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Effect of Seasonal and Altitude Variations in the Reactive Oxygen Species Production and Antioxidant's Concentration in *Mangifera indica* L. (var. kottukonam)

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ABSTRACT: Reactive oxygen species produced as by-product of aerobic metabolism are toxic but plays an important signaling role in plants and controlling the processes like growth and development. Their production are regulated by various biotic and abiotic stresses. Free radicals like Superoxides ($O^{\cdot}{}_2^-$), Hydroxyl radicals ($OH^{\cdot}$) and non-radicals such as Hydrogen Peroxide (H_2O_2) and Singlet Oxygen ($^1O_2$) are the common ROS which make serious oxidative damages to cells. Mango (*Mangifera indica* L.), a member of Anacardiaceae is perhaps the most sweetest and nutritious fruit yielding tropical tree. Kottukonan is one of the varieties over 1500 varieties of *Mangifera indica* L. found globally and are commonly cultivated in the southern parts of Kerala state of India especially in Thiruvananthapuram and Kollam districts was the study material. The leaf samples of kottukonam have been collected from three altitudes in four seasons. The major challenge of the study was to learn the dominant machineries in mango tree to alleviate the serious effects generated by ROS in relation with seasonal and altitude variations. The study revealed that changing the concentration of enzymatic (Ascorbate Peroxidase, Catalase, Glutathione Reductase, Guaiacol Peroxidase, Mono Dehydro Ascorbate Reductase, Dehydro Ascorbate Reductase, NADPH Oxidase and Super Oxide Dismutase) and non-enzymatic (Proline, Ascorbic acid and Reduced Glutathione) antioxidants with respect to the fluctuation in the concentration of ROS is the considerable mechanism to reduce the significant damages by them in cells. The concentration of ROS, enzymatic and non-enzymatic antioxidant composition is higher in pre-reproductive winter and reproductive summer seasons. The coastal area samples showed more concentration of such antioxidants and ROS due to an additional environmental stress, high salinity. Results indicated that the flowering and fruiting is also the upshot of higher production of many antioxidants along with other hormonal changes in relation with high intensity of major abiotic stresses in *Mangifera indica* L. (var. kottukonam). High antioxidant property of mango fruits is the reflection of such abiotic stresses in winter and summer seasons.

Keywords: Reactive Oxygen Species, Antioxidant compounds, *Mangifera indica,* Kottukonam, abiotic and biotic stresses.

Abbreviations: ROS, Reactive Oxygen Species; APX, Ascorbate Peroxidase; GR, Glutathione Reductase; GPX, Guaicol Peroxidase; MDHAR, Mono DehydroAscorbate Reductase; DHAR, DehydroAscorbate Reductase; SOD, Superoxide Dismutase; $O^{\cdot}{}_2^-$, Superoxide radicals; H_2O_2 Hydrogen Peroxide; $^1O_2$, singlet oxygen; $OH^{\cdot}$, Hydroxyl radical; vzm, Vizhinjam; kza, Kulathuppuzha; vjd, Venjaramoodu

INTRODUCTION

Antioxidants, Reactive Oxygen Species (ROS) scavengers are phytochemicals which can inhibit the oxidation of biomolecules like DNA, proteins and lipids by scavenging the free radicals and alleviating stress (Raja *et al*., 2017; Marcioand Isabel 2013; Jeremy and Alan 2012; Jin-Jian *et al*., 2012). ROS are produced constantly due to aerobic respiration, various biotic and abiotic stresses in the chloroplast (Dietz *et al*., 2016), mitochondria (Sako *et al*., 2020; Honglin *et al*., 2019), peroxisomes (Sandalio and Romero-Puertas 2015) and also by apoplast (Jan *et al*., 2020; Amna and Frank 2018; Aryadeep and Supratim 2012; Tana *et al.,* 2009) which cause damages to the plant cells. But generally they are unable to cause damage, as they are being scavenged by different antioxidant mechanisms (Christine and Graham 2005). The different stress factors like drought, pathogen infection, extreme temperature, salinity, high irradiance, pollution and heavy metals may imbalance the ROS generation and ROS scavenging. Here the survival of plants depends on some other factors such as severity and duration of stress, change in growth conditions and the capability of plants to suddenly adapt to changing energy equation (Gad *et al*., 2010).

