

THE SIGNIFICANCE OF CHLOROPHYLLS AND CAROTENOIDS IN ENHANCING SEED TOLERANCE TO ABIOTIC STRESS

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Abstract: Chlorophylls and carotenoids play critical roles in seed physiology, particularly in seed tolerance to abiotic stresses. Despite the degradation of chlorophylls during seed ripening, residual levels are detectable in mature seeds, affecting their stress tolerance. Carotenoids, predominantly lutein and β -carotene, accumulate in seeds under accelerated aging conditions and high-temperature germination, influencing stress response. The ratio of carotenoid to chlorophyll content (Car/Chl) emerges as a potential indicator of seed stress tolerance, with higher ratios correlating with enhanced resilience to stressors. **Objective:** This study aims to elucidate the significance of chlorophylls and carotenoids in enhancing seed tolerance to abiotic stressors by investigating their roles and interactions within seeds under stress conditions. **Methods:** Physiologically mature seeds containing residual chlorophylls were subjected to stress treatments, and carotenoid levels were assessed during seed aging and germination experiments. The Car/Chl ratio was calculated to evaluate its relationship with seed stress tolerance. **Results:** Seeds with elevated Car/Chl ratios exhibited higher tolerance to stress treatments, suggesting a protective role of carotenoids against oxidative stress induced by chlorophylls under abiotic stress conditions. Carotenoid accumulation during aging and germination further underscored their role in stress response, influencing seed resilience. **Conclusion:** although residual in mature seeds, chlorophylls contribute to oxidative stress under abiotic stressors. Conversely, carotenoids act as antioxidants, mitigating stress-induced damage and enhancing seed tolerance. The Car/Chl ratio is valuable for assessing seed stress resilience, providing insights into seed physiology under adverse environmental conditions.

Keywords: Chlorophylls, carotenoids, seed tolerance, abiotic stress, oxidative stress, Car/Chl ratio

Introduction

All seeds, irrespective of the conditions they are stored under, undergo time-dependent aging, a general deterioration in seed quality, and a decrease in viability with time (1). Relative humidity and temperature enhance the rate at which the seeds deteriorate and decrease the possible storage in years (2). On the other hand, greater humidity and temperature result in the more speedy development of injuries and speed up seed ageing. This concept is applied in the accelerated seed aging method that entails the exposure of seeds to high temperatures and humidity for days (3). A recent study shows that seeds that can remain resistant to the effects of accelerated aging are usually more capable of withstanding the abiotic factors of stress during the period of germination. Seeds' aging, and consequently, the decrease in stress tolerance, the main factor is stress-induced damage, resulting from free radical production through lipid peroxidation and non-enzymatic glycosylation (4). These processes can occur even at shallow seed moisture content; the reaction products end up in air-dried seeds, leading to oxidative destruction of membranes, proteins and DNA. It can be assumed that because specific combustion products remain inside the seeds, the

appearance of reactive oxygen species (ROS) leads to premature aging of the seeds (5). The defence of seeds from the attack of ROS is mainly covered by antioxidants such as tocopherols, ascorbate and glutathione (6, 7). Carotenoids may also have a similar protective function since it is well documented that they shield leaves against photo-oxidative injury (8, 9) and ripening fruits against OTA (10). The present work aimed to determine the part played by chlorophyll and carotenoids in improving seed tolerance to abiotic stress factors.

Methodology

The kinds of seeds utilised in this study involved tomato (*Solanum lycopersicum*), sunflower (*Helianthus annuus*), spinach (*Spinacia oleracea*), pea (*Pisum sativum*), peanut (*Arachis hypogaea*) and lentil (*Lens culinaris*). This was done after putting the seeds in a rotary shaker; % absorbance at 650 nm and chlorophyll fluorescence at 730 nm were determined. Seeds from the spring rape variety were manually sorted into three groups based on seed coat colour: black, brown and greenish-brown. Tomato, spinach and pea seeds were allowed to be incubated for one week in a

