EARLY FRUIT SET IN DWARF x TALL CROSSES OF COCONUT (*Cocos nucifera* L.) UNDER RECIPROCAL POLLINATION BETWEEN HEAT AND DROUGHT STRESSED AND NON STRESSED FLOWERS

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ABSTRACT

Production of climate resilient and high yielding Dwarf x Tall hybrids is the main strategy to meet increasing demand of coconut in a changing climate. Heat and drought stress (HTDS) at meiosis stage of gametophyte development reduces the early fruit set of coconut. In hybrid seed nut production, emasculated Sri Lanka Green Dwarf (SLGD) female flowers are crossed with the pollen of Sri Lanka Tall (SLT) or San Ramon Tall (SR) produced in the same month under field condition. Thus, crossing between HTDS parents and consequent reductions in fruit set could not be avoided. This could be overcome by using non/low stressed (NS/LS) pollen to pollinate HTDS female flowers which has not been evaluated for coconut so far. This study was, therefore, conducted to assess the early fruit set (FS %) of SLGD × SLT (CRIC65) and SLGD × SR (Kapruwana) hybrids using reciprocal pollination of HTDS and NS/LS male and female parents. The FS % was determined two months after pollination. FS % varied significantly (P < 0.05) among different periods and the type of pollen used for controlled pollination. The overall mean % FS of CRIC65 was higher (56 %) compared to that of Kapruwana (48 %). When LS/NS female flowers (SLGD) were pollinated with LS/NS pollen (SR and SLT), the FS% of hybrids was higher (75% to 85%) compared to that when both parents were heat and/or water stressed at meiosis stage (31% to 54%). Instead, when NS/LS pollen was used to pollinate those stressed SLGD female flowers, the FS% was increased by 19% to 62% compared to HTDS parents. Nevertheless, non/low stressed female flowers crossed with HTDS pollen reduced FS% by 25% to 65% compared to LS/NS parents. These results revealed two important aspects; one is the importance of quality of pollen for a successful fruit set in dwarf x tall coconut hybrids, and the other is an important strategy to increase the fruit set during stressed months by using non or low stressed pollen to pollinate the stressed female flowers in controlled hybridization of coconut. The fate of these hybrid seed nuts during maturation and germination needs to be further evaluated.

Key words: Coconut inflorescence, reciprocal pollination, fruit set, heat and drought stress, hybridization of coconut.

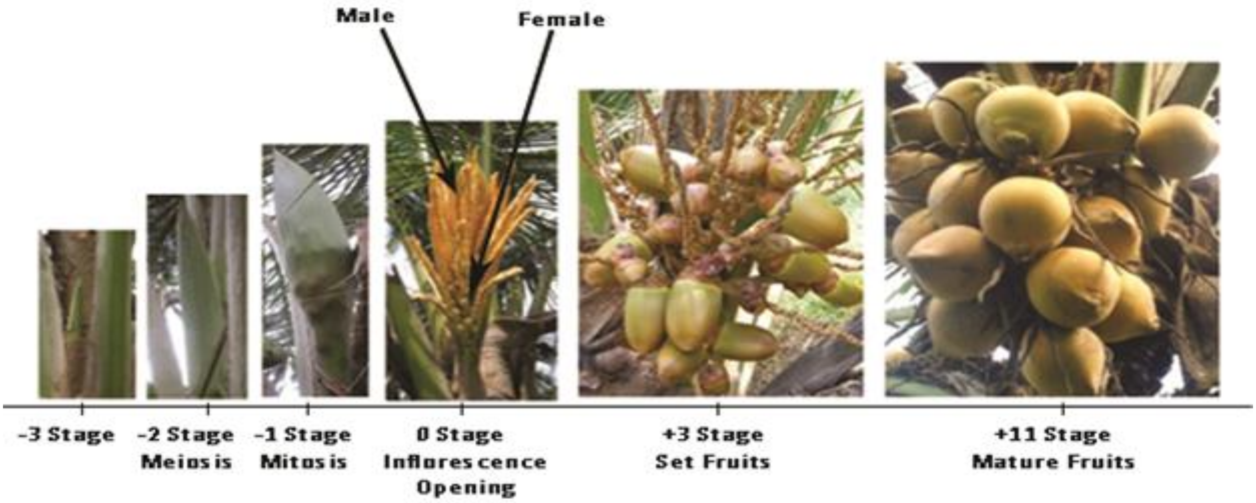
Introduction

Coconut palm is referred as the “tree of life” in the tropics due to its versatile uses. It is one of the three major plantation crops grown in Sri Lanka and the kernel (solid endosperm) of the fruit is used as a part of daily diet of people and the products of whole fruit act as a source of foreign exchange earnings (Gunasena and Gunathilaka, 2013).With the increasing global demand for coconut products, all coconut growing countries encounter a considerable gap between coconut production and the requirement to meet the demand. The yield reduction in coconut due to frequent heat and drought stress (HTDS) has further expanded this gap and it significantly affects the coconut based industries and livelihood of people engaged in these industries. Therefore, one of the main targets ahead of all coconut growing countries is to increase the production of coconut. Production of hybrid coconuts between dwarf and tall varieties are highly successful in terms of higher yields compared to pure tall forms

(Everard, 2004; Dissanayaka et al., 2012). Therefore, production of climate resilient and high yielding Dwarf x Tall hybrids is the main strategy to meet increasing demand of coconut in a changing climate.