The $^1O_2$ (singlet oxygen) (Hasanuzzaman *et al*., 2019), $O^{\cdot-}_2$ (Superoxide radicals), H_2O_2 (Hydrogen Peroxide) and $OH^{\cdot}$ (Hydroxyl radical)are the lethal ROS which cause damages to DNA, lipids and proteins and affects normal functioning of cells (Huang *et al*., 2016; Christine and Graham 2005; Changbin and Martin 2000). They are formed in phototrophs as a result of photosynthesis which is important for the existence of life on earth. Singlet Oxygen ($^1O_2$) is generated by the reaction of triplet state of Chlorophylls with Oxygen in the antenna system. Stomatal closure due to various environmental stresses like drought, heavy metals and high salinity leads to insufficient CO_2 concentration in plant cells which in turn favours the formation of singlet oxygen (Sheikh *et al*., 2022). This is an atypical ROS which can cause severe damage to Photosystem l and Photosystem ll. These singlet oxygen plays a key role in regulating genes that are meant for giving protection from photo-oxidative stress (Anja *et al*., 2008). Super oxide radical ($O^{\cdot-}_2$) is constantly generated in the chloroplast due to the oxygen partial reduction or the result of energy transfer to oxygen. It never cause severe damage to the cells by itself but its transformation into hydroxyl radical and singlet oxygen is more toxic and reactive which results in membrane lipid peroxidation (Barry 2006).

The moderately reactive ROS such as Hydrogen peroxide (H_2O_2) produced in plant cells in normal condition and also by oxidative stress due to intense light, chilling, drought, UV radiation, pathogen infection and wounding (Pallavi *et al*., 2012) is damaging at higher concentrations in plant cells. Hydroxyl radical ($OH^{\cdot}$) is the most toxic and most reactive ROS capable of damaging cellular components by lipid peroxidation, membrane damage and destruction of proteins. Cellular death is the result of excess accumulation of this ROS, which is due to the absence of enzymatic system to scavenge them in plant cells. The functioning of the antioxidant machinery contains (1) antioxidant enzymes such as APX (Ascorbate Peroxidase), GPX (Guaiacol Peroxidase), CAT (Catalase), SOD (Superoxide Dismutase), GR (Glutathion Reductase), MDHAR (Monodehydroascorbate Reductase), DHAR (Dehydroascorbate Reductase) and NADPH Oxidase (Ahanger *et al*., 2019), and (2) the non-enzymatic antioxidants like Ascorbic acid, Reduced Glutathione, α-tocopherol, Carotinoids, Phenolics, Flavonoids and Prolineto overcome the stressful conditions in plants (Naz *et al*., 2016; Jeremy and Alan 2012; Sarvajeet *et al*., 2010; 2011). The survival of plants depends on the counteractions of these antioxidant machineries against the ROS produced.

Mango (*Mangifera indica* L.), a member of Anacardiaceae is perhaps the most sweetest and nutritious fruit yielding tropical tree and support the economy of many developing countries. It is commonly designated in India as 'The king of fruits'. Kottukonan is one of the variety over 1500 varieties of *Mangifera indica* L. found globally and are commonly cultivated in the southern parts of Kerala state of India especially in Thiruvananthapuram and Kollam districts. Since the variation in the quantity of ROS especially superoxide anions and hydrogen peroxide, and antioxidant compounds in relation with seasonal and altitude variations in *Mangifera indica* L. (var. kottukonam) is an underexplored area of research. The present study focused to quantify the antioxidant compounds from the leaves of plants which are collected in four seasons (summer, south west monsoon, north east monsoon and winter) from three altitudes [Vizhinjam (coastal area), Kulathuppuzha (semi high range area) and Venjaramoodu (normal sea level area)].

MATERIALS AND METHODS

Planting materials and sampling. *Mangifera indica* L. (var. kottukonam) was the experimental material. The leaves of experimental plants were collected from (1) Venjaramoodu, normal sea level area (Latitude: 8.68 N, Longitude: 76.91 E, Altitude: 53.00m above sea level) (2) Vizhinjam, coastal area (Latitude: 8.38 N, Longitude: 77 E, 45.00m above sea level) and (3) Kulathuppuzha, semi high range area (Latitude: 8° 54′32.46″N Longitude: 77° 3′33.57″E Altitude: 203.6m above sea level). The first two sampling areas belong to the Thiruvananthapuram district and the last one from Kollam of Kerala state in India. The samples were collected in four seasons such as summer (February – May, average temp. of 32 to 36°C and 135mm pptn.), South West Monsoon (June – September, average temp. of 19 to 30°C and 2250 to 2500mm pptn.), North East Monsoon (October – November, average temp. of 29 to 35°C and 450 to 500mm pptn.) and Winter (December – beginning of February, average temp. of 18 to 28°C and 25mm pptn.). All the mango trees selected for the study are about 50 to 55 years old.

