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Physiological and Biochemical Basis of Thermotolerance in Selectively Fertilized Coconut Hybrids

Aisha Renju N. A. [a++*], Roy Stephen [a#], R. V. Manju [a#] and Viji M. M. [b#]

[a] Department of Plant Physiology, College of Agriculture, Vellayani, Trivandrum, India.
[b] Department of Plant Physiology, RARS, Ambalavayal, Wayanad, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The aim of the study was to physiologically and biochemically assess selectively fertilized coconut hybrids for their tolerance to high-temperature stress. Conducted using a two-factorial Completely Randomized Design, the research took place at the Department of Plant Physiology, College of Agriculture, Vellayani, Trivandrum, between October 28, 2023, and December 28, 2023. Methodology involved cultivating six-month-old selectively fertilized hybrid seedlings of three coconut varieties, Kerasree, Keraganga, and Kerasankara, in a polyhouse with a pre-set temperature of 40±1°C for two months. Various physiological and biochemical parameters, including Relative Water Content, Chlorophyll Stability Index, Epicuticular Wax Content, Lipid

[++] *Ph.D Scholar;*
[#] *Professor;*
**Corresponding author: E-mail: aishiyas221109@gmail.com;*

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Peroxidation Level, and antioxidant enzyme activity, were measured to assess stress tolerance. Results showed that for the physiological parameters RWC and CSI, selectively fertilized hybrids varied significantly under high temperatures from their corresponding non-fertilized hybrids, with average mean values of 88.53% and 83.27%, respectively. High-temperature-induced solute leakage was lowest in Kerasankara selectively fertilized hybrids with a mean value of 3.58±0.63, and wax deposition values were on par for selectively fertilized and non-fertilized hybrids. MDA accumulation due to lipid peroxidation was significantly lowest in selectively fertilized hybrids with a mean value of 2.47µg g-1. Antioxidant enzymatic activity in selectively fertilized hybrids was remarkably higher, with significant percent increases of 97.1% for Peroxidase and 96.1% for SOD in the Keraganga selectively fertilized genotype. The study concluded that coconut hybrid accessions developed through pollen selection demonstrated significant superiority over regular hybrids in terms of physiological and biochemical stress tolerance indices. Thus, pollen selection followed by selective fertilization is a reliable, cost-effective, and time-saving approach for breeding stress-tolerant crops, including perennials.

Keywords: Selective fertilization; high temperature stress; pollen selection; stress tolerance.

ABBREVIATIONS

N.F : Normally Fertilized
S.F : Selectively Fertilized
HT : High Temperature
AT : Ambient Temperature
RWC : Relative Water Content
CSI : Chlorophyll Stability Index
ECW : Epicuticular Wax
SOD : Superoxide dismutase
ROS : Reactive Oxygen Species
MDA : Malondialdehyde
IPCC : Intergovernmental Panel on Climate Change
Term : Definition for the term

1. INTRODUCTION

The coconut palm, often referred to as the "tree of life," plays a vital role in the livelihoods of smallholders, providing cash income, nutrition, and materials. It primarily thrives in the humid tropics, located between 23°N and 23°S of the equator, and can grow at altitudes up to 600 meters above sea level for nut production. According to the FAO statistical database, global coconut production is about 63.6 million nuts, covering a planted area of 11.6 million hectares [1]. Optimal production requires well-distributed rainfall and an annual mean temperature of 27-29°C [2]. A minimum temperature above 10°C promotes flowering, while temperatures below 10°C for a month cause nut fall. Conversely, temperatures above 40°C from April to July in the tropics reduce the functional leaf area index, dry matter production, and nut yield [3].

According to projections, the Earth's surface temperature would rise by 1.5°C to 11°C by 2100 as a result of an increase in greenhouse gases [4]. It is predicted that brief bursts of extreme weather, such as high temperatures, may occur more frequently, which will affect fruit set and production. The temperature has an important impact on plant growth and development, especially during the flowering period [5]. Sexual reproduction in plants is more sensitive to high temperature than vegetative processes, and therefore, plant reproductive organs will be more vulnerable to changes in short episodes of high temperature prior to and during early flower stage [6]. As reported, the no. of female flowers/inflorescence and the no. of nut set/inflorescence were the main yield components in coconut affected by temperature and moisture stresses [7]. It is imperative to develop types that can withstand high temperatures in order to maximize coconut production under these circumstances.

