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CHAPTER 7

Functional Role of Pigments in Maintaining the Health of Pomegranate (Punica granatum L.)

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Abstract

Punica granatum L., commonly known as pomegranate, is a fruit of considerable agronomic and nutritional interest due to its rich phytochemical composition, including high chlorophyll content, as well as its qualities and cultural significance. Chlorophyll concentration in the leaves plays a critical role in maintaining efficient photosynthetic activity, thereby supporting carbohydrate synthesis essential for fruit development. In this study, the influence of pigment content on fruit integrity was investigated by comparing two different varieties — healthy and cracked fruits—of the same cultivar, Bhagwa. Leaf samples were collected during two distinct phenological stages: the early fruit development phase and the harvest stage. Results indicated that during the initial stage, chlorophyll and associated pigment levels were comparable between plants bearing healthy and cracked fruits. However, at the harvest stage, a significant reduction in leaf pigment concentrations, across all solvent extracts, was observed in plants producing cracked fruits compared to those with healthy fruits. These findings suggest a potential link between diminished chlorophyll content and fruit cracking. So, identifying the factors contributing to chlorophyll degradation and implementing timely intervention strategies may be critical in mitigating fruit cracking and enhancing overall fruit quality and yield in pomegranate.

Keywords: Punica granatum L., UV, Cracking, Chlorophyll, Carotenoids

1. Introduction

Punica granatum L. (pomegranate), a small tree belonging to the Punicaceae family, is native to a region extending from Iran to the Himalayan of India [1]. Renowned for its nutritional value, pomegranate has rapidly gained significance as a health-promoting fruit. Since the chemical composition of the fruit changes during maturation, affecting its nutritional content and health benefits, it is crucial to harvest it at a stage when these bioactive components are at their peak [2]. However, fruit cracking presents a major challenge, significantly reducing both the quality and yield of pomegranate crops [3]. This not only decreases market value but can also render the fruit unsuitable for consumption [4, 5]. Cracking is a common issue across all cultivars and growing regions, though its severity is influenced by several factors, including climate, genetic traits, nutrient deficiencies, variety, fruit development stages, and cultivation practices[6]. Photosynthesis, the fundamental process driving energy and material flow in nature, plays a crucial role in plant development. During the light-dependent phase, the chlorophyll-protein complex captures solar energy and transfers it as excitation energy to the reaction centers of photosystems II and I (PS II and PS I) [7].

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Chlorophyll serves as a key indicator of chloroplast abundance, making the periodic assessment of photosynthetic activity and overall plant metabolism essential. It is primarily located in the chloroplasts of green tissues such as leaves, stems, flowers, and roots, and also functions as an antioxidant compound [8, 9]. Within plant photosystems, chlorophyll a and b play crucial roles in capturing and converting light energy [10]. Of these, chlorophyll a is the primary pigment responsible for energy production, typically present at concentrations two to three times higher than chlorophyll b [8]. The concentration of chlorophyll in leaves is influenced by factors such as plant genotype, species, salt concentration, and developmental stage [11]. Furthermore, chlorophyll content serves as an indirect indicator of a plant's nutrient status, as a significant proportion of leaf nitrogen is integrated into chlorophyll molecules [12]. Additionally, chlorophyll levels are closely associated with plant stress, making them a valuable parameter in assessing plant health [13].

The function of carotenoids in higher plants is largely determined by their site of synthesis. Within chloroplasts, carotenoids serve two primary roles in photosynthesis: aiding in light harvesting and providing protection against photo-oxidative damage [14, 15]. Their ability to absorb light in the visible spectrum is attributed to their conjugated double bonds, which expand the range of wavelengths utilized during light capture [14]. Efficient energy transfer from carotenoids to chlorophyll necessitates a close spatial association between the two pigments [16].

This study investigates the seasonal variation in leaf pigment content—specifically chlorophyll a, chlorophyll b, and carotenoids—and its relationship to the occurrence of healthy versus cracked fruits in pomegranate plants. Understanding these pigment dynamics is crucial, as they directly influence photosynthetic efficiency and overall plant development.

