

A Novel Approach to Minimize Failures in Fruit Set in the Production of Dwarf × Tall Hybrid Seeds of Coconut (*Cocos nucifera* L.) Under Heat And Water Stress

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ABSTRACT

High sensitivity of pollen and ovules to stress, particularly around meiosis, is one of the major attributes for failures in fruit set in many crops. This study assessed the influence of heat (monthly mean $T_{max} > 33^{\circ}\text{C}$) and water stress (monthly rainfall < 90 mm) at stages around meiosis on the quality of female (Sri Lanka Green Dwarf, SLGD) and male (Sri Lanka Tall (SLT) and San Ramon (SR)) flowers and their fruit set (SLGD × SLT and SLGD × SR) with similarly stressed parents and with stressed vs non-stressed parents (reciprocal pollination) under controlled hand pollination. Flowers were sampled to represent their development under eight different stress levels; six with heat and/or water stress at stages around meiosis (flowers opened in March, June and September in two years) and two controls without stress around meiosis (flowers opened in December). Female flowers of emasculated SLGD palms opened in selected eight months were pollinated with SLT and SR pollen produced in the same month and produced before three months to accomplish reciprocal pollination. Quality of flowers, number of female flowers and fruit set (FS) were monitored. Unstressed pollen had significantly higher germination (PG %), tube growth (PTL) and starch and, female flowers had higher starch content compared to flowers stressed at any stage around meiosis. Water stress particularly at the meiosis stage increased the total soluble sugars (TSS) in pollen and female flowers. The FS was significantly higher (80%) in unstressed parents compared to stressed parents. The failures in fruit set between stressed parents could be minimized by using non stressed pollen to pollinate stressed female flowers. Of the two crosses the early fruit set of SLGD × SR hybrid was greater compared to that of SLGD × SLT. Whilst female flower number ($R^2=0.62$) and PTL ($R^2=0.54$) were significantly ($p<0.05$) influenced by the cumulative rainfall during final four months prior to flower opening, pollen and female flower starch ($R^2=-0.61$, $R^2=-0.67$) was affected by mean T_{max} of the same period. FS% showed the best correlation with starch of female flowers ($R^2=0.78$). The study concluded that heat and/or water stress around meiosis is very critical for reproductive organs and early fruit set in hybrid seeds. It also revealed an important strategy to minimize the failures in fruit set of dwarf × tall seed coconuts during stressed months, by using non stressed pollen to pollinate the stressed female flowers in controlled hand pollination.

Keywords: *Climate change, Heat and water stress, Coconut hybrids, Flower carbohydrates, Fruit set, Pollen germination*

INTRODUCTION

With the increasing global demand for coconut products, coconut growing countries encounter a considerable gap between coconut production and the requirement to meet the demand and, this gap is being widened due to the effects of climate change. Production of hybrid coconuts between dwarf (*nana*) and tall (*typica*) varieties are highly successful in terms of higher yields compared to tall forms (Everard, 2004). In the production of *CRIC65* and *Kapruwana* hybrid seed nuts, emasculated Sri Lanka Green Dwarf (SLGD) female flowers are crossed with the pollen of Sri Lanka Tall (for *CRIC65*) or San Ramon Tall (for *Kapruwana*), produced in the same month in the seed gardens of Coconut Research Institute. The critical temperature and rainfall for reproductive success in coconut are 33 °C and 90 mm / month (considering the water requirement of 3 mm / day), respectively, and the months exceeding these critical values cause heat or water stress on the developing reproductive organs (Thomas *et al.*, 2012; Ranasinghe *et al.*, 2015). The coconut palms frequently expose to these stress levels in the warm /drought seasons. As a result, significant failures in fruit set, especially in flowers opened in February, March, April, August and September, are already witnessed in the coconut seed gardens and, frequency of these extreme events is in the increasing trend due to effects of climate change (IPCC 2007).

Coconut palm generally produces one inflorescence, with both male and female flowers, every month. The sex determination of flowers takes place four months prior to inflorescence opening (-4 month) (Perera *et al.*, 2010). Thereafter, the critical development stages of male/female flowers take place during the final four months; differentiation of anther /development of ovule in the ovary (-3 month, three months prior to opening), meiosis of

microspore/megaspore mother cells (-2 month), pollen mitosis/development of the embryo sac in the ovule (-1 month) and pollen/ovule maturation (0 month, month of flower opening) (Perera *et al.*, 2010). Therefore, short-term climatic variability at 2-3 months prior to inflorescence opening has a vital role in determining fruit set.

The reductions in hybrid fruit set under stress can be due to reduced quality of pollen, female flowers or impaired pollination process. Heat and water stress around meiosis stage in flowers can alter the carbohydrate balance in pistils and pollen grains inhibiting male and female gametophyte development, pollen germination, pollen tube growth and fertilization (Zinn *et al.*, 2010; Hedhly 2011; Snider *et al.*, 2009). There is considerable number of reports published on the effects of either heat or water stress around meiosis on reproductive development of crops under controlled conditions (Barnabas *et al.*, 2008; Zinn *et al.*, 2010), however, the studies under field condition are limited and none so far in coconut.

To develop a pollination strategy to minimize stress-affected fruit set failures, information on the influence of climatic variability around meiosis on the fertility of flowers is vital. According to the existing practice of hybrid seednut production, both male and female reproductive structures are simultaneously subject to stress during heat and water stressed months. Therefore, consequent reductions in fruit set due to crossing between stressed parents cannot be avoided. This could be overcome by using non/low stressed pollen to pollinate stressed female flowers (reciprocal pollination). However, this strategy has not been evaluated for coconut so far. This study, therefore, focused to assess (a) the possible influence of heat and water stress prevailed during last four months prior to flower opening on the quality of female

(dwarf) and male (tall) flowers and their fruit set (dwarf x tall) and (b) to assess the fruit set of hybrid seeds using reciprocal pollination of stressed and non/low stressed parents under controlled hand pollination. Since the palms were managed with similar agricultural practices throughout the experimental period and the solar radiation intensity was always above the sufficiency level for coconut, we hypothesized that the measured parameters may vary in response to heat (monthly mean $T_{max} > 33$ °C) and water stress (monthly total rainfall < 90 mm) prevailed at meiosis, pre-meiosis and post-meiosis stages of flowers (last four months prior to opening).