In vitro pollen selection, followed by selective fertilization, is the most feasible and cost-effective approach. By applying the appropriate selective pressure during the pollination and fertilization processes, only pollen grains that are resistant to the pressure will be able to germinate and fertilize the ovule. Pollen genotype selection has shown promise for a number of variables, including biotic [8] and abiotic stress tolerance [9,10] reported effectiveness of pollen selection to develop moisture stress tolerance in Sorghum.

Stephen et al. [11] developed water stress tolerance in coconut via invitro pollen selection [12]. Evaluated the water stress tolerance of selectively fertilized coconut hybrids using a range of physiological and biochemical indices.

The results showed that S.F hybrids fared better during dry spells. Selectively fertilized hybrids of Kerasree and Keraganga assessed as thermotolerant recorded maximum critical temperature for pollen germination [13]. Therefore, the current study was carried out to confirm the effectiveness of the pollen selection technique in developing genotypes that are stress resistant and to investigate and assess the thermotolerance of the selectively fertilized coconut hybrids based on physiological and biochemical characteristics.

2. MATERIALS AND METHODS

2.1 Experimental Setup

To assess the resistance of the selectively fertilized coconut hybrids created through pollen selection to high temperature stress, a polyhouse-based experiment was carried out at the College of Agriculture, Vellayani from October 28, 2023, to December 28, 2023. In the polyhouse facility, where the temperature was set to a higher known value compared to the ambient condition, three varieties of hybrids—Kerasree (West Coast Tall x Malayan Yellow Dwarf), Keraganga (West Coast Tall x Ganga Bondam), and Kerasankara (West Coast tall X Chowghat Orange Dwarf)—were laid out under three replications. The hybrids were fertilized both normally and selectively. Selectively fertilized hybrids and normally fertilized hybrids used in this study were developed in a previous study by the author via pollen selection and conventional method of assisted pollination respectively. Six-month-old seedlings were sown in polybags of size 40x24x24cm filled with 1:2:1 potting mixture and maintained as per POP recommendation [14].

2.2 Physiological Evaluation

Following two months of high temperature stress (40 ± 1^0C) on hybrid seedlings, an evaluation was performed for a range of physiological observations. Leaf samples were obtained from the third index leaf of the seedlings for each parameter that was taken into account.

2.2.1 Relative water content (Percentage)

A leaf's hydration status is assessed by comparing its actual water content to its maximum water-holding capacity when fully turgid (a process known as "relative turgidity," or relative water content, or RWC [15]. The sample's turgid weight was measured by immersing it in distilled water for three hours and then weighing it; the sample's dry weight was measured by drying the turgid leaf samples in a hot air oven at 70^0C until they reached a constant weight.

RWC (%) was calculated using the formula:

RWC = (Fresh weight - dry weight) / (turgid weight - dry weight) X 100

2.2.2 Membrane Integrity (Percentage of solute leakage)

The quality or state of the entire membrane in ideal condition is known as membrane integrity. Cell membrane thermostability can be effectively determined by measuring electrolyte leakage, which has been used as a direct heat injury indicator [16]. Using the following formula, the membrane integrity of leaf tissues was determined and expressed as a percentage of leakage.

$$\% \text{ leakage} = ((EC_b - EC_a) / EC_c) \times 100$$

where EC_a is the initial value of electrical conductivity caused by immediate leakage from leaf samples in distilled water, EC_b is the value of electrical conductivity caused by leakage from samples incubated for 30 minutes and EC_c is the value of electrical conductivity caused by leakage from samples treated in boiling water bath for 10 minutes.

