



A comprehensive study on the physio-chemical responses of camellia plants to abiotic stresses

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DOI: <https://doi.org/10.33545/2664844X.2019.v1.i3a.87>

Abstract

Camellia species, being a woody perennial with more than 100 years of life span, it experiences several abiotic stresses throughout its life. Conventional breeding is protracted and restricted principally to selection, which escorts to taper down of its genetic base. Predominantly being strict monoculture cultivation, the plants countenance pest populations that are dreadful and unique. Young leaves are economically important parts and abiotic stresses are extremely detrimental to production. For instance, drought alone accounts for 40% loss of yield of tea per annum. Despite constraints, commendable efforts have been perpetrated to appreciate the physiological as well as biochemical alterations of an assortment of abiotic stresses encountered by these plants. Thus, this review presents a consolidated account of the accomplishment and inadequacy of these tools and techniques hitherto applied to the plants. Expectedly, this will form a foundation for making further advances intended for improvement of tea and other economically important wild relatives, in particular, belongs to *Camellia* sp. To the best of our understanding, this is the first comprehensive compilation of such study in *Camellia* species with particular reference to tea [*Camellia sinensis* L. (O) Kuntze].

Keywords: Abiotic stress, camellia, physiological parameters, biochemical analysis

Introduction

Tea [*Camellia sinensis* (L.) O. Kuntze], of family Theaceae and an evergreen shrub, is indigenous to Indo-China region and is cultivated in humid and sub-humid tropical, sub-tropical, and temperate regions of the world, in about 52 countries, which grow mainly on acidic soils. Apart from tea, a number of its wild species bear enormous significance. For instance, *C. japonica*, a wild species of tea, is cultivated due to its excellent floricultural splendor. Quite a few other wild species such as *C. reticulata*, *C. sasanqua*, and *C. siamensis* are also popular owing to their ornamental value. Species such as *C. oleifera*, *C. semiserrata*, and *C. chekiangolomy* produce oil from mature seeds destined to pharmaceutical industry, albeit to a limited scale. Stress evaluation and exploration of alleviatory actions are the two most active and kinetic research fields of tea science. In this study, progress in the research of abiotic stresses of tea plants have been appraised.

Abiotic Stress

Abiotic stresses are responsible for at least 65% diminution of tea yield. Being perennial, tea plants experience manifold abiotic stresses adversely influencing growth and yield, and therefore they need to tackle an array of multi-pronged stresses at some unspecified junctures of life cycle. To facilitate adaptation and survival, they have evolved counteractive mechanisms to endure stresses. Nevertheless, stresses beyond tolerance levels will unavoidably culminate in oxidative damage attributable to intensive production of reactive oxygen species (ROS). Oxidative stress is a phenomenon surfaces as a consequence of unevenness concerning the ROS production and competence of antioxidative defensive devices.

Macronutrients

The perennial plantation crops such as tea encounter typical predicament of element deficiency due to extended life span,

unrelenting harvest without proper replenishment of nutrients and deterioration of soil fertility owing to human interference. Apart from deficiency, heavy metal contaminations due to constant handling and industrial emission have become a critical alarm to the tea plantation worldwide.

Enormous improvements have been made till date in unveiling a handful of elements that result in an exponential amount of crop loss.

Nitrogen (N)

N is an essential macro element for plants. Tea plants require immense quantity of N not only for amino acid biosynthesis, but also for yield of secondary metabolites. Tea is low pH tolerant and favors ammonium (NH_4^+) because of quicker absorption. But N, as nitrate, acted as a stressor and reduced glutamine synthetase (GS) activity, N content, free amino acids, glucose and leaves became yellow under deficiency. Likewise, in coffee plants, the rate of superoxide (O_2^-) formation increased under sunlight indicating increased photo protective capacity per amount of photons absorbed. However, ascorbate peroxidase (APX) activity reduced with hydrogen peroxide (H_2O_2) accumulation. Unsaturation of chloroplast membranes was higher in N deficient coffee plants. The generation of ROS such as; O_2^- , H_2O_2 and hydroxyl radical (OH) damaged cellular components, protein, and membrane lipids. Plants responded to ROS by increased defensive enzymes production under N deficiency suggesting the role of N as stress inducer. A positive correlation between peroxidase (POD) activity and tolerance to N deficiency affected water relations of *C. sinensis* by increasing stomatal resistance and transpiration rate (E). Similarly, *Picea sitchensis*, N deficiency reduced chlorophyll (chl) and carotenoid (car), photosynthetic rate (PN) and stomatal conductance (GS), and increased intercellular partial pressure of CO_2 (C_i). Similarly, under N deficiency, *Fraxinus* seedlings decreased uptake of N and phosphate, reduced leaf

mass ratio and biomass and conversely, increased N concentration, reduced chl, soluble protein and ribulose biphosphate carboxylase oxygenase (Rubisco) of red pine trees drastically indicating the probable role of N as stressor in excess also. However, problems with excess N are not very frequent in the natural ecosystems; it is widespread in areas with agricultural malpractices.

