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Dynamics of anthocyanin and chlorophyll content in red fruited okra var. Kashi Lalima

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Abstract

An experiment was conducted with the objectives to study the dynamics of anthocyanin and chlorophyll content at various stages of fruit growth and development in the red fruited okra variety Kashi Lalima and also to evaluate nutritional quality at harvestable maturity stage. Anthocyanin and total chlorophyll content of the fruits of Kashi Lalima studied with the help of spectrophotometer at various stage of fruit growth i.e., starting from zero days after anthesis (0 DAA) to 35 DAA. It has been observed that chlorophyll is the only pigment detected in the mature ovary at the time of anthesis, while no anthocyanin is detected at 0 DAA. Traces of anthocyanin detected only at 2 DAA and the content in gradually increased up to 8 DAA (3.90 mg/100g FW) i.e., during harvestable stage for fresh vegetable. At this point of time fruits are scarlet red in colour. Fading of coloration start at 10 DAA and significant discoloration observed at 35 DAA (1.99 mg/100g FW). On the other hand significant amount of chlorophyll was also reported from 0 DAA to 35 DAA.This study provides the insight of the red coloration of the fruit of Kashi Lalima, pattern of anthocyanin pigmentation and identification of the appropriate stage of harvesting for better nutritional quality.

Keywords: Red okra, anthocyanin, chlorophyll, fruit growth, nutritional quality

Introduction

Okra (*Abelmoschus esculentus* L. Moench.) is a warm season vegetable crop and native to Africa. It is also familiar as ladies finger and bhendi, belong to the family Malvaceae. It is important vegetable of the tropical countries and very popular in India, Nigeria, Pakistan, Cameroon, Iraq and Ghana. In the world, India ranked 1st in the okra production and contribute more than 60% to the global production. Usually immature tender fruits are edible part of this crop and either consumed in fresh or dried form, even thoughthe leaves and seeds are also known for their sporadic culinary use in various part of the world. Apart from its culinary use, okra fruit extracts widely utilized in the food and pharmaceutical sector as emulsifiers and binding agent.The edible immature fruits of okra are considered as highly nutritious and are rich in dietary fibers, carbohydrates, flavonoids, vitamins and minerals. Okrafruit also exhibit appreciable antioxidant activities, solely due to their richness for vitamin C, carotenoids and flavonoids (Gemede et al. 2014, Petropoulos et al. 2018).Fruits are helpful to strengthen the digestive and immune systems and utilized widely as food additives owing to their antigastric acid, antifatigue, antioxidation, and anti-inflammation properties (Zhang et al. 2021). Okra fruits are reported for their therapeutic use against diabetes, hyper-lipidaemia, microbes, ulcers and neurodegenerative diseases (Petropoulos et al. 2018).

The colour of okra fruits varies from green to red and later being the most predominant in the market. Being a newer market segment, the demand of red okra is growing day by day in India. Red fruited okra received much attention from consumers due to the appealing color and health-promoting constituents (Zhang et al. 2021). In red fruited okra, reddish purple coloration is mainly due to the accumulation of anthocyanin which present in combination with chlorophyll. In general unfertilized ovaries of red okra are green in colour

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and devoid of anthocyanin pigmentation. Sparse red pigmentation starts mostly 2 days after fertilization and concentration increase subsequently leading to dark reddish purple fruit at harvestable maturity. After certain period of fruit growth and development, the intensity of reddish purple pigmentation in okra fruits exhibits declining trend. Anthocyanin pigmentation is localized on the epidermis of the fruits and pericarp tissues are pigmented with chlorophyll. On the other hand in green fruited okra pigmentation is due to chlorophyll and intensity enhanced with respect to fruit growth and development. The information related to the pigmentation pattern, dynamics of pigment content and nutritional quality at edible maturity of red fruited okra is meager. Therefore the present study was undertaken to reveal the dynamics of anthocyanin & chlorophyll and to study the nutritional content at harvestable maturity in red fruited variety Kashi Lalima in comparison with green fruited varieties Kashi Chaman.

