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# Comparative studies of pigment and nutrient contents of various castor genotypes

(Kajian perbandingan pigmen dan kandungan nutrien dalam pelbagai genotip kastor)

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**Abstract**

The selection of castor genotypes is an important factor for better growth and development of eri silkworm (*Philosamia ricini*) for higher productivity in terms of cocoon yield. This study has been made to find out the pigment and nutrient content of leaves of various castor genotypes (non bloomy green (NBG), non bloomy red (NBR) and DCS-107, DCH-519, DCH-177). The chlorophyll-*a* was significantly higher in tender leaves of DCH-177 (0.52 mg/g); in middle leaves of DCS-107(0.48 mg/g) and in mature leaves of NBR (0.46 mg/g) and chlorophyll-*b* was significantly higher in tender leaves of NBG (0.69 mg/g); middle leave of DCH-177(0.87 mg/g) and in mature leaves of DCH-177 (0.82 mg/g). Carotenoid content was significantly higher in tender leaves of NBG and DCH-177(0.19 mg/g); in middle leaves of DCH-177(0.18 mg/g) and also in the mature leaves of NBG (0.17 mg/g). The total protein and carbohydrate were highest in NBR (49.85 and15.87 g% respectively). The major nutrient content of castor genotypes viz. nitrogen, phosphorous, kalium were more in local NBR genotype in comparison to hybrid genotypes. The study reflects wide variability among different genotypes with respect to pigment and nutrient content.

Keywords: castor genotypes, *Ricinus communis*, eri silkworm, nutrient, pigment

## Introduction

Castor (*Ricinus communis* L.) belonging to the family Euphorbiaceae, is mainly cultivated as an intercrop with agricultural and horticultural crops. In India, castor is cultivated in an area of 10,96,000 ha with a production and productivity of 16,44,000 tonnes and 1500 kg/ha, respectively Manjunath and Sannappa (2014). Castor, being the primary host plant of non-mulberry silkworms i.e. eri silkworm (*Philosamia ricini*), the nutritional status of leaves has been considered as a major factor for their survival.Its leaf quality is of much importance to the healthy growth of worms and ultimately the cocoon harvest (Govindan et al. 2003 b; Pandey 1995). Better the quality of leaves, greater would be the chances of getting the good cocoon harvest (Ravikumar 1988). Directorate of Oil Seed Research (DOR), Hyderabad,India has been maintaining more than 2,856 accessions of castor in their germplasm bank. High yielding castor varieties/hybrids viz., Jyothi, GCH-5, GCH-4, DCH-519, DCH-177, DCH-32, etc. have been evolved and released for commercial exploitation (Nagraj 2002). Castor is rich in varietal composition and many local and high yielding varieties/hybrids are widely grown in Assam and other parts of the country (Sannappa 1997). The selection of castor genotypes is an important factor for better growth and development of eri silkworm for higher productivity in terms of cocoon yield. This study aimed at a comparative study of pigment and nutrient contents of leaves of

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various castor genotypes for sorting out the best castor genotype which in turn will give high cocoon yield of eri silkworm reared on it.

**Materials and methods**

The seeds of castor genotypes of local non bloomy green (NBG), non bloomy red (NBR) were collected from local areas and the hybrid genotypes DCS-107, DCH-519, DCH-177 were procured from the Directorate of Oilseeds Research, Rajendranagar, Hyderabad. The seeds of the five genotypes were planted during the last week of June in 2013 in Sambalpur University campus, Odisha, India which is located at latitude of 20.473ºN and longitude of 85.891″ E. The leaf samples at three different heights of the plant viz., top, middle and bottom, were collected in polythene bags at 90 days after sowing (Chandrashekhar et al.2013). Leaves were shade dried for three days (± 25 ºC) and then dried in hot air oven at 70 ºC until constant weight was obtained (Chandrashekhar et al. 2013). The dried leaf samples were ground into fine powder and preserved in butter paper bags for biochemical analysis

***Estimation of pigments***

For estimation of pigments, fresh leaves of different ages from each variety were taken in five replicates. Chlorophyll 'a', 'b', carotenoid and total clorophyll were estimated as per (Arnon 1949). For pigment estimation, 1 g of fresh green leaves were taken. These leaves were homogenised with acid washed sand and then with 10 ml of pre chilled 80% acetone. Centrifugation was carried out for 10 min. This process was carried out for 3 to 4 times and the each time supernatant (i.e. green solution) was collected in a measuring cylinder. It was kept inside an ice bucket. Absorbance was taken at 665 nm (total chlorophyll), 645 nm (chlorophyll-*a*), 663 nm (chlorophyll-*b*) and 475 nm (total carotenoid). The result was expressed in mg/g.

