Original Research Article

PHYSIOLOGICAL ASSESSMENT OF TEMPERATURE STRESS TOLERANCE IN SELECTIVELY FERTILZED COCONUT HYBRIDS

Abstract

In vitro pollen germination study is one of the important technologies for understanding the functions of pollens as well asin many areas of pollen biotechnology particularly in pollen selection. Ability to separate germinated and non-germinated pollen offers selective treatments and can be used for identifying stress tolerant and sensitive alleles carried by their pollen grains. The present study was carried out in Kerasree and Keraganga selectively fertilized (S.F) coconut hybrids produced through pollen selection. Pollens were subjected to germination in specific media maintainedat different levels of water stress induced by PEG 6000 and the critical water potential for pollen germination was identified. The water stress tolerant pollen grains at critical level were selected and used for fertilization. Evaluation of temperature stress tolerance of these two selectively fertilized hybrids along with their normal hybrids and west coast tall(WCT)was carried out by assessing their critical temperature stress for pollen germination. Both Kerasree selectively fertilized and Keraganga selectively fertilized hybrids recorded highest critical temperature of 42^0C for pollen germination with germination percentage of 20.17 and22.77 respectively compared to their normal hybrids (38^0C) and WCT (40^0C).

Key words; coconut, drought tolerant, temperature stress, *invitro* pollen selection

Introduction

Cocos nucifera (2n=32) is an important tropical plant species and is benevolent provider of the basic needs of millions of people. It is often eulogized as “tree of life” or Kalpavriksha because of its multiple uses [13]. Coconut is cultivated in 13Mha area over 90 countries, including India with an estimated production of million nuts. India is the largest producer of coconut in the world followed by Indonesia and Philippines. India stands first in production (20309 million nuts) and second in productivity (9346 nuts/ha) and third in area of cultivation (2373 ha) under coconut [25]. Coconut hold a vital role in contributing India’s GDP of about 15,000 crore and 72% of total world’s production is from India. In India, Kerala tops the list in area but Karnataka holds premier in production and Tamil Nadu tops in productivity [18]. Coconut plays very important role in social, cultural and economic life of people especially in Kerala.Coconut can be grown successfully under optimum temperature conditionbetween 27°C to 30 °Cin moderate or well aerated areas. But unpredictable climate change interferes the production and as per the reports of IPCC, 2014[9]atmospheric temperature is likely to increase by 1.1 to 2.6 °Ctowards the end of 21st century [16].Successful fruit set in coconut depends on several reproductive processes including pollen germination and pollen tube growth. High temperature (>33 °C) during flowering reduces fruit set in coconut. Therefore, identification and development of coconut varieties or hybrids with high reproductive heat tolerance will benefit the coconut industry in view of the climate changes [20].

Climate change is the major concern and vulnerability in the field of coconut sector. Climate change adaptation technologies can provide a buffer against the risks brought by climate change and can increase the economic growth and food security.Development of heat and drought tolerant coconut cultivars has been acknowledged as a major adaptation measure to reduce the impact of climate change. In many crops, reduction in pollen quality and adverse effects on pollination process have been identified as the principal deleterious effects of high temperature and water stress on fruit set [28].*In vitro* pollen germination at

different temperatures [19] and field evaluation of cultivars for reproductive performance [26]has been used to select heat and drought tolerant crop varieties. However, quantitative data revealing information on heat and water stress impacts on pollen quality, pollen germination, female flower production and fruit set and their variation with cultivars is very scanty in coconut.

Selective fertilization is a process, in which selective pressure is intentionally imposed during pollen germination, so that the pollen grains which can withstand the selection pressure only will germinate and fertilize the ovule. The resultant progeny will be tolerant to that particular stress [22].Water stress tolerant coconut hybrids (Kerasree selectively fertilized and Keraganga selectively fertilized) were developed through *invitro* selection of pollen grains at critical water potential followed by selective fertilization [21] and evaluated selectively fertilized coconut hybrids in the seedling stage and it was reported that selectively fertilized hybrids were drought tolerant and had higher water use efficiency compared to normal coconut hybrids.Afna and Roy, 2023[1] conducted a study for evaluating matured selectively fertilized palms for water stress tolerance and reportedthat they were water stress tolerant while comparing with their normal hybrids in terms of epicuticular wax, relative water content, chlorophyll content and amount of proline accumulated during water stress period. The *in vitro* pollen germination screening has been successfully used to select extreme temperature-tolerant cultivars in several plants [24, 8, and 2]. Additionally, differences in pollen germination ability under extreme temperatures vary between and within species [14, 4 and 27]. Such differences have enabled researchers to group cultivars into heat-tolerant or heatsensitive, and cold-tolerant or cold-sensitive types based on the temperature response of the pollen [24].