2.2.3 Chlorophyll Stability Index (Percentage)

Plants' ability to withstand stress is indicated by the chlorophyll stability index, or CSI. A high CSI value indicates that the stress had little impact on the plants' chlorophyll content. Through improved chlorophyll availability, a higher CSI increases a plant's ability to withstand stress [17]. By calculating the ratio of the total chlorophyll content of the heated leaf samples to the control samples and multiplying the result by 100, CSI (%) was calculated.

$$\text{Chlorophyll Stability Index (\%)} = \left\{ \frac{\text{Total Chl. Content in Heated lot}}{\text{Total Chl. Content in Control lot}} \right\} \times 100$$

2.2.4 Epicuticular wax content (mg 10cm^{-2})

Epicuticular wax (EW) coats aerial surfaces to form a barrier between the environment and the plant to protect plants from biotic stressors like excessive radiation, water loss, high temperatures, and drought [18]. Leaf bits of 10cm^2 area were immersed in pre-weighed beakers and chloroform solvent sufficient to immerse them. Following a chloroform solvent wash, the wax that had been deposited on the leaf surfaces was collected by evaporating the solvent.

$$ECW\ (mg) = W_2 - W_1$$

Where ECW is the Epicuticular wax content, W_1= Initial weight of the beaker, W_2= Final weight of the beaker.

2.3 Biochemical Evaluation

Evaluation for various biochemical observations were performed after two-month exposure of hybrid seedlings to high temperature stress. For all the parameters observed, leaf samples were taken from third index leaf of the seedlings

2.3.1 Malondialdehyde Content (µg g^{-1})

The ROS-induced peroxidation of membrane lipid (measured by MDA content) is a sign of cellular damage brought on by stress. MDA content was determined by the Thiobarbituric acid (TBA) reaction as described by [19]. The absorbance of the assay extract was observed at 532 and 600 nm, and MDA concentration (µg g^{-1}) was estimated by subtracting the non-specific absorption at 600 nm from the absorption at 532 nm.

2.3.2 Activity of enzymatic antioxidants

2.3.2.1 Peroxidase (activity g^{-1}min^{-1})

Plant cells' enzymatic defense system includes peroxidases, which initiate the transformation of H_2O_2 into water and oxygen [20]. The peroxidase activity in plants was estimated following Pyrogallol method described by [19]. One unit of peroxidase is defined as the change in absorbance/minute at 430nm.

2.3.2.2 Superoxide dismutase (activity g^{-1}min^{-1})

In plants, the enzyme SOD is directly related to stress, which initiates the first line of defense, converting O_2^- into H2O2 [21]. SOD activity of plants was quantified following the method described by [22]. One unit of enzyme activity is defined as the amount of enzyme that gave 50% inhibition of NBT reduction in one minute.

3. RESULTS AND DISCUSSION

The experimental data was statistically analyzed in two factorial completely randomized design using grapes Agri1 software [23]. Results for physiological and biochemical parameters are presented in Tables 1 to 4 and Figs. 1 to 3. In the following section, normally fertilized and selectively fertilized hybrids are referred as N.F and S.F respectively.

3.1 Relative Water Content (%)

The impact of ambient and high temperatures on the Relative Water Content (RWC) of N.F. and S.F. hybrid coconuts are illustrated in (Table 1). Under high temperature (40$\pm$1^{0}C), the mean values of RWC for S.F hybrids ranged from 85.73% to 94.67% and for N.F hybrids from 79.54% to 91.03%. Kerasankara S.F. distinguished itself significantly from the other hybrids with the highest mean value of 94.67%, a 15.9% increase over its N.F counterpart. At higher temperatures, the lowest RWC value (79.54%) was recorded in Kerasankara N.F. The highest RWC mean value (91.67%) under ambient conditions (28$\pm$2^{0}C) was found in Kerasankara N.F., which was comparable to the mean values of Kerasankara S.F. and Kerasree N.F. The Keraganga N.F., which differed significantly from its S.F. hybrid, had the lowest value. The RWC expresses the precise amount of water that a plant needs to reach full saturation, making it a valuable indicator of the state of the plant's water balance. Data on tomato cultivars also showed that, in stressed conditions, tolerant genotypes had significantly higher RWC than susceptible ones [24,13] observed higher leaf water status in selectively fertilized, drought-tolerant coconut hybrids: Kerasree and Keraganga.

