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Identification of High Beta Carotene Lines in F_5 Generation of Pearl Millet [*Pennisetum glaucum* (L.) R. Br.]

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A B S T R A C T

Pearl Millet is a principal food cereal cultivated in drought prone semi-arid regions of Africa and Indian subcontinent. The present investigation was carried out in F_5 generation of pearl millet population at Department of Millets, Tamil Nadu Agricultural University, Coimbatore-03. Attempts were made to estimate total carotenoids and β carotene content of single cross combination, PT 6029 x PT 6129 and its selected promising progenies of F_5 generation. The parent PT 6129 showed higher total carotenoids (8.99 µg/g) and β carotene content (1.71 µg/g) than the other parent and it is having yellow grain colour, while, the parent PT 6029 recorded grey colour grain with low β carotene content (0.61 µg/g) which indicated that yellow colour contributed to β carotene content. The progenies, TNBG-06-53-1-4-7 recorded high total carotenoids (8.10 µg/g) and β carotene (1.73 µg/g) followed by TNBG-06-81-10-9-9, TNBG-06-127-3-3-1, TNBG-06-81-4-8-6 and TNBG-06-3-3-6-1. Hence, these progenies provide a valuable basis for increasing β carotene through conventional breeding.

Introduction

Pearl millet is one of the sixth most important cereal crops grown globally and is being grown in arid and semi arid regions of the world including West Africa, India and Pakistan with the rainfall ranging from 150-700 mm (Rai *et al.,* 2009). In India it is the fifth most important grain crop next to rice, wheat, maize and sorghum (Singh and Sehgal, 2008).

Pearl millet has more than 16 times the iron content of rice and is primarily consumed in the states of Haryana, Rajasthan, Gujarat and Madhya Pradesh in India and Sahel, northern Nigeria, Niger, Mali and Burkina Faso, Namibia of Africa. Pearl millet is a good source of fat soluble vitamins with a vitamin A content of 0.22mg/100g (Aykroyd *et al.*, 1963 and Adrian and Jacquot, 1964). A golden pearl millet germplasm originating from Burkina Faso has been identified at ICRISAT having ß-carotene level of 137 µg/100g grain, which is comparable to that in golden rice (Rai *et al.,* 2009). The scientists at ICRISAT found that these yellow coloured pearl millet grain and they called it as naturally available golden millet. Golden millet is thus an existing new alternative that deserves further deployment. Pearl millet grains provide a low-cost solution to

combating malnutrition. Also, it has additional health related advantages because of its higher levels of insoluble dietary fibre and more balanced amino acid profile. Thus, pearl millet with enhanced nutritional quality could contribute significantly to improve the nutritional value of the diets of people, who dependent on pearl millet as a major energy source.

Materials and Methods

The experimental material includes two hundred progenies of F_5 generation which were developed from the cross PT 6029 × PT 6129. The materials for the field experiments were obtained from the Pearl millet unit at the Department of Millets, Tamil Nadu Agricultural University, Coimbatore. The parent PT 6129 is a golden millet parent has yellow coloured grains contain highest amount of β carotene (2.417 µg/g) and the other parent PT 6029 is grey in grain colour and has a good agronomic value which showed low β carotene content of 0.059 µg/g (Plate 1) (Aarthy *et al.*, 2011).

At F_4 generation, ten plants were selected based on grain colour, compactness of ear head and other agronomical features. These selected plants were received from pearl millet unit at Department of Millets at TNAU, Coimbatore forwarded to F_5 generation during *kharif* 2012. The single ear head of each plant were raised in a single progeny row as per ear to row method. The spacing was maintained at 45 x15 cm. Observations on total carotenoids and β carotene content were estimated for ten plants which were selected in F_5 generation based on their grain colour, compactness of ear head and other agronomically desirable features along with parents (Plate 2). The details of the selected individual progenies with pedigree are presented below.

