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SUMMARY OF DOCTORAL THESIS

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Title: Phytochemical Accumulation in Coffee (*Coffea arabica* L.) Plants as a Photoprotective Mechanism during Oxidative Stress Conditions

（酸化ストレス条件下における光保護メカニズムとしてのコーヒー(*Coffea arabica* L.)へのファイトケミカルの蓄積）

This study aimed at profiling and reinforcing the nonenzymatic antioxidant system in young coffee plants through exogenous applications of several elicitor compounds to enhance coffee cultivation under oxidative stress conditions.

In chapter two, the phytochemical composition of coffee plant organs and their corresponding antioxidant capacities compared to green and roasted coffee beans was investigated. HPLC analysis indicated that the compounds were present in all organs, except mangiferin which was absent in roots, stems and seeds and caffeine which was absent in stems and roots. Total phytochemicals were highest in the green beans (GB) at 9.70 mg gDW^{-1}, while roasting caused a 66% decline in the roasted beans (RB). Amongst the leaves, the youngest (L1) contained the highest content at 8.23 mg g DW^{-1}, which gradually reduced with leaf age to 5.57 mg gDW^{-1} in the oldest (L6). Leaves also contained the highest TPC (over 60 mg GAE gDW^{-1}) and exhibited high antioxidant capacity expressed as trolox equivalent antioxidant capacity (TEAC), the latter being highest in L1 at 328.0, 345.7 and 1097.4 and least in L6 at 304.6, 294.5 and 755.1 µmol Trolox gDW^{-1} for DPPH, ABTS and FRAP assays, respectively. Phytochemical accumulation, TPC and TEAC was least in woody stem (WS) at 1.42 mg gDW^{-1}, 8.7 mg GAE gDW^{-1}, 21.9, 24.9 and 110.0 µmol Trolox gDW^{-1} while herbaceous stem (HS) contained up to 4.37 mg g DW^{-1}, 27.8 mg GAE gDW^{-1}, 110.9, 124.8 and 469.7 µmol Trolox gDW^{-1}, respectively. Roots contained up to 1.85 mg g DW^{-1}, 15.8 mg GAE gDW^{-1} and TEAC of 36.8, 41.5 and 156.7 µmol Trolox gDW^{-1}. Amongst the organs therefore, coffee leaves possessed higher values than roasted beans on the basis of phytochemicals, TPC and TEAC.

In chapter three, the response of the leaf phytochemicals in responses to changes in the ambient environmental conditions during; (i) 24hr cycle; (ii) summer to winter seasons and (iii) in response to high light intensity was investigated. Diel fluctuations in the environmental conditions caused no significant changes in the contents of pigments and antioxidant compounds. Nevertheless, daylight conditions tended to elicit higher concentrations of carotenoids causing a significantly least ratio of chlorophylls to carotenoid by evening. This was accompanied by higher concentrations of non–enzymatic compounds such as 5–caffeoylquinic acid (5–CQA) especially at midday. On the other hand, both chlorophyll and carotenoid content were significantly highest during summer season. This was followed by a remarkable reduction in their content with reduction in the ambient temperatures from the onset of winter at the beginning of November. This resulted into a reduction in the ratio of chlorophylls to carotenoid. The content of the antioxidant compounds and the ratio of 5–CQA/caffeine were generally least during autumn conditions while both summer and winter conditions elicited higher amounts of these protective compounds. Similarly, exposure of previously low light acclimated coffee plants to high sunlight intensity caused remarkable reductions in both chlorophylls. Extreme sunlight conditions elicited higher concentration of carotenoid pigments resulting into lower chlorophyll to carotenoid ratio. In addition, direct sunlight conditions caused rapid accumulation of antioxidant compounds especially 5–CQA and mangiferin while caffeine and trigonelline were not

significantly affected. This resulted into a higher ratio of 5–CQA/caffeine and total HPLC metabolite content in sunlit plants compared to the shaded counterparts. The results presented in the current study demonstrate the protective role of antioxidant compounds especially 5–CQA, mangiferin and carotenoids against reactive oxygen species that reduce chlorophyll efficiency during abiotic stress conditions.