Phosphorus (P)

Due to a constituent of DNA, RNA, ATP and phospholipids, P is essential for plants. Tea is cultivated mainly on acidic soils where P deficiency is ubiquitous and P deficiency led to lower activities of superoxide dismutase (SOD), APX, glutathione reductase (GR), catalase (CAT), monodehydroascorbate reductase (MDAR) dehydroascorbate reductase (DHAR) together with ascorbic acid (ASA) and reduced glutathione (GSH). But malondialdehyde (MDA) levels indicating active participation of antioxidant system against photo-oxidative injury. P induced generation of singlet oxygen (1O_2) and other ROS, exerted several harmful effects. Therefore, decrease in ASA, probably exposed P deficient plants to oxidative damages that compounded by decreased antioxidative enzyme activity. Conversely, P deficient treatments altered internal use efficiency. P deficiency, in tea, inhibited growth due to reduction of PN and GS that caused photoinhibition and photodamage of photosynthetic apparatus, and induced malate and citrate release accompanied by increased activity of PEP carboxylase, PEP phosphatase, citrate synthase (CS) and NAD-malic enzyme (NAD-ME) in addition to decreased pyruvate kinase (PK), NADP-malic enzyme (NADP-ME) and NADP isocitrate dehydrogenase activities in roots. In the tea leaves, NADP-ME, NAD-ME and PK activities enhanced. In *Fraxinus* sp., P deficiency, reduced 5-aminolevulinic acid biosynthesis and porphobilinogen synthase activity, N and P content, that in turn, decreased maximum quantum efficiency of PS-II, electron transport rate and PN. In tea, P deficiency decreased the nutrients, and root Mg content. Leaves showed lower N and higher C and C/N ratios under deficiency. P deficiency reduced ATP content terminating in a smaller amount of RuBP regeneration and CO₂ assimilation. Energy dissipation increased to facilitate protection of P-deficient tea plants. Excess light can be fatal for plants due to harmful ROS generation in the photochemical reaction centers. This happens when energy absorption exceeds the rate of carbon fixation. Hence, in order to survive, plants have evolved several protective processes by which energy dissipation has improved. Therefore, decrease in ASA and other antioxidative enzymes are liable to predispose plants to oxidative damages under P stress.

Iron (Fe)

Fe is an important macroelement of plants. Fe toxicity, in tea, declined chl and polyphenols along with decreased amylase, invertase, aspartate aminotransferase, glutamate synthase activity. Polyphenols, the major alkaloids, decreased under Fe toxicity suggesting the gradual loss of protection to overcome oxidative damage and declined quality of tea. In shade trees severe Fe deficiency resulted in chlorosis, reduced leaf as well as shoot growth leading to dieback of twigs. Fe deficiency, in *Quercus*, also decreased chl and car contents, but decrease of chl was more. Fe deficiency caused 8% decrease in the dark-

adapted efficiency of PS-II and 43% decrease in PS-II efficiency at steady-state photosynthesis.

Potassium (K)

K is one of the major macro elements that play a key role in a variety of movements of individual cells in certain tissues. K deficient tea plants demonstrated increased stomatal diffusive resistance, reduced night opening and lower cuticular resistance. In K-deficient Hibiscus, leaf water content, turgor potential, and PN, E, as well as GS were consistently lower, compared to K-sufficient plants. Consistent lowering of gas exchange related parameters indicate that K deficiency may be fatal to plants because K⁺ is involved in guard.

Magnesium (Mg)

Mg is another crucial macroelement of plants and deficiency of Mg reduced glutathione and increased MDA in *Citrus sinensis* and *C. grandis*. Hence, increase in MDA with deficient Mg is indicative of free radical generation. Mg toxicity induced development of coppery color all over the leaf surfaces and led to defoliations in tea plants, prevented Ca uptake because both are divalent cations with similar radius to each other. Synthesis and transport of amino acids in tea plants decreased under toxicity that also inhibited polyphenol synthesis of tea. Activities of nitrate reductase, glutamine synthetase, aspartate and alanine aminotransferase decreased under toxicity. Mg deficiency, in *Citrus reticulata*, decreased leaf CO₂ assimilation, carbohydrate contents, chl, car, CAT and Rubisco activity and impaired photosynthetic electron transport chain indicating its pivotal role as a central molecule of chl.

Micronutrients

Trace elements are required in very little quantity but their importance for the growth of the plant is no way less than the major elements. Due to deficiency of these minor elements leaves, branches and fruits may not grow properly and they may even affect quality of products as well as production. These elements help in synthesis of hormones, enzymes, chl and absorption.

Boron (B)

B is an essential mineral nutrient for plants. Under starvation, NO₃ concentrations diminished while NO₂ increased in the young tea leaves and APX and POD activity increased in leaf and roots respectively.