Coconut palm generally produces one inflorescence, with both male and female flowers, every month. In hybrid seed nut production, emasculated Sri Lanka Green Dwarf (SLGD) female flowers are crossed with the pollen of Sri Lanka Tall (SLT) or San Ramon Tall (SR) produced in the same month in seed gardens of Coconut Research Institute. Sex differentiation of male and female flowers in coconut inflorescence takes place approximately four months prior to inflorescence opening (-4 stage) and the critical development events of flowers take place within the final four months prior to inflorescence opening. For instance, in the male flower, differentiation of anther and filaments (-3 stage, three months prior to opening), meiosis of micro spore mother cells (-2 stage), formation of microspores by mitosis (-1 stage) and pollen maturation (0 stage, month of flower opening) and in the female flower, development of ovule in the ovary (-3 stage), meiosis of megaspore mother cells (-2 stage), development of the embryo sac in the ovule (-1 stage) and ovule maturation (0 stage) (Perera, 2003; Perera et al., 2010) (Figure 1).

Figure 1: Critical development stages of a coconut bunch



Three (-3 stage), two (-2 stage) and one (-1 stage) months prior to inflorescence opening, month of inflorescence opening (0 stage) and three (+3 stage) and eleven (+ 11 stage) months after inflorescence opening. Male: male flowers, female: female flowers.

In Sri Lanka, the hybrid seed coconut production (yield) is severely affected by heat and drought stress that prevails during March to August in almost every year. Early fruit set is the main yield determinant in coconut and monthly variation in fruit set is controlled mainly by female flower production, total dry matter requirement of reproductive organs and the number of heat stressed days (≥ 33 ^{o}C) during the first three months after inflorescence opening (Ranasinghe et al., 2015).Thus, initial fruit set can be low due to unfavorable environmental factors including high temperature, low light conditions or water stress in coconut (Thomas et al., 2012; Ranasinghe et al., 2012), oil palm (Legros et al., 2009) and other fruit crops (Wubs et al., 2009). Furthermore, viability of pollen and receptivity of the stigma are essential pre-requisites for pollination success of fruit crops (Barnanbas et al., 2008; Snider and Oosterhuis, 2011).

The current strategy of hybrid seed coconut production allows controlled hybridization between female flowers of SLGD and pollen of SR or SLT produced in the same month. Thus, during heat and drought stressed months, both male and female reproductive structures of coconut inflorescence are simultaneously subjected to stress. Therefore, consequent reductions in fruit set due to crossing between stressed parents could not be avoided. This could be overcome by using non/low stressed (NS/LS) pollen to pollinate stressed female flowers. Similar studies have been conducted for tomato, where reciprocal crosses were carried out between complementary reproductive organs of the different temperature regimes (control/heat stressed pollen x control/heat stressed pistil) and the study revealed that when pollen donor plants were exposed to heat stress before and during pollen release, the fruit set decreased more severely compared to the heat stress applied to the developing ovule and to the style after pollen application (Peet et al., 1998). In *Brassica napus* the most acute reduction in seed set resulted from crossing gametes of heat-stressed pollen donor plants with heat-stressed female receptor plants. Further, when pollen donor plants treated with heat stress were crossed with unstressed emasculated female plants the seed set was severely reduced compared to unstressed parents (Young, et al., 2004).

In Sri Lanka, the seed gardens in which mass production of improved cultivars (Dwarf x Tall hybrids) is carried out are located in drought prone areas. Therefore, effect of heat stress can't be controlled even though the effects of water stress could be mitigated by irrigation depending on the availability of water. Thus in the seed gardens, both male and female flowers are simultaneously exposed to the stress during dry spells and consequently, decline the quality of flowers and reduce the nut yield significantly. If the pollen grains produced under favourable conditions with better quality are used for pollinating the stressed female flowers, instead of using stressed pollen, it would minimize the reduction of yield considerably. However, this reciprocal pollination strategy has not been evaluated for coconut so far. Therefore, the main objective of this study was conducted to assess the pollination success (early fruit set (FS %)) of two hybrids; SLGD × SLT and SLGD × SR using reciprocal pollination of heat and/or drought stressed and non/low stressed male and female parents.

Materials and methods

Sites, plant material and design

Adult coconut palms of Sri Lanka Tall (SLT), San Ramon Tall (SR) and Sri Lanka Green Dwarf (SLGD) cultivars grown in the Isolated Seed Garden (ISG) at *Ambakelle* and *Pallama* Seed Garden (PSG) of the Coconut Research Institute of Sri Lanka (CRISL) (latitude 7° 07' N, longitude 79° 87' E) were used (Table 1). These three coconut forms are currently used in controlled hybridization for production of CRIC65 (SLGD x SLT) and *Kapruwana* (SLDG x SR) with SLGD as female parent at *Ambakelle* (ISG) and *Pallama* (PSG) seed gardens. As 100% of hybrid seed nuts (*CRIC 65 and Kapruwana*) is produced in ISG and PSG these seed gardens were selected for the study. The distance between the two seed gardens is approximately 4 km. The plantations are located in the lower elevation (0 – 600 m above mean sea level) sub-humid zone in the North-West of Sri Lanka, which is known as the 'low country intermediate zone (IL_{1a}), according to the classification of Agro-Ecological Regions of Sri Lanka (Punyawardena, 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 cultural practices recommended by CRISL.

Table 1: Origin, stature, reproductive behaviour and tolerance to abiotic stress of three coconut varieties used in the study

Variety	Origin	Stature	Reproductive behavior	Tolerance to stress
Sri Lanka Tall (SLT)	Sri Lanka	Tall (*typica*)	Cross pollinating	tolerant
San Ramon (SR)	Philippines	Tall (*typica*)	Cross pollinating	tolerant
Dwarf Green (SLDG)	Sri Lanka	Dwarf (*nana*)	Self-pollinating	sensitive

Six representative palms each from SLT and SR (pollen parents) which are already being used as pollen parents in the development of tall x dwarf hybrids under controlled pollination were selected for pollen collection and 48 SLGD palms (female parent), 24 each from ISG and PSG were selected for pollination with selected pollen. The experimental design was a completely randomized design (CRD) with factorial arrangement (SLGD from ISG and PSG).