Since the production and productivity of the coconut interfere with the impact of high temperature, development of climate smart coconut is increasingly an urgent requirement of today. In this juncture this study was proposed with the objective of evaluation of selectively fertilized coconut hybrids for temperature stress tolerance.

Materials and methods

Location:

The study was carried out in the Regional Agricultural Research Station, Pilicod and College of Agriculture Vellayani, Thiruvanthapuram. Selectively fertilized Kerasree, selectively fertilized Keraganga, Kerasree normal hybrid, Keraganga normalhybid and WCT planted in RARS Pilicode were used as experimental plants. Selectively fertilized (SF) hybrids of Kerasree (WCT X MYD) and Keraganga (WCT X GB), were produced by pollen selection. Pollens were subjected to germination in specific media with different levels of water stress induced by PEG 6000 and critical water potential was identified. Those water stress tolerant pollen grains at critical level were selected and used for fertilization and selectively fertilized palms of Kerasree and Keraganga were produced during the year 2008, under a project funded by the International Foundation for Science IFS, Sweden in the Department of Plant Physiology, College of Agriculture, Vellayani.

The present study focused on evaluatingthe temperature stress tolerance of selectively fertilized Kerasree and Keraganga along with normal hybrids of Kerasree, Keraganga and WCT by assessing their critical temperature for pollen germination. This experiment wasundertaken in 15 year old coconut palms of the following varieties planted at Regional agricultural research station, Pilicode, Kerala.

Kerasree (WCT X MYD)-Selectively Fertilized

Keraganga (WCT X GB)-Selectively Fertilized

Kerasree (WCT X MYD) Hybrid

Keraganga (WCT X GB) Hybrid

West Coast Tall

To assess the temperature stress tolerance, critical temperature for pollen germination and protein content of pollen were analyzed in this experiment.

Critical temperature

To assess the critical temperature for pollen germination, spikelet from the inflorescence (5 to 6 days after inflorescence opening) were collected at 9.00 am, immediately transferred to thepolythene bag and brought to the laboratory. The male flowers collected were tapped with nylon brush so that they shed pollen and collected in a petriplate spread with butter paper. The pollen grains collectedwere transferred to germination medium. Initially pollen germination was tried in different identified media and maximum germination was noted in the following media (200 mg L-1 MgSO4 .7H2O; 300 mg L-1 Ca(NO3)O2 .4H2O; 100 mg L-1 KNO3 ; 100 mg L-1 H3BO3 ; 40 g L-1 of sucrose)given by Lora et al., 2006 [12] and then this medium was used for the entire study. 2 ml of germinating media was spread on a glass slide which was then place in a petridish and incubated in BOD incubator for two hours with temperature ranging from 35 °C to 45 °C with 4 replication. Pollen germination was observed after 2 hours of incubation as revealed under 10X magnification of microscope connected with computer. A pollen grain was considered as germinated when the length of the germinated pollen tube was equal or longer than the diameter of the pollen [11]. The temperature at which only 20-30 % of pollen germinated wasidentified as critical temperature stress for pollen germination. Pollen germination percentage was calculated as follows. The percentage pollen germination was averaged. Critical temperature was identified from the data. Linear regression analysis was carried out, taking germination percentage as dependent variable and temperature as independent variable for deriving mathematical relationship between temperature and germination percentage.

Pollen germination %= (No. of pollen grains germinated **/** Total No. of pollen grains) X100

Protein estimation:

Pollen grains were collected from mature male flowers during summer month. Pollens were first stirred in a solution of 30 mM Tris/ EDTA, pH 8.5. Then 0.025 g of pollen in 2 mL buffer Tris/EDTA) was then spun for 20 minutes at 45000 × g. The supernatant was collected in 0.1 mL aliquots and the protein level was determined (Bradford, 1976) [3]. The Bradford assay works by the action of Coomassie brilliant blue G-250 (CBBG) dye. The CBBG (100 mg) was dissolved in 50 mL 95% ethanol. To this solution 100 mL 85% (w/v) phosphoric acid was added. The resulting solution was diluted to a final volume of 1 litre with water. For the preparation of the standard protein, 10 mg bovine serum albumin crystallised was dissolved in 10 mL Tris/EDTA buffer. The pollen load protein values were determined against the absorbance at 595 nm which was calibrated from several dissolutions of control protein at different concentrations. The results are expressed in mg of protein extracted from g of sample (mg g^{-1}).

Statistical analysis: Statistical analysis were carried out using R software version 4.3.0. and the procedure followed was RBD ANOVA with five treatmentsKerasree, Kerasree S.F, Keraganga, Keraganga S.F and WCT each with four replications. Post hoc analysis was carried out using LSD test with 5% level of significance.