Antioxidant compounds and enzymes. Analysis of antioxidant compounds and enzymes in the samples from three altitudes in four seasons were performed by standard protocols. Non-enzymatic antioxidants namely ascorbic acid (Jadhao *et al*., 2016), glutathione (Nadia *et al*., 2022) and proline (Bates *et al*., 1973); antioxidant enzymes such as NADPH Oxidase (Evan and Federica 2018), Guaiacol peroxidase (Angela and Sabine 2003), Ascorbate peroxidase (Gad *et al*., 2010), Catalase (Amna *et al*., 2010), Superoxide dismutase (Chris *et al*., 2011), Monodehydroascorbate reductase (Vincent *et al*., 2017) and Dehydroascorbate reductase (Zhongand Daniel 2006) was studied.

Quantification of signaling ROS.

H_2O_2. 1gm fresh tissue was homogenized with 10 ml of 10 mM KH_2PO_4 buffer with pH 7. Filtered extract were centrifuged at 10000 rpm for 10min, collected the supernatant and made up to known volume. Took 1.5 ml extract and added 1.5 ml assay reagent (500 µm ammonium ferrous sulphate, 0.05M conc. H_2SO_4, 200 µm xylenol orange and 0.2M sorbitol) and kept it for 45 min. Took the absorbance at 560 nm against the blank.

Superoxide anion. 1gm fresh tissue was homogenized with 10 ml of 50 mM Sodium acetate buffer (pH 6.5), centrifuged the filtered extract at 10000 rpm for 10 min. Collected the supernatant and made up to a known volume. Took 0.1 ml aliquots and mix with assay reagent (0.01M Potassium phosphate buffer (pH 7.8),

0.05% Nitrobluetetrazolium, and 10 mM Sodium azide). After 30 min of incubation took the OD at 580 nm. Put the mixture in a water bath at 85°c for 15 min and took the final OD at 580 nm. OD difference was taken and calculated the amount of superoxide anion in 1gm tissue.

Statistical analysis. The data obtained were analyzed statistically by one-way analysis of variance (ANOVA) and t- test ($p < 0.05$). The results were average of five replications and represented as mean ± SD.

RESULTS AND DISCUSSION

Amount of ROS (superoxide anions and hydrogen peroxide) (Table 1a& b) and antioxidant compounds present in methanol extract of *Mangifera indica* L. (var. kottukonam) from three altitudes in four seasons were quantified. The results revealed that the quantity of superoxide anions and hydrogen peroxide, enzymatic and non-enzymatic ROS scavengers varied in accordance with the variation in altitude and season (Table 2a & b). The amount of Reactive Oxygen Species such as hydrogen peroxide and superoxide anions were high in Pre- flowering winter season in all the samples followed by flowering and fruiting season samples in summer. During summer, in addition to the common abiotic stresses like high temperature, high light intensity and water scarcity, the plant again stressed due to flowering and fruiting. These stresses resulted the over production and accumulation of superoxide anions and hydrogen peroxide in mango which are counter balanced by producing various secondary metabolites, enzymatic and non-enzymatic antioxidants. Winter season is the pre-flowering season during which the plants prepared for the reproductive face, much more quantity of ROS were produced than the south west and north east monsoon seasons. Additional stress, high salinity resulted higher ROS concentration in coastal (Vizhinjam) samples of all the seasons (Table 2a and b).