Collection of Pollen and Storage

Male flowers (with mature pollen) were collected at eight sampling events representing flower development under different heat and water-stressed levels (Table 2). Spikelets with ready-to-open male flowers were sampled from the middle of the inflorescence (to minimize the variation of pollen germination gradient along the spikelet) three to eight days after opening of the spathe (male phase is started within this time period), between 9.00 – 10.00 am. Male flowers were separated, processed to obtain pollen using fluidized bed dryer (FBD), immediately stored at -4 °C until they used for pollination. Germination ability (percentage germination PG %) and pollen tube length (PTL)) (Ranasinghe et al., 2010) were measured prior to pollination.

A colour code system was used for labeling the four different types of processed pollen and each pollinating inflorescence was tagged with the same colour code and respective numbers for easy identification (Table 3). Accordingly, there were eight set of pollinations during the entire experimental period (September 2013, December 2013, March 2014, June 2014, September 2014, December 2014, March 2015 and June 2015).

Table 2: Description of the heat and water stress identified during the experiment period

Month of Flower opening	**-3 stage**		**-2stage**		**-1stage**		**0 stage**	
	HS	**WS**	**HS**	**WS**	**HS**	**WS**	**HS**	**WS**
Sep 2013								
Dec 2013								
Mar 2014								
June 2014								
Sep 2014								
Dec 2014								
Mar 2015								
June 2015								

0stage: flower opening month, -1 stage: first month prior to flower opening, -2 stage: second month prior to flower opening, -3 stage: third month prior to flower opening). WS: Water stressed (rainfall <90 mm/month) and HS: Heat stressed ($T_{max} \geq 33^oC$) stages are highlighted (horizontal bars).

Table 3: Pollen treatments for reciprocal pollination of SLGD female flowers

Treatment	Colour code	Description
T1	White	No / Low stressed pollen of SR (Collected in December and June)
T2	Red	Stressed pollen of SR (Collected in March and September)
T3	Yellow	No / Low stressed pollen of SLT (Collected in December and June)
T4	Purple	Stressed pollen of SLT (Collected in March and September)

Reciprocal crosses between stressed and non/low (NS/LS) stressed flowers

Emasculation of SLGD female parents and pollen collection from SLT and SR parents were done four times (months) in a year (as described earlier), representing two stressed months (March and September) and two non- or low-stressed (NS/LS) months (December and June). Mature inflorescences of the selected SLGD palms that were about to burst-open, were emasculated (male flowers removed) to prevent self-pollination and the initial number of female flowers at the time of emasculation was recorded. Then the inflorescences were covered with specially prepared bags (pollination bags) until female flowers become receptive. Then the receptive female flowers were applied with respective pollen treatment (Tables 3 and 4) and the inflorescences were kept covered with a pollination bag for 15 days (until pollination process is completed) to avoid pollination with unknown pollen sources.(one person was used to pollinate all the experimental palms to avoid personal errors)This was repeated for two years and thus, there were eight set of emasculated inflorescences (48 inflorescence per month: 24 in ISG, 24 in PSG) and four treatments (type of pollen) applied during each month (six inflorescence per treatment).

Table 4: Treatment combinations of reciprocal hand pollination

Month of Inflorescence opening (stress level) – Female	X	Month of pollen collection (stress level) Male
March (stressed)		March (stressed), December (non stressed, NS)
June (low stressed, LS)		June (low stressed, LS), March (stressed)
September (stressed)		September (stressed), June (low stressed, LS)
December (non stressed, NS)		December (non stressed. NS), September (stressed)

Palms were exposed to the same growth conditions, except rainfall, and temperature in the eight pollination events and, therefore, it was assumed the variations in quality of pollen in SLT and SR and SLGD female flowers is influenced by the inflorescence development stage exposed to water stress (rainfall < 90 mm /month) and/or heat stress (T_{max}> 33°C) (variation of the quality of pollen and female flowers in eight sampling events was studied and reported separately, therefore it is not given in this report (Amarasinghe et al., not published)).

Fruit set and abortion

The pollination success was measured in terms of early fruit set (%).The number of fruits retained in each pollinated inflorescence was recorded two months after pollination and early fruit set percentage (FS%) was calculated using initial number of female flowers and number of set fruits after two months.

Climate Data

Daily rainfall and maximum temperature (T_{max}) were collected from the Agri-Meteorological Station at the experimental site.

Data Analysis

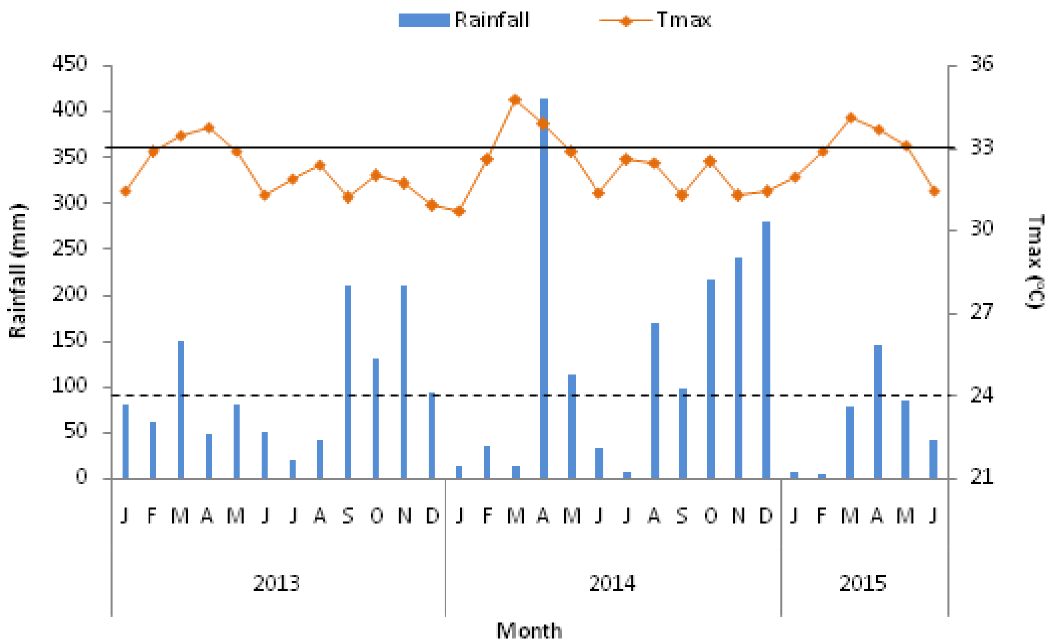
The statistical differences among eight months and treatments (4 types of pollen) were determined by Analysis of Variance (ANOVA) following the General Linear Model (GLM) procedure using the SAS statistical package version 9.1. Means were separated using Duncan's New Multiple Range Test.