2. Materials and Methods

2.1. Sample Collection

This study was done with two variants of the same cultivar, bhagwa of pomegranate, in the Kachchh district of Gujarat state, India. Leaf samples of the same cultivar were collected from two different orchards, which were 1.5km apart from the same region. As the study examines pigment levels in healthy and cracked fruits, samples were collected from the orchard that contained almost all the nutritious fruits. In contrast, the selected second orchard contained plant-bearing cracked fruits. The geographical location of both orchards lies at 23°09'N latitude and 69°86'E longitude. The soils of the orchards were slightly alkaline and non-saline (pH 7.85 and EC 1.07 dS m-1) and contained approximately 1.68% organic carbon. Fresh green leaves around the plant were collected in two seasons; the first season included a stage in which fruits were in the initial development stage (season 1), while in the second season, the fruits were at the ready-to-harvest stage (Season 2). Season 2 contained cracked fruits for the second orchard. Leaf sampling for the first season started on 14th August, and 25th January for season 2.

2.2. Pigment Study in leaves of Punica granatum L

Accurately weigh 0.5g of fresh plant leaves of both the varieties of Punica granatum L. Using tissue homogenizer, the leaves were homogenized with 10 cm3 of different extracting solvents (95% ethanol, methanol, 80% acetone, and DMSO). The homogenized sample mixture was centrifuged for 15 minutes at 40 °C at 10,000 rpm. The supernatant was isolated from the residue, and 1 ml of supernatant was diluted with 4 ml of the respective solvent [17–19]. Solutions were analyzed for Chlorophyll-a, Chlorophyll-b, and carotenoid content in a UV-Visile Spectrophotometer (Shimadzu).The concentration of pigments in various solvents was calculated from the following equations (Table 1)1.

The experiment was designed in a randomized complete block design with three replications. In each replication, one tree was used and collected at intervals of 5 days. Statistical analysis of all data was performed with SPSS,20 version. Differences between the means were compared, and analysis of variance (ANOVA) was carried out at a significance level of $P<0.05$.

Table 1: Calculation of magnitude of pigments concentration for various extracting Solvents

Extracting Solvent	Equations
95% Ethanol	Ch-a=13.36A664 – 5.19 A649 Ch-b=27.43A649 – 8.12 A664 Cx+c=(1000A470–2.13Ca-97.63Cb)/209
Methanol	Ch-a=16.72A665.2 – 9.16A652.4 Ch- b=34.09A652.4 – 15.28A665.2 Cx+c=(1000A470 – 1.63Ca – 104.96Cb)/221
80% Acetone	Ch-a=12.25A663.2 – 279A646.8 Ch-b=21.5A646.8 – 5.1A663.2 C x+c=(1000A470– 1.82Ca –85.02Cb)/198
DMSO	Ch-a=12.47A665.1 – 3.62A649.1 Ch-b=25.06A649.1 – 6.5A665.1 C x+c=(1000A480– 1.29Ca-53.78Cb)/220

3. Results and Discussion

The plant's photosynthetic activity is among the vital parameters that influence yield, and it can be detected by measuring leaf chlorophyll content and net photosynthetic rate [20]. For plants containing healthy fruits, during early development stage of fruit (Season 1) (Table 2.) average chl-a content was 8.073,11.518,12.513 and 15.810 mg/L, respectively, in various solvents. For plants containing cracked fruits, during season 1 average chl-a content was 8.671,10.686,12.734 and 17.505 mg/L during season 1. No drastic variation was observed in season-1 for both the varieties.

Table 2: Quantification of Chlorophyll a, Chlorophyll b, and Carotenoids (mg/L) in leaves of Punica granatum L. in various extracting solvents for (season -1)