Materials and Methods

This experiment was conducted at ICAR-Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh during 2020-21. The experimental materials of the present study were consisting of red fruited variety Kashi Lalima and green fruited variety Kashi Chaman. These two okra varieties were developed at ICAR-IIVR, Varanasi and released & notified by CVRC in 2019 for cultivation in the state of Uttar Pradesh. Kashi Lalima is the first notified variety in red fruited segment in India. These two varieties differed significantly with respect to their agro-morphological characteristics including fruit colour, former being reddish-purple and later is dark green. Crop was raised during the rainy season of 2020-2021 at the Research Farm, ICAR-IIVR, Varanasi, Uttar Pradesh located at 25°10’55’’ N latitude and 82°52’36’’ E longitude.

For the laboratory experiment to analyze total monomeric anthocyanins, chlorophyll-a, chlorophyll-b, total chlorophyll, total phenolics, total carotenoids and ascorbic acid content triplicate fruit sample of sufficient quantities collected from both the varieties at various stages of fruit growth, starting from days of anthesis/ 0 days after anthesis (DAA) to 35 DAA including harvestable maturity. These pigments were analyzed using UV–Visible spectrophotometer. Total anthocyanin was estimated as per Ranganna (2001) and the content was expressed as mg/100g FW. Chlorophyll-a, chlorophyll-b, total chlorophyll were measured as defined by Arnon (1949) and calculated as mg/100g FW. Total phenol was analyzed spectrophotometrically using Folin-Ciocalteu reagent (Singleton et al. 1999) and results were computed as gallic acid equivalent (mg GAE/100 g FW). Total carotenoids and ascorbic acid content estimated according to Ranganna (2001) and Albrecht (1993), respectively; and expressed as mg/100g FW.

Data regarding total monomeric anthocyanins, chlorophyll-a, chlorophyll-b, total chlorophyll, total phenolics, total carotenoids and ascorbic acid content of Kashi Lalima and Kashi Chaman were statistically analysed and compared using standard error bars with probability of less than 5% ($p<0.05$).

Results and Discussion

The color of fruit is considered as a key exterior and commercial parameterhaving significant influence in the market valueand consumer choice. It has been observed that most purple or red fruits are reported for their richness in anthocyanin pigment and grapes, strawberries, apples and eggplants are few examples of anthocyanin rich fruit (Paauw et al. 2019).The fruit colour of okra varies from green to red or even whitish green. Though the green fruited okra is most common in the Indian vegetable market, but the consumer’s reference toward red fruited okra is increasing day by day with a perception of their nutritional richness. The colour of red/reddish-purple fruit of okra is solely due to the anthocyanin and spread over the epidermis of the fruit. The genotypes of okra with red/reddish-purple fruit contain significantly high anthocyanin pigment and capable of attracting more consumers than the genotypes with green or whitish green fruit epidermis. The ovary of the red fruited okra is green in colour and does not possess any anthocyanin pigmentation. Nevertheless, the reddish purple color of okra fruit peel gradually enhance after fruit set and fading of colour starts after the commercial maturity stage. Therefore, the investigation of pigmentation dynamics is of great importance in determining the product quality of red okra.

Dynamics of anthocyacnin content: Anthocyanin content in the fruits of red fruited variety Kashi Lalima and green fruited variety Kashi