3.2 Chlorophyll Stability Index (%)

The impact of both ambient and high temperatures on the Chlorophyll Stability Index (CSI) of N.F. and S.F. hybrid coconuts is displayed in Table 2. At high temperatures (40$\pm$1^{0}C), the mean CSI for N.F and S.F hybrids is 80.04% and 83.27%, respectively. The Kerasree S.F genotype outperformed all other

Table 1. RWC of coconut hybrids under High and Ambient temperature conditions

Sl.No	Hybrid genotypes	Relative Water Content (%)					
		HT			AT		
		NF	SF	Percent difference	NF	SF	Percent difference
1.	Kerasree	91.03^{b}	85.73^{c}	5.8	90.35^{ab}	86.49^{cd}	4.3
2	Keraganga	85.22^{c}	85.19^{c}	0.04	82.50^{e}	83.33^{e}	0.9
3	Kerasankara	79.54^{d}	94.67^{a}	15.9	91.67^{a}	90.00^{ab}	1.82
Mean		**85.26**	**88.53**		**88.17**	**86.60**	
SE±	**0.98**				**0.68**		
CD at 5%	**2.95**				**2.03**		

CD=critical difference, SE=standard error, NF=Normally Fertilized, SF= Selectively Fertilized

Table 2. Chlorophyll Stability Index of coconut hybrids under High and Ambient temperature conditions

Sl. No	Hybrid genotypes	Chlorophyll Stability Index (%)			
		HT		AT	
		NF	SF	NF	SF
1.	Kerasree	70.31^{de}	97.83^{a}	85.02^{de}	97.29^{a}
2	Keraganga	92.15^{ab}	65.19^{e}	92.68^{bc}	91.05^{c}
3	Kerasankara	77.65^{cd}	86.78^{bc}	72.43^{f}	82.49^{e}
Mean		**80.04**	**83.27**	**83.38**	**90.28**
SE±	**3.05**			**1.53**	
CD at 5%	**9.15**			**4.58**	

CD=critical difference, SE=standard error, NF=Normally Fertilized, SF= Selectively Fertilized

genotypes by a significant margin, and its NF hybrid had the highest CSI value (97.83%) in high temperatures. All S.F genotypes displayed greater CSI values in ambient conditions, except Keraganga, which displayed values comparable to its NF hybrid. A higher CSI makes a plant more resilient to stress by increasing the availability of chlorophyll. Chlorophyll resilience leads to greater dry matter production, higher productivity, and a higher photosynthetic rate in a salinity stressed rice plant. [17]. Similar information supporting the current investigation was shown for wheat cultivars [25].

3.3 Epicuticular wax content (mg 10cm^{-2})

The impact of ambient and high temperatures on the Epicuticular wax content (ECW) of coconut hybrids is shown in Fig. 1. In comparison to its N.F hybrid, the S.F hybrid of Keraganga displayed the highest wax content under high temperatures. Significantly, Kerasankara S.F had the highest ECW value in ambient conditions. Similar findings were reported in wheat cultivars [26] where significantly greater wax deposition was noted under higher temperature stress. The presence of ECW protects plants from environmental stresses and water loss [27].

3.4 Lipid peroxidation - Malondialdehyde content (µg g^{-1})

Reactive oxygen species (ROS), including superoxide radical, hydrogen peroxide, hydroxyl radical, and singlet oxygen, are produced in excess when there is heat stress. The lipid peroxidation caused by reactive oxygen species (ROS) generated in plant tissue under heat stress damages membranes and forms microscopic hydrocarbon fragments like malondialdehyde (MDA), one of the key markers of oxidative stress [28]. The amount of MDA accumulated in the N.F and S.F coconut hybrids at high and ambient temperatures is shown in Fig. 2. All of the SF hybrids showed lower MDA levels than the corresponding N.F hybrids at higher temperatures, but the SF hybrids performed differently in terms of lipid peroxidation at ambient conditions. Coconut hybrid seedlings observed tolerant at elevated temperature conditions exhibited lower MDA levels [29]. The decrease in MDA content in S.F hybrids is a manifestation of reduced damage at cellular level due to lipid peroxidation which is desirable for thermotolerance.