RESULTS

The variation of maximum temperature and rain fall throughout the experimental period

The monthly variation in maximum temperature(mean T_{max}) and rainfall (monthly total) during experimental period is shown in Figure 2. In all the years, there was a temporal variation in the maximum temperature (T_{max}) which was equal or higher than the critical limit (33➡C) during March, April and May. Further, in 2014 the T_{max} of March reached 35➡C. There were two distinct peaks of rainfall and the rainfall during September to December was always higher than 90 mm /month.

Figure 2: Variation of monthly total rainfall (RF) and maximum temperature (T_{max}) during the experimental period.

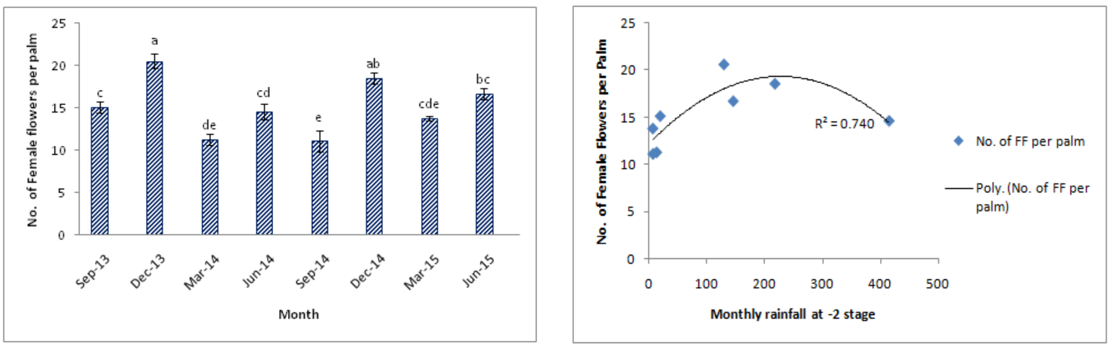


Two straight lines indicate critical temperature limit (33➡C) (above straight horizontal line) and critical rainfall (90 mm) (below dotted horizontal line). Y axis represents monthly total rainfall (mm) (left) and monthly mean of maximum temperature (T_{max}) (right).

Variation of number of female flowers produced in SLGD palms among the sampling months

There was a significant difference ($p < 0.05$) in production of female flowers (number /palm/ month) among the eight months. The highest number of female flowers was observed in December 2013 (21) and it was significantly higher compared to all other sampling months except December 2014 (Figure 3a). In other months, female flower production showed changes in varying magnitudes depending on the intensity of stress prevailed during final four months of inflorescence development. For instance, when female flowers were developed under severe water stress (rainfall < 14mm) during meiosis (-2 stage) (September 2014, March 2014 and 2015) (Table 2), those inflorescences had significantly lower number of female flowers compared to June 2015 in which inflorescences developed without water stress at -2 stage irrespective to the heat and/or water stress prevailed in other development stages (Figure 3a). The rainfall received at -2 stage showed a strong positive relationship ($R^2 = 0.74$) with number of female flowers produced /inflorescence in a given month (Figure 3b).

Figure 3: Variation of number of female flowers produced in SLGD palms among the experimental months



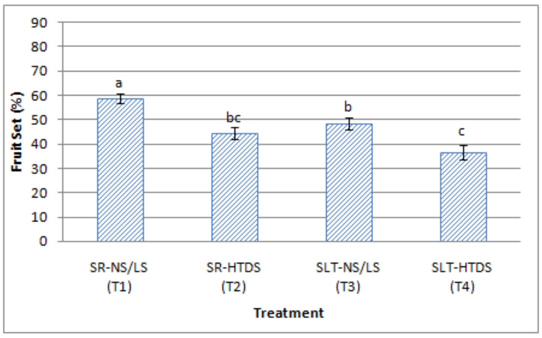
(a) **(b)**

Means with the same letter are not significantly different at P<0.05, bars indicate ± SE (a). FF: Female Flowers (b)

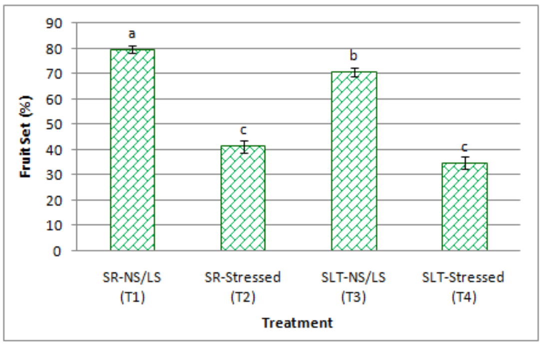
Effect of the type of pollen on early fruit set of stressed or non/low stressed female flowers