***Estimation of total protein***

Total protein content was estimated following the procedure of Lowry et al.(1951). For estimation of protein content, 1g of sample was taken and homogenised it with 10 ml of 10% TCA. The paste was centrifuged for 10 min in 4000 rpm. The supernatant was discarded and the residue was collected. It was dissolved in 1N NaOH solution. Then the extract was diluted 10 times with 0.3N NaOH solution for measurement. An amount of 0.2 ml diluted extract was taken and volume was made 2 ml by adding 1.8 ml distilled water. Then 3 ml of protein reagent was added. Then it was incubated for 30 min in room temperature. After incubation 0.5 ml of folin's reagent was added. Then incubated for 20 min in room temperature. Absorbance was taken at 750 nm. A standard graph was plotted by taking BSA (mg/ml) as standard and the protein content was calculated from the graph. It was expressed in gram percentage.

***Estimation of total carbohydrate***

Total carbohydrate content was estimated by phenol sulphuric acid method as per Dubois et al.(1956). A total of 1g of dry power sample was taken and homogenised with distilled water. Centrifuged at moderate speed for 10 min in 4,000 rpm and the supernatant were collected. The process was carried out 3 to 4 times and each time the supernatant was collected. Total volume of the supernatant was measured. From the collected supernatant 0.2 ml extract was taken and to it 0.8 ml distilled water was added. Then 1 ml of 18% phenol and 5 ml of sulphuric acid was added. Then, it was incubated for 20 min and absorbance was taken at 490 nm. Standard graph was plotted by taking glucose (µg/ml). The carbohydrate content was calculated from the standard graph. It was expressed in gram percentage.

***Estimation of total nitrogen***

Total nitrogen was measured by Kjeldahl method (Jackson1973). A total of 10 g of the air dried sample was weighted in a round bottomed Pyrex Kjeldahl flask and 30 ml of concentrated $H_2SO_4$ was added. Immediately 1g of salicylic acid was added (to include $NO_3^-$ N) and the reaction was allowed to place in cold for half an hour with occasional shaking. An amount of 5 g of sodiumthiosulphate was added and then a mixture of 8 g of kalium sulphate and 0.2 g of selenium were added to hasten the reaction. The flask was heated gently in fume chamber. When the frothing subside and the flask was fall of white fumes, raise the flame continue heating the flask strongly until the contents of the flask become colourless or pale yellow (pale straw). The flask was cooled and immediately 200 ml of hot distilled water were to prevent the crystallisation of the sulphate. At this stage $NH_3$ released due to disintegration reaction with $H_2SO_4$ to form $(NH_4)_2SO_4$. The supernatant or fluid part was collected to 1-litre flask. The sand residues were washed five to six times with hot distilled water and transferred to the flask. The distillation unit was set as per the protocol. The modified method involved the mixing of extract and alkali in a separate chamber which after reaction forces out $NH_3$ vapour due to the pressure exerted by a steam. The $NH_3$ is distilled in the condenser and collected in a flask containing 10 ml of 0.1N $H_2SO_4$ with 2 drops of methyl red indicator. Usually 10 ml of supernatant and 20 ml of 40 % NaOH was added through the funnel and heat was applied (alkali used here to neutralise the $H_2SO_4$ present during digestion). The $NH_3$ was collected in the receiving flask and was titrated against 0.1N NaOH. NaOH was to be standardised with 0.1N succinic acid to know its exact strength. A blank was run in the same manner. It was expressed in terms of percentages (%) of N in dried plant tissues.

***Estimation of total phosphorus***

Total phosphorus was estimated by colorimetric method (Murphy and Riley 1962). An amount of 1.0000 g + 0.0005 g of dried and ground plant tissue was weighted and transferred into 150-ml beakers. Digestion of samples was done using the wet oxidation procedure. Quantitatively the samples were transferred into 100-ml volumetric flasks and diluted with distilled water. Using a dilutor-dispenser, the samples was diluted and the 20, 40, 60, and 80 mg P/litre standards 1:100 with the working solution. It was allowed the colour to develop for at least 30 min before reading. Reading was recorded at 660 nm with a visible spectrophotometer. It was expressed in terms of percentages (%) of P in dried plant tissue.