Tea plantlets reduced growth, gas exchange parameters, pigments, dry weight (DW) and fresh weight (FW). Total and reducing sugar, starch, ASA and phenolics enhanced, but protein contents declined. Quantity of O₂⁻, MDA, electrolyte leakage and H₂O₂ increased under deficiency. B deficiency up-regulated activities of POD, SOD and APX; a similar observation was made in mulberry which indicated that B deficiency provoked oxidative stress and the enzymes associated with the antioxidant system may be considered as stress markers. Oxidative metabolism, the prime defense mechanism, brought about a cascade of biochemical transformations and implied the essence of B in tea. Besides, closing of stomata, increased abscisic acid (ABA) and proline indicated moisture stress under B deficiency. Likewise, B deficiency increased phenolic compounds in leaves of olive

plants. Transmission electron micrograph (TEM) of cell walls elucidated thickened middle lamellae of leaf cells; a key deficiency marker because of its nonexistence in B-adequate cells. Under deficiency, the B-rhamnogalacturonan-II complex influenced the solidification of the middle lamella. Likewise, due to B deficiency, phenol metabolites accumulated in olive leaves and free amino acids increased in Lupinus.

Zinc (Zn)

Zn is a microelement, deficiency of which affects plants. Apart from other reasons, increase of P in the soil due to excessive phosphate applications induced Zn deficiency. Zn stress (deficiency or toxicity) led to inhibition of growth, and FW and DW. Zn stress reduced P_N , E and G_S in conjunction with chl a and b. Excess Zn *in vivo*, altered photosynthetic parameters in *Populus x euramericana*. O_2^- , MDA, H_2O_2 and electrolyte leakage elevated in deficient and toxic plants whereas total and reducing sugar and starch increased in optimum plants. Phenol content and of antioxidative enzyme activities intensified in Zn deficient plants indicating inflated ROS production under Zn stress. Similarly, activities of APX, CAT, POD and SOD were increased in Zn-deficient and -sufficient mulberry indicating their protective role. In sycamore maples, small quantities of Zn set off oxidative stress in older leaves because Zn reached sensitive sites inside leaf blade. They accelerated cell senescence resulting in chlorosis and necrosis. Typical toxicity symptoms of Zn included leaf browning. UPASI-9 cultivars, under toxicity of 2000 mg kg⁻¹ died within 15 d, while 1000 mg kg⁻¹ took the toll by 36 d. Zn toxicity antagonized P, Mg and Fe absorption. In Zn-scarce tea, youngest leaves turned narrow, strap-like, erect and formed rosette. Leaf blades became yellow and severely affected leaves became stunted with inward-curling margins; apical growth and branch extension retarded. Chloroplast ultrastructures transformed under Zn excess and chloroplast membrane ruptured, thylakoid collapsed, adversely influencing photosynthesis. Similarly, mitochondria swelled and cristae disintegrated implying the failure of antioxidants to scavenge surplus ROS.

Aluminum (Al)

Al phytotoxicity is well-known on acidic soils in temperate and tropical regions. However, tea plants are Al hyper-accumulator and accumulate huge quantity in mature leaves which is toxic for other crops. Al uptake by roots reduced the concentration of Ca, Mg and P. Al led to Mg deficiency in Norway and red spruce, Ca deficiency in loblolly pine and P deficiency in American and European beech.

In tea, Al at toxic concentration induced oxidative damage and Al contents increased in mature leaves. Pigments, P_N , E and G_S decreased but activated oxygen metabolism was evidenced by increasing MDA, O_2^- , H_2O_2 and membrane injury. Phenol, total sugar and starch decreased under toxicity but APX, GR, POD, CAT and SOD activities increased with elevated Al. Citrate and malate production also increased to minimize toxicity. Besides, increased callose formation was a frequent response under toxicity. TEM analysis of Al treated leaves, revealed significant membrane damage at 0.53 mM, due to loss of equilibrium between ROS formation and detoxification. Capacity of Al accumulation depends on genotype because two Chinese cultivars, Pingyangtezao and Wuniuzao, performed differently

as indicated by differential accumulation of chl and defensive enzyme activities. Al and F, interaction had noteworthy effects on physiochemical parameters of tea because, Al alone enhanced root systems and SOD activity, but electrolyte leakage, POD vis-a-vis CAT activities declined; whereas, F increased enzyme activity but decreased permeability. Proline, POD, CAT and SOD activities remained lower under high Al: F, but when F increased, enzymes escalated, signifying injury. Higher Al concentration drastically inhibited root elongation in *Picea abies* seedlings.

Al contents generally increased from young to mature leaf and the 6th mature leaf divulged that Al progressively increased from the centre towards the periphery. Earlier studies revealed that, Al was detoxified by exclusion mechanism forming Al-ligand complexes and oxalate was a key chelating compound in tea roots and oxalate exudation along with small quantity of malate, citrate and oxalate metabolisms were coupled to Al accumulation in roots. Poly-L-aspartate and poly-L-glutamate protected growth of pollen tube, in tea plants, from toxicity of Al by either interfering with Ca²⁺ transport or binding to calmodulin. Cell suspension cultures of *Picea rubens*, to Al toxicity resulted in a loss of cell viability, inhibition of growth and a significant decrease in mitochondrial activity along with increased soluble protein. Al reduced water permeability of red oak root cortex, and diffusion resistance of *Picea* and *Abies*. Reduction of stomatal aperture was observed in many species because Al altered membrane permeability, stomatal aperture and root surface area. Al-induced shoot damage in peach and coffee. In coffee shoots, toxicity symptoms included chlorosis and necrosis of younger leaves and spotty chlorosis of older leaves.