There was a significant effect of treatments (type of pollen) on early fruit set (FS %) in stressed (Fig 4a) or non/low stressed (Fig 4b) SLGD palms ($p<0.05$). Use of stressed pollen (T2 and T4) for pollination, resulted lower percentage of fruit set in SLGD palms compared to use of LS/NS pollen (T1 and T3). Application of Low/non stressed SR pollen (T1) for pollination, showed the highest FS% in both stressed (Figure 4a) and LS/NS female flowers (Figure 4b) of SLGD (59% and 80% respectively). From the crosses between non/low stressed pollen and female flowers (T1 and T3 of Fig 4b), the FS% of SLGD x SR was significantly higher (80%) than that of SLGD X SLT (71 %)(Figure 4b).Similar pattern was shown when the heat and/or drought stressed female flowers were crossed with LS/NS pollen. For instance, the FS% of SLGD x SR was 59% and that was significantly higher than the FS% of SLGD x SLT (49 %)(Figure4a). However, this difference in FS% could not be observed when heat and/or drought stressed pollen was used to pollinate either stressed or non/low stressed female flowers (Figure 4a and b).

Figure 4: The variation of Fruit Set (%) with type of pollen used to pollinate SLGD flowers



(a)



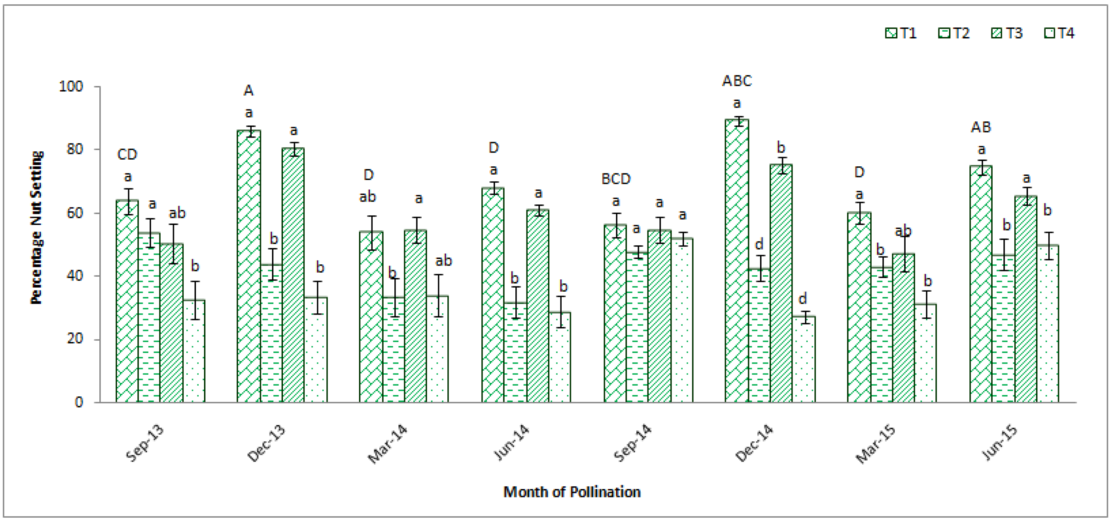
(b)

Means with the same letters are not significantly different at P<0.05, bars indicate ± SE. Fruit set (%) in (a) stressed SLGD flowers, (b) Low/Non stressed SLGD flowers when pollinated with stressed or non/low stressed (NS/LS) pollen of SR and SLT.

Effect of Reciprocal pollination on Early Fruit Set in Sri Lanka Green Dwarf female Flowers (SLGD)

Fruit setting (%) of SLGD palms significantly varied among the eight months and the type of pollen (treatment) used for pollination (p<0.05). When the SLGD female flowers developed under no stress (December 2013 and 2014) were pollinated with the pollen developed under same condition (T1 and T3)the palms showed significantly higher FS% in both crosses (88% in SLGD x SR, 78% in SLGD x SLT) compared to those pollinated with stressed(severe drought stress at meiosis stage)pollen (T2 and T4) (44% in SLGD x SR, 30% in SLGD x SLT). Similarly low stressed SLGD flowers (June 2014 and 2015) crossed with low stressed pollen of SR and SLT (T1 and T3) resulted higher FS% in SLGD x SR (71 %) and SLGD x SLT (63 %) compared to those were crossed with HTDS pollen collected in March (T2 and T4) (40% in both crosses). The heat and drought stressed female flowers of SLGD that were opened in March (2014 and 2015) and crossed with non stressed (T1 and T3) pollen, showed higher FS% in SLGD x SR (57 %) and SLGD x SLT (51 %) compared to those crossed with heat and drought stressed pollen produced in March (T2 and T4) (39% and 33%) respectively. Similar pattern shown in the reciprocal crosses in September (2013 and 2014), where stressed female flowers pollinated with low stressed pollen of SR and SLT (T1 and T3) showed higher FS% (60% and 53% respectively) compared to those pollinated with stressed SR and SLT pollen (51 % (T2) and 43 % (T4) respectively) (Figure 5).

Figure 5: Variation of early fruit set (%) in SLDG palms pollinated with four different types of pollen in eight events



T1: NS/LS pollen of SR, T2: Stressed pollen of SR, T3: NS/LS pollen of SLT, T4: Stressed pollen of SLT. Capital letters indicate significance among the months of pollination and lowercase letters between types of pollen used within a month. Means with the same letter are not significantly different at P<0.05, bars indicate ± SE.

Discussion

Effect of climatic variability during flower development on female flower production of SLGD palms

The number of female flowers produced in an inflorescence is one of the important yield determinant factors in coconut (Dissanayake et al. 2012; Ranasinghe et al., 2015). Female flower production was severely affected by the rainfall received at meiosis stage (-2 stage; two months prior to open the inflorescence) of mega spore mother cells. Severe water stress prevailed at -2 stage reduced female flower production in SLGD palms by 26% - 46% compared to non- stressed flowers, irrespective of the heat and/or water stress prevailed in other development stages.