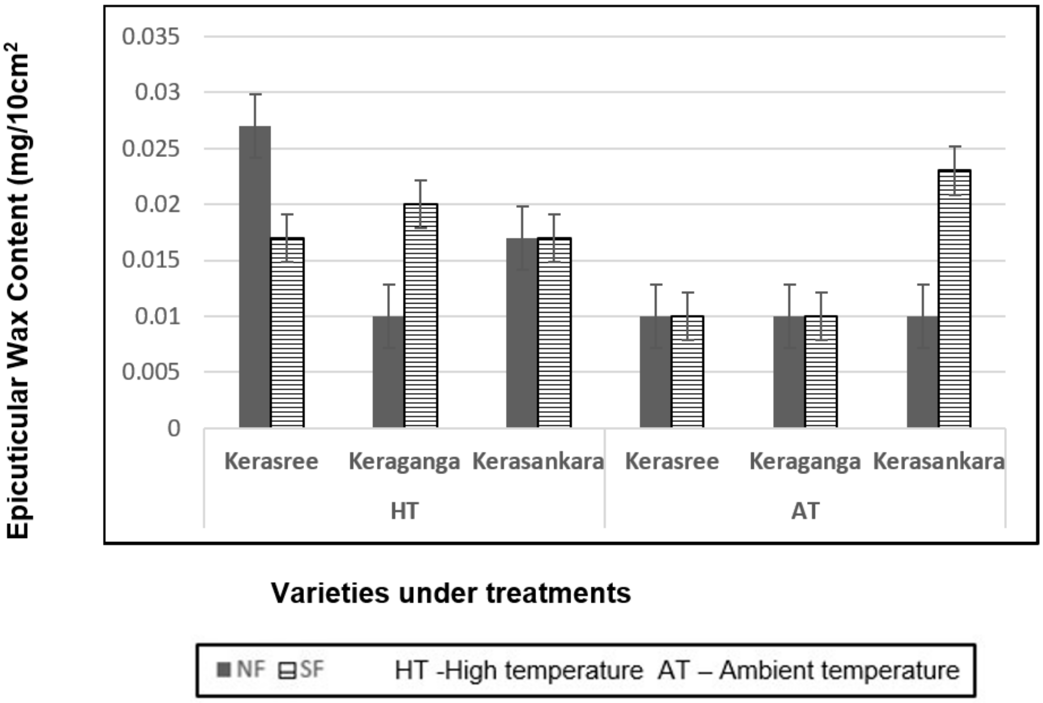

Fig. 1. Epicuticular Wax Content of Coconut hybrids under High and Ambient temperature conditions