Chromium (Cr)

Cr is a widespread natural contaminant and its accumulation in soil creates a disparaging effect on tea plants. It not merely affects production and quality, in addition jeopardizes human health through food chain. Cr toxicity decreased root and shoot length and protein, chl, car, proline and nitrogenase in *Pongamia glabra*, *Cassia auriculata*, *Pithecellobium dulce*, *Azadirachta indica* and *Tamarindus indicus* and induced metabolic modifications, alterations in photosynthesis in *Genipa americana*. Cr diminished chl content, P_N , G_S and E along with the number of new buds, and weight of tea. Increasing Cr toxicity lowered photosynthesis indicators, along with altered antioxidation and modified resistance. Activities of defensive enzymes like, SOD, POD and CAT diminished, while proline, MDA and conductivity increased in *C. sinensis*. Probably Cr increased free radical generation, as indicated by increased MDA production. The content of MDA, a peroxidation product of membrane lipids, is often used as an indicator of oxidative damage. Thus, pigment and protein destruction indicate inefficacy of antioxidants to scavenge excessive ROS generated because of Cr toxicity.

Lead (Pb)

Pb is a well-known pollutant having the ability to function as a stressor. Pb substantially affected seed germination, seedling growth and DW in *Thespesia populnea*. In Pb contaminated soil, phytotoxic symptoms appeared in tea plants along with reduction in biomass, caffeine and free amino acid but catechin

content increased and the roots accumulated higher quantity of Pb. Pb toxicity impaired photosynthesis and decreased chl, P_N , G_S and E significantly. Nevertheless, tolerance to Pb toxicity depends upon the genotypes of tea. Longjing showed an enhancement in P_N when both Longjing and Zhenong were subjected to Pb toxicity. POD and SOD activities increased under toxicity in Zhenong but in longjing, POD activity boosted up initially while SOD activity increased gradually. Besides, Pb inhibited seed germination of *Pinus helipensis*. Pb toxicity increased the growth of Longjing (a tea variety), but reduced in Zhenong. Conversely, at a lower level, Pb decelerated the net P_N of Zhenong, but enhanced that of Longjing. In Zhenong, POD and SOD activities increased with increasing Pb whereas in Longjing POD activity of increased initially and then decreased.

Fluorine (F)

F is phytotoxic as it can alter a series of metabolic pathways. *Amygdalis communis* seedlings, under increasing F, decreased chl, Ca, Mg, and starch and sugar content of leaves. Mineral concentration, mainly Mn, showed a major decrease at 2.5 mM F. Tea plants accumulated large quantity of F innately, but under increasing Ca, the uptake was affected possibly due to the effect of Ca on the properties of cell wall or membrane permeability or altered F speciation. Under high F stress (>0.32 mM), chloroplast membranes ruptured, thylakoid expanded and degenerated and reduced P_N . F also mitigated the toxic effect of Al and stimulated pollen tube growth in tea. In conifers, F and SO_2 initially induced chlorosis on needle apices and brachyblasts that became greyish-brown due to the death of cells and gradually fell down. This defoliation process ensued rapidly in the presence of HF and gas mixtures that contained HF. Small leafed Chinese tea variety accrued very high F compared to their large leafed equivalent while the Assam variety contained the lowest F. Toxic F concentrations decreased FW, DW, chl and P_N whereas CAT and guaiacol peroxidase (GPX) activities increased considerably along with H_2O_2 , MDA and proline. Increased H_2O_2 in the F toxic leaves probably triggered Fenton/Haber-Weiss reaction associated with generation of $OH\cdot$ radical and lipid peroxidation. However, increased activities of antioxidative enzymes minimized stress to an extent beyond which growth retarded.

Mercury (Hg)

Hg pollution has become a vital concern due to its environmental impact on a universal scale. Germination of seeds of *Platanus occidentalis*, *Pinus echinata*, and *Pinus taeda* under mercuric nitrate and methyl mercury chloride solutions were decreased, with *P. echinata* being the least sensitive. Germination was inhibited more when seeds were exposed to methyl mercury chloride than the other. Organic species of mercury was found to be more toxic than inorganic counterparts. Hg can also induce oxidative stress in tea. Upon exposure, Hg decreased phenol contents, PPO and chl along with Hill reaction, but, on the contrary, PAL activity and proline contents increased under Hg toxicity. Tea plants exposed to high Hg reduced P_N , growth and led to root browning as well as leaf chlorosis. Thus, it may be recommended that antioxidative defence system did not adequately protect the tissues under severe Hg stress.