In chapter four, due to cold–stressed leaves possessing an elevated risk of photodamage because of an inefficient photosynthetic apparatus during winter conditions, recovery of cold–stressed coffee seedlings treated with different concentrations of nitrogen applied as foliar urea sprays; Control (0), 5, 10, 20 and 40 mM, was investigated under optimum growth chamber conditions (25/20°C) for 3 months. Concentrations of nitrogen and photosynthetic pigments in the leaves increased with increasing concentration of the foliar sprays up to 20mM. This was accompanied with a recovery of the photosynthetic apparatus and increased net carbon assimilation rate. In addition, 10 mM and 20 mM treated plants also had the highest photosystem II maximum efficiency compared to their lower or higher nitrogen concentration treated counterparts. Conversely, these plants contained lower concentrations of 5–caffeoylquinic acid, mangiferin, trigonelline and caffeine than control plants. It was concluded therefore, that foliar sprays of appropriate nitrogen concentration were adequate for recuperating the photosynthetic apparatus and improved the photosynthetic performance of the cold–stressed coffee seedlings.

In chapter five, evaluation of several elicitor compounds on reinforcement of the nonenzymatic antioxidant system of coffee plants with concomitant effects on the photosynthetic physiology during cold stress conditions was evaluated. Exogenous foliar application of kinetin, salicylic acid, melatonin and TiO_2 NPs improved the antioxidative capacity of the coffee plants by upregulating the metabolism of the nonenzymatic antioxidant compounds. This was associated with increased ROS scavenging capacity. Exogenous application of the substances also increased the photochemical and mesophyll efficiency for CO_2 fixation in addition to maintaining higher gas exchanges parameters under cold stress conditions. Therefore, on the basis of the results presented in this chapter, it is suggested that exogenous application of elicitor compounds has a potential to modulate the growth of the coffee plants under cold stress conditions.

In chapter six, by combining the results of chapter 4 and 5, the current chapter evaluated the effects of nitrogen and melatonin on improvement of cold tolerance of coffee plants. The results indicated that nitrogen improved the photosynthetic physiology of the coffee plants by enhancing both the net and gross maximum CO_2 assimilation rates. This was associated with a higher nitrogen partitioning for both the metabolism of photosynthetic pigments, RuBisCO enzymes and other nitrogenous compounds such as trigonelline. Moreover, nitrogen treated plants exhibited a high capacity for RuBP carboxylation and regeneration under optimum conditions. Nevertheless, cold stress conditions caused strong reductions in the photosynthetic physiology of coffee plants which caused a tremendous damage to the cell membranes. This damage was however less prominent in melatonin treated plants which also had high contents of phenolic nonenzymatic antioxidant compounds such as anthocyanins, flavonoids, chlorogenic acids, mangiferin resulting into a higher ROS scavenging capacity in coffee plants. Therefore, the combined effects of nitrogen and melatonin indicate a possible remedy for mitigating cold–induced decline in the photosynthetic physiology of coffee plants.

In chapter seven, the mechanism through which the nonenzymatic antioxidant system is modulated during cold stress conditions by either oxidative stress or RBOH induced ROS in coffee plants was evaluated. The results indicated that excessive production of ROS instigated by cold stress conditions caused membrane damaged and suppressed the metabolism of nitrogen. However, accumulation of ROS initiated by both cold stress and RBOH also triggered nonenzymatic antioxidant protective mechanism which led to increase in the levels of especially ascorbic acid and 5–caffeoylquinic acid which restored ROS to homeostatic levels and thereby preventing further oxidation of the cellular membrane.

The results of the current study therefore revealed that phytochemicals that accumulate in coffee plants play an important role in protection of the photosynthetic apparatus during oxidative stress conditions. Thus, for successful cultivation of coffee plnats during abiotic stress conditions, the combined effects of nitrogen and elicitor compounds such as melatonin indicate a possible remedy for mitigating cold–induced decline in the photosynthetic physiology of coffee plants with the former improving the efficiency of the photosynthetic apparatus while the latter reinforcing the nonenzymatic antioxidant system of the coffee plants.