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Analysis of morphological and biochemical features in pink-husked coconut (*Cocos nucifera* L.)

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Abstract

A comparative analysis was conducted to characterize the pink-husked type in the San Ramon Tall (SNRT) coconut population. In terms of floral traits, the pink-husked palms produced two types of male flowers, distinguished by their dark pink and yellow anther filaments, while no differences were observed in the size of male flowers compared to the non-pink husked SNRT palms. Additionally, no significant differences were found in the quantitative and qualitative traits of tender nuts and their water between the pink-husked and non-pink husked SNRT palms. Biochemical analysis of the leaf and husk tissues revealed that pink-husked palms contained notably higher levels of phenols, flavonoids, and anthocyanins compared to the non-pink husked palms. Phenol content was measured at 9.24 mg GAE/g in leaves and 22.22 mg GAE/g in the mesocarp of 7-month-old fruits for pink-husked palms, compared to 7.85 mg GAE/g in leaves and 19.56 mg GAE/g in the mesocarp of non-pink husked palms. Similarly, flavonoid levels in pink-husked palms were 19.69 mg QE/g in leaves and 37.20 mg QE/g in mesocarp tissues, compared to 18.11 mg QE/g in leaves and 34.51 mg QE/g in husk tissues of non-pink types. Anthocyanin content was also higher in pink-husked palms, measuring 8.50 mg/100g in leaves and 13.89 mg/100g in mesocarp of 7-month-old fruits, while non-pink types recorded only 6.69 mg/100g in leaves and 2.74 mg/100g in mesocarp. Notably, in 4-month-old pink-husked fruits, phenol, flavonoid, and anthocyanin levels were 26.64 mg GAE/g, 38.79 mg QE/g, and 17.59 mg/100g, respectively, which were higher than the levels recorded in 7-month-old pink-husked fruits. This study suggests that pink-husked coconuts are a valuable source of phenols, flavonoids, and anthocyanins, contributing to human health. These findings underscore their potential in breeding programs, providing a distinctive marker for creating new, visually appealing coconut varieties with higher bioactive compounds.

Key words: Coconut, *Cocos nucifera*, pink husk, non-pink husk, morphology, biochemical, anthocyanin, coconut breeding

Introduction

Coconut (*Cocos nucifera* L.), is an essential palm species widely cultivated in tropical regions around the world. Often called the "Tree of Life", nearly every part of the coconut palm—from its roots to its terminal bud—serves a variety of human needs. It is grown in over 93 countries, providing livelihoods for millions and playing a key role in preserving vulnerable island ecosystems. The global area under coconut cultivation spans approximately 12.25 million hectares, with an estimated annual production of 66.67 billion nuts. India, Indonesia, and the Philippines are the leading producers, collectively accounting for more than 75% of the world's coconut supply (CDB, 2023).

Efforts to improve coconut varieties have traditionally focused on exploiting geographic diversity. However, the challenges posed by quarantine restrictions in germplasm exchange make it essential to maximize the use of morphological diversity within specific regions. Germplasm collections in major coconut-producing countries have identified several unique traits, including coconuts with sweet, fragrant, or jelly-like endosperms, edible husks, and fruits with unusual ring or horn-like structures. Other notable morphological traits include albinism, plicata, and variations in sex expression (Arunachalam *et al.*, 2001). Despite their economic potential, many of these traits remain underutilized.

Characterizing germplasm resources is crucial for identifying economically valuable traits. Among these, pink-husked coconuts stand out, with their tender mesocarp exhibiting a distinct pinkish hue. This unique trait has been observed in accessions like West Coast Tall, Guam Tall, and Malayan Green Dwarf (Chowdappa *et al.*, 2017; Thomas *et al.*, 2020). However, pink-husked coconuts are rare, with only a small percentage of palms within the accessions displayed the characteristic, while most exhibit the typical cream-colored husk.

The demand for pink-husked coconut water is particularly notable among traditional medicine practitioners, who use it for treating hepatitis. Studies have shown that pink-husked coconuts contain higher anthocyanin levels than regular tender nuts (Thomas *et al.*, 2020). Similarly, Cungap Red coconuts have been identified as rich sources of anthocyanins, β-carotene, and minerals, offering significant health benefits (Karouw *et al.*, 2019).