Chaman studied with the help of spectrophotometer at various stage of fruit growth i.e., 0 DAA, 2 DDA, 4 DDA, 6 DDA, 8 DDA, 10 DDA, 15DDA, 20 DDA, 30 DDA and 35 DAA. At the time of anthesis, the colour of ovary was green in both Kashi Lalima and Kashi Chaman. It had been observed that chlorophyll was the only pigment detected in the mature ovary at the time of anthesis, while no anthocyanin was detected at the time of anthesis. Traces of anthocyanin (0.045mg/100g FW) detected only 2DAA in the fruits of Kashi Lalima and the content was gradually increased up to 8 DAA (3.90 mg/100g FW) i.e., during harvestable stage for fresh vegetable. At this point of time of fruit growth, the fruit colour of Kashi Lalima was scarlet red in colour. Up to 8 DDA, a positive correlation was observed between fruit growth and anthocyanin content. Fading of coloration start at 10DAA and significant discoloration observed at 35 DAA; and at this point, anthocyanin content was reduced to 1.99mg/100g FW from 3.90 mg/100g FW. A negative correlation was observed between anthocyanin content and fruit growth after 10 DAA and same pattern continued up to 35 DDA i.e., at the time of seed maturity stage. On the other hand no anthocyanin pigment was detected at various stages of fruit growth in the fruits of green fruited variety Kashi Chaman (Figure 1). The results were in accordance with the finding of Wang et al. (2022) in brinjal and Heleys et al. (2006) in tomato.

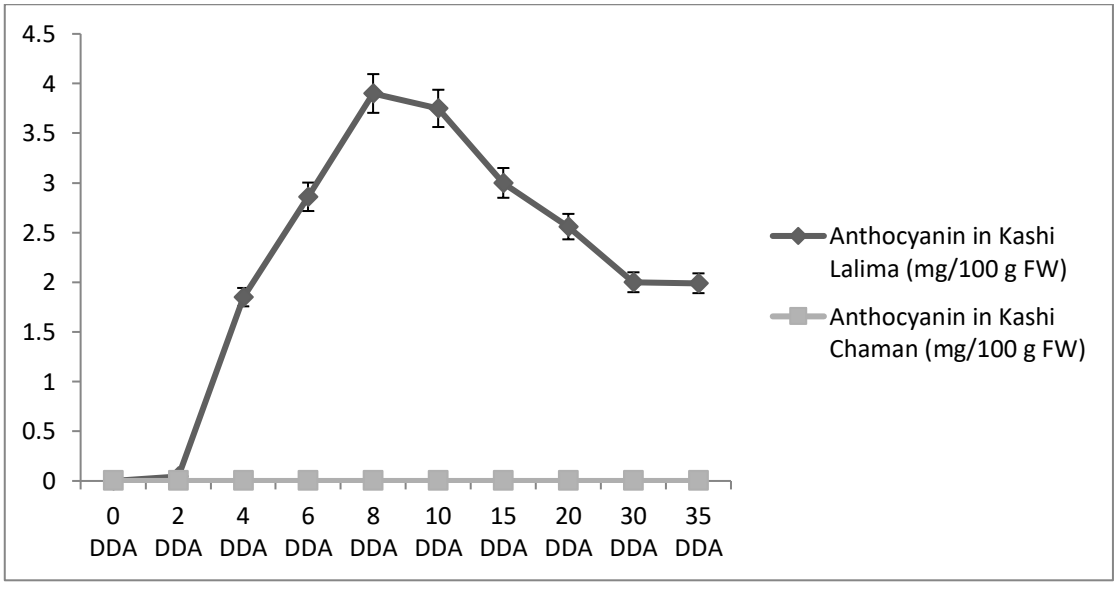

Figure 1: Anthocycnin content in Kashi Lalima and Kashi Chaman at various stage of fruit growth

Dynamics of chlorophyll content: Chlorophyll is the predominant pigment in the okra fruit, be it a green or reddish purple. Like anthocyanin pigment chlorophyll-a, chlorophyll-b and total chlorophyll content at various developmental stage of Kashi Lalima fruit was quantified using spectrophotometer.

It was found that only pigment present in the mature ovary at the time of anthesis is chlorophyll in the form of Chlorophyll-a, and chlorophyll-b. In the mature ovary of Kashi Lalima at 0 DAA chlorophyll-a, chlorophyll-b and total chlorophyll were estimated 0.68, 0.27 and 0.95 mg/100g FW, respectively. After 2 DAA, chlorophyll-a, chlorophyll-a, and total chlorophyll content of the growing fruit of this genotype showed decreasing trends up to 8 DAA, when the fruits totally scarlet red in colour. But the content of these three pigments showed increasing trends from 10 DDA and continued up to fruit ripening stage, when they