***Estimation of total kalium***

Total kalium was determined by flame emission photometer (Hanlon and De Vore 1989) by taking 10 g of dry ashweight of tissue, the resulting product was wetted with a small amount of deionised water and brought into solution using 2 ml concentrated HCl. It was diluted to 100 ml final volume with deionised water. After bringing to final volume, the solution was mixed by inversion of the volumetric flask for several times. Serial dilutions were made until the kalium concentration reading was within the standardised range of the instrument using 0.1 to 0.3 MHCl diluents. The actual linear range of K is between 0 and 10 mg K/litre. However, commercial instrumentation can be programmed with three to five serial dilutions of the K standard to extend the upper limit of the working range to between 50 – 100 mg K/litre. It will be expressed in terms of percentages (%) of K in dried plant tissue. The data obtained were subjected to two way ANOVA in EL STAT, 2014.

**Results and discussion**

The data on biochemical content (chlorophyll, carotenoid, total protein, total carbohydrate, nitrogen, kalium and phosphorus) of leaves of five castor genotypes are provided in the *Table 1*.

The significance of difference in pigment content and nutrient content among genotypes and different parts of the leaves have been illustrated in *Table 2* and *3* respectively.

Table 1. The pigments content of tender, middle and matured leaves of different castor genotypes (mean ± SD). As we have to compare the average values of more than two types of leave parts, among five genotypes, it is better to go for ANOVA, as't' test is applicable when we have two compare the mean values of two variables

| Pigments | LEAVES | NBR | NBG | DCS-107 | DCH-177 | DCH-519 |
|---|---|---|---|---|---|---|
| Chlorophyll-*a* | Tender | 0.40 ± 0.07 | 0.50 ± 0.03 | 0.40 ± 0.05 | 0.52 ± 0.04 | 0.38 ± 0.07 |
| | Middle | 0.45 ± 0.03 | 0.47 ± 0.03 | 0.48 ± 0.04 | 0.35 ± 0.04 | 0.46 ± 0.01 |
| | Matured | 0.46 ± 0.05 | 0.44 ± 0.05 | 0.45 ± 0.04 | 0.41 ± 0.04 | 0.46 ± 0.04 |
| Chlorophyll-*b* | Tender | 0.30 ± 0.08 | 0.69 ± 0.08 | 0.11 ± 0.02 | 0.42 ± 0.06 | 0.16 ± 0.02 |
| | Middle | 0.68 ± 0.08 | 0.72 ± 0.08 | 0.30 ± 0.03 | 0.87 ± 0.10 | 0.66 ± 0.02 |
| | Matured | 0.66 ± 0.09 | 0.58 ± 0.07 | 0.21 ± 0.02 | 0.82 ± 0.09 | 0.75 ± 0.08 |
| Total Chlorophyll | Tender | 0.82 ± 0.10 | 1.19 ± 0.08 | 0.55 ± 0.06 | 0.94 ± 0.07 | 0.52 ± 0.08 |
| | Middle | 1.12 ± 0.07 | 1.18 ± 0.06 | 0.78 ± 0.07 | 1.17 ± 0.06 | 1.10 ± 0.03 |
| | Matured | 1.12 ± 0.08 | 1.04 ± 0.06 | 0.66 ± 0.05 | 1.23 ± 0.06 | 1.18 ± 0.08 |
| Carotenoid | Tender | 0.14 ± 0.05 | 0.19 ± 0.01 | 0.15 ± 0.02 | 0.19 ± 0.01 | 0.11 ± 0.05 |
| | Middle | 0.17 ± 0.01 | 0.17 ± 0.01 | 0.16 ± 0.01 | 0.18 ± 0.02 | 0.17 ± 0.01 |
| | Matured | 0.16 ± 0.04 | 0.17 ± 0.01 | 0.17 ± 0.01 | 0.17 ± 0.02 | 0.17 ± 0.02 |

Table 2. 'F' values of two-way ANOVA with replication to find out the significance difference of pigment contents between leaves of castor genotypes and leaves of different age

| Sources of variation | Chlorophyll –*a* | Chlorophyll-*b* | Total chlorophyll | Carotenoid |
|---|---|---|---|---|
| Castor genotypes | 3.41* | 118.18*** | 95.23*** | 3.68* |
| Age of leaves | 2.94 NS | 187.04*** | 172.78*** | 9.98*** |
| Castor genotypes × age of leaves | 11.17*** | 36.10*** | 37.11*** | 5.40** |