Proline. Proline, an amino acid capable of mitigating the effect of abiotic and biotic stresses includes drought, high salinity, high temperature and pathogen attack. It is a major part of structural protein and suggests its role as stress amino acid in plants. The exposure of plants to abiotic stresses leads to the production of Reactive Oxygen Species (ROS) that enhances the programmed cell death (Sheikh 2022; Klaus and Heribert 2004). To defeat such a crisis the plants over produced the stress aminoacid namely proline which act as an active ROS scavenger and prevent them from programmed cell death. Concentration of proline accumulation in plant cells during different seasons in the present study might be related to their responses to various abiotic and biotic stresses [S1vzm, 685.48±13.48mg/gm tissue; S1kza, 455.18±1.96mg/gm tissue; S1vjd, 550.30±1.40mg/gm tissue; S2vzm, 469.35±1.44mg/gm tissue; S2kza, 341.96±0.54mg/gm tissue; S2vjd, 388.68±0.86mg/gm tissue; S3vzm, 488.49±0.86mg/gm tissue; S3kza, 325.57±2.15mg/gm tissue; S3vjd, 377.94±0.88mg/gm tissue; S4vzm, 701.14±0.55mg/gm tissue; S4kza, 467.80±0.56mg/gm tissue; S4vjd, 556.98±1.56mg/gm tissue] (Table 2a & b). The more stressful conditions in pre reproductive winter and reproductive summer seasons indicated the direct correlation between the production of ROS and antioxidant proline during during such seasons. The effect of additional stress called high salinity can be noticed in the result of coastal samples.

Ascorbic acid. Ascorbic acid is the most extensively studied and abundant ROS scavenging compound in plants. Ascorbate produced in plant cells by Smirnoff-Wheeler pathway is powerful antioxidant compound counteract the ROS produced as it can donate electrons to enzymatic and non-enzymatic reactions. These are also involved in retrograde signaling (Konig *et al*., 2018). The present study also revealed that the ascorbic acid concentration varied along with the change in altitude and season. The highest content of ascorbic acid was noted in pre-flowering winter season [S4vzm, 57.10±0.84mg/gm tissue; S4kza, 44.11±0.62mg/gm tissue; S4vjd, 50.96±0.59mg/gm tissue] and then in the flowering and fruiting summer season [S1vzm, 48.12±0.85mg/gm tissue; S1kza, 41.10±0.11mg/gm tissue; S1vjd, 44.90±0.33mg/gm tissue] than the other two seasons (south west monsoon and north east monsoon) (Tables 2a & 2b). The major reason behind this in winter and summer seasons is due to abiotic stresses. High salinity acted an additional stress in coastal area (Vizhinjam) resulted much more Ascorbate content in the samples from there. Konig *et al*. (2018) and Seung and Adel (2000) reported that high light intensity and water scarcity increases the Ascorbic acid content of plant tissues.

Reduced Glutathione (GSH). Glutathione is a low molecular weight thioltripeptide. Present in the cell components like Cytoplasm, Mitochondria, Endoplasmic Reticulum, Vacuoles, Chloroplast, Peroxisomes and Apoplast. The high reductive potentiality of glutathione resulted their involvement in a wide range of processes in plants like growth, cell division, senescence, cell death, nucleotide and protein synthesis, detoxification of xenobiotics and the expression of stress responsive genes (Philip and Thomas 2005). GSH protects the plant cells from ROS like H_2O_2, $^1O_2$, OH^-, and O^-_2 formed by various biotic and abiotic stresses due to its reducing power by the presence of a cysteine residue with nucleophilic character. Earlier studies revealed that the glutathione content varied along with seasonal variations. In *Mangifera indica* L. (var. kottukonam), glutathione is high in the winter samples [S4vzm, 56.00±0.34mg/gm tissue; S4kza, 47.71±0.42mg/gm tissue; S4vjd, 50.71±0.40mg/gm tissue] during which the plant prepared for flowering. In addition to the preparations for flower production the plant also faced other abiotic stresses especially high light intensity and temperature in day time, water scarcity etc. also act as key reasons for the formation of ROS in higher quantity during winter. High quantity of glutathione were also observed in the samples of summer season [S1vzm, 55.19±0.74mg/gm tissue; S1kza, 44.09±0.17mg/gm tissue; S1vjd, 49.14±0.21mg/gm tissue] during which flowering and fruiting occurs. Due to the high salinity

stress in coastal area along with other abiotic stresses, Vizhinjam samples in all the seasons showed much more glutathione content than the others (Tables 3a & b). The result of the study have a correlation to the previous work of Konig *et al.* (2018).