Details of selected progenies in F_5 generation for β carotene estimation

Sl. No	Selected plants in F_5 generation
1	TNBG-06-3-3-6-1
2	TNBG-06-3-7-9-4
3	TNBG-06-53-1-4-7
4	TNBG-06-53-2-4-8
5	TNBG-06-53-6-4-10
6	TNBG-06-81-1-1-4
7	TNBG-06-81-4-8-6
8	TNBG-06-81-5-4-2
9	TNBG-06-81-10-9-9
10	TNBG-06-127-3-3-1

Extraction of carotenoids from pearl millet grains

Extraction of carotenoids was done based on the principle of liquid-liquid extraction using separation funnel. The carotenoid extract prepared as per the Harvest plus method given by Rodriguez Amaya and Kimura, (2004) was used for estimation of total carotenoid content with spectrophotometer (450 nm) and β-carotene content using HPLC. Beta carotene is sensitive to light, oxygen and temperature so the extraction procedure was carried out in dim light at low temperature, never with direct light**.** The extract of the samples was analyzed by Spectrophotometer and HPLC just after extraction.

Representative samples of pearl millet grains were homogenized into powder and 3 g of ground representative sample were weighed. The extraction was done in dark at low temperature as β carotene is light sensitive and to prevent oxidation of carotenoids. The weighed ground sample of 3g was hydrated at room temperature with 10 ml of water for 30 min. Ice cold acetone of 20 ml was added to it and left for 15 min to precipitate proteins. Further 50 ml acetone was used to grind the extract using pestle and mortar. The extract was filtered through Buchner funnel equipped

with Whatman filter paper No 2. One third of the filtrate is added to 20 ml of petroleum ether in separating funnel and shaken well by adding 300 ml of water. Partition of petroleum ether to organic phase and acetone, water to aqueous phase occurs, thereby carotenoids get partitioned to organic phase and proteins get precipitated in acetone. The aqueous phase was discarded and organic phase was collected. The process was repeated two times for the entire filtrate so that the carotenoids extract in acetone gets partitioned to petroleum ether phase. The carotenoids in petroleum ether was obtained as light yellow colour extract which is washed with water of 200ml in separating funnel for three times to remove traces of acetone. It is then passed over funnel containing 25 g Anhydrous Sodium sulphate to remove traces of water.

Quantification of total carotenoids

The absorbance of blank (petroleum ether) and the extract was taken at 450 nm using UV- Spectrophotometer and the total carotenoid content was calculated using absorption coefficient of 2500 that is recommended for mixtures.

Extraction of β carotene

The extract of total caroteniods obtained in petroleum ether was concentrated in Rotary Evaporator at about 35ºC. It was then reconstituted using HPLC grade methanol of 2ml and filtered through 0.22µm PTFE (Poly Tetra Fluoro Ethylene) syringe filter directly into sample vials. The samples were then sonicated using sonicator to remove dissolved air bubbles. Samples were kept at 4ºC to avoid degradation of carotenoids.

Preparation of standards

The standard of β carotene was purchased from Sigma Inc, USA. β carotene standard solutions were prepared from range 1-1000 ppm concentrations by dissolving them in HPLC grade acetonitrile and filtered through 0.22µm PTFE (Poly Tetra Fluoro Ethylene) syringe filter directly into sample vials. It was then stored at 4ºC.

Screening of β carotene content through HPLC

The analysis of the extract was done in High Performance Liquid Chromatography system (HPLC) equipped with LC8A pump, SPD-M 10 A vp diode Array Detector in combination with Class LC 10 A software (Shimadzu). The chromatographic conditions used for the HPLC analysis as per the Harvest plus protocol were followed. The mobile phase was HPLC grade of Acetonitrile: Ethyl acetate: Methanol (80:10:10) and stationary phase column of ODS (Octadecyl silane) C18, 5µ size, Detector: SPD-M 10 A vp diode array detector with wave length of 450 nm, flow rate of 1ml/min, injection volume of 20µl. The solvents were sonicated for two cycles in sonicator to break intermolecular interactions. HPLC analysis was performed with Acetonitrile at pump B and equal mixture of Ethyl acetate and Methanol at pump A in the ratio 40:60.

The chromatogram of HPLC shows the Retention time, Area and the Wavelength absorbed readings. Instrument baseline was stabilized before starting the actual run and it was checked frequently after injecting 5 samples. The standard concentration of 10µg, 1µg were injected initially and were run for a period of 30 min. The samples were then injected followed by the standards. The presence of β carotene in the sample was detected by comparing with the retention time of the standard. The area of the standard was compared with the area of the sample and the amount of β carotene in the extracts was calculated. Observations were recorded and β carotene content was calculated.