Copper (Cu)

Cu is an essential element for all forms of life, acting as the prosthetic group of many enzymes. However, Cu toxicity inhibited root elongation of both *P. pinea* and *P. pinaster* seedlings within 3 d of exposure. Root cortex thickened significantly and lignin synthesis increased in *P. pinaster*, and decreased cell elongation caused by increased permeability and cell-wall lignification was the main mechanisms of Cu toxicity in *Pinus*. Cu ions, in tea plants, oxidized thiol bonds causing disruption in protein structure and functions. Cu stress increased phenol, proline and PAL activity in tea but PPO and chl reduced whereas glutamine synthase activity decreased. Chinary cultivars were more tolerant than Assamica, because the later accumulated more ROS, showed higher activity of phytochelatin synthase. Excessive soil Cu hindered the extension of young branches of apple, decreased the content of active Fe and chl significantly, and declined the activity of CAT in young leaves.

Selenium (Se)

Although Se is a trace element, but toxic if present in excess. *Prunus* species showed reduced growth with incremental Se treatments. Se induced a partial stomatal closure, as evidenced by G_S , resulting in a reduction in net assimilation, and thus a decrease in dry-mass production. In green tea, vitamin C content remained lower and during storage decline was much higher in Se deficient plants. The color of green tea extract in Se-enriched green tea was more stable compared to the counterpart during the storage period. The sweetness and aroma of Se-enriched green tea were also significantly higher. It also indicated that Se may play positive role in increasing the quality parameters.

Nickel (Ni)

Ni is an essential nutrient deficiency of which culminated in mouse ear disease in pecan and biochemically disrupted the ureide pathway, the urea cycle, the citric acid cycle, and the shikimic acid pathway. *Pinus sylvestris*, upon exposure to Ni, augmented higher sucrose accumulation, signifying interruptions in carbohydrate metabolism. Trees exposed to Ni had higher content of condensed tannins compared with controls. Ni executed the role of a stressor in tea plants also, because it decreased phenols, PPO and chl content along with Hill activity but proline contents and PAL activity were increased in tea. PAL had widely been studied concerning its induction by various environmental factors such as light, wounding excision and infection that may be considered as stress generators. In plants, PAL is an important intermediate in the metabolic pathway to phenylpropanoid metabolism and thus performs defense-related functions. In presence of excess Ni the activity of PAL as well as other stress markers increased indicating the potent role of Ni in inducing stress.

Arsenic (As) and cadmium (Cd)

As concentrations elevated in soils due to geochemistry or human activities. As toxicity-decreased chl in *Pistacia lentiscus* and *Tamarix gallica*, but MDA increased considerably. Enhanced As^{++} decreased germination and twig growth rigorously along with instantaneous reduction in chl, P_N , G_S and

E. In As and Cd polluted soil, feeder roots played the buffering role and minimum quantity of As was transported to the shoots. Cd persuaded growth, photosynthesis and chl synthesis when present in a tolerable quantity but surplus quantity impaired the plants but *P. tremula* exhibited growth inhibition and changed gene expressions and a decreased level of proteins. Similarly, Cd exposure decreased P_N , E and biomass, chl and protein in tea, but enhanced MDA, expression of γ - glutamylcysteine synthetase, glutathione synthetase, and GR, which implied the defensive activity of these enzymes under excess Cd. In spruce needles, the soluble fractions of GPX initially increased and decreased subsequently. Increased cell wall bound POD activity was marked under extended treatment. Effect of Cd on tea indicated that, chl remained higher in spring than summer and reduced with an increment in Cd, which enhanced MDA and soluble sugar in spring tea indicating the role of Cd as a potent stressor. Cd concentration changed phenol metabolism in callus culture depending on the origin of the tissues. In leaf and stem originated callus, it decreased biomass and flavans but uniqueness remained unaffected in calli derived from roots. Simultaneously lignin content in the calli of root and stems increased, while remained unaltered in the leaf. Appreciable inhibition of the nitrate reductase activity was found in *Silene cucubalus*. Growth, APX and CAT activities inhibited along with H_2O_2 accumulation in response to Cd in poplar roots. A significant reduction in plant height, biomass, stem diameter, leaf area and weight of leaves in hybrid poplar was observed under Cd toxicity. CAT activity and proline accumulation decreased significantly, but SOD activity reduced in roots and increased in the leaves under toxicity. A gradual decrease in growth of *Dalbergia sissoo* with increasing Cd levels was reported.