Antioxidant enzymes. Various abiotic and biotic stresses persuade the photosynthetic system of leaves to photo-inhibition, resulting in a light-dependent inactivation of the primary photochemistry associated with photosystem II, which often persists after re-watering (Deeba *et al*., 2012). Photosynthesis to be affected by water deficits and high salt contents, via decreased CO_2 diffusion to the chloroplast and metabolic constraints (Pinheiro and Chaves 2011). Acclimation of plants to drought, temperature, light intensity, salinity, diseases and pathogen attack is often associated with increased levels of reactive oxygen species (ROS). During stressful conditions the production of ROS become transcend and the action of antioxidative system to scavenge ROS resulted in oxidative stress (Paul and Panneerselvam 2013). Antioxidant enzymes such as Ascorbate peroxidase, Catalase, Glutathione Reductase, Mono DehydroAscorbate Reductase, DehydroAscorbate Reductase, Guaicol Peroxidase, NADPH Oxidase and Superoxide Dismutase protected plants from the damage caused by ROS Zeng *et al*. (2017); Ron *et al*. (2011); Ron Mitrtler (2002).

Ascorbate Peroxidase (APX). APX is an efficient H_2O_2 scavenger during stress periods in plants commonly located in the chloroplast, peroxisomes, mitochondria and cytoplasm (Zeng *et al*., 2017; Pallavi and Dubey 2004). It is an unavoidable component of Ascorbate-Glutathione cycle. In the present study the highest quantity of APX noted in the most stressful seasons such as in winter (pre-flowering) and summer (flowering) in *mangifera indica* L. (var. kottukonam than that in south west and north east monsoon seasons (S1vzm, 600.57±0.36 unit/gram tissue; S1kza, 323.60±2.20 unit/gram tissue; S1vjd, 585.66±3.08 unit/gram tissue; S2vzm, 420.81±0.79 unit/gram tissue; S2kza, 257.18±1.48 unit/gram tissue; S2vjd, 291.54±4.06 unit/gram tissue; S3vzm, 418.14±2.51 unit/gram tissue; S3kza, 247.59±0.38 unit/gram tissue; S3vjd, 215.04±3.63 unit/gram tissue; S4vzm, 678.61±0.38; S4kza, 342.09±0.20 unit/gram tissue; S4vjd, 599.69±1.47 unit/gram tissue) (Table 3a & 3b).

Catalase (CAT). Catalase, a unique antioxidant enzyme having greater affinity to H_2O_2, located in mitochondria, chloroplast, per oxisomes and cytosol. Oxidative stress lead to the over production of H_2O_2 (Sheikh, 2022; Ron Mittler, 2002) which are removed efficiently by CAT in an energy efficient way. Maximum amount of CAT resulted in *Mangifera indica* L. (var. kottukonam) during the most stressful seasons namely summer and winter (S1vzm, 2214.81±2.18 unit/gram tissue; S1kza, 1082.45±2.79 unit/gram tissue; S1vjd, 1981.54±1.23 unit/gram tissue; S4vzm, 2561.06±12.01 unit/gram tissue; S4kza, 1023.85±0.39 unit/gram tissue; S4vjd, 2086.64±2.37 unit/gram tissue) (Table 3a & 3b). Caverzan (2016); Paul and Panneerselvam (2013); Pinhieroand Chaves (2011) reported that the strength of stresses were overcome by the plants by producing the antioxidant catalase enzyme in much more concentration.

Glutathione Reductase (GR). GR is a flavoproteinoxidoreductase used to reduce Glutathione disulfide (GSSG) to reduced Glutathione (GSH) commonly found in chloroplast and small amount in cytoplasm and mitochondria. They are the scavenger of detrimental ROS, $^1O_2$ and OH^-. The result of the present study indicated the amount of GR is highest in the samples of winter season (S4vzm, 1245.55±1.24 unit/gram tissue; S4kza, 810.94±1.01 unit/gram tissue; S4vjd, 1154.39±2.88 unit/gram tissue) and then in the summer season (S1vzm, 1117.54±0.56 unit/gram tissue; S1kza, 788.01±2.03 unit/gram tissue; 1057.42±1.18 unit/gram tissue) because of pre-flowering and flowering stresses to the tree in addition to the common environmental stresses. Caverzan (2016) reported that the stressful conditions leads to the over production of antioxidant enzymes including GR.

