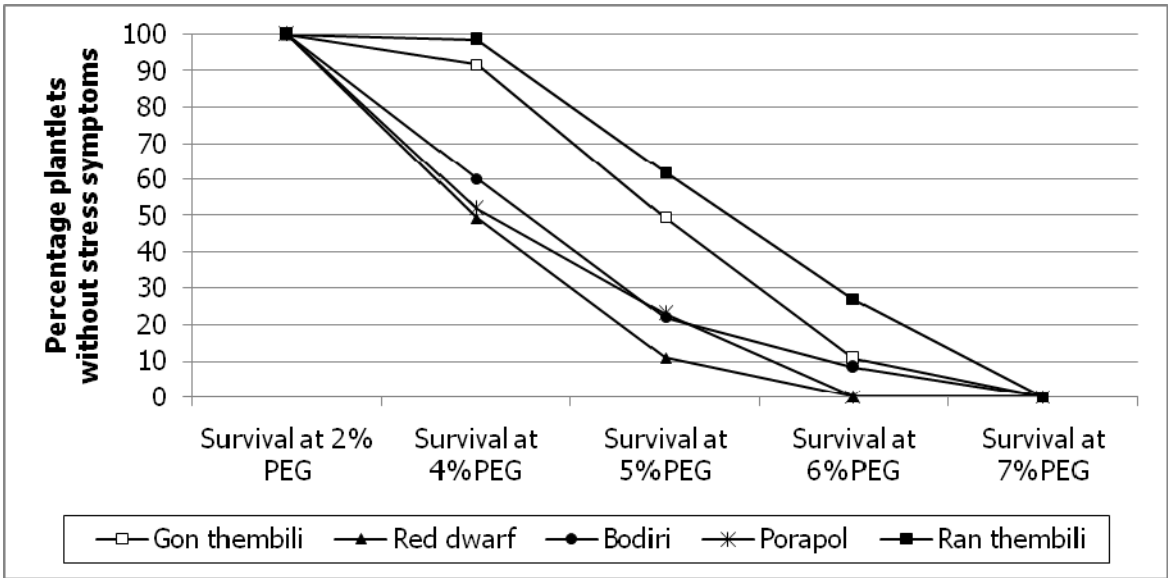
| Coconut varieties | Plant height (cm) | Number of leaves per plant | Number of primary roots per plant |
|---|---|---|---|
| Gon thembili | 16.2[b] | 1.1[b] | 2.2[b] |
| Red dwarf | 15.3[b] | 1.4[a] | 1.9[bc] |
| Bodiri | 14.6[b] | 1.0[b] | 1.7[c] |
| Porapol | 15.0[b] | 1.1[b] | 2.0[bc] |
| Ran thembili | 20.7[a] | 1.1[b] | 2.9[a] |
| Significance | P<0.0001 | P<0.0001 | P<0.0001 |
| CV (%) | 40.4 | 29.4 | 46.1 |

*Mean in the same column followed by a common letters are not significantly different at 5% level by DMRT. ns refers to non significant, * refers to significant, **refers to highly significant.*

**Table 3. Growth performance of plants under PEG induced stress conditions**

| Coconut varieties | Plant height (cm) | Number of leaves per plant | Number of primary roots per plant |
|---|---|---|---|
| Gon thembili | 28.3 c | 1.9 b | 2.7 b |
| Red dwarf | 30.5 b | 2.3 a | 2.7 b |
| Bodiri | 26.7 c | 1.4 c | 2.6 b |
| Porapol | 28.9 bc | 2.0 b | 2.6 b |
| Ran thembili | 34.2 a | 2.3 a | 3.2 a |
| Significance | p< 0.0001 | p< 0.0001 | p< 0.0001 |
| CV (%) | 16.3 | 32.1 | 26.9 |

**Figure 3. Percentage of plantlets survived water stress caused by different PEG levels**



reports of Bloch and Hoffimann (2005) and Manjoj and Uday (2007) who reported deep rooting and higher root: shoot ratios in drought tolerant plants compared to drought susceptible plants respectively. Further when the growth of plants before and after applying stress conditions were compared (Tables 2 and 3), it could be noted that the coconut variety having plants with the best initial growth (Ran thembili) produced the significantly vigorous plants even under stress conditions and survived the highest level of PEG (6%).

During the present study, 93 plants that survived water stress caused by 4-6% PEG were successfully acclimatized. The plants are maintained in poly-bags until they are ready to be field planted. These seedlings will be planted in green house and will be compared for their response to drought with a known drought susceptible variety as a control for their physiological and phenotypic response under a simulated water stress condition.

## Conclusion

All the five coconut forms could be successfully germinated and developed into plants *in vitro*. PEG induced *in vitro* water stress conditions enabled to characterize the drought tolerance capacity of the endogenous coconut varieties evaluated. Among the coconut forms evaluated, zygotic embryos of Ran thembili showed the highest drought tolerance potential.

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