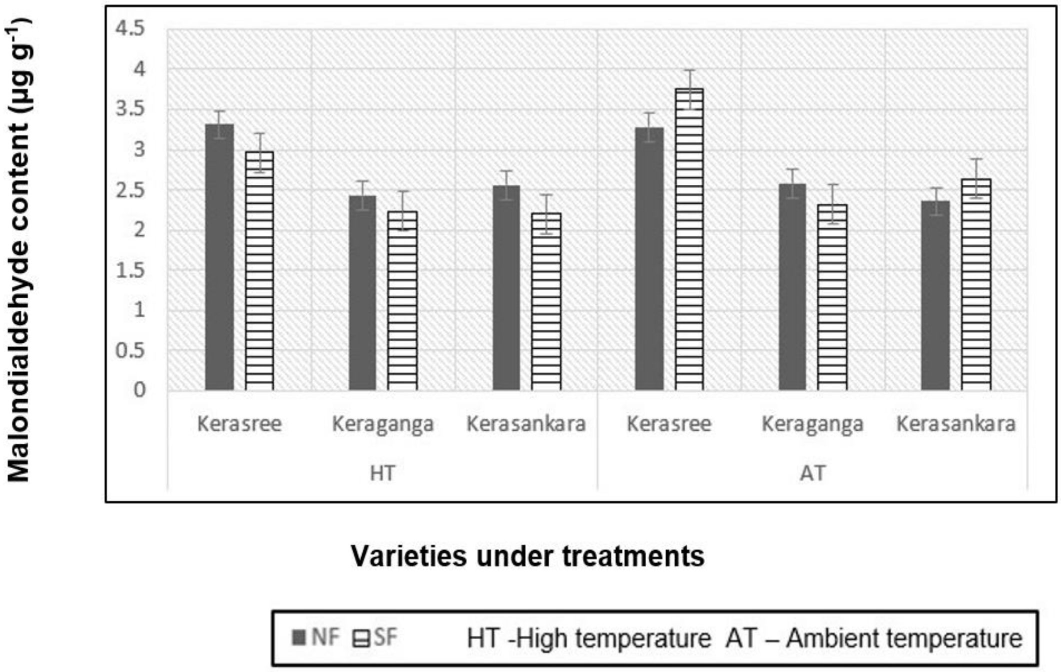

Fig. 2. Malondialdehyde Content of Coconut hybrids under High and Ambient temperature conditions

Table 3. Peroxidase activity of coconut hybrids under High and Ambient temperature conditions

S.No	Hybrid genotypes	Peroxidase				
		HT			AT	
		NF	SF	Percent Increase	NF	SF
1.	Kerasree	0.18^{f}	3.53^{d}	94.9	0.56^{de}	0.51^{e}
2	Keraganga	0.12^{f}	4.07^{c}	97.1	0.25^{f}	0.99^{c}
3	Kerasankara	3.30^{e}	11.13^{a}	70.4	0.49^{e}	1.87^{a}
Mean		**1.2**	**6.24**		**0.43**	**1.12**
SE±	**0.04**				**0.04**	
CD at 5%	**0.12**				**0.12**	

CD=critical difference SE=standard error NF=Normally Fertilized SF= Selectively Fertilized

Table 4. Superoxide Dismutase activity of coconut hybrids under High and Ambient temperature conditions

Sl.No	Hybrid genotypes	SOD				
		HT		Percent increase	AT	
		NF	SF		NF	SF
1.	Kerasree	0.36^{f}	3.47^{c}	89.6	0.44^{e}	3.58^{b}
2	Keraganga	0.22^{f}	5.59^{b}	96.1	0.22^{f}	5.26^{a}
3	Kerasankara	1.54^{e}	7.41^{a}	79.2	0.38^{ef}	2.79^{c}
Mean		**0.71**	**5.49**		**0.35**	**3.88**
SE±	**0.12**				**0.07**	
CD at 5%	**0.35**				**0.21**	

CD=critical difference SE=standard error NF=Normally Fertilized SF= Selectively Fertilized