MATERIALS AND METHODS

Sites and plant materials

Adult coconut (*Cocos nucifera* L.) palms of SLGD (Sri Lanka Green Dwarf, variety *nana*, female), and two tall forms, SLT (Sri Lanka Tall, variety *typica*,) and San Ramon Tall (variety *typica*, SR) (male), the parents of CRIC65 (SLGD x SLT) and *Kapruwana* (SLGD x SR), established in two seed gardens (Ambakelle and Pallama) of the Coconut Research Institute were used. The experimental sites are located in the low country intermediate zone (IL_{1a}) (Punyawardane, 2008). Generally, these areas receive the highest rainfall during October to December and are prone to moderate to severe droughts during February to September (Peiris *et al.*, 2008). The plantations are maintained with agricultural practices recommended by CRISL. Twelve representative tall palms (six each from SLT and SR (pollen parents)) and 48 dwarf palms (SLGD, female parent) were selected for data collection under eight selected stress levels.

Collection of flowers developed under different stress levels

Times (months) of sample collection were decided based on the historical climatic data on rainfall and maximum temperature prevailed in the experimental site over last 30 years and monthly fruit set data in the experimental area over last five years. Accordingly, sampling of male and female flowers was done at eight occasions to represent flower development under different stress levels at the stages around meiosis (pre-meiosis, meiosis, post meiosis and spore maturation - final four months prior to opening). There were six occasions under heat and/or water stress during final four months (flowers opened in March, June and September in two years) and two occasions (controls) without heat or water stress during the final four months (flowers opened in December) (Table 1). The newly opened inflorescences in the selected eight months were used for sampling and the same inflorescences of dwarf palms were tagged for recording fruit set.

Collection and processing of pollen

Spikelets with ready-to-open male flowers were sampled from SLT and SR palms between 9.00-10.00 am (Ranasinghe *et al.*, 2010) and processed to obtain pollen using fluidized bed dryer (FBD) and immediately stored at -4 °C until used for analysis (this pollen was used to measure pollen germination (PG%), pollen tube length (PTL), total soluble sugar (TSS) and starch concentration of pollen grains).

Collection of female flowers

Female flowers at 'receptive stage' were collected from emasculated SLGD palms, between 9.00-10.00 am. The number of female flowers present in each sampling inflorescence was recorded, one

flower from each inflorescence was sampled on ice and stored at -4 °C until used for analysis. Quality of female flowers was measured by means of total soluble sugar (TSS) and starch concentrations.

In-vitro pollen germination (PG %) and pollen tube growth (PTL)

PG% and PTL were determined by incubating pollen in the germination medium at 28°C for 3hrs (for measurement of PTL) or 24hrs (for %PG) as described in Ranasinghe *et al.*, (2010). For estimating the PG% and PTL of each month, nine microscopic fields /palm (54 fields each from SLT and SR) and 18 pollen tubes/palm (108 tube lengths each from SLT and SR), respectively, were used.

TSS and starch in mature pollen and receptive stage-female flowers

The TSS of pollen (0.1g) and female flowers (0.5g) were extracted with 80% ethanol and concentrated supernatants were analyzed for total soluble sugars using Phenol Sulfuric Method (Dubois *et al.*, 1956). The residue was digested using 1% α -amylase and the starch content (sugar equivalents) was measured by the same photometric method at 490 nm wavelength (UV/VIS Spectrophotometer).

Pollination and fruit set (similarly stressed parents and reciprocal pollination)

Female flowers produced (opened) in the selected eight months were subjected to hand-pollination with pollen produced in the same months or to reciprocal pollination (stressed female flowers with non-stressed pollen and *vice versa*). For instance, the female flowers opened in March (stressed) were hand pollinated with pollen of

March (stressed) and that of previous December (non-stressed), female flowers opened in June (stressed, but no water stress at meiosis) were pollinated with pollen of June (stressed, but no water stress at meiosis) and that of previous March (stressed), female flowers opened in September (stressed) were pollinated with pollen of September (stressed) and that of previous June (stressed, but no water stress at meiosis) and female flowers opened in December (non-stressed) were pollinated with pollen of December (non-stressed) and that of previous September (stressed). The survival of fruits in each pollinated inflorescence was recorded monthly and the number of surviving fruits at the third month after pollination (early fruit setting) was used to determine pollination success (beyond that the pollinated fruits can fall due to factors other than flower quality).

Climate and other supportive soil and plant data

Daily rainfall and temperature were collected from the Agri- Meteorological Station at the experimental site. Of the temperature components, the maximum temperature (Tmax) was found to be the most influential on coconut fruit setting (Ranasinghe *et al.*, 2015) and therefore, monthly mean of Tmax values and monthly total of rainfall were considered as the mean parameters causing climatic variability among months. Soil moisture percentage adjacent to experimental palms (six sampling points per variety at 25 cm soil depth using gravimetric method) and leaf stomatal resistance of parent palms (six palms / variety using LI-COR 6200 photosynthesis meter) which are the most appropriate indicators to express the stress level of the palms, were measured during the eight sampling months to ascertain the soil and plant stress levels.