Effect of pollen type on early fruit set in Dwarf x Tall hybrids

From the two crosses of SLGD x SR and SLGD x SLT, the highest percentage of early fruit set could be observed when the SLGD female flowers crossed with SR pollen. Past studies revealed that, among the two types of pollen parents (SR and SLT), pollen germination ability and pollen tube growth of SR was greater compared to SLT (Ranasinghe et al. 2010, Amarasinghe et al., 2014). San Ramon (SR) is a variety introduced to Sri Lanka imported from the Philippines, presumably had gone through several generations of natural selection in the Mindanao islands, where frequent fluctuations in climatic conditions are prevalent. The variety, as a consequence of environmental pressure and also due to domestication over a long period of adaptation, may have accumulated favourable genes to withstand harsh environment conditions (Fernando, 1999) inheriting the ability of producing pollen with higher germination ability. Further, Everard (2004) has also observed better combining ability of SLGD (female) with SR than SLT.

Pollination success (early fruit set %) of respective Dwarf x Tall hybrids under reciprocal pollination

Pollination is a crucial stage in the reproduction of most flowering plants and also it is prerequisite for fertilization which is the process of fusion of nuclei from the pollen grain with nuclei in the ovule. Viability of pollen and receptivity of the stigma is essential for pollination success (Barnanbas et al., 2008; Snider and Oosterhuis, 2011). The maximum temperature and number of heat stress days (T_{max}>33^{0}C) prevailed at pollen maturation is found to be important on FS% in both SLGD x SLT and SLGD x SLT crosses as it significantly and negatively correlated with FS%. The FS% in Dwarf x Tall hybrids reduced up to <30% when the T_{max} was increased greater than 33^{0}C. Literature revealed that, high temperatures mainly affect on the quantity and morphology of pollen, anther dehiscence, pollen wall architecture, the chemical composition and metabolism of pollen (Koti et al., 2005). Further studies revealed that, mild increases in temperature negatively affect on pollen viability (Aloni et al., 2001; Erickson et al., 2002), pollen germination ability (Koti et al., 2005 and Prasad et al., 2003), pollen tube growth rate (Hannsson and Stephenson, 1998), seed and fruit set in plants (Erickson et al., 2002).Snider et al, (2011) reported that high temperature resulted in slower pollen tube growth through the style in field grown *Gossypium hirsutum* pistils. Amarasinghe et al., (2014) in their study, observed that pollen germination percentage and pollen tube length of San Ramon (SR) and Sri Lankan Tall (SLT) varieties of coconut were significantly reduced at 34^{0}C compared to 28^{0}C.

Further, in this study, when both parents were developed under severe drought stress at meiosis stage, irrespective of the heat and/or drought stress prevailed at other stages, the resulted fruit set in both crosses were significantly low compared to that of non/low stressed parents. Past studies revealed that, abiotic stresses during the early reproductive stage lead to abnormal development of reproductive organs that results in failure of fertilization or abortion of fruits and seeds (Thakur et al., 2010), thereby dramatically decreasing crop yield (Setter et al., 2011; Kakumanu et al., 2012). The fertility of both male and female reproductive organs is vital for successful fertilization. Successful pollen tube growth through the transmitting tissue of the style (female flower) is an essential pre-requisite for ovule fertilization and fruit set. Further, stress induced floral defects included delaying of female organ development in maize and ovule abortion in Arabidopsis have been reported (Damptey et al., 1976). An insufficient supply of nutrients to the reproductive structures as a result of inhibition of the photosynthesis process under drought leads to block the development of reproductive structures, causing abortion (Westgate & Boyer 1986). Female gametophytes which developed under long period of water stress resulted severe structural and functional abnormalities in the ovary, however their female gametophyte showed greater stress adaptability compared to the male in cereals (Saini and Lalonde, 1998). Westgate and Boyer, (1986) observed that development of 15-45% of abnormal ovules under water stress in maize compared with the irrigated plants where only 2.5% abnormal ovules have been observed. In addition to that, it was mentioned that changes in carbohydrate availability, metabolism (Saini and Westage, 1999), distribution within anthers and inhibition of key sugar cleaving enzyme, acid invertase (Saini, 1997) appear to be involved in the effect of stress during anthesis and meiosis. Heat and drought stress induced these changes in the carbohydrate balance in pistils and pollen grains may inhibit male and female gametophyte development, pollen germination, pollen tube growth and fertilization (Dorian et al., 1996; Pressman et al., 2002; Reddy and Kakani, 2007; Zinn et al., 2010; Snider and Oosterhuis, 2011).

According to this study, reduction of FS% due to hybridization between stressed parents could be overcome by using non/low stressed pollen to pollinate stressed female flowers where FS% could be increased by 19% to 62%. Nevertheless, When low/no stressed SLGD female flowers crossed with stressed pollen, acute reduction of FS% was shown by SLGD x SR (36% - 53%) and SLGD x SLT (25% -64%). These results in line with the reciprocal pollination studies conducted by Peet et al., (1998) (Tomato) and Young et al., (2004) (*Brassica napus*) in which they suggested that the susceptibility of male gametophytes for heat stress than female gametophyte and pollen maturation is more severely affected by the heat stress than pollen germination, tube growth, and fertilization. This pattern of reduction in fruit set revealed that importance of quality of pollen for a successful fruit set in dwarf x tall coconut hybrids and more importantly, the ability to use non/low stressed pollen to pollinate stressed female flowers is a positive sign for varietal development in coconut in a changing climate.