Pigments	**Leaves of Plant bearing Healthy fruit (Season -1)(ppm)**				**Leaves of Plant bearing Cracked fruit (Season -1)(ppm)**			
95% Ethanol								
Pigments	14/08	19/08	24/08	Average	14/08	19/08	24/08	Average
Chlorophyll a	8.519	8.2	7.5	**8.073**	9.913	8.3	7.8	**8.671**
Chlorophyll b	4.5	4.1	3.988	**4.196**	5.0	**4.846**	4.2	4.682
Carotenoids	2.340	1.8	1.2	**1.780**	2.116	2.0	1.5	**1.872**
Methanol								
Chlorophyll a	12.454	11.3	10.8	**11.518**	11.2	10.5	10.358	**10.686**
Chlorophyll b	1.116	1.1	1.0	**1.072**	1.7	1.2	1.441	**1.447**
Carotenoids	3.4	4.1	3.585	**3.695**	4.0	3.5	2.403	**3.301**
80% Acetone								
Chlorophyll a	12.0	14..219	11.5	**12.573**	11.87	13.902	12.5	**12.734**
Chlorophyll b	7.396	6.8	7.5	**7.232**	8.0	6.8	7.703	7.501
Carotenoids	4.1	3.353	3.2	**3.551**	5.2	3.1	1.879	**3.393**
Dimethyl Sulphoxide (DMSO)								
Chlorophyll a	16.930	16.0	14.5	**15.810**	18.015	16.5	18	**17.505**
Chlorophyll b	9.0	11.930	8.5	**9.810**	10.5	10.0	12.232	**11.244**
Carotenoids	5.096	5.057	5.432	**5.195**	5.466	5.378	5.65	**5.498**

As Chlorophylls are essential pigments for photosynthesis. In green plants, chlorophyll is the primary pigment used in photosynthetic processes to transport light energy to a chemical acceptor [21]. The amount of chlorophyll varies in the leaves of higher plants as they go through distinct developmental phases. For plants containing healthy fruits, average carotenoid content was 1.780, 3.695, 3.551, and 5.195 mg/L, respectively, in various solvents during the early development stage of fruit (season-1), and for plants containing cracked fruits, average chl-a content was 1.872,3.301,3.393 and 5.498 mg/L during season 1. As not much deviation was observed in pigments for both varieties, the photosynthetic rate is similar during the early development stage for both varieties.

The average chl-a content for a plant containing healthy fruit during season 2 when fruits were in a ready-to-harvest state were 7.734, 11.291,10.855, and 10.667 mg/L, respectively, in various solvents. Similarly, the chl-a content for a plant containing almost all cracked fruits

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Table 3: Quantification of Chlorophyll a, Chlorophyll b, and Carotenoids (mg/L) in leaves of Punica granatum L. in various extracting solvents for (season -2)

Pigments	Leaves of Plant bearing Healthy fruit (Season -2)(ppm)				Leaves of Plant-bearing Cracked fruit (Season -2)(ppm)			
95% Ethanol								
Pigments	25/01	30/01	04/02	**Average**	25/01	30/01	04/02	**Average**
Chlorophyll a	8.1	7.902	7.2	**7.734**	8. 0	7.5	5.782	**7.094**
Chlorophyll b	3.497	2.8	2.1	**2.799**	2.5	1.660	2.2	**2.120**
Carotenoids	0.45	0.55	0.422	**0.474**	0.788	0.8	0.65	**0.746**
Methanol								
Chlorophyll a	11.873	11.2	10.8	**11.291**	11	9.5	11.363	**10.621**
Chlorophyll b	-0.2	-0.1	-0.564	**-0.288**	-0.715	-0.55	-0.85	**-0.705**
Carotenoids	2.8	2.2	1.744	**2.248**	3.1	2.1	1.703	**2.301**
80% Acetone								
Chlorophyll a	11.50	11.0	10.065	**10.855**	10.348	9.0	9.5	**9.616**
Chlorophyll b	5.8	6.1	4.498	**5.466**	3.138	3.258	4.35	**3.582**
Carotenoids	4.333	3.120	2.462	**3.305**	2.035	0.697	1.159	**1.297**
Dimethyl Sulphoxide (DMSO)								
Chlorophyll a	11.026	9.997	10.978	**10.667**	9.230	8.253	8.407	**8.630**
Chlorophyll b	6.097	5.329	6.025	**5.817**	5.195	4.458	3.892	**4.515**
Carotenoids	3.4	2.9	2.799	**3.033**	2.546	2.172	1.78 6	**2.168**

during season -2 was 7.09,10.621,9.616 and 8.630 mg/L in multiple solvents (Table 3)3. Thus, all chlorophyll parameters changed during the season. Both varieties' chl-a, chl-b, and carotenoid content were wide in early August (season 1). They decreased throughout development periods and reached the lowest level during harvest (season 2). A drastic decrease was observed in pigment content for plants containing cracked fruits during season 1 and season 2, indicating reduced photosynthetic activity from the early development stage to the matured stage.