Calculation of total carotenoid and β carotene concentrations

Total carotenoids and β carotene concentration in the samples were calculated by the following formula

Total carotenoid content (µg/g) =

$$\frac{A_{total} \text{ X Volume of extract (ml) X } 10^4}{A^{1\%}_{1cm} \text{ X Sample weight (g)}}$$

Where,

A_{total} = absorbance;
$A^{1\%}_{1cm}$ = absorption coefficient of 2500, which is recommended for mixtures.

β carotene concentration was calculated using the formula given by Harvest plus protocol

$$C_x\ (\mu g/g) = \frac{A_x . C_s . \text{Total Volume of extract}}{\text{Sample weight in g} . A_s}$$

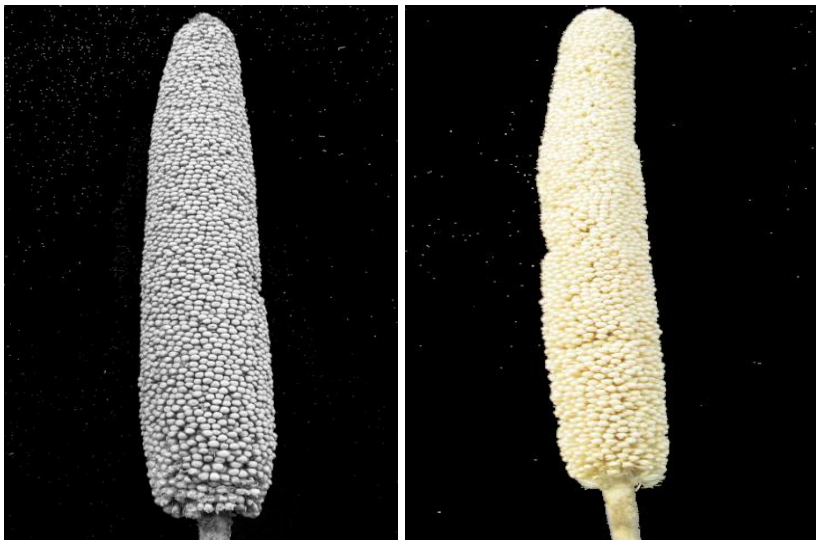
C_x – Concentration of β carotene
A_x – peak area of β carotene
C_s – concentration of the standard
A_s – peak area of the standard

Results and Discussion

Total carotenoid and β carotene content (µg/g) was estimated for parental lines and ten selected progenies. The parents PT 6029 and PT 6129 recorded 3.15 µg/g and 8.99 µg/g of total carotenoid content, respectively. Among the progenies, the highest total carotenoid content was observed in TNBG-06-53-1-4-7 (8.10 µg/g) and lowest content was recorded by the plant TNBG-06-81-1-1-4 (3.29 µg/g). The total carotenoid content was ranged between 3.29 µg/g to 8.10 µg/g.

The β carotene content was also estimated for parents and the ten selected progenies. The parents, PT 6029 and PT 6129 recorded 0.61 µg/g and 1.71 µg/g (Figs. 1 and 2) of β carotene content, respectively. Among the progenies, the highest content of β carotene was recorded by the progeny TNBG-06-53-1-4-7 (1.73 µg/g) (Fig. 3) followed by TNBG-06-81-10-9-9 (1.68 µg/g) (Fig. 4), TNBG-06-127-3-3-1 (1.67 µg/g) (Fig. 5), TNBG-06-81-4-8-6 (1.63 µg/g). The lowest β carotene content was recorded by the progeny TNBG-06-81-1-1-4 (1.29 µg/g) (Fig. 6).