To fully utilize these variants, it is essential to understand their morphology, biochemical properties, inheritance patterns, and distribution. Such insights can facilitate their integration into coconut improvement programs and promote exploration of their nutraceutical potential. In this context, ICAR-CPCRI, Kasaragod, identified palms within the San Ramon Tall accession that exhibit pink-colored mesocarps. To harness this variation, a preliminary study was conducted to compare pink-husked and non-pink husked palms from the same accession. This study examined floral and fruit traits alongside biochemical properties, including

total phenol, flavonoid, and anthocyanin content in leaves and fruits at various growth stages.

Information on pink-husked palms, particularly regarding floral and fruit traits as well as biochemical constituents, is limited. To the best of our knowledge, this study is the first to document variations in floral traits and germinating fruits linked to pink coloration, alongside estimating phenol, flavonoid, and anthocyanin content in leaves and mesocarp tissues of fruits at different ages. The findings provide a foundation for further research into segregation patterns, gene expression, and the nutraceutical potential of these variants. Such insights are critical for developing high-value tender nut varieties with enhanced health benefits. Ultimately, this effort aims to meet consumer demand while improving farmers income.

Materials and methods

A unique pink-husked selection was identified within the San Ramon Tall population, maintained in the field gene bank at ICAR-CPCRI, Kasaragod, located at an altitude of 10.7 m above MSL (12.58 N latitude and 74.96 E longitude). The average maximum temperature ranges from 28 to 33.5°C, while the minimum temperature varies between 20 and 25°C. The site receives an average annual rainfall of 3, 400 mm, with 132 rainy days per year. The soil type is red sandy loam with a pH of 5.3.

Detailed observations were made on this palm's floral and fruit traits based on standard procedure (Ratnambal *et al.*, 1995).

Characterization of tender nut and its water: Seven-month-old tender nuts were collected from the pink-husked palm to analyze both quantitative and qualitative traits. Fruit color, volume of tender nut water, Total Soluble Solids (TSS) of the water were assessed. TSS of the tender nut water was estimated using a hand refractometer. Biochemical characterization of the tender nut water as per standard protocols; total sugars by phenol-sulphuric acid method, reducing sugars by Nelson-Somogyi's method and free amino acids by ninhydrin method. From the matured fruit copra yield was also recorded.

Biochemical characterization of leaf and husk: Biochemical analyses were conducted on leaf and husk samples from fruits of different ages (4 and 7 months) in pink-husked and 7 month fruit of non-pink husked palms from the SNRT population. For the analysis, one gram of husk sample and 0.5 grams of leaf sample were extracted in 80% ethanol through three successive extractions, which were then pooled. The resulting extract (0.1 mL) was used for total phenol analysis using Folin-Ciocalteu (FC) assay with gallic acid as the standard, and results were expressed as mg GAE/g of sample. Total flavonoid content was measured from 0.1 mL of the ethanol extract, using quercetin as the standard, and results were expressed as mg QE/g of sample. Total anthocyanin content was determined following the method described by Sims and Gamon (2002).

Statistical analysis*:* Paired t-test was used to statistically analyse the data to evaluate its significance at different levels (1% and 5%) using WASP 2.0 software.

Results and discussion

The pink-husked coconut is a unique variant characterized by its distinct pink-colored husk in tender and developing fruits. To explore the potential of this novel trait for coconut breeding programs, a preliminary study was conducted to examine the morphological and biochemical characteristics of palm parts displaying the pink coloration, including male flowers and fruits.