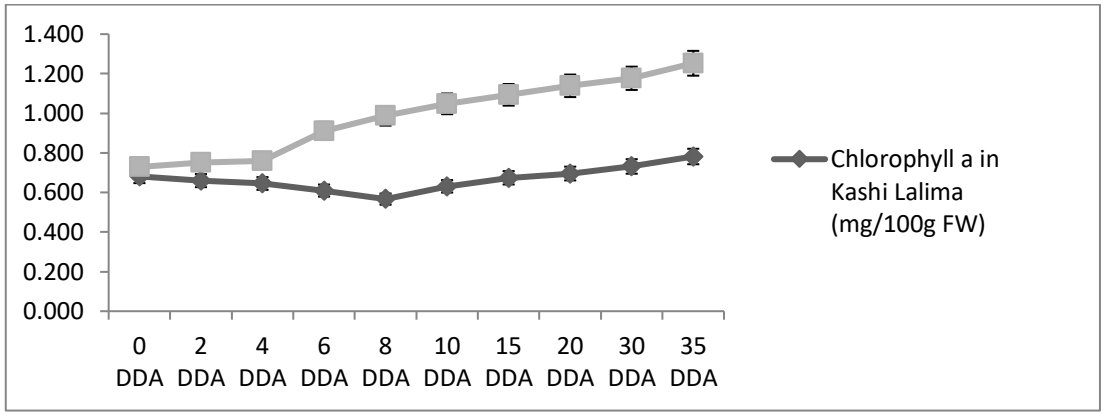

Figure 2: Chlorophyll-a content in Kashi Lalima and Kashi Chaman at various stage of fruit growth

started splitting. The maximum content of these three green colour imparting pigment in the fruits of Kashi Lalima was reported at 35 DAA (chlorophyll-a: 0.78 mg, chlorophyll-b: 0.31mg, and total chlorophyll: 1.09 mg per 100 g FW). Though the chlorophyll-a, chlorophyll-b and total chlorophyll content in the fruits of this variety initially exhibited negative correlation with the age of fruits up to 8 DAA, but positive correlation between the content of these three pigments and age of the fruit started after 10 DDA and same pattern continued up to fruit ripening.

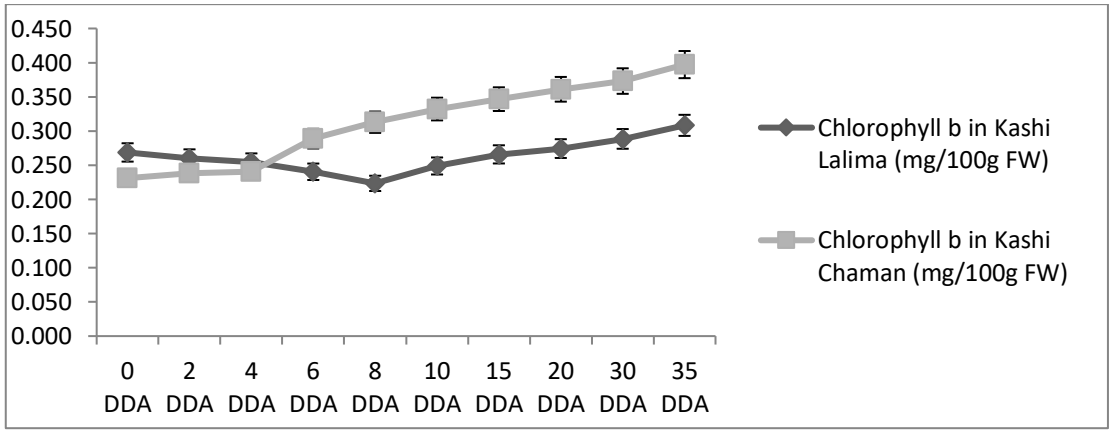

Figure 3: Chlorophyll-b content in Kashi Lalima and Kashi Chaman at various stage of fruit growth