The symbol* indicates significant at $p < 0.05$ ** indicates significant at $p < 0.01$ , *** indicates significant at 0.001

Table 3. 'F' values of two-way ANOVA with replication to find out the significance difference of nutrient contents between leaves of castor genotypes and leaves of different age

| Sources of variation | Total protein | Total carbohydrate | Nitrogen | Kalium | Phosphorus |
|---|---|---|---|---|---|
| Castor genotypes | 2842.17*** | 20.12*** | 569.07*** | 258.10*** | 593.71*** |
| Types of leaves | 2445.34*** | 81.24*** | 13391.99*** | 1782354.61*** | 11322.92*** |
| Castor genotypes × Types of leaves | 1476.16*** | 13.88*** | 171.35*** | 10.47** | 29.81*** |

The symbol** indicates significant at $p$ <0.01, the symbol*** indicates significant at $p$ <0.001

***Pigment content***

Marked differences were observed among the leaves of castor genotypes with respect to pigment contents *viz*., chlorophyll *a*, *b*, total chlorophyll and carotenoid as shown in the *Table 1*. The maximum chlorophyll *a* was recorded in DCH 177 (0.52 mg/g) in tender leaves and minimum in the middle leaves of DCH 177 (0.35 mg/g). The difference of chlorophyll a were statistically significant among the different castor genotypes (F = 3.41, $p$ < 0.05). However, the difference between leave parts was not significant. The chlorophyll-*b*contents of tender, middle and mature leaves of castor genotypes have been represented in *Table 1*. Chlorophyll-*b* was significantly higher in tender leaves of NBG (0.69 mg/g); middle leave of DCH-177(0.87 mg/g) and in mature leaves of DCH-177 (0.82 mg/g). The difference in chlorophyll-*b* content was highly significant with respect to different castor genotypes (F = 118.18, $p$ <0.001). Significant variation was also observed between chlorophyll-*b* contentof tender, middle and mature leaves of castor genotypes (F = 187.04, $p$ <0.001). Total chlorophyll was significantly higher in tender leaves of NBG (1.19 mg/g); in middle leaves of NBG (1.18 mg/g) and mature leaves of DCH-177 (1.23 mg/g). The total chlorophyll content was highly significant with respect to different castor genotypes (F = 95.23, $p$ <0.001) and with respect to leavesof different age (F = 172.78, $p$ <0.001). The carotenoid content of different castor genotype leaves. The carotinoid content was 0.19 mg/g in tender leaves of NBG and DCH-177; 0.18 mg/gin middle leaves of DCH-177 and 0.17 mg/g in the mature leaves of NBG. Significant difference of carotinoid content among different castor genotypes (F = 3.68, $p$ <0.05) as well as between tender, middle and mature leaves (F = 9.98, $p$ <0.001) was observed in the present study. Chandra et al. (2000) observed variation in chlorophyll '*a'*, '*b*' and chlorophyll a/b ratio in leaves of host plants of eri silkworm. Chandrashekhar et al. (2013) also reported variation in chlorophyll- *a,b* and total chlorophyll content of leaves of castor genotypes. The local varieties in the present study showed maximum pigment in tender leaves. However, the pigment content of middle and matured leaves were highest in hybrid varieties. We fed matured leaves to the 5$^{th}$ in star larvae which are at the verge of forming cocoon. In this aspect the hybrid variety of castor leaves have much contribution towards the production of cocoons in comparison to local varieties.

***Total protein***

*Figure 1* indicates the total protein content of leaves (tender, matured) of different castor genotypes. The result indicated that

total protein content varied markedly among the leaves of different castor genotypes. Notably higher protein content was recorded in leaves of NBR (13.39 and 49.85 g%) in comparison to other genotypes. The total protein content was highly significant with respect to different castor genotypes ($F = 2842.17$, $p < 0.001$) as well as between tender and matured leaves ($F = 2445.34$, $p < 0.001$). Kaleemurrahaman and Gowri (1982); Basaiah (1988_; Sannappa and Jayaramaiah (2002); Govindan et al. (2003a, b); Chandrappa et al. (2005) recorded variation in crude protein content among castor genotypes raised under rain fed situation. Protein content in leaf is a major source for silkworm to synthesise the silk which consists of two proteins namely fibroin and sericin (Rangaswami et al. 1976).