Reduced photosynthesis results in a decrease in the number of accessible metabolites needed for plant growth. The crops' ability to photosynthesize is one of the critical elements affecting the yield that may be observed by physiological characteristics such as leaf chlorophyll concentration, net Stomatal conductance and photosynthetic rate [22]. This study examined the effect of leaf chlorophyll content in two different seasons for plants bearing healthy and cracked fruit. Chl-b content showed a similar trend to chl-a. It was higher at the beginning than at the season's end (Table 2). The highest chl-b content, 9.810mg/L, was obtained from plants containing healthy fruit during season 1 in DMSO solvent. A drastic decrease in chl-b content was observed for both varieties during season 2 (Table 3).

Carotenoid content followed a similar trend as chl-a and chl-b. A drastic decrease was found in carotenoid content during season 2 for both varieties. Average carotenoid content decreased to 0.474,2.248, 3.305, and 3.033 mg/L, respectively, in various plant solvents containing healthy fruit during season 2 when fruit was ready to harvest. Similarly, carotenoid content also decreased for plants containing almost all cracked fruits during season -2, and it decreased to 0.746,2.301, 1.297, and 2.168 mg/L, respectively, in various solvents (Table 3).

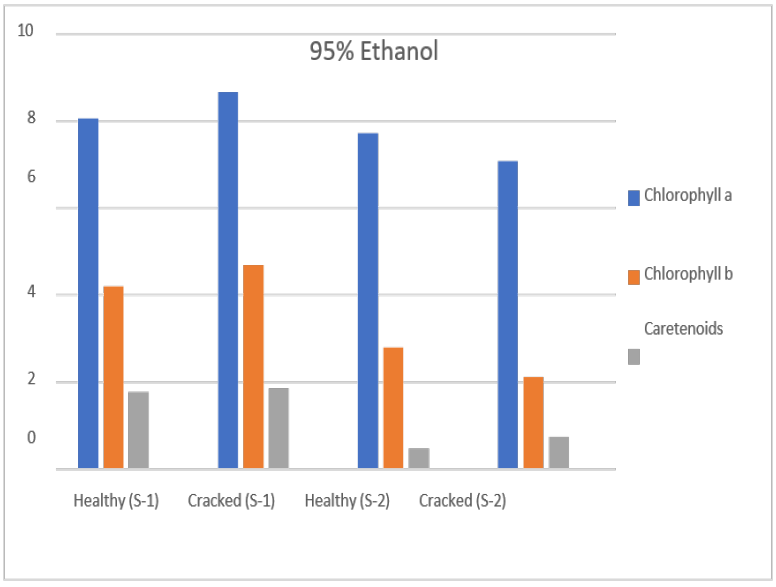

Figure 1: Pigment levels in leaves of Pomegranate in 95% Ethanol in seasons 1&2

As shown in Fig. 1, Chlorophyll a,b, and carotenoid levels in leaves extracted with 95% ethanol declined significantly between Season 1 and Season 2 for both healthy and cracked fruits; more variation was observed for cracked fruits, suggesting reduced photosynthetic activity as fruits mature.