Result and Discussion

Critical temperature (^{0}C)

Data on percentage germination of pollen grain for identification of critical temperature is depicted in Table 1. Percentage of germination and critical temperature varied between the treatments. Among the treatments,Kerasree S.F and Keraganga S.F genotypes showed highest critical temperature of 42 ^{0}C for pollen germination. Pollen germination was 20.17

percentage and 22.77 percentage in Keraganga SF hybrid andKerasree SF hybrids respectively. In WCT 40^0C was identified as critical temperature with 21.25 % of germination. Lowest critical temperature for pollen germination was observed in Keraganaga (38 ^{0}C) with 20.03 percentage of germination, which was on par with Kerasree (20.21 % germination). When the temperature was increased to 45^0C, germination in all the treatments was very poor, and following germination percentage were noted, WCT(0.50%), Kerasree SF(0.16%), Keraganga SF(0.03%) , Kerasree(0.5%) and Keraganga (0.16%). Selectively fertilized hybrids performed better than Kerasree, Keraganga normal hybrids and WCT for temperature stress tolerance.

Exploitation of pollen selection method for imparting abiotic stress tolerance in plants there should be a selection system and selection pressure applied to the pollen before pollination [7] or during pollen tube growth in the style [17] and selection response must be observed in the seedlings developed [5]. Kerasree S.F and Keraganga S.F hybrids were developed by pollen selection at critical water potential during the year 2008 in the department of Plant Physiology, College of Agriculture, Vellayani. So in this study we focussed on the evaluation of their temperature stress tolerance by assessing critical temperature for pollen germination.

The pollen grains of twelve genotypes comprising five tall, five dwarf and two hybrids were screened for their cardinal temperature for pollen germination and pollen tube growth and pointed that cultivar variation existed for cardinal temperature [8]. The genotypes with higher temperature for maximum pollen germination (T_{max}) and pollen tube growth were identified as more tolerant to high temperature. Here in this study, there was genetic diversity in selected genotypes for critical temperature for pollen germination. Maximum critical temperature was noted in Kerasree SF and Keraganga SF. Accordingly, selectively fertilized hybrids can be assumed as more temperature stress tolerant among the treatments. The percentage of germination decreased with increase in the temperature and at 45^0C it was very less and approaching zero. Differences in pollen germination and tube growth during stress is due to their genetic variation between individuals [15] and low pollen germination and tube growth are highly correlated with low fruit set [30, 32, 33].

From this experiment it is clear that susceptibility of the coconut palm to stress is a pollen transferable trait and the palms which were developed through pollen selection at critical water potential also had maximum critical temperature and temperature stress tolerance trait. This emphasis the possibility of using them as a pollen parent in coconut hybridization programme as well as manifestation of *in vitro* pollen selection technology widely in different crop species for inducing abiotic stress tolerance such as water stress and temperature stress. .

Linear regression analysis was done, taking germination percentage as dependent variable and temperature as independent variable and itrevealed the mathematical expression for the prediction of germination percentage at higher/lower temperature (Table 2). Since the accuracy percentage from the analysis was greater than 70 percentage, this can be used as a good fit model for prediction of germination percentage in other lower and higher temperature.

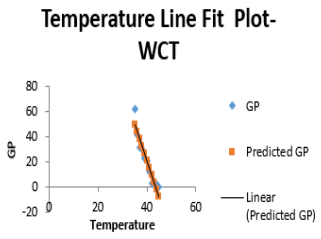
Table 1. Germination percentage at different temperature for identification of critical temperature

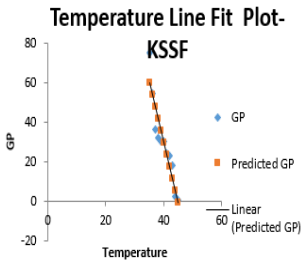
Temperature(^{0}C)	**Percentage germination (%)**				
	WCT	Kerasree SF	Keraganga SF	Kerasree	Keraganga

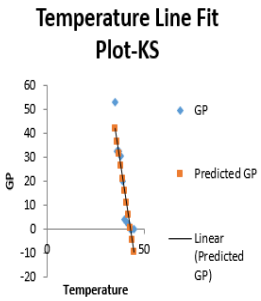
35	62.26	75.23	63.44	52.82	53.94
36	42.16	54.39	55.71	32.42	32.45
37	31.36	36.40	51.52	32.39	30.8
38	30.60	32.03	42.07	30.30	25.65
38	22.77	30.08	33.41	**20.21**	**20.03**
40	**21.25**	30.33	31.28	4.08	14.69
41	12.93	23.99	30.03	3.16	8.08
42	2.77	**22.77**	**20.18**	1.93	4.74
43	2.14	17.69	4.90	0.31	1.59
44	1.73	2.14	0.76	0.12	1.17
45	0.50	0.50	0.165	0.03	0.165