Morphological characterization: The pink-husked selection from the SNRT population, both male and female flowers, along with fruits from all bunches of the palm, displayed a ring of pink coloration beneath the tepals, slightly extending outward (Fig. 1, 2 and 3). Notably, the male flowers on this palm showed distinctive traits that may support marker-assisted selection in developing tender nut varieties with appealing pink husks. Specifically, the palm produces two types of male flowers, differentiated by the color of their anther filaments—one with dark pink and the other with yellow. Although there were no size differences between these two types in the male flowers and anthers, the pink-filamented flowers were easily identifiable even at the unopened stage, as a pink hue was visible at the base of the tepals (Fig. 4). The tender fruit husk also displayed color gradients from intense

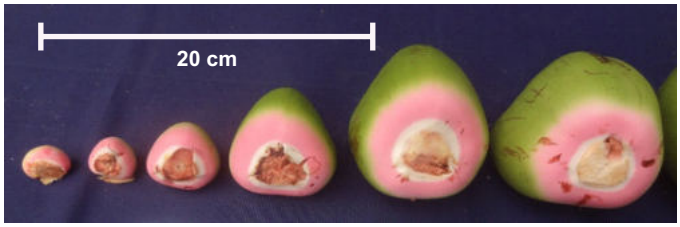

Fig 1. Pink fruits from SNRT population at various stages of development


Fig 2. Pink husked tender fruit


Fig 3. Unopened male flowers with pink tinge over the tepals

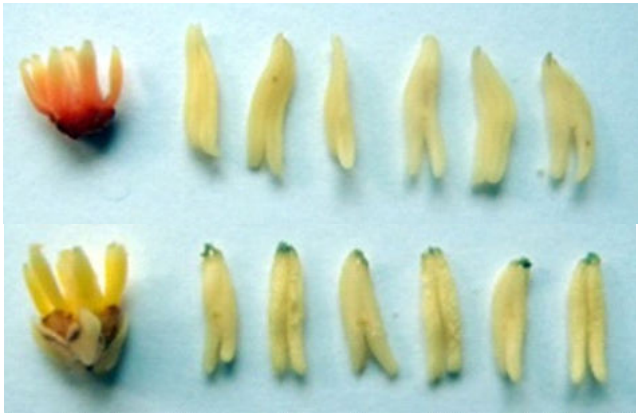

Fig. 4. Two types of male flowers with pink and yellow anther

Fig. 5. Sprout of pink husked SNRT Nut

to light pink, moving from the outer to the inner layers. This pink coloration appeared on the inner surface of the shells in young fruits aged 3 to 5 months. Additionally, a pink hue was observed in the sprout of germinating fruits (Fig. 5), providing an opportunity to select seedlings with pink-colored traits at the nursery stage.

Characterization of tender nut and its water: Quantitative and qualitative parameters were assessed in the pink husked tender fruits (Table 1). Characteristics such as fruit color (Green), tender nut water content (605 mL), TSS (5.9°Brix), total sugar content (4.65%), reducing sugar content (2.74%), free amino acid content (0.04%) of tender nut water and copra content in mature fruits (260g) were found to be nearly comparable with the SNRT non-pink husked population except for amino acid content which is comparatively high in pink husked tender nut (Niral *et al.*, 2016).

Table 1. Quantitative and qualitative fruit component traits of pink and non-pink husked fruits from SNRT population

Tender nut traits	Pink husked fruit	Non-pink husked fruit
Colour of the fruit	Green	Green
Volume of tender nut water (mL)	605	612
TSS of tender nut water (°Brix)	5.9	6.2
Total sugar (%)	4.65	4.64
Reducing sugar (%)	2.74	2.52
Free amino acid (%)	0.04	0.02
Copra content in mature fruits (g)	260.0	272.9

Thomas *et al.* (2020) studied pink-husked types in the Guam Tall population and noted that pink color intensity among Guam Tall fruits varies, with some showing a light pink hue only at the calyx end, while others displayed a more intense pink across both the calyx and mesocarp. No morphological differences were noted in nut size, shape, or water content between pink and non-pink husked types.

