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Research Article

Estimates of genetic parameters and quantification of β carotene in Pearl Millet (*Pennisetum glaucum* L.) segregating population

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Abstract
An attempt was made to study the genetic variability and inter-relationship analysis in F_3 and F_4 generations for ten quantitative characters. The phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV) for all the characters studied in both F_3 and F_4 generation, indicating environmental influence on expression of all characters. High heritability combined with high genetic advance for the trait ear head girth and moderate heritability with high genetic advance noticed in single ear head grain weight and grain yield per plant indicated that these characters were governed largely through additive effects of genes and improvement in these traits may be achieved through phenotypic selection. Association studies revealed that, selection based on the traits, single ear head grain weight, ear head girth, single ear head weight, number of productive tillers per plant, plant height and grain yield per plant will be effective. In path coefficient analysis, single ear head grain weight and total number of productive tillers showed direct effect to account for yield. The effect through ear head girth, single ear head weight and 1000 grain weight was high in F_3 and F_4 generations indirectly. These revealed that the true relationship of these characters with grain yield. Selection based on these characters may be helpful in planning efficient breeding programme. Total carotenoids and β carotene content was estimated in parents and ten F_4 progenies. Among them, five progenies *viz.*, TNBG-06-81-9-13 (1.698 µg/g), TNBG-06-81-10-9 (1.624 µg/g), TNBG-06-127-3-3 (1.585 µg/g), TNBG-06-53-6-6 (1.460 µg/g) and TNBG-06-81-7-10 (1.420 µg/g) were identified for high β carotene and these lines were used for future breeding programme for producing high yield with β carotene rich pearl millet inbreds.

Key words
Pearl millet, β carotene,Genetic variability, Correlation and Path analysis

Introduction

Pearl millet (*Pennisetumglaucum* (L.)R. Br.) is a cross pollinating, diploid (2n=2x=14) annual crop and belongs to section Paniceae of family Poaceae. It is world's sixth and India's fourth important cereal crop and one of the most suitable dual purpose, drought resistant fodder crop; its cultivation extends widely from well irrigated area to the most arid regions of the world in Asia and Africa (Kapila *et al.*, 2008). Pearl millet grain has higher levels of nutritional value and it has high protein content with balanced amino acids, carbohydrate and fat which are important in the human diet, and its nutritive value is considered to be comparable to rice and wheat. It is also rich in B vitamins, potassium, phosphorus, magnesium, iron, zinc, copper and manganese. Besides, Pearl millet contain sufficient amount of β carotene which is the precursor of vitamin A (Curtis *et al.*, 1966). Vitamin A deficiency (VAD) causes about 70 percent of childhood deaths worldwide and blindness in 0.25-0.50 million children every year.Vitamin A is an important micronutrient that plays vital role in vision, growth and development of humans. Vitamin A Deficiency, widely prevalent worldwide, affects people predominantly dependent on cereals diet that are mostly deficient in β carotene (Vignesh *et al.*, 2014). A stable food with rich β carotene would be the easiest way to alleviate vitamin A deficiency which is one of the most important nutritional problems in developing countries.Grain yield as a character is quantitative in nature and is polygenetically controlled. Selection on the basis of grain yield character alone is usually not very effective and efficient.The nature and magnitude of variability, genetic variations among yield attributes, inter relationship of different characters are required to initiate an effective breeding programme (Izge *et al.*, 2006).

Correlation and path analysis aid in the selection of superior genotypes from the breeding population. The correlation between yield and other component traits should therefore be given importance in the selection of genotypes for more yield in pearl millet. The partitioning of correlation coefficient into the measure of direct and indirect effects determines their contribution towards yield.

The present investigation was conducted with the objectives to study the genetic parametersin the F_4 population which was developed involving beta carotene lines and to identify agronomically superior progeny with rich β carotene for developing β carotene rich inbreds in biofortification breeding.

Materials and methods

Two hundred F_3 progenies were chosen for the present studies which were derived from a cross between PT 6029 × PT 6129 to develop RIL population for β carotene. The parent PT 6129 (yellow grain type) contained a high amount of β-carotene content of 1.71 µg /g and PT 6029 (grey grain type) contained low β-carotene content of 0.61µg/g. The 200 F_3 progenies were raised during summer 2011 and were forwarded to F_4 generation during *kharif* 2011 at Department of Millets, Centre for Plant Breeding and Genetics, TamilNadu Agricultural University, Coimbatore, which lies between 11° North latitude and 77° East longitude. All the recommended package of practices was carried out to grow a healthy crop. Each selfed ear head was raised in a single progeny row with spacing of 45 cm between rows and 15 cm between plants. Ten plants were selected randomly in each row for recording observation in both the F_3 and F_4 generations. Data were recorded on tenquantitative characters *viz*., days to 50 per cent flowering (days), days to maturity (days), plant height (cm), number of productive tillers per plant (nos.), ear head length (cm), ear head girth (cm), single ear head weight (g), single ear head grain weight (g), 1000 grain weight (g) and grain yield per plant (g). The various variability parameters were worked out as per Burton (1952). The broad sense heritability and genetic advance was estimated according to Johnson *et al.* (1955), correlation and Path analysis (Deway and Lu, 1959) were calculated as per statistical method.