Data Analysis

The main analysis, statistical differences of measured parameters among the eight months (flowers exposed to six different stress levels and two control conditions) was conducted by Analysis of Variance (ANOVA) following the General Linear Model (GLM) procedure using the SAS statistical package version 9.1. Means were separated using Duncan's New Multiple Range Test (DMRT). For pollen parameters, the differences between two male parents within each month were compared by a 't test'. For reciprocal pollination, within each month, the differences in fruit set were analysed by ANOVA following the GLM procedure and means were separated using DMRT. Regression analysis was performed to analyze the relationships between fruit set and tested parameters.

RESULTS AND DISCUSSION

Climatic conditions during flower development

The monthly variation in rainfall (monthly total) and maximum temperature (mean Tmax) during the experimental period is shown in Figure 1. The Tmax was higher than the critical limit (33°C) during March and April (heat stress) in all three years and the rainfall during September to December was always higher than 90 mm /month (no water stress). Flower development under eight stress levels during final four months prior to opening is depicted in Table 1.

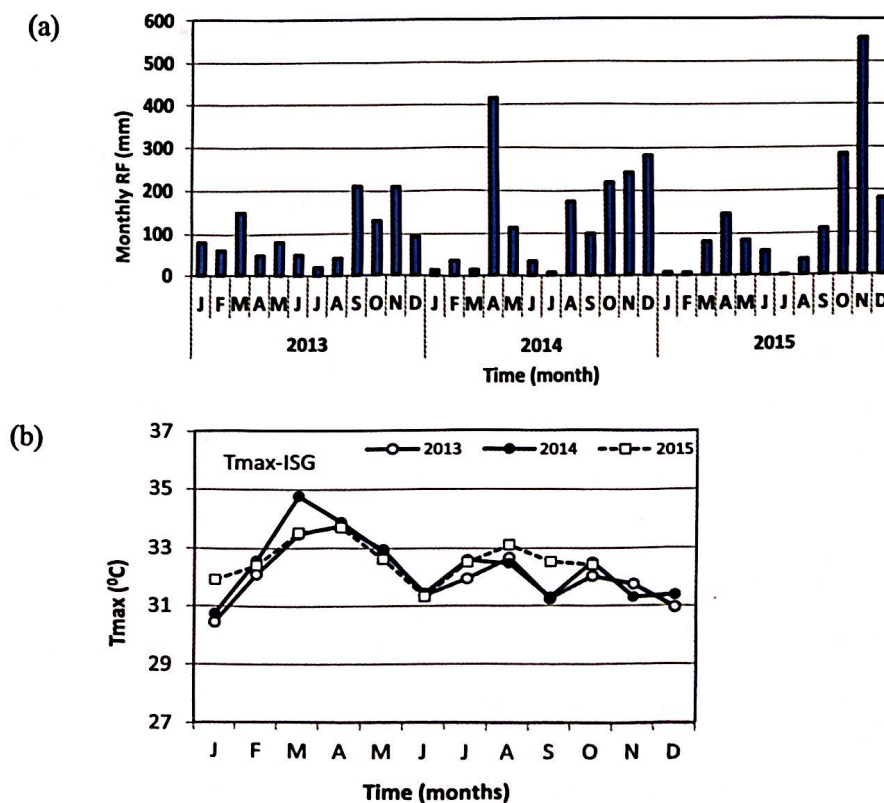


Figure 1. Variation in (a) monthly total rainfall (RF, mm) and (b) maximum temperature (Tmax, °C) during the experimental period (2013-2015)

Table 1. Description of the heat and water stress prevailed in eight sampling events (months) based on rainfall and maximum temperature (T max) during final four months prior to flower opening

-3 month Pre-meiosis		-2 month Meiosis		-1 month Post-meiosis		0 month Spore maturation & flower opening		Month of flower opening & sampling
HS	WS	HS	WS	HS	WS	HS	WS	
	■		■		■			Sep 2013
								Dec 2013
			■		■		■	Mar 2014
■	■						■	June 2014
	■		■					Sep 2014
								Dec 2014
			■		■		■	Mar 2015
■	■				■		■	June 2015

0 month: flower opening month, -1 month: first month prior to flower opening, -2 month: second month prior to flower opening, -3 month: third month prior to flower opening). WS: Water stressed (rainfall <90 mm/month) and HS: Heat stressed (mean monthly Tmax ≥ 33°C) stages are highlighted (horizontal bars).

The variation in soil moisture (SM%) of the plantation at eight sampling occasions clearly showed the intensity of stress prevailed due to rainfall and temperature during respective months and the leaf stomatal resistance (r_s), the best indicator of palm water stress, revealed the different intensities of stress levels perceived by the palms at eight sampling months (data not shown).

Variation of pollen germination (PG%) and tube growth (PTL)

There was a significant ($p < 0.05$) difference in PG (%) and PTL between stressed and non-stressed (control) flowers during final stages and both varieties showed the similar pattern of variation

(Figures 2a, 2b). For instance, pollen developed without stress during all four months around meiosis (control) (pollen of December 2013 and 2014) had significantly higher PG (50-60%) and PTL (638 μm) than those developed under stress at any stage around meiosis (pollen of March, June and September of both years). Among the stressed pollen, the PG (%) and PTL varied according to the stage of exposure to stress, though the pattern of response was not the same. PG% showed a very high sensitivity to continuous water stress at pre-meiosis, meiosis and post-meiosis stages (pollen produced in September 2013, Table 1), and had the lowest PG (18%).