Monodehydro Ascorbate Reductase (MDHAR) and Dehydro Ascorbate Reductase (DHAR). By using the reducing agent, MDHAR regenerating Ascorbic acid from the short lived Monodehydro Ascorbate, resulted the restoring of Ascorbate in the Ascorbic acid pool of cells. MDHAR occurred in the peroxisomes and mitochondria along with Ascorbate peroxidase and helped for scavenging the ROS especially Hydrogen Peroxide (Sheikh, 2022; Ron, 2002). The present study revealed that the most stressful condition faced by *Mangifera indica* L. (var. kottukonam) is the winter and summer, and thus the quantity of Antioxidant enzyme MDHAR in these two seasons is also comparatively more than the other two seasons (Table 3a & b). The samples from coastal area in all seasons showed more quantity of MDHAR due to an additional stress, high salinity.

Using Reduced Glutathione as an electron donor DHAR reduces Dehydro Ascorbate to Ascorbic acid (Amin *et al*., 2007) to enriched the Ascorbic acid pool of cells and maintaining the redox state of the cells (Zhong and Daniel 2006). This indicated that the stressful conditions led to the higher production of DHAR (S1vzm, 1252.20±2.22 unit/gram tissue; S1kza, 984.66±5.32 unit/gram tissue; S1vjd, 1176.37±1.95 unit/gram tissue; S4vzm, 1286.80±1.31 unit/gram tissue; S4kza, 901.73±5.28 unit/gram tissue; S4vjd, 1116.96±1.40 unit/gram tissue) like that of MDHAR and the present study also an agreement to this facts (Table 3a & b).

Guaicol Peroxidase (GPX). GPX is a heme containing protein seen associated with many biosynthetic processes and defense against biotic and abiotic stresses. They oxidise aromatic electron donors at the expense of hydrogen peroxide (Pallavi *et al*., 2012). The present study in *Mangifera indica* L. (var. kottukonam) indicated that like that of other antioxidant enzymes GPX content also more during winter and summer seasons and much more in coastal samples (S1vzm, 653.81±1.75 unit/gram tissue; S2vzm, 457.23±1.48 unit/gram tissue; S3vzm, 463.30±0.36 unit/gram tissue; S4vzm, 678.67±0.40 unit/gram tissue)

(Table 3a & b). The result also an agreement to the previous studies of Caverzan (2016); Andre *et al.* (2006); Jingxian and Khirkham (1996).

NADPH Oxidase. NADPH Oxidases in plants are commonly called Respiratory Burst Oxidase Homologues (RBOHs) which act as a catalyst in the physiological generation of short - lived superoxide radicals (David, 2004). In the present study, the concentration of NADPH Oxidases is more in winter and summer seasons especially in coastal samples due to over environmental stresses (Table 3a & 3b) and this stimulate the production of ROS. The earlier studies revealed that the over presence of this enzyme is a signal to the plants to the environmental stresses and the present study is also an agreement to that findings (Smirnoff and Arnaud 2018).

Superoxide Dismutase (SOD). Metalloenzyme common in all aerobic organisms is the first line of defense against ROS induced damages under abiotic stresses (Boguszewska, 2010). SOD generally localised in cytoplasm, chloroplast, mitochondria and peroxisomes. In *Mangifera indica* L. (var. kottukonam), the SOD content is higher in winter season (S4vzm, 457.36±3.81 unit/gram tissue; S4kza, 287.66±3.82 unit/gram tissue; S4vjd, 433.08±0.78 unit/gram tissue) and then in flowering and fruiting summer season (S1vzm, 418.32±1.08 unit/gram tissue; S1kza, 223.66±0.43 unit/gram tissue; S1vjd, 376.30±0.51 unit/gram tissue) than other two seasons (south west monsoon and north east monsoon) due to the over stress faced by the plant from environment (Table 3a & b). The coastal samples (vzm) in all seasons showed much more content of SOD than other altitude samples in different seasons because of the additional salt stress. The present study results were also revealed that the earlier studies of Lu *et al*. (2017); Zlatev *et al.* (2006); Kukreja *et al.* (2002) was truthful.

Table 1a: Quantification of ROS, superoxide anions and hydrogen peroxide in the leaf samples of *Mangifera indica* L. (var. kottukonam) from three altitudes [Vizhinjam- coastal area (vzm): Kulathuppuzha- semi high range area (kza) and Venjaramoodu- normal sea level area (vjd)] in two seasons [sample 1, summer; sample 2, south west monsoon] in mg/gm fresh wt. Values with ± SD from five independent experiments.