Correlation between climatic parameters during pollen development and fruit set (FS %)

The regression analysis proved that the rainfall received at meiosis stage of pollen development had significant and positive correlation on early fruit set in Sri Lanka Green Dwarf palms. Saini, (1997) also reported that, water deficit during meiosis in the microspore mother cells is more critical for plants as it is more sensitive to drought and it causes morphological, structural and metabolic alterations in male gametophytes that are leading to meiotic defects or premature spore abortion and male reproductive sterility (Storme and Geelen, 2014). Thus when these pollen grains applied on the stigma of a SLGD flower, fertilization would not be success resulting lower fruit set and higher abortion.

Further, maximum temperature and number of heat stressed days prevailed during pollen maturation negatively and significantly correlated with the FS%. This revealed the importance of maximum temperature on pollen quality and fertility. Reproductive failures may occur even under a single hot day or cold night (Zinn et al., 2010). Kakani et al., (2005) has suggested that pollen grains on the exposed surface of the stigma would be more sensitive to high temperature than the more deeply seated ovules.

Conclusions

The reduction in early fruit set in Dwarf x Tall coconut hybrids during heat and drought stressed periods can be minimized by using low/no stressed pollen, that were developed under favourable conditions during the final four months,to pollinate the stressed female flowers. As coconut palms are perennial in nature and tall in size, provision of controlled conditions (glass houses) with optimum growing environment to produce hybrid seed nuts is impractical and economically not feasible. Therefore, under field conditions, this can be considered as a good strategy to reduce stress induced fruit abortion. However, the fate of these hybrid seed coconuts during maturation and germination needs to be further evaluated as they can be altered with climatic condition prevailed during fruit development.

Acknowledgements

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References

Aloni, B., Peet, M., Pharr, M. & Karni, L. (2001). The effect of high temperature and high atmospheric CO_2 on carbohydrate changes in bell pepper (*Capsicum annum*) pollen in relation to its germination. *Physiol. Plant,* 112, 505-512.

Amarasinghe, K.G.A.P.K., Ranasinghe, C.S., Abeysinghe, D.C. & Perera, A.A.F.L.K. (2014) Heat and drought stress effect on pollen quality of major pollen donors in coconut (*Cocos nucifera*L.) used for artificial hybridization programme in Sri Lanka. Paper presented at Wayamba University International Conference, held on29-30 August 2014 (pp. 245).

Barnabas, B., Jager, K., & Feher, A. (2008). The effect of drought and heat stress on reproductive processes in cereals. *Plant, Cell and Environment,* 31, 11-38.

Damptey, H. B., Coombe, B. G. and Aspinall, D. (1976). Apical dominance, water deficit and axillary inflorescence growth in *Zea mays*: the role of abscisic acid. *Annals of Botany,* 42, 1447-1458.

Dissanayaka, H.A.M.A.C., Attanayake, R.B., Fernando, A.A., Jayathilake, R., Padmasiri, M.H.L., Herath, H.M.N.B., Chandrasiri, S.A.S., Perera, S.A.C.N., Meegahakumbura, M.K. & Perera, L. (2012). Development of promising coconut hybrids utilizing Novel Brown Dwarf coconut variety. (In) Plant breeding and propagation. *Proceeding of the forth symposium on plantation crop research,* Taj Samudra Hotel, Colombo, 20-21 September, 2012.

Dorion, S., Lalonde, S. and Saini, H. S. (1996). Induction of male sterility in wheat by meiotic-stage water deficit in invertase activity and changes in carbohydrate metabolism in anthers. Journal of Plant Physiology, 111 (1), 137-145.

Erickson, A. N. & Markhart, A. H. (2002). Flower developmental stage and organ sensitivity of bell pepper (*Capsicum annum* L.) to elevated temperature. *Plant Cell Environ,* 25, 123-130.

Everard, J.M.D.T., (2004). From Ceylon Latin Square to coconut genome frame work: A relentless journey. In: Proceedings of the International conference of the Coconut Research Institute of Sri Lanka –Part 1. (Eds. T.S.G. Peiris and C.S. Ranasinghe), Coconut Research Institute of Sri Lanka, Lunuwila, Sri Lanka, 10-40.

Fernando, W. M. U., 1999. Genetic improvement of the coconut cultivar San Ramon. COCOS 13, 30-39.

Gunasena, H. P. M. & Gunathilake, H. A. J. (2013). Present status of the coconut industry of Sri Lanka. In: Gunasena, H. P. M., Gunathilake, H. A. J., Fernando, L. C. P., Everard,, J. M. T. D. & Appuhamy, P. A. H. N. (2013). Weligama coconut leaf wilt disease: six years after. Coconut Research Institute, Lunuwila, Sri Lanka, 1-12.

Hannsson, M. H. & Stephenson, A. G. (1998). Effects of temperature during microsporogenesis on pollen performance in *Cucurbitapepo* L. (Cucurbitaceae). *Int. J. Plant Sci,* 159, 616-626.

Kakumanu, A., Ambavaram, M. M. R., Klumas, C., Krishnan, A., Batlang, U. & Myers, E., (2012). Effects of drought on gene expression in maize reproductive and leaf meristem tissue revealed by RNA-Seq. *Plant Physiol.* 160, 846–867.

Kakani, V. G., Reddy, K. R., Koti, S., Wallace, T. P., Prasad, P. V. V., Reddy, V. R. & Zhao, D. (2005). Differences in in vitro pollen germination and pollen tube growth of cotton cultivars in response to high temperature. *Annals of Botany,* 96, 59-67.

Koti, S., Reddy, K. R., Reddy, V. R., Kakani, V. G. & Zhao, D. (2005). Interactive effects of carbon dioxide, temperature, and ultraviolet – B radiation on soybean (*Glycine max* L.) flower and pollen morphology, pollen production, germination, and tube lengths. *J. Exp. Bot*, 56, 725-736.