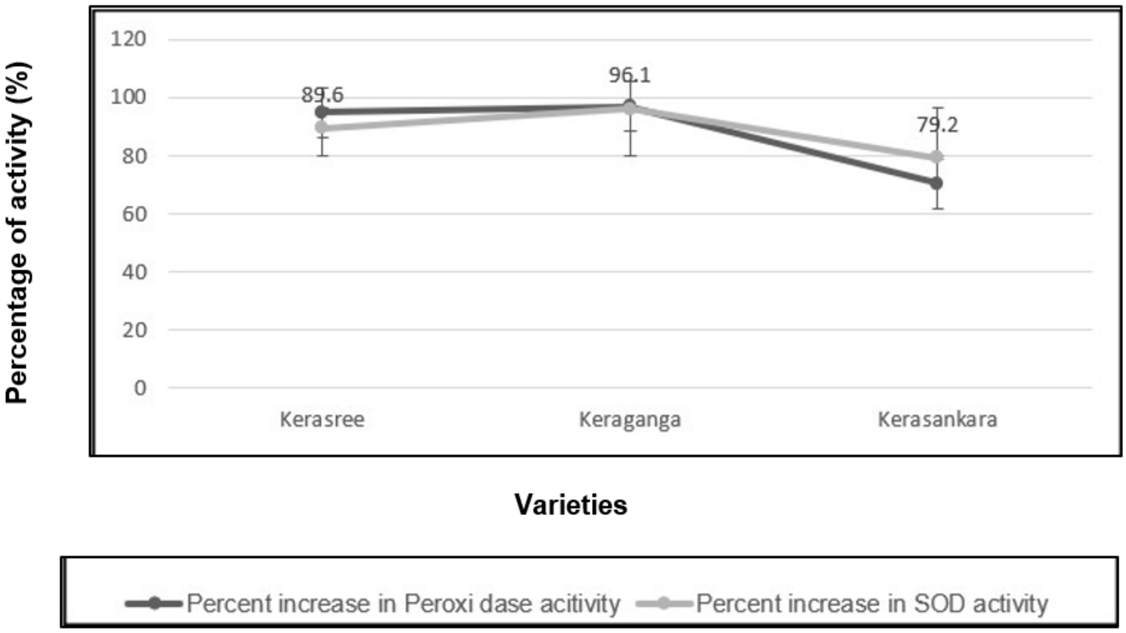

Fig. 3. Percent difference of Peroxidase and SOD activity in coconut hybrids under high temperature

3.5 Activity of Peroxidase ($g^{-1}min^{-1}$)

Table 3 shows the impact of both ambient and high temperatures on the peroxidase activity of coconut hybrid seedlings. Under high temperatures, all genotypes of SF hybrids showed significant variation from their NF hybrids, with a mean value of 6.24 activity $g^{-1}min^{-1}$. In high temperatures, Kerasankara SF displayed the highest mean value of 11.13 activity g-1min-1, while Keranganga N.F recorded the lowest mean value of 0.12 activity $g^{-1}min^{-1}$. At high temperature, all of the SF hybrids showed a notable percentage increase in peroxidase activity (94.9,97.1,70.4%) (Fig. 3). With a mean activity of 1.12 $g^{-1}min^{-1}$, the S.F hybrids outperformed their N.F hybrids in ambient conditions, recording higher mean values. Peroxidase enzyme activity was increased by high-temperature stress, and it has been linked to the emergence of physiological injuries brought on by thermal stress in plants [30]. Plants increase their thermotolerance by recruiting the antioxidant enzymes superoxide dismutase, ascorbate peroxidase, catalase, glutathione reductase, and peroxidase [31]. Drought tolerance in coconut varieties were characterized by high activities of SOD, POX and CAT and consequent lower level of MDA content[32]. Increased peroxidase activity exhibited in S.F hybrids under stress condition might be an adaptive strategy to save the membrane system from oxidative stress and remain tolerant.

3.6 Activity of Superoxide dismutase (activity $g^{-1}min^{-1}$)

Table 4 shows SOD activity in coconut hybrid genotypes affected by various temperature conditions. Under high temperatures(40 ± 1^0C), S.F hybrids demonstrated an average mean value of 5.49 activity $g^{-1}min^{-1}$ for SOD activity, with Keraganga demonstrating a 96.1 % increase in enzyme activity (Fig. 3) in comparison to its N.F counterpart. In comparison to their N.F hybrids, none of the N.F hybrids displayed as much SOD activity at high temperatures. The enzyme activity's highest mean value (7.41 activity $g^{-1}min^{-1}$) was found in Kerasankara S.F, while Keraganga S.F had the lowest mean value (0.22 activity $g^{-1}min^{-1}$. According to [17] the enzyme SOD in plants is directly linked to stress and starts the first line of defense by changing O^{2-} into H_2O_2. Increased activity of SOD was observed in oxidative stress-tolerant cultivars and hybrids of coconut under elevated temperatures [29]. SOD, together with Peroxidase activity, was part of a strong defense antioxidant mechanism to support seedlings in minimizing cell injury, and maintain growth under elevated temperatures. Tomato genotypes that can withstand higher temperatures have been shown to have higher SOD sensitivity [33].

4. CONCLUSION

All genotypes of SF hybrids exhibited superior performance in the current study for all physiological parameters associated with stress tolerance, including epicuticular wax content, chlorophyll stability index, and relative water content. S.F hybrids outperformed their N.F hybrids in mean values for biochemical observations such as lipid peroxidation and enzymatic antioxidant activity. Together, these indices confirm that S.F. hybrids are more temperature-tolerant. In order to create high temperature tolerant hybrids in coconuts, the current evaluation experiment thus validates the effectiveness of the pollen selection technique followed by selective fertilization.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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