Reactive Oxygen Species (ROS)	Sample 1 [S1]			Sample 2 [S2]		
	Vzm	kza	vjd	vzm	Kza	vjd
Superoxide Anion	19.45±0.46	9.39±0.32	13.96±0.37	4.27±0.26	6.14±0.17	8.35±0.34
Hydrogen Peroxide	5.09±0.069	3.25±0.207	3.31±0.196	3.38±0.115	1.90±0.137	2.35±0.279

Table 1b: Quantification of ROS, superoxide anions and hydrogen peroxide in the leaf samples of *Mangifera indica* L. (var. kottukonam) from the three altitudes [Vizhinjam- coastal area (vzm): Kulathuppuzha- semi high range area (kza) and Venjaramoodu- normal sea level area (vjd)] in two seasons [sample 3, north east monsoon; sample 4, winter] in mg/gm fresh wt. Values with ± SD from five independent experiments.

Reactive Oxygen Species (ROS)	Sample 3 [S3]			Sample 4 [S4]		
	Vzm	kza	vjd	vzm	kza	vjd
Superoxide Anion	3.81±0.209	6.30±0.368	7.81±0.726	20.44±0.563	10.71±0.653	15.35±0.743
Hydrogen Peroxide	6.40±0.334	3.60±0.234	3.75±0.209	6.05±0.088	3.57±0.329	3.60±0.365

Table 2a: Amount of Non enzymatic Antioxidant compounds in the leaves of *Mangifera indica* L. (var. kottukonam) from the three altitudes [Vizhinjam- coastal area (vzm): Kulathuppuzha- semi high range area (kza) and Venjaramoodu- normal sea level area (vjd)] in two seasons [sample 1, summer; sample 2, south west monsoon] [Values are with ± SD from five experiments each] (in mg/gm fresh wt.).

Antioxidant compound	Sample 1 [S1]			Sample 2 [S2]		
	vzm	kza	vjd	vzm	kza	vjd
Proline(µmol/gm fresh wt.)	685.48.±13.48	455.18±1.96	550.30±1.40	469.35±1.44	341.96±0.54	388.68±0.86
Ascorbic Acid (mg/gm fresh wt.)	48.12±0.85	41.10±0.11	44.90±0.33	42.25±0.65	38.54±0.33	41.19±0.73
Reduced Glutathione (mg/gm fresh wt.)	55.19±0.74	44.09±0.17	49.14±0.21	49.05±0.42	39.73±0.90	41.28±0.61

Table 2b: Content of Non enzymatic Antioxidant compounds in the leaves of *Mangifera indica* L. (var. kottukonam) from the three altitudes [Vizhinjam- coastal area (vzm): Kulathuppuzha- semi high range area (kza) and Venjaramoodu- normal sea level area (vjd)] in two seasons [sample 3, north east monsoon: sample 4, winter] [Values are with ± SD from five experiments each] (in mg/gm fresh wt.).

Antioxidant compound	Sample 3 [S3]			Sample 4 [S4]		
	vzm	kza	vjd	vzm	kza	Vjd
Proline (µmol/gm fresh wt.)	488.49±0.86	325.57±2.15	377.94±0.88	701.14±0.55	467.80±0.56	556.98±1.56
Ascorbic Acid (mg/gm fresh wt.)	44.05±0.11	37.87±1.85	44.14±0.76	57.10±0.84	44.11±0.62	50.96±0.59
Reduced Glutathione (mg/gm fresh wt.)	46.99±0.45	38.07±0.11	39.05±1.94	56.00±0.34	47.71±0.42	50.71±0.40

Table 3a: Specific activity of Antioxidant Enzymes in the leaf samples of *Mangifera indica* L. (var. kottukonam) from the three altitudes [Vizhinjam- coastal area (vzm): Kulathuppuzha- semi high range area (kza) and Venjaramoodu- normal sea level area (vjd)] in two seasons [sample 1, summer; sample 2, south west monsoon] [Values are with ± SD from five experiments each] (in unit/gm fresh wt.)