On the other hand the chlorophyll-a, chlorophyll-b and total chlorophyll content in the mature ovary of Kashi Chaman were 0.73, 0.23 and 0.96 mg/100 g FW, respectively at 0 DAA or at the time of anthesis. Total chlorophyll content in the mature ovary of green fruit variety Kashi Chaman reported to be higher as compare to Kashi Chaman. The content of these three pigments in the fruits of Kashi Chaman showed increasing trends and exhibited positive correlation with the age of growing fruits even up to its ripening. The maximum chlorophyll-a, chlorophyll-b and total chlorophyll content of 1.25 mg, 0.40mg and 1.65 mg/100 FW, respectively were analyzed at 35 DAA in the fruits of this variety. Besides, the fruits of Kashi Chaman reported to have contained more amount of chlorophyll-a, chlorophyll-b and total chlorophyll (0.99 mg, 0.31 mg and 1.30 mg/100g FW, respectively) at harvestable maturity i.e., 8 DAA then the red fruited variety Kashi Lalima and also exhibited contrasting colour of green and reddish purple (Fig. 2, 3 & 4). The finding of this study was supported by the results of Yora et al. (2018) in okra.

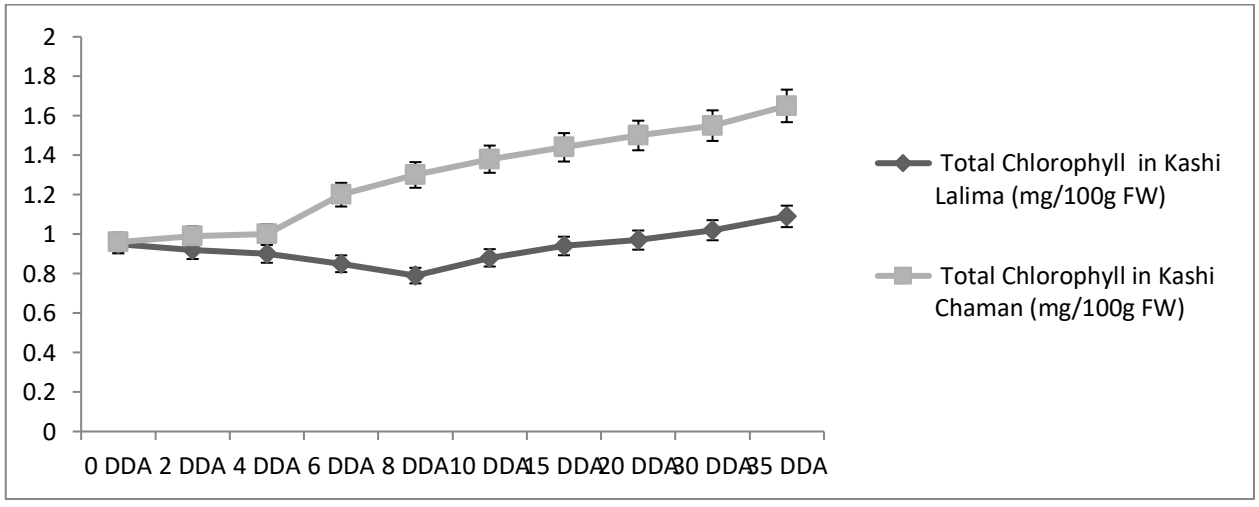

Figure 4: Total chlorophyll content in Kashi Lalima and Kashi Chaman at various stage of fruit growth

Nutritional quality at harvestable maturity: Different nutritional parameters of okra fruits viz., anthocyanin, chlorophyll-a, chlorophyll-b and total carotenoids, total phenolics and ascorbic acid content in Kashi Lalima and Kashi Chaman