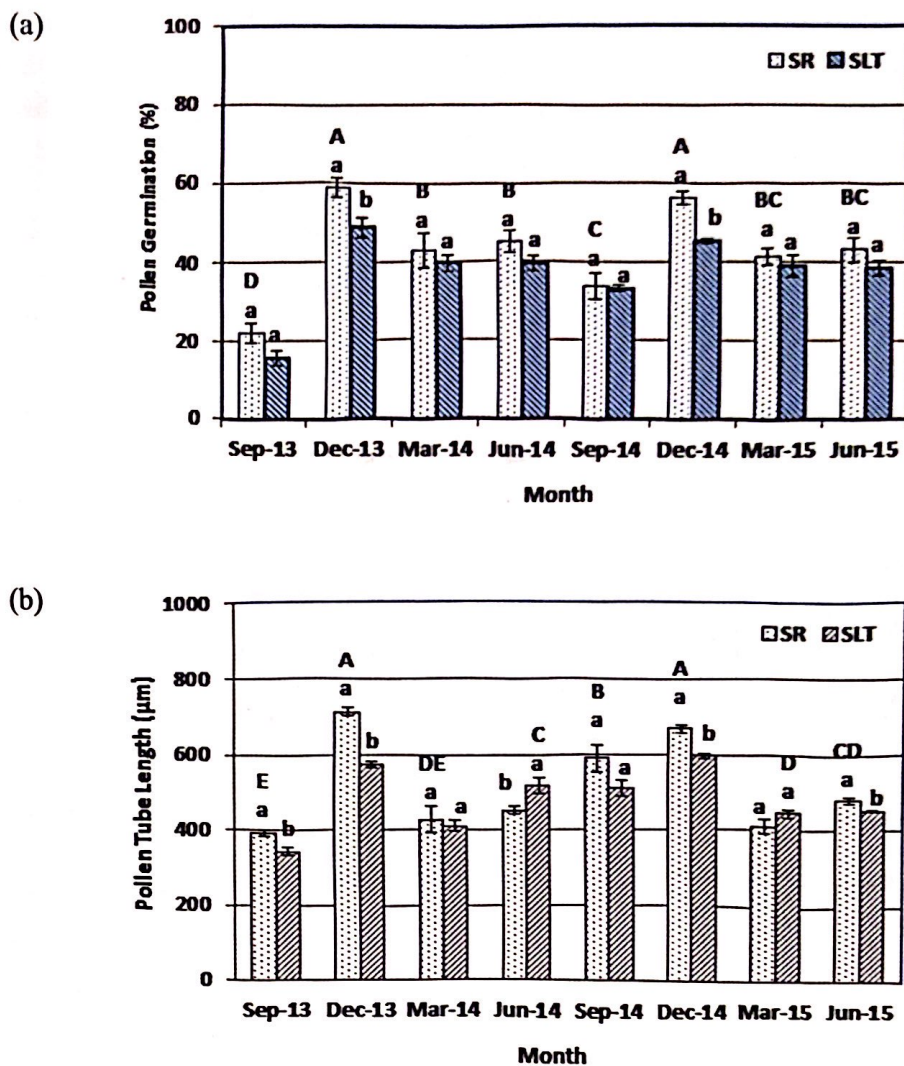


Figure 2. Germination (PG%) (a) and tube length (PTL) (b) of mature pollen grains sampled in selected eight months (exposed to different stress levels)

Capital letters indicate significance among the months and lowercase letters between varieties within a month. Means with the same letters are not significantly different at $P < 0.05$, bars indicate \pm SE of mean.

Variation of starch and total soluble sugars (TSS) in pollen and female flowers

Starch of pollen and female flowers showed a significant variation not only in response to water and/or heat stress around meiosis but also to their intensity (Figure 3a, 3b). For instance, the pollen and female flowers produced under no stress (in December) had significantly higher

starch contents compared to all other stressed flowers around meiosis (except the pollen starch in September 2013), and there was a significant difference between two controls; pollen starch in December 2013 and 2014; the former was higher than the latter. This may be mainly attributed to the intensity of rainfall at pre-meiosis stage of pollen produced in December (higher in 2013 than 2014) (Figure 1). Similarly, the pollen produced under

stress in March, June and September in 2014 had lower starch content than the pollen produced in the same months in other two years. This may be mainly attributed to the higher heat stress (35 °C) at spore maturation stage (0 month, Table 1) of March flowers, the same heat stress at pre-meiosis stage of June flowers (-3 month) and, higher water stress at meiosis and pre-meiosis stage of September flowers in 2014, compared to flowers opened in the same months in other two years (Figure 1). Of the stressed female flowers,

the flowers exposed only to water stress during all four stages around meiosis (September opened flowers) had significantly higher starch than the stressed flowers produced in March and June, 2014, but had similar starch to flowers produced under stress in March and June, 2015 (Figure 3b). This may again due to the same reason explained for pollen, the higher temperature stress at mega spore maturation (in March pollen) and pre-meiosis stages (in June pollen) in 2014 compared to the same months in 2015.