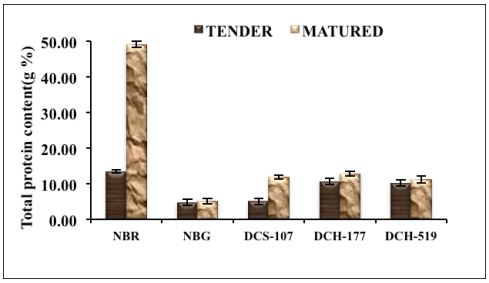


*Figure 1. Total protein content (g %) of tender and mature leaves of different castor genotypes*

***Total carbohydrate***

Significant variations also observed in total carbohydrate content among the leaves of various castor genotypes (*Figure 2*). The local NBR showed highest (1.59 and 15.87 g%) and DCH-177 lowest (0.45 and 1.02%) carbohydrate content. The total carbohydrate content was highly significant with respect to different castor genotypes ($F = 20.12$, $p < 0.001$). Significant variation was observed within the types of leaves ($F = 81.24$, $p < 0.001$). Sugar plays an important role in determining the quality of leaf that in turn influences growth, development and health of silkworm. Kaleemurrahaman and Gowri (1982); Basaiah (1988); Sannappa and Jayaramaiah (2002); Govindan et al. (2003a, b) and Chandrappa et al. (2005) also observed variation in the total carbohydrate content among the castor genotypes. As per Horie (1978), more carbohydrate content in leaves is helpful in producing fat body glycogen in eri silk worm which in turn increases the trehalose content in haemolymph.

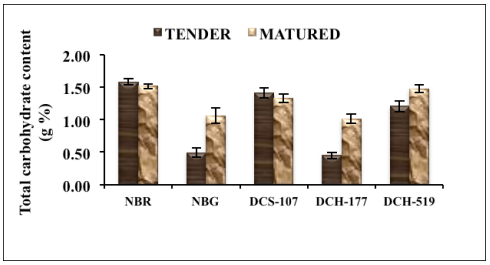


*Figure 2. Total carbohydrate content (g%) of tender and mature leaves of different castor genotypes*

***Total nitrogen***

Marked variations were noticed for nitrogen content among the leaves of different castor genotypes (*Figure 3*). In leaves (tender, matured) of NBR variety showed significantly highest nitrogen content (4.97 and 5.375% in tender and mature leaves respectively). Lowest nitrogen content was found in DCH-519 (0.97 and 1.11%). The total nitrogen content was highly significant with respect to different castor genotypes ($F = 569.07$, $p < 0.001$). Further, it was observed that the nitrogen content was highly significant within the types of leaves ($F = 13391.93$, $p < 0.001$). Shankar (1997) opined that nitrogen content in leaf influences the quality of leaf especially its protein content, in addition, it also controls the plant reproduction cycle. In this connection the local variety of castor genotypes is a good choice as a feed for eri silk worm.

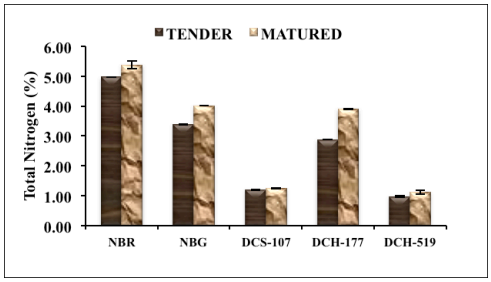


*Figure 3. Total nitrogen content (g%) of tender and mature leaves of different castor genotypes*

***Total kalium***

The kalium content (*Figure 4*) varied significantly among leaves of selected castor genotypes with more being in local NBR genotype leaves both in tender and matured (4.02 and 4.03%). Less kalium content was found in leaves of DCH-177 (0.12 and 0.15%). Total kalium content was highly significant with respect to different castor genotypes (F = 258.1039, $p$ <0.001) and also within the types of leaves (F = 1782354.61, $p$ <0.001). Shankaret al. (1999) suggested that the silkworms fed on leaves with higher content of kalium are known to increase the body weight of silkworm consequently enhances the cocoon production.