Manganese (Mn)

Mn is an essential micronutrient required for the normal growth of higher plants and may become toxic when present in excess. Mn deficiency depressed leaf photosynthetic capacity in plants of *Carya illinoensis*, primarily by reducing the number of PSII units per unit area of leaf whereas *Populus cathayana*, under humid condition accumulated more Mn, decreasing growth, chl and activities of antioxidant enzymes than the dry climate population. In Citrus, Mn-excess decreased CO_2 assimilation, G_S , increased intercellular CO_2 , whereas Rubisco activity decreased. Chl a fluorescence (O-J-I-P; chl fluorescence kinetics) transients from Mn-excess leaves showed increased O- and decreased P-steps, accompanied by positive L- and Kbands, indicating Mn-excess leaves had increased damage of oxygen evolving complex and less energy exchange between independent PSII units. Mn-excess decreased light energy transformation efficiency (F_v/F_m) but increased relative variable fluorescence at I-steps (VI) and energy dissipation. These leaves displayed higher MDAR, GR, SOD, CAT and guaiacol peroxidase (GPX) activities and contents of antioxidants. Thus, it indicated that Mn excess leaves were damaged by oxidative stress, which was possibly due to the overreduction of the photosystem as a result of slower dark reactions resulting from decreased Rubisco and other enzymes. Hence, it can be suggested that the defensive system of plant regulated the changes of enzyme activities to facilitate the defensive function against toxic Mn.

Humidity and Drought

Moisture stress is a major limitation for plant growth, and to counteract, several physiological and antioxidative mechanisms have evolved. Tea plants under drought or inundation declined water potential (ψ) and decelerated growth. Inherently, even tea seeds are desiccation sensitive. Activities of PPO and POD decreased along with P_N and metabolism, but conductivity increased. Tender leaves had high diffusion resistance whereas drought tolerant clones showed increased relative water content but clones that minimized water loss, endured drought. Soil moisture deficiency severely affected PS-II reaction centre but P_N increased in irrigated tea compared to drought-affected. Similarly, maximal fluorescence (F_m), variable fluorescence (F_v), F_v/F_m and activity potential of PS-II (F_v/F_o) decreased leading to non-photochemical quenching. Moisture deficiency reduced shoot: root ratio, leaf area, stem diameter, net CO_2 assimilation and rearranged dry matter partitioning to roots. Soil water content influenced G_S that affected E and played a key role in shaping water status of leaves. Decreased G_S in response to increased irradiance, leaf temperature (T_L) and air vapor pressure deficit played a key role in photoinhibition of tea. Similarly, in *Corylus avellana* L., G_S progressively decreased with increased long-term water deficiency. At T_L , of 20-30 °C in tea, P_N decreased at a rate of $0.053 \mu\text{mol m}^{-2}\text{s}^{-1} \text{ } ^\circ\text{C}^{-1}$. However, under water deficiency, optimum T_L remained around 20-24 °C while P_N reduced significantly. Total chl content decreased under dehydration. High humidity generated maximum P_N whereas low temperature along with low soil moisture eased P_N . Under inundation, photosynthetic photon flux density (PPFD) decreased prominently. Under drought, the amount of dry matters partitioned to leaf, stem and harvested shoots decreased by 80-95%. Drought increased conductivity in tea. Solute leakage is the indicator of stresses such as metal toxicity; drought etc., that gives rise to increased ROS, which in turn, increases membrane permeability and culminates in electrolyte leakage.

Water deficiency led to increase in car, ABA, GSH, and GPX activities but reduced proline, H_2O_2 and O_2^- , however subsequent rehydration led to recovery. A positive correlation documented that the activity of Rubisco, POD and PPO enzymes served as markers to monitor drought tolerance. Tea cultivars (TV-18, -26, T-78 and HV-39), under water stress, enhanced phenol and proline along with increased phenylalanine ammonia lyase (PAL), but prolonged drought exhibited reverse trend. Drought augmented accumulation of proteins but protracted stress, diminished them in T-78 and HV39. Drought stress followed by rehydration treatments, reduced ASA and GSH in TV-1, 20, 29 and 30, and among them, TV-1 had the highest activities of GR and CAT indicating the severe effect of drought. China cultivars, namely; Zhengong, Fudingdabai, Yunqi and Zisun under drought, increased proline. P_N and E was the highest in Zhengong, and Zisun was the most affected with lowest P_N and E. Tea seeds under desiccation increased proline, H_2O_2 content, APX and SOD activities indicating that the level of osmoprotectants increased. Before the appearance of visual symptoms of drought, dehydrin, ABA ripening protein, calmodulin-binding protein, GPX and cinnamoyl CoA reductase increased in tea leaves. Similarly, under desiccation APX and SOD activities increased in tea seeds. Increased activity of CAT, ASA, and

other antioxidants, scavenged ROS that altered redox status to become deleterious. CaCl_2 application thwarted drought induced oxidative damage by increasing DW, proline, phenol, activity of SOD, POD, CAT and GR enzymes and plummeted H_2O_2 and MDA. Accumulation of K, Ca, Mn and B accelerated during drought recovery and they positively modulated enzymes like, SOD, CAT, POX, PPO and GR that scavenge ROS suggesting that drought caused oxidative damages to plants.