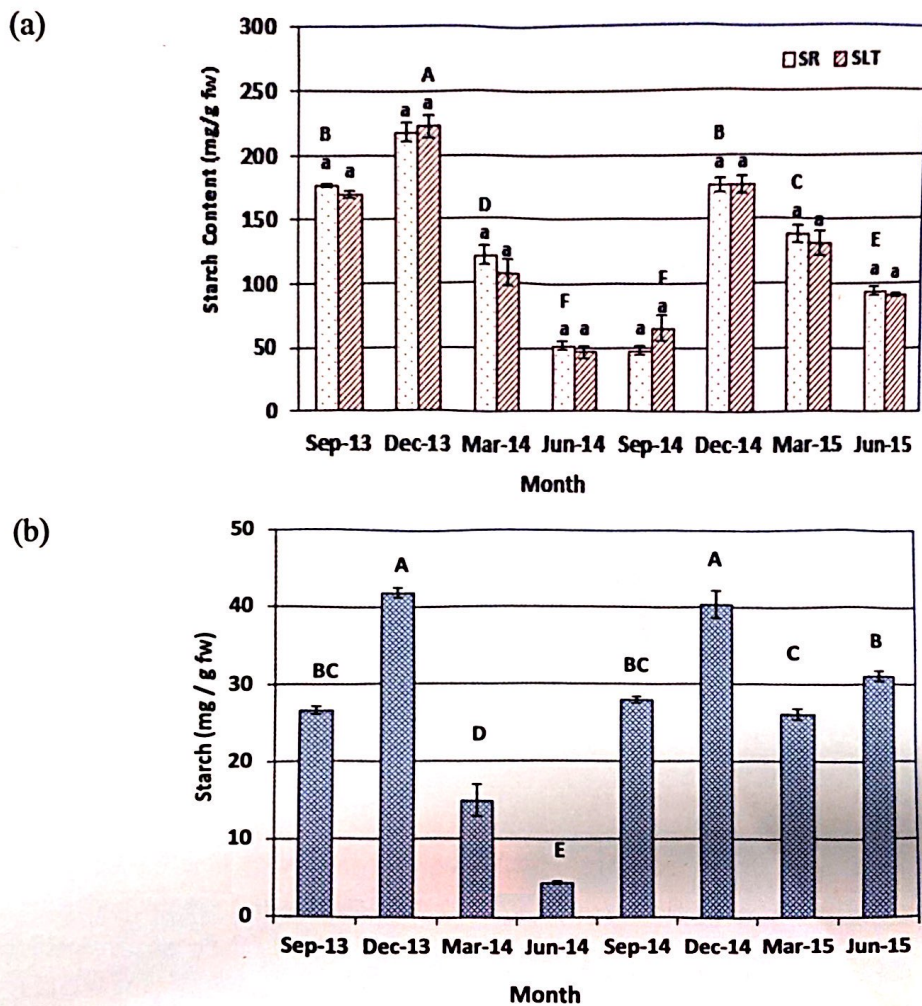


Figure 3. Starch concentration of mature pollen grains of male (SR and SLT) (a) and female flowers at receptive stage (SLGD)(b) sampled in selected eight months

Capital letters indicate significance among the months and lowercase letters between varieties within a month. Means with the same letters are not significantly different at $P < 0.05$, bars indicate \pm SE of mean.

Pollen and female flowers exposed to water stress at the stage of meiosis (-2 month, Table 1) (flowers of March and September), had higher TSS compared to flowers that were not exposed to water stress at meiosis (flowers of June and December), except for female flowers of September in 2014. Further, when pollen was not water stressed at meiosis, the TSS of pollen was

not significantly different to each other (flowers of June and December), irrespective of heat or water stress at other sensitive stages, or even heat stress at meiosis (pollen of June). However, in female flowers, there was a difference between TSS of December- and June-produced flowers (Figure 4b).

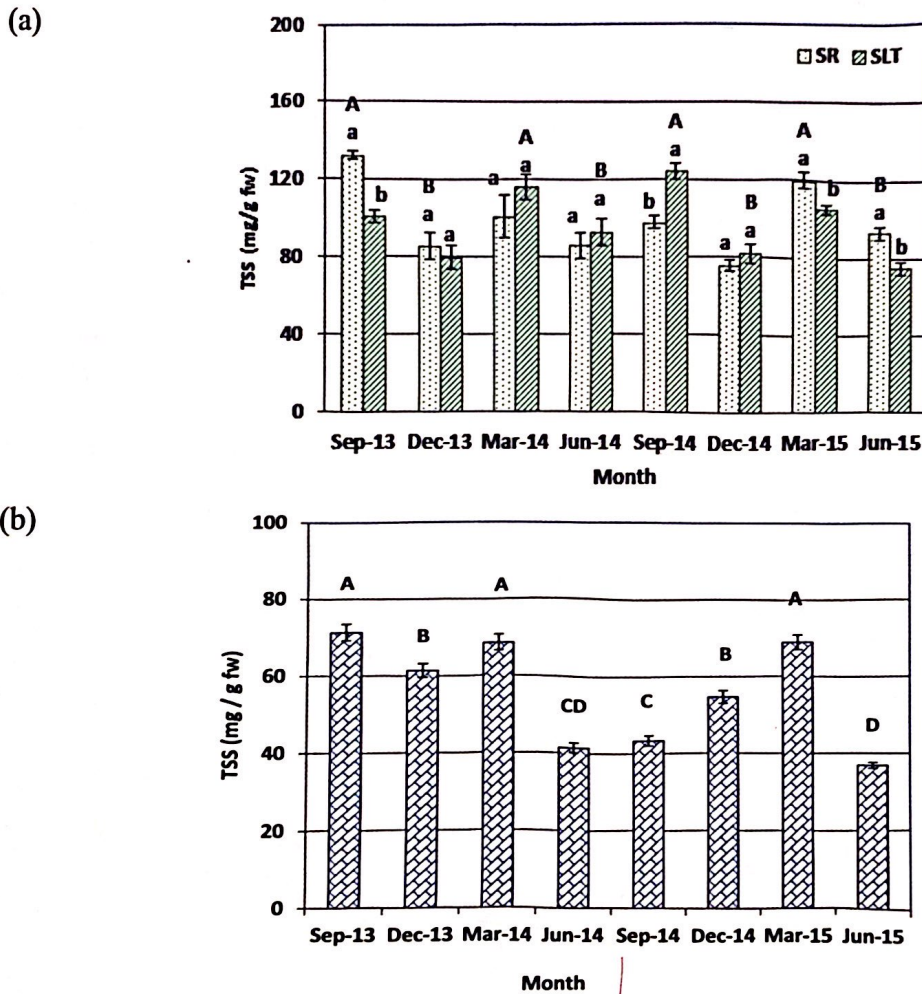


Figure 4. Total soluble sugar (TSS) concentration of mature pollen grains of male (SR and SLT) (a) and female flowers (SLGD) (b) sampled in selected eight months

Capital letters indicate significance among the months and lowercase letters between varieties within month.

