# Determination of Cultivar Differences of Coconut on Heat Tolerance by *In Vitro* Pollen Germination

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## Introduction

Coconut as one of the main commercial crops in Sri Lanka, it mainly grows in intermediate zone (around 50% of total land), wet zone (around 25% of total land) and the balance in the dry zone. Heat and drought stress are the main impacts of climate change on coconut production. Therefore, development of heat and drought tolerant coconut cultivars has been recognized as a major adaptation measure to climate change. Plant reproductive organs are more vulnerable to changes in short episodes of stress prior and during early stages of flowering (Ranasinghe et al., 2010). The major cause for failures in pollination under high temperature is reduced pollen germination at temperatures as high as 35 °C to 39 °C during some seasons. Therefore, it is imperative to develop tools for screening coconut for high temperature tolerance with respect to pollen germination. Several recent studies have used the *in-vitro* pollen germination and pollen tube growth under different temperatures to screen genotypes for high temperature tolerance. This type of pollen characteristics will provide useful insight into the reproductive tolerance of coconut to anticipated climate change. *In-vitro* pollen germination and pollen tube growth of *typica* and *nana* varieties were studied by Ranasinghe et al., (2010) and suggested that the response of *in-vitro* pollen germination to temperature will be an accurate method to screen coconut varieties to high temperature tolerance,. Therefore, this study focused on identifying the effect of temperature on pollen germination and pollen tube growth of new coconut hybrids.

## Methodology

Six healthy coconut palms of Tall X Tall (TT), Dwarf Green X Tall (DGT), Tall x San Ramon (TSR), Brown Dwarf x Tall (DBT), Tall x Brown Dwarf (TBD), Brown Dwarf x Sa n Ramon (DBSR), Dwarf Green X San Ramon (DGSR) forms were selected randomly from Raddegoda and Mawathagama sites in Kurunegala, IL1a. The experimental design was Complete Randomized Design (CRD). Male flowers were collected from six palms of each cultivar and pollen of three randomly selected flowers was dusted into microfuge tubes with germination media and allowed to germinate in incubators. Incubators were maintained at predetermined temperatures from 16 °C to 39 °C within 2 °C intervals (3 tubes per temperature regime). Pollen grains were counted for pollen germination (3 slides from each microfuge tube) after 22 hrs of incubation under light microscope. Germination percentage (% PG) was determined. The *in-vitro* elongation of pollen tubes was measured after 3 hrs of incubation by using an ocular micrometer fitted to the eye-piece of the microscope under a high power (x40). There were 18 pollen tubes per temperature regime for each variety. Maximum pollen germination percentage and pollen tube length recorded after incubation, at each temperature were analyzed using linear and non linear regression models (Ranasinghe et al., 2010). The bilinear equation (Equation 1) was used to estimate cardinal temperatures ($T_{min}$ ,$T_{opt}$ and $T_{max}$) of all the varieties. Where; t is actual treatment temperature, and a, $b_1$ and $b_2$: equation constants. $T_{opt}$: the optimum temperature for pollen germination or tube growth. $T_{min}$, and $T_{max}$ were determined by Eqs (2) and (3) as shown below;

$$PG\ (\%) \text{ or } PL = a + [b_1 (t - T_{opt})] + [b_2 (ABS\ (t - T_{opt})] \quad \text{- Equation 1}$$

$$T_{min} = \frac{[a + T_{opt}(b_2 - b_1)]}{(b_1 - b_2)} \quad \text{Equation 2}$$

$$T_{max} = \frac{[a - T_{opt}(b_2 + b_1)}{(b_1 + b_2)} \quad \text{Equation 3}$$

**Results and Discussion**

The maximum pollen germination and pollen tube length after incubation were attained at temperatures between 27 $^0$C and 28 $^0$C. For pollen germination, cultivars differed for both pollen germination percentage (%PG) and cardinal temperatures (Table 1). Maximum percentage of germination ranged from 54 % (TSR) to 68% (DGT) with a mean of 61% (Table 1). Cardinal temperatures differed greatly among cultivars. Values of $T_{min}$ ranged from 15 $^0$C (TSR) to 17.5 $^0$C (DGSR) with an average of 16.7 $^0$C. Optimum temperature ($T_{opt}$) ranged from 27.5 $^0$C for DGT to 30.2 $^0$C for DBT with an average $T_{opt}$ of 28.3 $^0$C. The $T_{max}$ values ranged from 37.5 $^0$C for DBT to 41 $^0$C for DGT with an average $T_{max}$ of 39.2 $^0$C (Table 1).