Moisture deficiency reduced PAL activity in UPASI-2, -8 and 9 clones and reduced the synthesis of EGCG and ECG due to molecular rearrangement with elevated leaf temperature. Similarly, flooding deteriorated tea quality, due to reduction of gallic acid and caffeine contents leading to reduced synthesis of Phthalamic acid, Phthalamic acid-3'-gallate and theaflavic acid. Drought tolerant clones had been found to translocate more photosynthates to shoots under moisture stress. Downpour for longer period led to lowering of EGC, epicatechin, ECG, EGCG but increased catechins. However, relative humidity significantly deteriorated EGC, TC and caffeine. Thus, it seems that flooding as well as drought influences the quality of made tea because the prime components for the supremacy of tea i.e., the quality parameters were declined.

Continuous evapo-transpiration eventually became fatal. Hence, application of antitranspirants minimized the effect and 'film type' antitranspirants were more competent than 'stomatal type'. Phenyl mercuric acetate (PMA) is useful due to its inefficient translocation and short life span. Alongside ABA, commercial antitranspirants such as Raliidhan and Antistress are effective due to increased stomatal diffusion resistance, ψ , relative turgidity, and reduced E. A linear relationship between total DW and the ratio between E and mean saturation vapor pressure deficit in clonal tea plants manifested marked significance.

Leaf necrosis and defoliation in *Eucalyptus pilularis* under severe drought stress indicated the effect of drought. In apple, emissions of α -pinene, β -pinene and limonene were negatively correlated with rainfall which supported that drought resulted in higher formation of secondary metabolites.

Temperature

Temperature is an important abiotic stress, which below 13°C adversely affect various biosynthetic activities of tea. Low temperature is one of the key stresses that tea bushes necessitate to cope with. They are also susceptible to frost damage that culminates in scorching of young pluckable shoots and defoliations. During winter, low temperatures alone or in combination with low irradiation induced oxidative stress in plants. It had been found that genotypes having shorter dormancy period displayed elevated activities of defensive enzymes due to accrual of ROS. Efficient scavenging of toxic oxygen species led to lower accumulations during winter months coupled with reduced winter dormancy. Photosynthesis can be a potential marker to understand the effects of low temperature stress on plants. Low temperature tolerant tea genotypes were found to possess higher MDA, proline and car content along with enhanced ROS scavenging machinery than the susceptible clones. Therefore Netto recommended that clones, for instance; TTL-1, TTL-4 and UPASI-9 being low temperature and frost tolerant were more suitable compared to

clones like, TTL-6, SM/ OM/54 and SMP1 for a region having recurrent low temperature situations because plantation of these clones will ensure the production of augmented yield and quality. Low temperature induced albino tea cultivar, reversed the albino phenomenon when the temperature was above 20°C , resulting sharp decline in amino acids content. In albino tea, the development of chl from etioplast and the accumulation of chl a and b continued to be the same at 15°C ; thus culminating in albino shoots. Experimental evidences specified that chl content reduced with decrease in temperature and leaves were in need of mature chloroplasts at 15°C ; rather, etioplasts developed. Cold tolerance of grafted and non-grafted tea plants were correlated to P_N *in vivo*. Exposure of tea buds to proline and betaine decreased cold stress, enhanced methylglyoxal and MDA, and increased GR and glutathione-S-transferase activity. Tea growing under the shade of *Grevillea robusta* had substantially lower transpiration rates than unshaded tea because shades reduced E by minimizing irradiance that strike the canopy and also by reducing canopy temperature. P_N and G_S of *Quercus alba* decreased between 35 and 40°C , whereas in *Q. rubra* the parameters increased over this range, thus indicating vulnerability of white oak to temperature. Therefore, temperature stresses can be detrimental for growth and development of tea and other woody plants. Nevertheless, tea plants have evolved complex mechanisms to cope with such unfavorable conditions and thus increased levels of ROS that trigger changes in plants are scavenged by antioxidants up to a certain extent.

Ultraviolet-B (UV- B) Irradiation

UV- B is harmful to plants as it reduced expression and synthesis of chl a/b-binding protein and the D 1 polypeptide of PSII and inhibited growth. Short-term irradiation of UV- B stimulated accumulation of total catechins. Conversely, excessive UV- B irradiation suppressed catechin accumulation. EGCG increased more rapidly than other catechins under irradiation. UV- B impeded size of callus forming cells and promoted deposition of phenolic compounds on cell-wall and intercellular spaces. UV- B induced chl-bearing cell formation and PS-II activity stimulated in the phototrophic cells of callus culture. P_N , E, and T_L of tea leaves were affected by photosynthetic photon flux densities (PPFD) and experimental evidences revealed that PPFD of $2,200 \mu\text{mol m}^{-2} \text{s}^{-1}$ affected P_N but E and T_L of maintenance leaves linearly increased from $200,200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD. B deficient tea plants mitigated the reduction of photosynthetic energy conversion under intermediate and highly illuminated circumstances. Under intermediate light intensity, CO_2 assimilation was the highest and produced lower activity of APX, SOD and proline content. Environmental factors acts upon the contents of tea plants. Darkness inhibited synthesis of flavanols, declined galocatechin and leucoanthocyanin contents. The leucoanthocyanins content of darkened stems decreased much less than the leaves. Leaves appreciably gained phenyl propanoids, benzenoids and phenylalanine under shade, whereas upstream metabolites like, shikimic acid, prephenic acid, and phenylpyruvic acid decreased.