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desiccator at a temperature of 22°C and 86%RH, put in an aluminium coated paper and placed in a thermostat at 40°C for another six days. An imbibition experiment was carried out with sunflower, peanut and lentil seeds, which were seeded on the filter paper and soaked in distilled water for 12 hours rpm at 220C in complete darkness. Seeds that imbibed were placed at 50°C for 4 or 8 hours while mock imbibed seeds were incubated at 22°C. After germination was conducted at the temperature of 22°C for ten days, with counts taken on the tenth day, the number of regular, abnormal and unswollen seeds was recorded. Plant tissue samples (500 mg) were homogenised in 2 µl of extraction solvent, a mixture of petroleum ether and tetrahydrofuran in a 4 1 1-volume ratio. The resulting homogenate was then filtered by a Kaipron 100 GDP RO membrane filter, and the absorption spectra of the filtrates were analysed with an ELISA reader from Elx, Japan. Pigments were extracted in a solvent containing methanol, ethyl acetate and acetonitrile in a ratio of 1:3:3. To perform chromatographic analysis, a

liquid chromatograph with a column featuring an internal diameter measuring at 4 is necessary. An Rf of 6 mm by 15 cm was used, packed with Separon C-18 containing 7µm silica gel. Furthermore, a pre-column of internal diameter 4. Vial: The vial used was the headspace vial with a headspace volume of 0 mm and a length of 4 cm filled with the same sorbent material. The mobile phase was a solvent combination of acetonitrile, ethyl acetate, methanol and water with a flow rate of 500µl/min. Using standard samples of lutein, zeaxanthin, lycopene and β-carotene, the efficiency of the chromatograph was determined.

Results

Pigment content analysis can be seen physiologically for seeds in diversity, revealed the presence of residual chlorophylls (a + b) in samples examined, as shown in Table 1

Table 1. Experimental Calculations (Mean ± S.E)

	Variant		Chl (a + b)	Carotenoids (Car)		Car/Chl ratio
				Carotenes	Xanthophylls	
Chlorophylls and Carotenoids, Phytochemical Analysis (µg/g dry wt)						
Tomatoes seeds	wax maturity stage	upper layer	116.0 ± 1.5	3.0 ± 0.1	49.0 ± 0.9	0.4
		middle layer	66.0 ± 0.7	3.0 ± 0.1	32.0 ± 0.6	0.5
		bottom layer	7.0 ± 0.1	3.0 ± 0.1	13.0 ± 0.1	2.1
	technical maturity stage (sorting by CFI)	high CFI	58.7 ± 1.4	1.5 ± 1.5	11.7 ± 0.4	0.4
low CFI		4.0 ± 0.4	0.6 ± 0.1	4.9 ± 0.4	1.4	
Rape seeds	wax maturity stage (sorting by seed-coat colour)	pale-brown	29.0 ± 0.2	6.0 ± 0.1	42.0 ± 0.3	1.6
		brown	18.0 ± 0.3	4.0 ± 0.1	32.0 ± 0.2	2.0
		black	12.0 ± 0.1	4.0 ± 0.1	26.0 ± 0.4	2.5
Effects of accelerated aging (AA)						
Tomatoes seeds	CFI (High)	before AA	60.7 ± 1.6	1.5 ± 1.5	11.7 ± 0.4	0.2
		after AA	36.0 ± 0.6	2.7 ± 0.4	18.6 ± 0.9	0.6
	CFI (Low)	before AA	4.0 ± 0.4	0.6 ± 0.1	4.9 ± 0.4	1.4
		after AA	3.9 ± 0.3	1.7 ± 0.2	12.0 ± 0.1	3.5
Analysis of Stress by Heat (50°C)						
22°C (12 h)	unheated		9.5 ± 0.3	0.6 ± 0.1	10.3 ± 0.9	1.1
	50°C (4 h)		7.5 ± 0.4	0.7 ± 0.1	25.2 ± 0.8	3.5
	50°C (4 h) → 22°C (3 h)		7.5 ± 0.3	0.2 ± 0.1	39.9 ± 0.5	5.3

Discussion

Our specific research objective in this study was to increase the understanding of the functions of remaining

chlorophylls in promoting seed tolerance to abiotic stresses in crop plants. There were considerable variations in chlorophyll (Chl) content when harvested seeds were observed as they came in different maturity metrics. The