Table 1. Maximum pollen germination percentage (max %PG), modified equation constants and cardinal temperatures for pollen germination of seven coconut cultivars.

| Cultivar | Max. %PG | Equation constants | | | | Cardinal temperatures ($^0$C) | | |
|---|---|---|---|---|---|---|---|---|
| | | a | $b_1$ | $b_2$ | $R_2$ | $T_{min}$ | $T_{opt}$ | $T_{max}$ |
| TT | 61 | 78.5296 | -0.5955 | -6.1768 | 0.98 | 17.0 | 28.9 | 40.0 |
| TSR | 54 | 54.9677 | -0.4659 | -3.4628 | 0.92 | 15.0 | 28.2 | 39.0 |
| TDB | 61 | 62.9035 | 0.2666 | -4.5827 | 0.93 | 17.1 | 27.5 | 38.4 |
| DBT | 60 | 60.893 | -1.1526 | -5.1873 | 0.85 | 16.5 | 30.2 | 37.5 |
| DBSR | 64 | -247.2 | 21.81 | -0.385 | 0.70 | 17.3 | 28.3 | 38.5 |
| DGSR | 64 | 63.8316 | -0.0489 | -4.2976 | 0.88 | 17.5 | 28.0 | 39.8 |
| DGT | 68 | 72.0458 | 0.9243 | -4.329 | 0.93 | 16.5 | 27.5 | 41.0 |
| Mean | 61 | | | | | 16.7 | 28.3 | 39.2 |

For pollen tube growth, the responses of seven coconut cultivars to temperature were significantly different. The length of pollen tubes ranged from 200 µm (TSR) to 680 µm (DBSR) with an average of 505 µm. Most genotypes had tube lengths between 440 to 640 µm (Table 2). The mean values for $T_{min}$, $T_{opt}$, $T_{max}$ for pollen tube growth after three hours of incubation were 18 $^0$C, 28 $^0$C, and 37.7 $^0$C, respectively.

Table 2. Maximum pollen tube length , equation constants and cardinal temperatures for pollen tube length of seven coconut cultivars in response to temperatures.

| Cultivar | Max.PL | Equation constants | | | | Cardinal temperatures ($^0$C) | | |
|---|---|---|---|---|---|---|---|---|
| | (µm) | a | $b_1$ | $b_2$ | $R_2$ | $T_{min}$ | $T_{opt}$ | $T_{max}$ |
| TT | 460 | -2484 | 207.9 | -3.67 | 0.79 | 17.9 | 28.3 | 39.1 |
| TSR | 200 | -3798 | 70.53 | -1.244 | 0.92 | 18.3 | 28.3 | 39.5 |
| TDB | 580 | -3533 | 298.2 | -5.326 | 0.89 | 18.0 | 28.0 | 37.0 |
| DBT | 640 | -3562 | 298.3 | -5.305 | 0.83 | 16.8 | 28.1 | 36.6 |
| DBSR | 680 | -3477 | 282.7 | -4.916 | 0.7 | 19.0 | 28.8 | 36.7 |
| DGSR | 440 | -1817 | 164.4 | -3.016 | 0.79 | 18.4 | 27.3 | 38.9 |
| DGT | 540 | -2798 | 242.9 | -4.41 | 0.82 | 17.8 | 27.5 | 36.3 |
| Mean | 505 | | | | | 18.0 | 28.0 | 37.7 |

Cultivars with the highest pollen tube length (DBSR) had the highest $T_{opt}$ and it could maintain relatively higher pollen tube growth after three hours of incubation, at 29 °C, compared to other tested cultivars (Table 2).

## Conclusions

Dwarf x Tall hybrids perform better than Tall x Tall hybrids with respect to *in vitro* pollen germination and pollen tube growth under high temperatures. Therefore, efforts should also be made to identify water stress tolerance of these cultivars to identify heat and drought tolerant cultivars which can be recommended for heat and drought prone areas and also as an adaptation measure to impacts of climate change.

## References

Ranasinghe, C.S.,Waidyarathna, K.P., Pradeep, A.P.C., Meneripitiya, M.S.K., 2010. Approach to screen coconut varieties for high temperature tolerance by *in-vitro* pollen germination. Cocos, 19, 1-11.