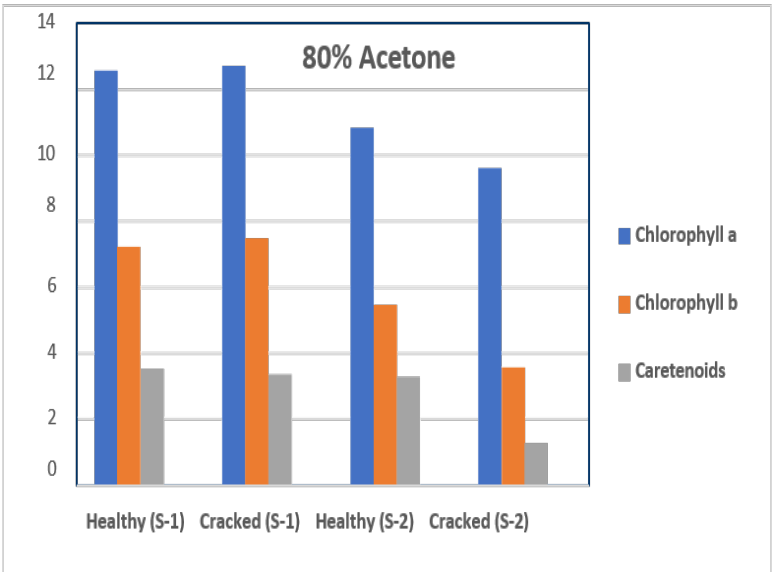

Figure 2: Pigment levels in leaves of Pomegranate in 80% Acetone in seasons 1 &2

In Fig. 2, pigment levels in 80% acetone show a similar trend to 95% ethanol with lower chlorophyll and carotenoid content as the season progresses, particularly in plants with cracked fruits.3

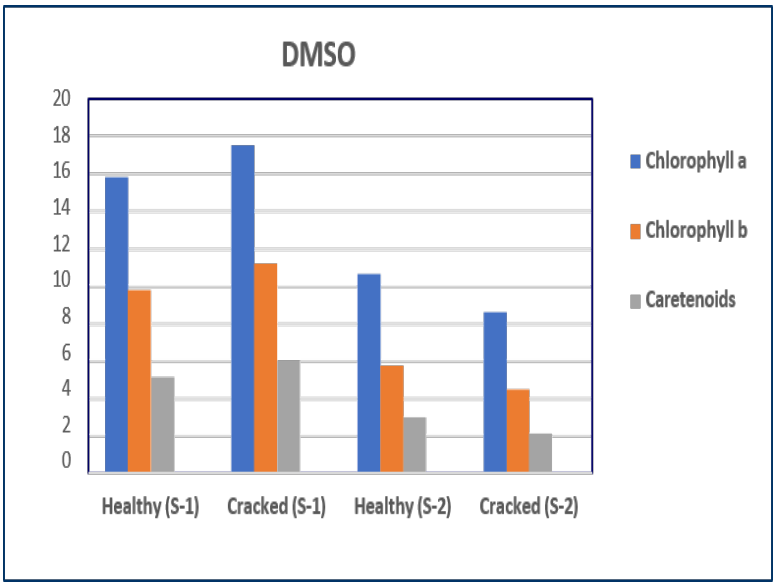

Figure 3: Pigment levels in leaves of Pomegranate in DMSO in seasons 1 &2

The highest amount of chl a, b, and carotenoid levels were found in leaves extracted with DMSO solvent, indicating more extraction than acetone and ethanol (Fig 3). A similar trend, 95% ethanol and acetone, were followed with lower chlorophyll and carotenoid content as the fruit reached matured, particularly in plants with cracked fruits. From Fig1,2,3, it can be observed that pigment level decreases more in all extracting solvents for cracked fruits than healthy fruits, as the fruit reaches from early development stage to matured one, indicating a decrease in photosynthetic activity.4

Table 4: Regression coefficient results from the data of experiments for pigment yield

Values	Mean	Std. Deviation	Coefficients	Standard error	t - value	p-value
Value of Pigment (mg/L)						
(Constant)	5.83	4.28	7.82	1.32	5.93	.000
Season	1.50	0.504	2.383	.508	4.691	.000
Health of the fruit	2.00	0.822	-.349	.311	-1.123	.265
Solvent	2.50	1.126	1.215	.227	5.348	.000
LevelPigments	2.00	0.822	-3.952	.311	-12.71	.000

Table 5: Analysis of Variance (ANOVA) for Pigment Study

SS	DF	f-value	P-value	Mean Square	R2	Adjusted R2
For Pigment Yield						
990.658	4	53.333	0.0010	247.664	0.761	0.747
SS: sum of squares; DF: Degree of Freedom						

Leaf average chl-a, chl-b, and Carotenoids affected pomegranate's health (healthy and cracked fruit). These differences were statistically significant. Average leaf chl-a, chl-b, and total chlorophyll of plants containing healthy fruit were higher than that of plants containing healthy fruit during both seasons. The leaf chlorophyll content was the lowest at season 2 when fruits were in a matured state. The highest values were found during the early development stage for both varieties.