Plate.1 Earheads of selected parents



PT 6029 **PT 6129**

TNBG -06-81-10-9-9

TNBG-06-3-7-9-4

TNBG-06-81-5-4-2

TNBG-06-53-1-4-7

Plate.2 Progenies with high β carotene content in F_5 generation

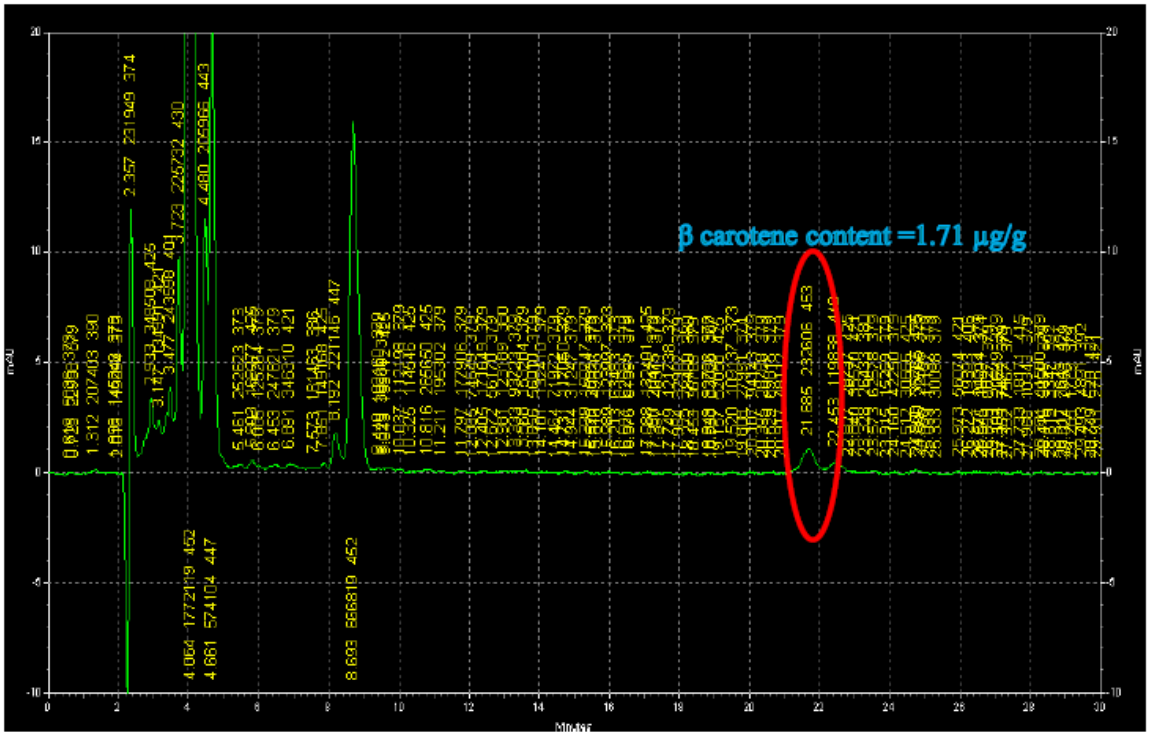


Fig 1. HPLC chromatogram for β carotene content in parent PT 6129

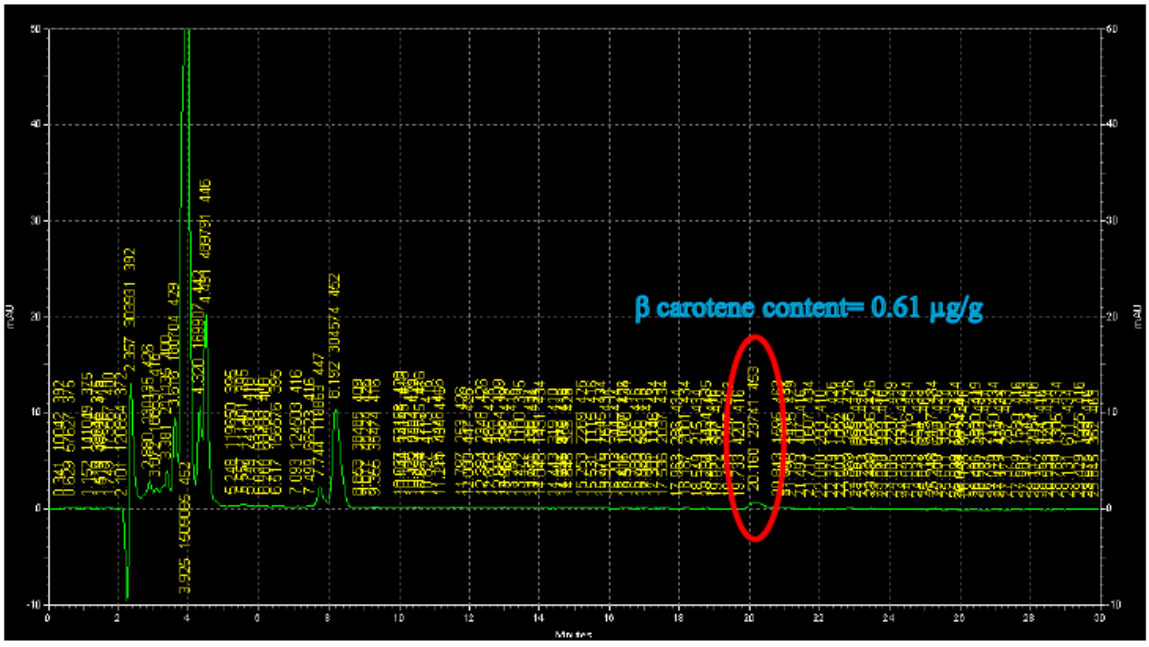


Fig 2. HPLC chromatogram for β carotene content in parent PT 6029