Production of female flowers

The highest number of female flowers was produced in control inflorescences (December opened flowers) followed by the flowers developed under no water stress at meiosis stage (-2 month, June flowers) (Table 2). The lowest number of flowers was produced in inflorescences opened in March and September 2014, and the numbers were significantly lower than inflorescences developed under no water stress at meiosis stage (December and June flowers in both years).

Table 2. Variation of female flower production among the eight months.

Month of flower opening	No. of female flowers produced palm / month
Sep 2013	14.42 ± 4.25 ^{bcd}
Dec 2013	20.75 ± 6.02 ^a
Mar 2014	10.21 ± 4.66 ^d
June 2014	16.08 ± 6.09 ^{bc}
Sep 2014	10.54 ± 8.25 ^d
Dec 2014	18.17 ± 3.49 ^{ab}
Mar 2015	13.58 ± 1.53 ^{cd}
June 2015	15.58 ± 3.57 ^{bc}

Means (± SE) with the same letter are not significantly different at P<0.05.

Early fruit setting (dwarf x tall hybrid) of similarly stressed parents

The 'pollination success' (% fruit set) of dwarf x tall hybrid seed nuts (SLGD x SR and SLGD x SLT) varied significantly (P < 0.05) among the months in response to the stress level faced by the parents. Both varieties showed almost the same response, except the flowers of September 2013 (Figure 5). The FS was significantly higher (80%) in pollination between unstressed parents (December female flowers with December pollen) compared to stressed parents. Of the stressed parents, hybrid between flowers that were not exposed to water stress at the meiosis stage (-2 month, Table 1) (June flowers) showed significantly higher FS (%) compared to those developed under water stress at the same stage (flowers of March and September). The hybrid between flowers exposed to water stress continuously during final three months with heat stress at the final month (flowers of March), had the lowest FS (%).

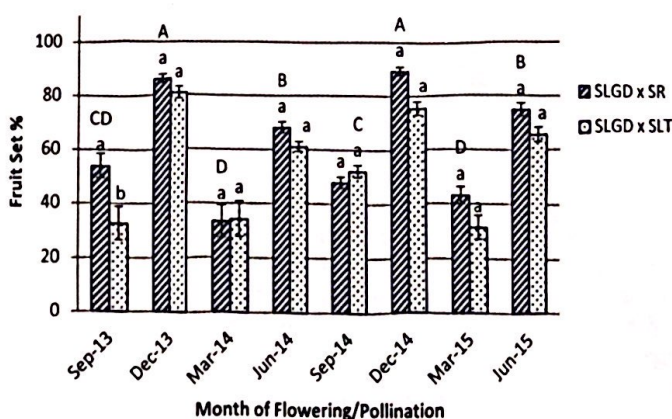


Figure 5. Variation in early fruit setting (%) of two hybrids when SLDG female flowers produced in eight selected months were pollinated with SR and SLT pollen produced in the same months. Capital letters indicate significance among the months of flower production and pollination and lowercase letters between variety of pollen used within a month.

Effect of reciprocal pollination on early fruit set of the two hybrids

The fruit set (%) of hybrid seed nuts varied significantly ($P < 0.05$) with the type of pollen (treatment) used for pollination. Both varieties showed almost the same response (Figure 6). When the female flowers developed under no stress (December 2013 and 2014) were pollinated with the pollen developed under same condition (pollen of same months), the palms showed significantly higher FS% in both crosses (88% in SLGD x SR, 78% in SLGD x SLT) compared to those pollinated with water-stressed pollen (pollen of September, 44% in SLGD x SR, 30% in SLGD x SLT) (Fig 6a, e). Similarly when the female flowers developed under stress, but without water stress at meiosis stage (pollen of June) were pollinated with the pollen developed under same condition (pollen of same months), the FS% was higher in both crosses (71 % in SLGD x SR and 63 % in SLGD x SLT) compared to those pollinated with water and heat stressed-pollen (pollen of March, 40% in both crosses) (Figure 6b, f). In contrast, when the heat and water stressed female flowers (opened in March) were pollinated with the pollen produced under same condition (pollen of March) the FS% was lower (39% SLGD x SR and 33% in SLGD x SLT) compared to those pollinated with non-stressed

pollen (pollen of December) (57% in SLGD x SR and 51% in SLGD x SLT) (Figure 6c, g). Similar pattern was observed in female flowers developed under continuous water stress in September, and pollinated with pollen produced under same condition (pollen of September) or pollen of June, but the improvements by June pollen (no water stress at meiosis stage) were not significant as it was shown with non-stressed pollen (Figure 6d, h).

When the overall response of FS to other parameters was analysed, FS% significantly ($p < 0.05$) and positively correlated with number ($R^2 = 0.64$) and starch of female flowers ($R^2 = 0.78$) and germination ($R^2 = 0.52$) and tube growth ($R^2 = 0.59$) of pollen and it negatively correlated with TSS ($R^2 = -0.65$) of pollen. With respect to the climatic parameters, female flower number ($R^2 = 0.62$) and pollen tube length ($R^2 = 0.54$) were positively and significantly ($p < 0.05$) influenced by the cumulative rainfall during the stages during the final four months of flower development. The starch content of pollen ($R^2 = -0.65$) and female flowers ($R^2 = -0.61$) were negatively influenced by the monthly mean Tmax during the final four months of flower development. PG% and TSS of pollen and female flowers were not significantly correlated with mean Tmax or cumulative rainfall over the stages around meiosis.