Biochemical characterization of leaf and husk: Biochemical analyses were conducted to measure the total polyphenol, total flavonoid, and anthocyanin content in the leaves and husks of both non-pink and pink-husked SNRT tender fruits. The results indicated that the leaves and husks of pink-husked types contained significantly higher levels of all three parameters compared to the non-pink husked types (Table 2). Phenol content in pink-husked palms was measured at 9.24 mg GAE/g in leaves and 22.22 mg GAE/g in the mesocarp of 7-month-old fruits, compared to 7.85 mg GAE/g in leaves and 19.56 mg GAE/g in the mesocarp of non-pink husked palms. Similarly, flavonoid levels in pink-husked palms were 19.67 mg QE/g in leaves and 37.20 mg QE/g in the mesocarp, while non-pink types recorded 18.11 mg QE/g in leaves and 34.51 mg QE/g in mesocarp tissues. Rodiah *et al.* (2018) reported a total phenolic content of 32.24 mg GAE/g in the dried mesocarp and 8.63 mg GAE/g in the exocarp of coconuts. Arivalagan *et al.* (2018) reported 146 ± 14.3 mg GAE/100 g of total phenolic acid content in dry coconut haustorium.

Anthocyanin content was also higher in pink-husked palms, with 8.50 mg/100 g in leaves and 13.89 mg/100 g in mesocarp tissues of 7-month-old fruits, compared to 6.69 mg/100 g in leaves and 2.74 mg/100 g in mesocarp tissues of non-pink types. Additionally, in 4-month-old pink-husked fruits, phenol, flavonoid, and anthocyanin levels in mesocarp tissues were 26.64 mg GAE/g, 38.79 mg QE/g, and 17.59 mg/100 g, respectively, surpassing the levels observed in 7-month-old pink-husked fruits. Thomas *et al.* (2020) observed higher anthocyanin content in the mesocarp of pink-husked Guam Tall coconuts (25.97 mg/100 g fresh weight) compared to a negligible amount (1.46 mg/100 g fresh weight) in non-pink husked palms of the same accession. Karouw *et al.* (2019) also reported higher anthocyanin content in various parts of the exotic Cungap Red coconut, measuring 8.01 mg/100 g in the husk, 1.5 mg/100 g in the kernel, and 0.8 mg/100 g in the coconut water compared to local tall coconuts. The purple-fleshed sweet potato (Var. Bhu Krishna) is a rich source of anthocyanins (85-90 mg/100g) and contains high levels of phenolics, flavonoids, tannins, and alkaloids, highlighting its potential as a nutraceutical food source (Behera *et al.*, 2024).

The high phenol and flavonoid content in pink-husked coconuts is valuable due to their antioxidant properties, which protect against free radicals and improve health. Anthocyanin-rich foods are also beneficial, potentially aiding in cancer, heart disease prevention and increased longevity. Coconut water, already renowned for its

Table 2. Estimation of biochemical constituents in leaf and mesocarp samples of non-pink and pink husked coconut

S No	.Parameters	Leaves		Paired t-test	Mesocarp of the 7 month old tender nut		Paired t-test	Mesocarp of the 4 month old tender nut
		Pink husk	Non-pink husk		Pink husk	Non-pink husk		Pink husk
1.	Total phenol content (mg GAE/g)	9.24±0.16	7.85±0.58	NS	22.22±0.08	19.56±0.02	*	26.64±0.13
2.	Total flavonoid content (mg QE/g)	19.67±0.12	18.11±0.01	*	37.20±0.24	34.51±0.18	*	38.79±0.62
3.	Anthocyanin content (mg/100 g)	8.50±0.02	6.69±0.04	*	13.89±0.31	2.74±0.34	*	17.59±0.70

hydrating and therapeutic properties in Ayurveda, could be further enhanced by utilizing variants with higher bioactive compounds. The pink coloration in tender coconuts adds visual appeal, increasing marketability while offering superior health benefits, making this trait promising for future coconut breeding programs.

This study on the morphology and biochemical properties of pink- and non-pink husked SNRT palms revealed that the pink-husked coconuts are potential sources of phytochemicals, which contribute to improved human health. Remarkably, this research is the first to report the occurrence of differently colored male flowers within a same inflorescence and the presence of a pink hue in germinating seed nuts. This distinctive pink hue can serve as an early marker for identifying pink-husked types at the nursery seedling stage. Such a trait holds significant potential as a breeding marker, enabling the development of novel varieties with enhanced aesthetic appeal and nutritional benefits. Further studies are recommended to fully harness the potential of the pink-husk trait.

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