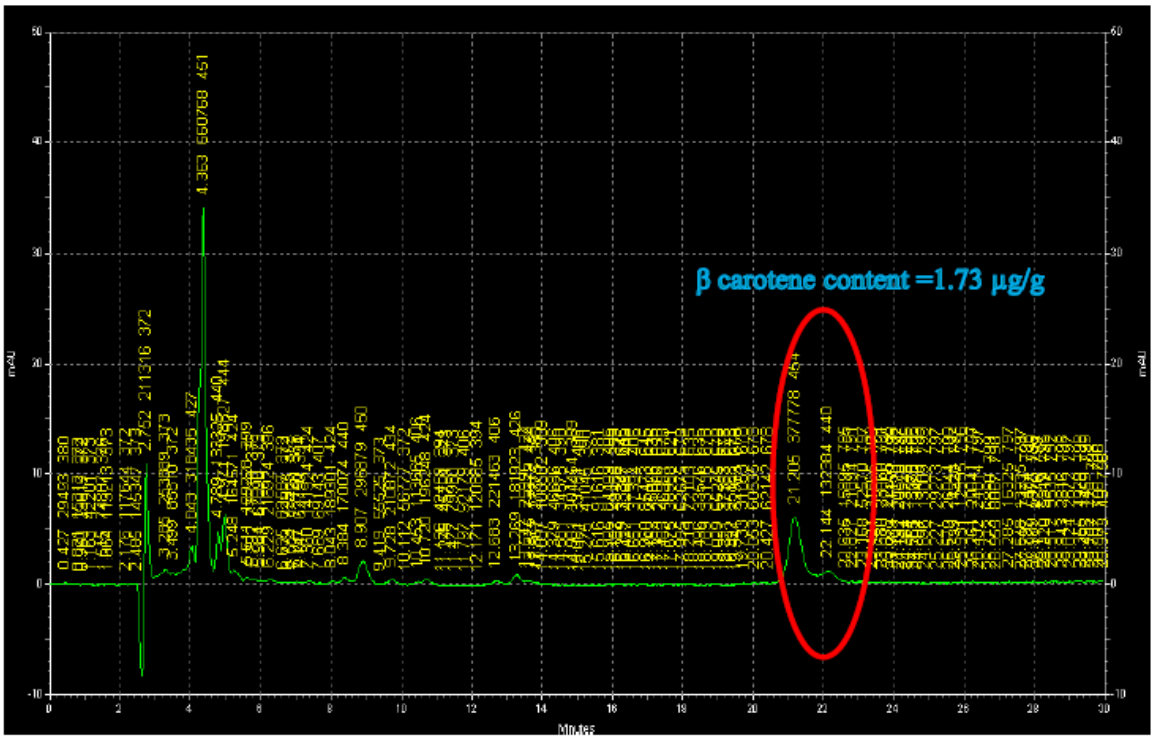


Fig 3. HPLC chromatogram for β carotene content in progeny TNBG-06-53-1-4-7

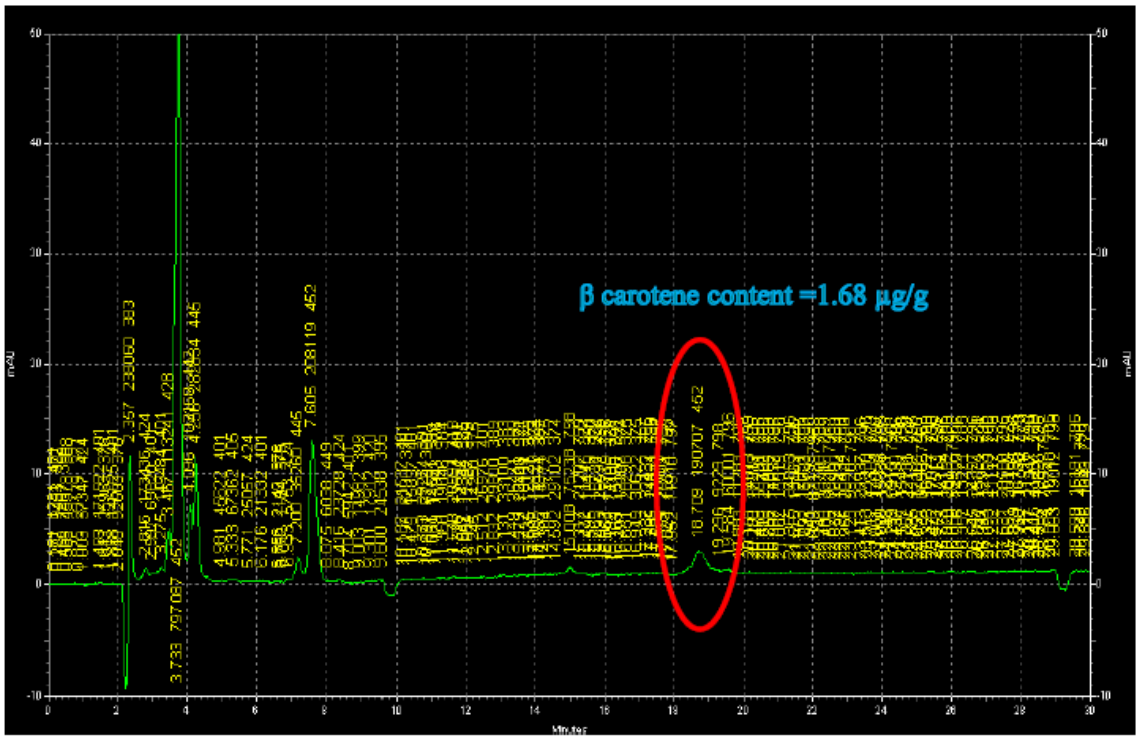


Fig 4. HPLC chromatogram for β carotene content in progeny TNBG-06-81-10-9-9

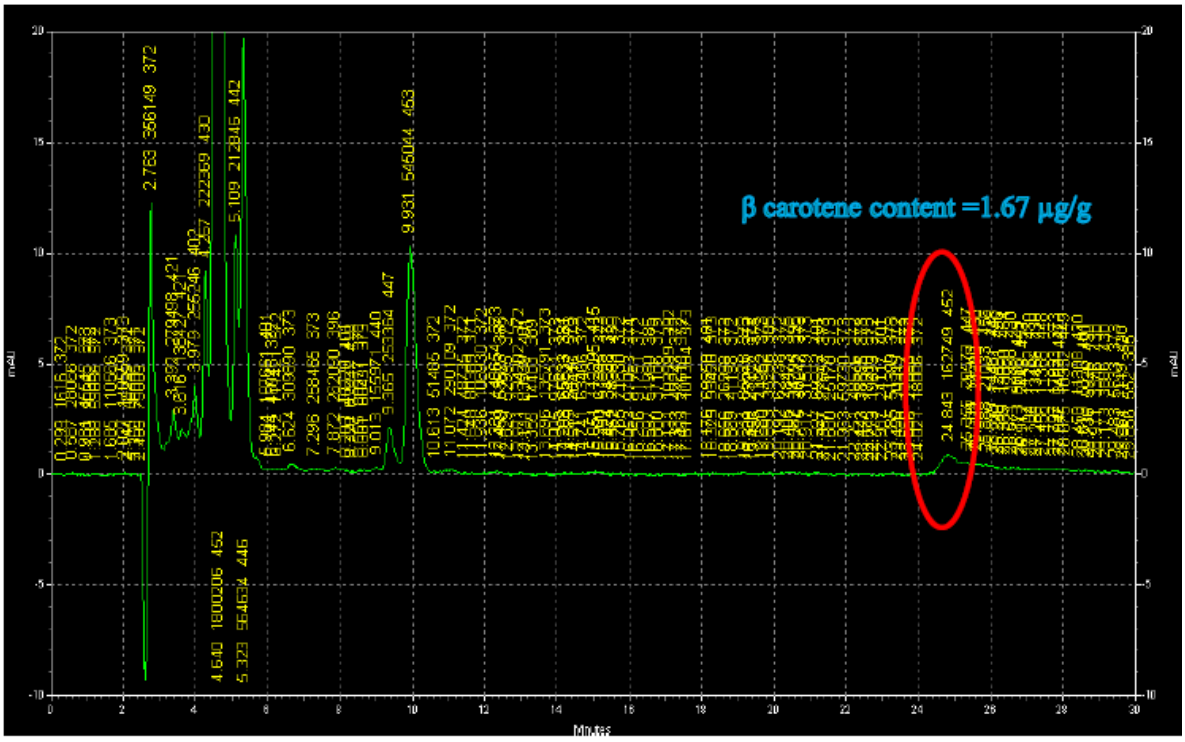


Fig 5. HPLC chromatogram for β carotene content in progeny TNBG-06-127-3-3-1

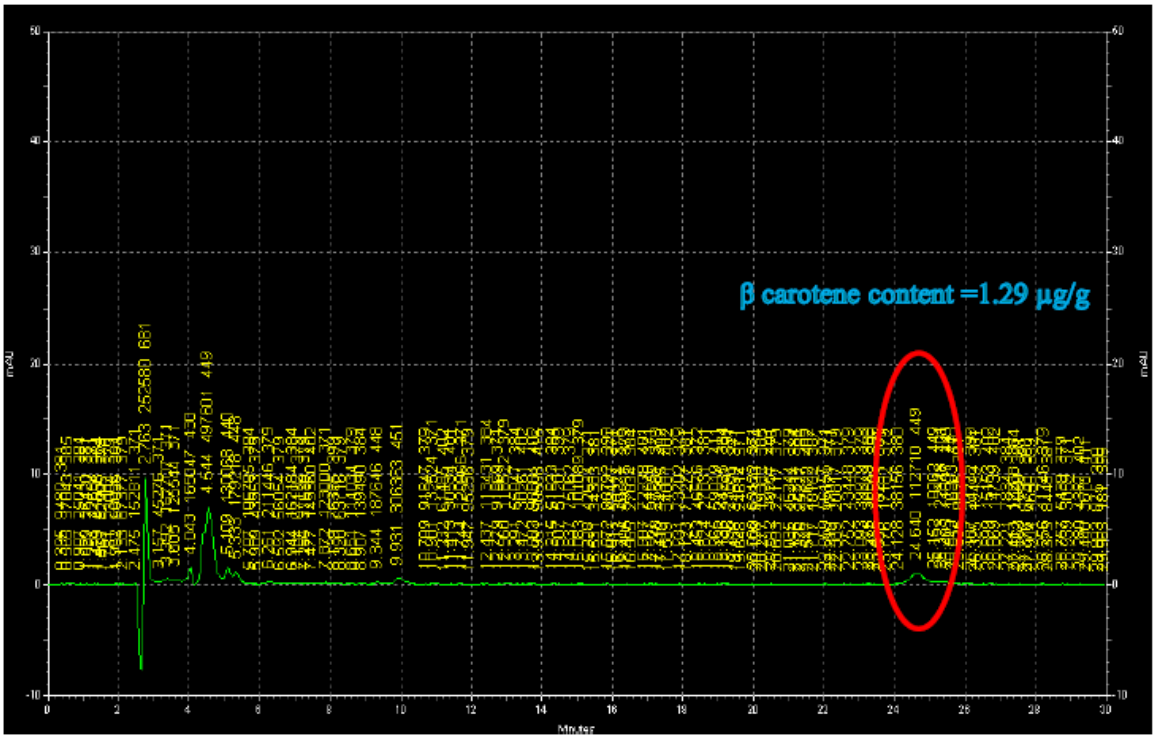


Fig 6. HPLC chromatogram for β carotene content in progeny TNBG-06-81-1-1-4

The selected progenies in F_5 generation, showed variations for grain colour *viz.*, cream, yellow and yellow brown grain colour. The progeny, TNBG-06-53-1-4-7 recorded high total carotenoids (8.10 µg/g) and β carotene (1.73 µg/g) which showed yellow brown grain colour followed by TNBG-06-81-10-9-9 (yellow brown grain colour), TNBG-06-127-3-3-1 (yellow brown grain colour), TNBG-06-81-4-8-6 (yellow grain colour) and TNBG-06-3-3-6-1 (yellow brown grain colour). The β carotene content was ranged between 1.29 µg/g and 1.73 µg/g among the progenies. This provides a valuable basis for increasing β carotene through conventional breeding and was in accordance with the findings of Khangura *et al.,* (1980), Aarthy *et al.*, (2011) and Sowmiya (2012).

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