Legros, S., Mialet-Serra, I., Caliman, J. P., Siregar, F. A., Clement-Vidal, A. & Dingkuhn, M. (2009). Phenology and growth adjustments of oil palm (*Elaeisguineensis*) to photoperiod and climate variability. *Annals of Botany*, 104, 1171-1182.

Peet, M. M., Sato, S. & Gardner, R. G. (1998). Comparing heat stress effects on male-fertile and male sterile tomatoes. *Plant, Cell and Environment*, 21, 225-231.

Peiris, T. S. G., Hansen, J. W., & Zubair, L., 2008. Use of seasonal climate information to predict coconut production in Sri Lanka. *International journal of climatology* 28, 103–110.

Perera, P. I. P. (2003). Cytological examination of microspore development for microspore and anther culture of coconut (*Cocos nucifera* L.) cv Sri Lanka Tall. *COCOS*, 15, 53-59.

Perera, P. I. P., Hocher, V., Weerakoon, L. K., Yakandawala, D. M. D., Fernando, S. C. & Verdeil, J. L. (2010). Early inflorescence and floral development in *Cocos nucifera* (L.) (Arecaceae: Arecoideae). *South African Journal of Botany*, 76, 482-492.

Prasad, P.V.V Boote, K. J., Jr, L. H. A. & Thomas, J. M. G. (2003). Super-optimal temperatures are detrimental to peanut (*Arachis hypogaea* L.) reproductive processesand yield at both ambient and elevated carbon dioxide. *Glob. Change Biol*, 9, 1775-1787.

Pressman, E., Peet, M. M., & Pharr, D. M. (2002). The effect of heat stress on tomato pollen characteristics is associated with changes in carbohydrate concentration in the developing anthers. *Annals of Botany*, 90, 631-636.

Punyawardane, B. V. R., 2008. Evolution of climatic zones in Sri Lanka. In: Agro-climatological Zones and Rainfall Pattern in Sri Lanka (in *Sinhalese*), Department of Agriculture, Sri Lanka. 44-113.

Ranasinghe, C. S., Waidyrathna, 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, 01-11.

Ranasinghe, C. S. & Thimothias, K. S. H. (2012). Estimation of carbon sequestration potential in coconut plantations under different growth conditions. *Journal of the NationalScience Foundation of Sri lanka*, 40 (1), 77-93.

Ranasinghe, C. S, Silva, L. R. S., & Premasiri, R. D. N., (2015). Heat stress, dry matter requirement of developing fruits and female flower production: major determinants of fruit set and yield fluctuation in coconut (*Cocosnucifera* L.), *Journal of National Science Foundation*, (in press).

Reddy, K. R & Kakani, V. G (2007). Screening Capsicum species of different origin for high temperature tolerance by in vitro pollen germination and pollen tube length. *ScientiaHorticulturae*, 112, 130-135.

Saini H. S. (1997). Effect of water stress on male gametophyte development in plants. *Sexual Plant Reproduction*, 10, 67-73.

Saini, H. S. and Lalonde, S. (1998). Injuries to reproductive development under water stress, and their consequences for crop productivity. *Journal of Crop Production*, 1, 223-248.

Saini, H. S. & Westage, M. E. (1999). Reproductive Development of Grain Crops during Drought. *Journal of Advances in Agronomy*, 68, 59-96.

Setter, T. L., Yan, J., Warburton, M., Ribaut, J. M., Xu, Y., & Sawkins, M.. (2011). Genetic association mapping identifies single nucleotide polymorphisms in genes that affect abscisic acid levels in maize floral tissues during drought. *J. Exp.Bot*, 62, 701-716.

Snider, J. L. & Oosterhuis, D. M., (2011). How does timing, duration and severity of heat stress influence pollen-pistil interactions in angiosperms? *Plant signaling and behaviour*, 6 (7), 930-933.

Snider, J. L., Oosterhuis, D. M., Kawakami, E. M. & Loka, D. A. (2011). High temperature limits in vivo pollen tube growth rates by altering diurnal carbohydrate balance in field-grown *Gossypium hirsutum* pistils. *Journal of Plant Physiology*, 168, 1168-1175.

Storme, N. D., & Geelen, D. (2014). The impact of environmental stress on male reproductive development in plants: biological processes and molecular mechanisms *Plant, Cell and Environment*, 37, 1–18.

Thakur, P., Kumar, S., Malik, J. A., Berger, J. D. & Nayyar, H. (2010). Cold stress effects on reproductive development in grain crops: an overview. *Environ. Exp. Bot*, 67, 429-443.

Thomas, R. J., Nair, R. V., Mathews, C., Ajithkumar, R., Sasikala, M. & Nampoothiri, C. K. (2012). Studies on fruit set in coconut upon artificial pollination in various cross combinations. *Indian Journal of Horticulture*, 69 (1), 7-12.

Westgate, M. E. & Boyer, J. S. (1986). Reproduction at low silk and pollen water potentials in maize. *Crop Science*, 26, 951-956.

Wubs, A, M., Heuvelink, E. & Marcelis, L. F. M. (2009). Abortion of reproductive organs in sweet pepper (*Capsicum annum* L.): a review. *Journal of Horticultural Science and Biotechnology*, 84 (5), 467-475.

Young, W. L., Wilen, R. W. & Bonham-Smith, P. C. (2004). High Temperature Stress of *Brassica napus* During Flowering Reduces Micro and Megagametophyte Fertility, Induces Fruit Abortion, and Disrupts Seed Production. *Journal of Experimental Botany*, 55 (396), 485-495.

Zinn, E. K., Tunc-Ozdemir, M. & Harper, J. F. (2010). Temperature stress and plant sexual reproduction: uncovering the weakest links. *Journal of Experimental Botany*, 61 (7), 1959-1968.