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figures provided are meant figures with their significant errors bar. It is used in seed development, and during this process, Diethyl hexyl phthalate induces disassembling of the protein complexes, and chloroplasts convert themselves into amyloplasts and elaioplasts (11, 12). However, chlorophylls are not photolysed in this process and, therefore, will remain in the plastids of the seed. As a result of this conclusion, seeds of many angiosperm species harbour small quantities of chlorophyll (13, 14). It has been ascertained that gene mutations for the ABA-dependent transcription factor include *abi3-1* and *grs* mutants that cause the Arabidopsis seeds to possess high Chlorophyll content (15, 16). Further, it has been reported that *abi3-1* mutants are associated with elevated levels of carotenoids (17, 18). As noted earlier, ABA controls several processes occurring during seed development, including synthesis of storage products, loss of water content, dormancy periods and degradation of chlorophyll. However, how ABA regulates chlorophyll degradation seems to differ from other genes regulated by ABA in ripening seeds (19). The data gathered also suggest a robust negative relationship exists between chlorophyll content and the seeds' stress tolerance levels, whereby the seeds with high chlorophyll content possess lower stress tolerance levels than seeds with low chlorophyll levels. Seeds were exposed to accelerated aging as a stress treatment for six days at 40 °C/ 86 % RH to test this. Seed aging is a gradual process and normally takes several years or even decades under favourable storage conditions. Hence, accelerated aging is used where aging has to be done within days (20). Therefore, I appreciate that this is a clear indication that the stress tolerance of seeds is not a function of the presence of chlorophylls only. Peculiarities of chlorophyll degradation in seeds have attracted much attention concerning biofuel production because chlorophyll dissolved with oils evokes oxidative reactions and decreases the storage stability of the oil. Interestingly, it was recently reported that the process of chlorophyll degradation in seeds is associated with the formation of pheophytin (21). Oxidative reaction dominate in most of the seed aging process. It has also been found that increased Chl content in seeds increases damage during aging; therefore, chlorophylls can accelerate the development of ROS. Chlorophyll is an effective photosensitiser, i.e. when it is excited by the light, it can transfer energy to different acceptor molecules (22). This may be because the chloroplast grana are dismantled, and a functional electron transport chain is no longer present in mature seeds; consequently, the chances of chlorophyll changing from its singlet state to its triplet state (³Chl) increase and the photooxidation processes singlet oxygen and free radicals being formed increase (23). Carotenoids found in tissues involved in photosynthesis are capable of quenching free radicals produced by chlorophyll in the presence of light. In seeds, carotenoids are associated with amyloplasts and elaioplasts (24). Lutein, a carotenoid located in the chloroplast's light-harvesting antenna complexes, has the critical function of energy dissipation connected with the conversion of chlorophyll to its triplet state (³Chl) (23). An area was utilised to compare stress tolerance in photosynthesising tissues where Car/Chl is an important measure. At a higher range of Car/Chl, more tolerance was found due to ROS, which scavenges the damage in autotrophic organs by carotenoids protecting the

photosynthetic machinery (25). In the next stage of our investigations, we analysed how stress conditions influenced the value of the Car/Chl ratio in germinating cabbage seeds. The increases found were that accelerated ageing resulted in the build-up of xanthophylls and two folds elevated the Car/Chl ratio level. It was five after the conventional test, and after the accelerated aging test, this ratio was 0.5 in seeds with high Chl content; the other five samples with low Chl content were included. The carotenoids associated with aging plant cells and ripening fruits are mainly xanthophylls stored in plastoglobuli, which are relatively stable and unaffected during Chl degradation (26). Moreover, it has been established that there is a similar pool of carotenoids in seed plastids. The situation in which xanthophyll content increases in accelerated aging and heat stress shows that these seed pigments can protect against stress conditions.

Conclusion

Although residual in mature seeds, chlorophylls contribute to oxidative stress under abiotic stressors. Conversely, carotenoids act as antioxidants, mitigating stress-induced damage and enhancing seed tolerance. The Car/Chl ratio is valuable for assessing seed stress resilience, providing insights into seed physiology under adverse environmental conditions.

Declarations

Data Availability statement

All data generated or analysed during the study are included in the manuscript.

Ethics approval and consent to participate

Approved by the department concerned.

Consent for publication

Approved

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Conflict of interest

The authors declared the absence of a conflict of interest.

Author Contribution

All authors contributed equally

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