Antioxidant Enzymes (unit/gm tissue)	Sample 1 [S1]			Sample 2 [S2]		
	vzm	kza	vjd	vzm	kza	Vjd
Ascorbate Peroxidase	600.57±0.36	323.60±2.20	585.66±3.08	420.81±0.79	257.18±1.48	291.54±4.06
Catalase	2214.81±2.18	1082.45±2.79	1981.54±1.23	1332.64±10.23	951.66±0.61	1118.10±2.49
Glutathione Reductase	1117.54±0.56	788.01±2.03	1057.42±1.18	884.79±8.98	679.95±2.96	900.69±0.47
Monodehydro Ascorbate Reductase	998.46±1.18	720.53±0.55	980.05±0.92	857.35±3.56	659.01±0.60	821.35±1.76
DehydroAscorbate Reductase	1252.20±2.22	984.66±5.32	1176.37±1.95	987.32±1.43	857.79±1.91	1055.88±2.51
Guaicol Peroxidase	653.81±1.75	400.77±0.34	535.78±2.55	457.23±1.48	377.81±0.90	490.71±1.02
NADPH Oxidase	489.49±0.36	330.16±1.56	427.74±1.47	387.24±0.81	317.84±0.98	353.84±0.89
Super Oxide Dismutase	418.32±1.08	223.66±0.43	376.30±0.51	370.21±1.14	197.08±1.65	229.005±0.83

Table 3b: Specific activity of Antioxidant Enzymes in the leaf samples of *Mangifera indica* L. (var. kottukonam) from three altitudes [Vizhinjam- coastal area (vzm): Kulathuppuzha- semi high range area (kza) and Venjaramoodu- normal sea level area (vjd)] in two seasons [sample 3, north east monsoon: sample 4, winter] [Values are with ± SD from five experiments each] (in unit/gm fresh wt.).

	Antioxidant Enzymes (unit/gm tissue)	Sample 3 [S3]			Sample 4 [S4]		
		vzm	kza	vjd	vzm	kza	vjd
	Ascorbate Peroxidase	418.14±2.51	247.59±0.38	215.04±3.63	678.61±0.38	342.09±0.20	599.69±1.47
	Catalase	1288.71±1.44	985.70±2.12	1104.38±3.61	2561.06±12.01	1023.85±0.39	2086.64±2.37
	Glutathione Reductase	867.66±0.92	723.02±1.17	908.05±1.17	1245.55±1.24	810.94±1.01	1154.39±2.88
	Monodehydro Ascorbate Reductase	808.32±0.87	674.79±3.91	797.50±5.29	1010.79±0.29	740.97±0.70	996.33±2.46
	Dehydro Ascorbate Reductase	884.74±0.92	720.84±2.46	998.01±1.30	1286.80±1.31	901.73±5.28	1116.96±1.40
	Guaicol Peroxidase	463.30±0.36	359.00±1.16	591.96±1.01	678.67±0.40	435.08±0.51	609.92±0.31
	NADPH Oxidase	368.78±0.28	321.39±0.41	375.19±0.88	495.86±0.48	361.25±2.23	461.16±0.99
	Superoxide Dismutase	367.69±0.87	201.11±0.28	248.62±0.59	457.36±3.81	287.66±3.82	433.08±0.78

CONCLUSIONS

In relation with seasonal and altitude variationsn on enzymatic and enzymatic antioxidants were produced in *Mangifera indica* L. (var. kottukonam). The environmental stresses like drought, high temperature, high salinity, high oxygen concentration and high light intensity leads to the over production of various ROS especially Hydrogen peroxide and Superoxide anions, and the plant defeated these injuries by the extra production of certain non enzymatic antioxidant compounds such as Proline, Ascorbic acid and Reduced Glutathione, and antioxidant enzymes such as Ascorbate Peroxidase, Catalase, Glutathione Reductase, Mono DehydroAscorbate Reductase, Dehydro Ascorbate Reductase, Guaiacol Peroxidase, NADPH Oxidase and Superoxide Dismutase. The quantity of these antioxidants varied in relation with the production of ROS which in turn related with the intensity and type of stresses faced by the plant in a specific season and altitude. The present result concludes that these antioxidant machinery seems to be sufficient in *Mangifera indica* L. (var. kottukonam) to fight against various environmental stresses.

FUTURE SCOPE

The leaves of *Mangifera indica* L. (var. kottukonam) was the material used in the present study and identified that the variation in the concentration of antioxidant compounds and triggering ROS in relation with seasonal and altitude variations. In addition to the fruits the leaves developed in the summer season is also a rich source of antioxidants. The present investigation suggested that the extraction of these antioxidants from leaves which are very useful to human health will be a promising one to the pharmaceutical industry.

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Conflict of Interest. None.

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