Leaf chlorophyll content was found to be lowest in cracked fruits during Season 2 (January-February, winter). Seasonal changes, particularly in summer and winter, influence both pomegranate fruit cracking and chlorophyll degradation. Cold winter conditions can slow metabolic activity, which delays chlorophyll degradation compared to warmer months [23]. Since the solar energy absorbed by a leaf largely depends on photosynthetic pigment levels, decreased chlorophyll content can directly impact photosynthetic efficiency and, ultimately, primary productivity [20].

According to [24], an increase in nutrient uptake from the roots correlates with higher leaf chlorophyll levels. Chlorophyll a, chlorophyll b, and total carotenoids were found to be higher in plants with healthy fruits in both seasons compared to those with cracked fruits. Both nutrient uptake and water availability are critical for maintaining fruit quality. A balanced nutrient management strategy, combined with adequate irrigation, helps minimize cracking and optimizes chlorophyll levels [25, 26].

Increased pigment levels, particularly chlorophyll, have been shown to enhance photosynthetic efficiency, promoting improved growth and fruit yield [27]. Additionally, higher concentrations of anthocyanins and carotenoids in fruits contribute to their antioxidant properties, thereby enhancing fruit quality and shelf life [28]. Elevated pigment levels can also strengthen a plant's resistance to diseases, reducing the incidence of pests and pathogens [29]. Plants with higher pigment content tend better to withstand environmental stresses, such as drought or salinity, ensuring consistent productivity [30]. This aligns with our findings, as plants bearing healthy fruits exhibited higher pigment levels in both seasons than those with cracked fruits.

Studies have shown that DMSO frequently results in higher yields of pigments due to its strong hydrogen-bonding capabilities, which enhance the solubility of plant pigments and make the extraction process more efficient than many other organic solvents [31]. DMSO also does not denature proteins or other biological molecules, helping to preserve pigment integrity during extraction [32]. Its ability to effectively penetrate cell membranes facilitates the release of intracellular pigments [33].

According to Gallardo, cracking in olives results in a loss of green pigments and color degradation [34]. The enzyme chlorophyllase, which facilitates chlorophyll degradation, is more active in saline conditions. High levels of Na+ and Cl- are likely contributors to the reduction of chlorophyll content in leaves [35]. Moran suggests that the nutrient status of a plant can be indirectly assessed by measuring chlorophyll content [36]. Low chlorophyll levels may indicate Fe deficiency or insufficient solar radiation penetration[37, 38].

From the analysis of variance, the model for pigment levels was found to be highly significant ($p < 0.01$), with an R^2 (coefficient of determination) value of 0.761. This R^2 value indicates that 76.1% of the total variation in the observed response can be explained by the model or by the experimental parameters and their interactions. The remaining 23.9% of variation was not accounted for by the model (Table 5). The coefficient estimates in the regression model for pigment analysis are presented in Table 4.

4. Conclusion

The study concludes that leaf chlorophyll content is strongly correlated with plant stress and senescence. The findings demonstrate that chlorophyll and carotenoid concentrations play a critical role in determining the overall health of pomegranate trees. Specifically, a marked decline in chlorophyll a, chlorophyll b, and carotenoid levels was observed from the early stages of fruit development to the harvest-ready phase. The lowest pigment levels were recorded in plants bearing cracked fruits, indicating that chlorophyll quantification may serve as a reliable indicator of plant vitality. Furthermore, fluctuations in chlorophyll a and b levels appear to be linked to the incidence of fruit cracking in pomegranates, suggesting that targeted management of influencing factors could help mitigate this issue. Regular monitoring of chlorophyll content is therefore recommended as a tool for evaluating nutrient status. Proactively identifying and managing the variables that contribute to chlorophyll depletion in leaves could play a pivotal role in reducing fruit cracking and associated economic losses for farmers.

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