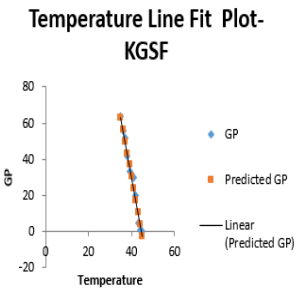
Table 2. Mathematical equation derived from linear regression

Genotype	Equation for germination percentage	Accuracy percentage of prediction
WCT	GP = -5.67 * Temperature + 247.73	89-90
KSSF	GP = -6.03 * Temperature + 270.84	86-87
KGSF	GP = -6.57 * Temperature + 293.29	97
KS	GP = -5.12 * Temperature + 220.98	83-85
KG	GP = -4.87 * Temperature + 212.25	89-90









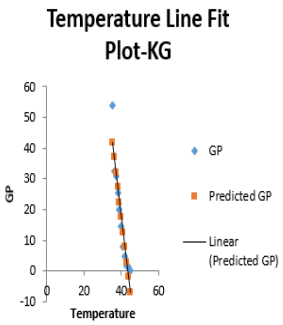


Fig.1 Temperature line fit plot for West coast tall (WCT), Kerasree selectively fertilized hybrid (KSSF), Kerasree (KS), Keraganga selectively fertilized hybrid (KGSF), and Keraganga (KG)

Protein

Amount of protein present in the pollen grains of the treatments became apparent only after they got germinated. Protein content recorded in different treatments are given in Table 3. WCT recorded highest amount of protein (18.24 mg g^{-1}) followed by Kerasree SF hybrids (16.94 mg g^{-1}), which was on par with Keraganga SF hybrids (16.78 mg g^{-1}). They were significantly different from their normal hybrids Kerasree (13.21mg g^{-1}) and Keraganga (14.25mg g^{-1}).

Table 3. Amount of protein present in the pollen grains of selectively fertilized hybrids

Treatment	Protein content a (mg g^{-1})
KSSF	16.94^{b} ±0.96
KGSF	16.78^{b} ±0.82
KS	14.21^{d} ±0.91
KG	12.65^{c} ±1.01
WCT	18.24^{a} ±1.5
CD(0.05)	**1.40**

Saraka et al (2017)[23] analysed the protein in the pollen grains of ComeroMoheli Tall (CMT) and Malayan Yellow Dwarf (MYD) genotypes of coconut, during 0-4 months of storage of pollen grainsin deep-freezer at -15°C and found variations in a range of 7.1 to 29.8 mg g^{-1} in CMT and 8 -30 mg g^{-1} in MYD. The amount of protein present in the pollen grains of different grass species and their mixtures like perennial rye grass, thimothy, and cocksfoot differed significantly between treatments [10]. Both these results were ratifying this experiment that all the treatments significantly varied in the amount of protein. During drought stress period pollen undergoing sterility may leads to reduced expression of genes and proteins. The functions of most of the drought induced or suppressed genes remains unclear [29]. In our experiment maximum protein content was noted in WCT followed by selectively fertilized hybrids. This can be correlated with the study of Rieu et al., 2017 [31], that high temperature can destroy the protein present in pollen grains. So high protein content of WCT and selectively fertilized hybrids at high temperature may indicatetheir temperature stress tolerance nature. A difference in pollen protein and carbohydrate content can result in genetic variation among genotypes because protein and carbohydrate present in the pollen grains are detrimental factors for pollen germination and pollen tube growth [6]. Temperature stress induced/suppressed genes in pollen grains have to be validated carefully for further understanding of protein present within them.

Conclusion

Kerasree selectively fertilized hybrid and Keraganga selectively fertilized hybrids have higher critical temperature stress for pollen germination and which means that they are more tolerant to temperature stress. They also retained higher amount of protein in their pollen grains than normal hybrids of Kerasree and Keraganga. Kerasree and Keraganga selectively fertilized hybrids have been developed through pollen selection at critical water potential and their water stress tolerance have alreadybeen reported [1 and 21].

However,the findings of the present study insinuate that the hybrids developed through pollen selection at critical water potential also have attributes of temperature stress tolerance. In the context of climate change,use of these hybrids will reduce the production losses associated with abiotic stresses. So it is concluded that selective fertilization can be used for adding traits like drought tolerance and temperature stress tolerance to the high yielding coconut hybrids. The potency of in vitro pollen selection technique is evinced. Further molecular evaluation and validation studies are needed.

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