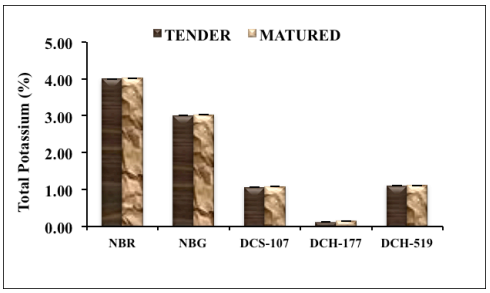


*Figure 4. Total kalium content (g %) of tender and mature leaves of different castor genotypes*

***Total phosphorus***

Notable difference existed in total phosphorus (*Figure 5*) among the castor genotypes with significantly highest content in leaves of local NBR (tender and matured) (0.53 and 0.611%). Lowest phosphorous content was found in tender and matured leaves of DCH-177 (0.09 and 0.12%). That total protein content was highly significant with respect to different castor genotypes (F = 593.71, $p$ <0.001) and it was observed that there was highly significant variation within the types of leaves (F = 11322.92, $p$ <0.001). Ray et al. (1973) suggested that the presence of higher phosphorus content in leaves enhances the levels of total sugars, besides responsible for improving the growth and development of silkworm larvae.

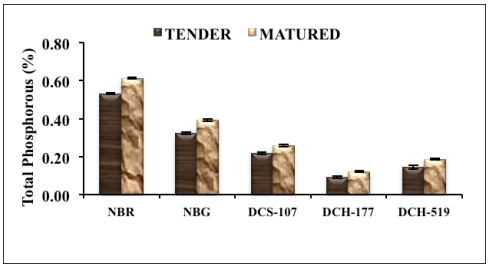


*Figure 5. Total phosphorous content (g %) of tender and mature leaves of different castor genotypes*

**Conclusion**

The present study showed that the nutritive value differed in different castor genotypes and in leaves types. The nutritive value/composition of leaves depends on the host plant variety and environmental conditions such as growing season, temperature, duration of sunshine hours and nature and type of soil, manures, fertilisers, soil moisture and method of leaf harvest, etc. Since the nutrition has been known to influence the growth as well as cocoon traits, it is necessary that certain care needs to be taken in selection of castor genotypes leaves to be fed to the worms to put up healthy growth and in turn to obtain better cocoon yield. The selection of castor genotype is an important criterion for better growth and development of eri silkworm for higher productivity in terms of cocoon and egg production. Almost all insects are host

specific and select their most preferred food in order to extract the maximum benefit out of it, although most of them eat, a great many of varieties (Brues 1946).
The variation in the nutrient content could be one of the attributable reasons for variability in the suitability of the castor genotypes as food of eri silkworm. The climate, edaphic factors of the plantation area seem to be unfavourable for the hybrid variety. On the basis of different nutrient contents of different castor genotype the present study strongly emphasised on the local NBR castor variety to be a better choice as a feed for eri silk worm.

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**Abstrak**

Pemilihan genotip kastor merupakan faktor penting untuk pertumbuhan dan pembangunan eri ulat sutera (*Philosamia ricini*) yang lebih baik dalam meningkatkan produktiviti dari segi hasil kepompong. Kajian ini telah dibuat untuk mengetahui pigmen dan kandungan nutrien daun dari pelbagai genotip kastor (*non bloomy green* (NBG), *non bloomy red* (NBR) dan DCS-107, DCH-519, DCH-177). Kandungan *klorofil-a* adalah ketara lebih tinggi dalam daun *tender* DCH-177 (0.52 mg/g); dalam daun tengah, DCS-107 (0.48 mg/g) dan dalam daun matang, NBR (0.46 mg/g) dan kandungan *klorofil-b* adalah jauh lebih tinggi dalam daun *tender* NBG (0.69 mg/g); daun tengah, DCH-177 (0.87 mg/g) dan dalam daun matang, DCH-177 (0.82 mg/g). Kandungan karotenoid adalah ketara lebih tinggi dalam daun *tender* NBG dan DCH-177 (0.19 mg/g); dalam daun tengah, DCH-177 (0.18 mg/g) dan juga dalam daun matang NBG (0.17 mg/g). Kandungan protein dan karbohidrat adalah paling tinggi dalam NBR (masing-masing 49.85 dan 15.87 g%). Kandungan nutrien utama genotip kastor, iaitu nitrogen, fosforus, kalium adalah lebih dalam genotip NBR tempatan berbanding dengan genotip hibrid. Kajian ini mencerminkan kepelbagaian luas antara pelbagai genotip berkenaan dengan pigmen dan kandungan nutrien.