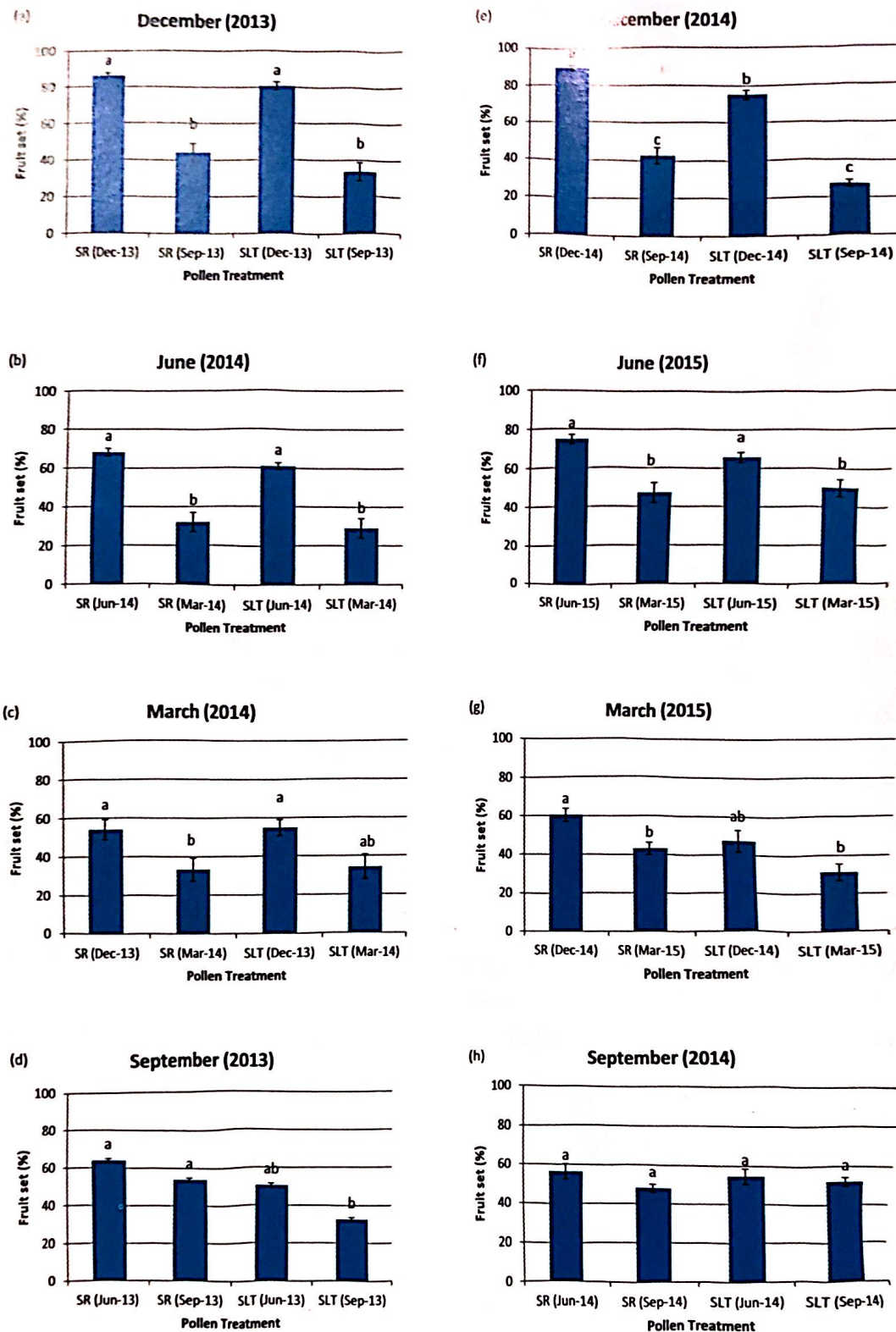


Figure 6. Variation in early fruit set (%) in SLDG palms pollinated with four different types of pollen under eight stress levels (December, June, March and September in two years). SR: San Ramon pollen, SLT: Sri Lanka Tall pollen (month of pollen production is given in the parenthesis). Means with the same letter are not significantly different at $P < 0.05$, bars indicate \pm SE of mean.

The present study revealed that when coconut male and female (dwarf) flowers developed without heat (monthly mean $T_{max} > 33$ °C) or water stress (monthly total rainfall < 90 mm) at the stages around meiosis (four month period prior to flower opening, Table 1), those male flowers had the highest pollen germination (%), pollen tube growth and pollen starch whilst female flowers had the highest number of flower production and starch contents. In contrast, the total soluble sugar (TSS) content in flowers developed without stress was low. Consequently, when these un-stressed SLDG female flowers were pollinated with un-stressed SLT or SR pollen, the hybrid fruit setting was the highest. However, when there was a heat and/or water stress at any stage around meiosis (Table 1) it reduced the pollen germination (%), pollen tube growth, pollen and female flower-starch and female flower numbers. In the stressed flowers, water stress at the stage of meiosis (-2 month, Table 1) increased the TSS substantially. Consequently the heat and/or water stress at any stage around meiosis reduced the hybrid fruit setting substantially and the intensity of reduction varied with type of stress and the stage of exposure. Further, the reduction in FS% due to pollination between stressed parents could be overcome by using non stressed pollen to pollinate stressed female flowers. When non stressed female flowers were pollinated with stressed pollen, there was an acute reduction of FS%. From the two crosses the early fruit set of SLGD x SR hybrid was greater compared to that of SLGD x SLT.