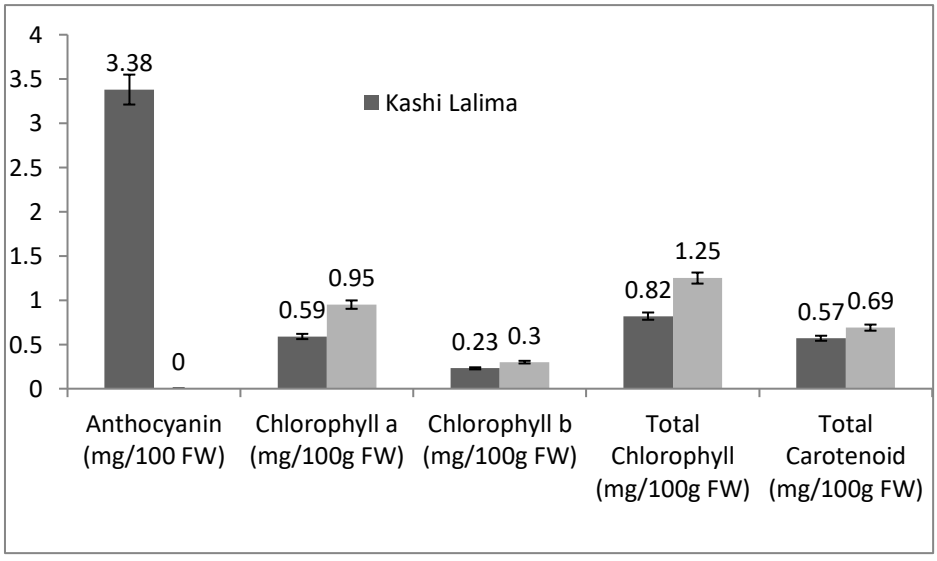

Figure 5: Anthocyanin, chlorophylls and total carotenoids content in Kashi Lalima and Kashi Chaman at harvestable maturity

were analyzed and compared. Kashi Lalima reported to have contained significantly higher amount (3.38mg/100g FW) of anthocyanin than the Kashi Chaman. Anthocyanin was not detected in the samples of Kashi Chaman. Total phenolics content were also found in appreciable higher concentration of 43.15 mg/100g FW in Kashi Lalima than 34.57 mg/100 FW in Kashi Chaman. This might be due to the higher content of anthocyanin in the fruits of Kashi Lalima. The ascorbic acid content was also reported to higher in Kashi Lalima (18.78mg/100g FW) than Kashi Chaman (15.33 mg/100g FW) at the time of fresh fruit harvesting. On the other hand, the total carotenoids, chlorophyll-a, chlorophyll-b and total chlorophyll content was higher in Kashi Chaman (0.69 mg, 0.95 mg, 0.30 mg and 1.25mg/100g FW, respectively) than the Kashi Lalima (Fig. 5&6). It has been observed that the

Kashi Lalima is more nutritious than the Kashi Chaman at harvestable maturity due to the higher contents of total anthocyanin, total phenolics and ascorbic acid in the fruits. Yora et al. (2018) and Petropoulos et al. (2018) also undertaken nutritional analysis and reported similar kinds of finding in okra in relation to harvestable maturity.