Impact of Various Stresses on Wild Relatives of Tea

Camellia is the largest genus of the family Theaceae that includes more than 325 species. In a conservative estimation, there are more than 3,000 cultivated varieties of ornamental Camellia worldwide of which more than 2,500 have been registered in the American Camellia Society. Camellia sp. has significantly different growth habits in different distribution areas due to diverse climatic conditions.

The relationship between the processing temperature and the cell injury rate showed that *C. oleifera* appeared as the best suitable species for high-temperature zones whereas *C. vietnamensis* suited for temperate regions. High temperature increased soluble protein, sugar and free proline in *C. oleifera* leaves. Conversely, under prolonged chilling, the leaves of *C. rusticana* showed no visible changes while *C. japonica* proliferated after 200 d. The decline in the rate of soluble carbohydrate content in *C. rusticana* remained about one-sixth of that in *C. japonica*. The P_N of *C. japonica* decreased to half of its initial value after 140 d, while that of *C. rusticana* remained same even after roughly one year.

Light intensity, radiation etc also affected physiological and biochemical characteristics of wild relatives of Camellia. *C. nitidissima* changed both qualitative and quantitative characters under changed light environment. Relative light intensity that was lesser than 20%; correlated leaf length and width, but beyond more than 20%, insignificant correlations occurred. Low light intensity induced development of shortnarrow leaves, while high light intensity stimulated longer and wider leaves. Under shade, the stomata of *C. rusticana* closed constantly, however japonica, remained open even after 90 d. γ radiation, increased SOD and POD activity in 'Ruanzhi 2' and 'Ruanzhi 3' upto 1000 rad but decreased under 3000 rad whereas 'Ruanzhi 11' and 'Yue 15' yielded the lowest under 3000 rad. Type of planting and density of plants also influenced P_N because low planting yielded higher P_N than higher planting population.

In *C. oleifera*, Al, under acidic conditions increased chl a, b, G_S and P_N but decreased when Al concentration increased. Simultaneously P_N also affected biomass accumulation. *C. oleifera* required suitable concentrations of Al and P for optimal growth but under Al toxicity and P inadequacy altered growth and photosynthesis rate. Lime in combination with P led to increase in P_N , G_S and decreased intercellular CO_2 concentration. Simultaneously car/chl changed significantly, suggesting role of car in photo-protection. Another study revealed that P deficiency led to higher activity of acid phosphatase in some varieties. In fact, leaf age and leaf position had been reported to be two important factors, which influenced P_N .

Leaf blight disease of *C. oleifera* is one of the major diseases, which causes premature defoliation. It had been found that *Bacillus subtilis* was able to control up to 84.7% of the diseases. *C. sasanqua* pollen grain produced shorter pollen tubes due to growth inhibitory substances but when soaked in acetone or diethyl ether produced longer pollen tubes suggesting that acetone or diethyl ether probably played as growth stimulant. Simulated acid rain produced inhibition of seed germination, increased MDA, but reduced POD, SOD, chl a, chl b, and their ratio (www. mt.china-papers.com; 2010). Spraying paclobutrazol on the leaves of *C. oleifera* seedling inhibited

shoot growth but root growth was promoted, and chl content, P_N and protein enhanced.

Dehydration also affected pivotal antioxidants because dehydrated seedlings increased the activity of PPO, POD and CAT activity along with MDA and relative conductivity. Under 0% -30% dehydration resistance value of leaf, decreased while the values of root and stem showed the same trend under 0% -25% of dehydration.

Conclusion and Future Challenges

Tea plants, being woody perennial, experience quite a lot of abiotic stresses. Although, conventional breeding is successful for varietal improvement of tea, yet, they are focused on to the improvement of yield, quality, drought and few diseases tolerance. While significant amount of achievement have been made in improving yield and quality parameters of tea, other attempts have marginally improved the objective. This is primarily due to the fact that most of the stresses are complex in nature, controlled by several genetic elements. Thus, prior to any attempt to progress further; it is essential to understand the physiological as well biochemical basis of such stresses, which later formulate the groundwork for studying the molecular basis. In tea, several studies have been accomplished which gives an apparent idea about the parameters involved. Tea is a hyper-accumulator and accumulates Al in such an extent that is toxic to other plants. Thus identification of the molecule behind such tolerance will facilitate to understand the mechanism which in turn will assist to develop the Al tolerant plant in other species. In general, detection of the key biochemical molecules associated with physiological parameters would aid nursery selection for agronomically important traits and will assist molecular breeding ultimately. Approaches like association mapping can only be successful if morphological, physiological as well as biochemical scoring are successful. In tea where conventional breeding is limited, association mapping shows tremendous scope for varietal improvement for complex traits.

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