There can be several reasons for the reduction in flower quality and fruit set under stress. In male flowers, since various phases of pollen development (differentiation of anthers, meiosis of mega-spore mother cells, pollen mitosis and pollen maturation) are sensitive to water stress (Liu *et al.*, 2006; Barnabas *et al.*, 2008), any factor that affects the pollen formation and function may

have caused loss of pollen germination ability. For example, in the present study the most deleterious effect on PG (%) was shown when there was a continuous water stress during pre-meiosis (-3 month, Table 1), meiosis (-2 month) and post-meiosis (-1 month) stages. That water stress may have altered the structural development of anthers such as tapetum and middle layer, subsequently limiting the nutrition supply to developing pollen grains and reducing the fertility of pollen at their maturity (Falasca *et al.*, 2013).

Another possible reason for reduced flower quality and fruit set under stress is the changes in carbohydrate metabolism. In the controlled pollination process for developing coconut hybrids, successful pollen tube growth through the transmitting tissue of the style (female flower) is an essential pre-requisite for ovule fertilization and fruit set. For that purpose, carbohydrate reserves in pollen as well as on the pistil of female flowers are of equal importance. Because, the pollen tube growth is initially fueled by the existing carbohydrate reserves in the pollen grain but later switches to use carbohydrates provided by the pistil (female flower) (Clement *et al.*, 1994). Sucrose, which is the principal sugar (TSS) transported to developing flowers, is generally converted to hexoses by invertase and / or sucrose synthase and these resulting hexoses are used for starch synthesis which will be later used as energy source for pollen germination in both flowers (Dorion *et al.*, 1996). In the present study, it was clearly shown that, water stress at meiosis (-2 month) appreciably increased the TSS in pollen and female flowers, irrespective of the stress level at pre- or post-meiosis stages. Therefore, it can be suggested that water stress mainly at the stage of meiosis of mega- or micro spore mother cells (-2 month) may have reduced the activity of acid invertase and thereby accumulated sucrose (the main component of TSS) while restricting the

starch biosynthesis and other critical processes involved in pollination. The study also revealed that the reduction of pollen- and female flower-starch with increasing mean T_{max} during the stages around meiosis, this may also support the reduction of acid invertase and/or starch synthase enzyme activity under heat stress. Similar results have been observed in previous studies. In rice anthers, water stress during meiosis inhibited starch accumulation, enhanced the accumulation of soluble sugars and induced male sterility (Sheoran and Saini 1996; Dorion *et al.*, 1996), in barley grains from heat-treated plants accumulated less starch due to reduced conversion of sucrose to starch (Wallwork *et al.*, 1998) and cotton flowers exposed to moderately high temperatures showed reduced carbohydrate reserves in the female flowers (pistils) (Snider *et al.*, 2009). In addition, in female flowers, ovary abortion under water stress has been reported and it is again associated with accumulation of non-reducing sugars and the failure of starch accumulation (Boyer and Westgate 2004). The study also highlighted that the response of coconut reproductive organs to heat stress are not necessarily the same as their response to water stress. This needs to be further evaluated.

It is also possible to argue that since the soil moisture condition during stressed months was comparatively low and leaf stomatal resistance (for water vapour) was high, there could be a possible reduction in plant assimilate production during the stressed months, and it may have also contributed to low starch accumulation in stressed pollen and female flowers. This reason could not be eliminated; however, the variation in rate of leaf photosynthesis of these palms during the sampling months did not follow the same pattern as the variation in flower carbohydrates (data not shown). In addition, there are reserved carbohydrates in coconut stem and fronds, which

may be used by the reproductive organs in the periods of short supply (Mialet-Serra *et al.*, 2008). Therefore, these overall results suggest that carbohydrate starvation was probably not the main factor responsible for the meiosis stage stress-induced low flower quality in coconut, and it could be the carbohydrate metabolism as observed in many other crops (Zinn *et al.*, 2010). In addition, the limitations to carbohydrate translocation from storage organs to reproductive sinks due to callus formation under stress could not be eliminated (Zinn *et al.*, 2010).

Initial number of female flowers produced in the inflorescence is also an important determinant of fruit set in hybrid coconut production. In our study, the cumulative rainfall received throughout pre-meiosis, meiosis, post-meiosis and ovule maturation stages (stages around meiosis) was a decisive factor of female flower numbers, and more specifically, female flower number showed a high sensitivity to water stress prevailed at meiosis stage. The variations in hybrid fruit set were associated with the quality of both male and female flowers (starch of female flowers at receptive stage ($R^2=0.78$), pollen germination ($R^2=0.52$), pollen tube growth ($R^2=0.59$) and total soluble sugars in pollen ($R^2=0.65$)) and female flower number ($R^2=0.64$). The results revealed two important aspects; one is the importance of quality of pollen for a successful fruit set in the production of dwarf x tall seed coconuts, and the other is an important strategy to minimize fruit set failures take place during stressed months, by using non stressed pollen to pollinate the stressed female flowers in controlled hand pollination. The applicability of this novel information under field condition can be explained as follows. In the production of hybrid seed coconuts by controlled hand pollination in drought prone areas, the pollen produced without any stress at the final four months prior to flower opening, could be used for

pollinating the female flowers produced during stressed months, instead of using the stressed pollen produced in the same month (which is the current practice).

In conclusion, water and/or heat stress at the stages around meiosis of male and female flowers altered total soluble sugars (TSS), starch and number in female flowers (dwarf), TSS, starch, germination (%) and tube growth of pollen (tall) and early fruit set of dwarf x tall hybrid coconut seeds. Flowers developed without heat or water stress around meiosis had the best quality pollen and female flowers and the highest number of hybrid fruit setting. The failures in fruit set of hybrid seed nuts during stressed periods can be minimized by using non stressed pollen to pollinate the stressed female flowers in controlled hand pollination.

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