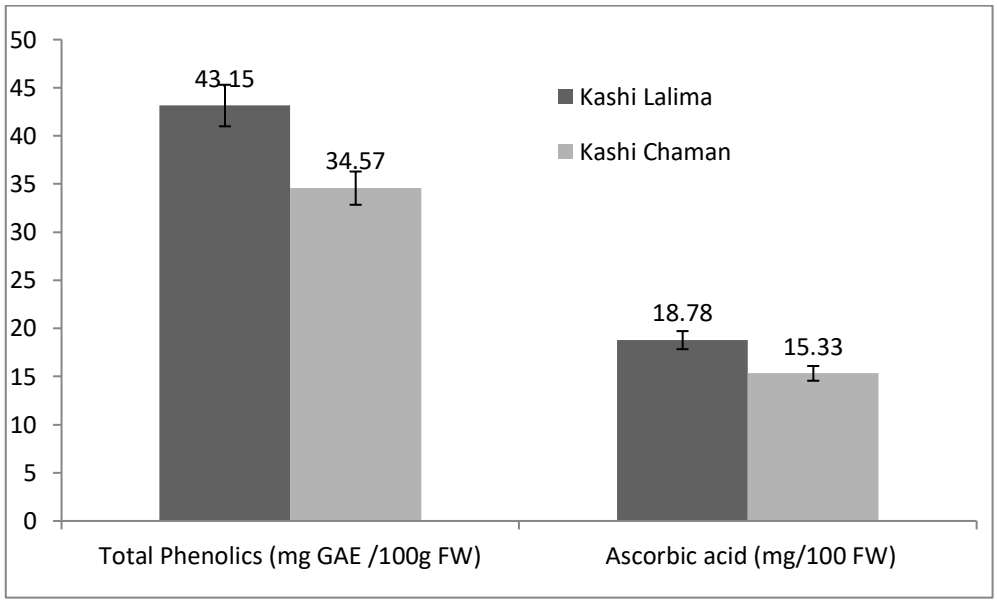

Figure 6: Total phenolics and ascorbic acid content in Kashi Lalima and Kashi Chaman at harvestable maturity

Conclusion

This study uncovered the dynamic of pigments content, more specifically anthocyanin, chlorophyll-a, chlorophyll-b and total chlorophyll content in the fruits of red fruited genotype Kashi Lalima at various stages of fruit growth and development in comparison with the fruits of green fruited variety Kashi Chaman. In the fruits of Kashi Lalima, anthocyanin pigment accumulated only 2-3 Days after anthesis and is solely responsible for reddish-purple color fruits of this variety. There was a decreasing trend of anthocyanin content and increasing trend of chlorophyll pigments observed after harvestable maturity in Kashi Lalima. Besides, the fruit of Kashi Lalima seems to be more nutritious than its counterpart green fruited variety Kashi Chaman at harvestable maturity due to higher content of anthocyanin, total phenolics and ascorbic acid. This study not only revealed the roles of anthocyanin in the red colour fruit development in Kashi Lalima, but also identified the proper stage of harvesting for superior nutritional quality.

सारांश

लाल फल वाली भिंडी किस्म का शीलालिमा में फलों के विकास और विकास के विभिन्न चरणों में एंथोसायनिन और क्लोरोफिल सामग्री की गतिशीलता का अध्ययन करने के उद्देश्य से एक प्रयोग किया गया था और कटाई योग्य परिपक्वता अवस्था में पोषण की गुणवत्ता का मूल्यांकन भी किया गया था। एंथोसायनिन और का शीलालिमा के फलों की कुल क्लोरोफिल सामग्री का अध्ययन फल विकास के विभिन्न चरणों में स्पेक्ट्रो फोटोमीटर की मदद से किया जाता है, यानी शून्य दिनों के बाद से शुरू होकर फूल खिलने से 35 दिन तक। यह देखा गया है कि एंथेसिस के समय परिपक्व अंडाशय में पाया जाने वाला एकमात्र वर्णक क्लोरोफिल है, जबकि फूल खिलते समय कोई एंथोसायनिन नहीं पाया जाता है। एंथोसायनिन के निशान केवल फूल खिलने के 2 दिन बाद पाए गए और मात्रा धीरे–धीरे फूल खिलने के 8 दिन बाद (3. 90 मिग्रा./100 ग्राम एफडब्ल्यू) तक बढ़ गई, यानी ताजी सब्जी के लिए कटाई योग्य अवस्था के दौरान। इस समय फलों का रंग सुर्ख लाल होता है। रंग का फीका पड़ना फूल खिलने से 10 दिन बाद शुरू होता है और फूल खिलने के 35 दिन बाद (1.99 मिग्रा./100 ग्राम एफडब्ल्यू) महत्वपूर्ण मलिन किरण देखा जाता है। दूसरी ओर फूल खिलने से 35 दिन

बाद तक क्लोरोफिल की महत्वपूर्ण मात्रा भी बताई गई। यह अध्ययन काशी लालिमा के फल के लाल रंग, एंथोसायनिन रंजकता के पैटर्न और बेहतर पोषण गुणवत्ता के लिए कटाई के उपयुक्तचरण की पहचान की अंतर्दृष्टि प्रदान करता है।

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