In F_4 generation, ten plants were selected based on grain colour and ear head compactness to β carotene analysis. Three qualitative characters *viz*., grain colour, total carotenoid content and β carotene were recorded in these selected progenies. Total carotenoid and β carotene estimation was done in triplicate to minimize handling error.

Extraction of carotenoid and quantification of β-carotene content: Representative samples of pearl millet grains were homogenized into powder and 3 g of ground representative samples were used for the assay. Since β-carotene is sensitive to light, oxygen and temperature, the extraction was done in dark at low temperature to prevent oxidation of carotenoids. The extraction was done as per the Harvest plus method (Rodriguez Amaya and Kimura 2004). Estimation of total carotenoid content was done with a spectrophotometer (450 nm) and quantification of β-carotene content was done using RP-HPLC (Shimadzu HPLC Analytical C18G 120A column 250 x 4.6 mm). Samples were eluted through YMC Carotenoid C18 column and detected with a photodiode array detector. The mobile phase consisted of Acetonitrile: Methanol: Ethyl acetate (80:10:10) at high pressure through the column, and the flow rate was 1 ml/min. The standard for β-carotene was purchased from M/s. Sigma Aldrich India, reconstituted in acetone to five different concentrations (0.1 µg/g; 1µg/g; 10µg/g; 50 µg/g; 100 µg/g) (Kurilich and Juvik 1999) and were used to make the standard curve for β-carotene. The concentration of β carotene in each inbred was measured by standard regression with external standards. Absorbance was measured at 453 nm to maximize the detection of β-carotene. The chromatogram was compared with that of the standards in order to calculate the amount of β-carotene in the samples.

Result and discussion

A wide range of variation was observed for all the traits studied (Table 1). In general, the mean value for almost all the traits in F_4 progenies was higher than the F_3 progenies in desirable direction. This shows the improvement of the traits in the advanced generation. The phenotypic and genotypic coefficients of variation are the measures of phenotypic and genotypic variances, respectively. In general, phenotypic coefficient of variation was found to be higher than genotypic coefficient of variation for all characters.

The phenotypic, genotypic variances, GCV and PCV were higher in F_3 generation than in the F_4 generation for all the traits studied except days to 50 per cent flowering. The PCV was higher than GCV for all the ten quantitative characters in F_3 and F_4 generations indicating higher environmental influence on expression of all the traits. The highest value of genotypic coefficients of variation was noted for single ear head grain weight (22.34 g and 19.85 g) and grain yield per plant (30.82 g and 24.51g) in F_3 and F_4 generation respectively. This revealed that the variation for these characters contributed markedly to the total variability. Similar result was reported by Kale *et al.,* 2011. Similarly moderate PCV and GCV was observed for the traits number of productive tillers per plant, ear head girth, 1000 grain weight, single ear head weight and plant height in F_3 and F_4 generations. Selection will be effective based on the heritable nature of these traits. Moderate PCV and Low GCV were observed for the trait ear head length in F_3 and F_4 generations. Low PCV and GCV were observed for the trait, days to maturity and days to 50 per cent flowering in F_3 and F_4 generations and hence selection based on these traits may not be rewarding. This was in accordance with the results of Meenakumari and Nagarajan, 2008 for days to 50 per cent flowering.

The estimate of heritability of a character provides a measure of the effectiveness of selection for that character. The high heritability was observed for plant height (67.99 and 62.81) and ear head girth (65.55 and 62.95) in both the populations (F_3 and F_4) whereas the remaining characters showed moderate heritability. Heritability alone will mislead the selection; hence, genetic advance should also be taken into consideration along with heritability during selection programme. High heritability combined with high genetic advance was observed for the trait earhead girth and moderate heritability with high genetic advance was noticed in single ear head grain weight and grain yield per plant. This indicates that these characters were governed largely through additive effects of genes and improvement in these traits may be achieved through phenotypic selection. A high estimate of genetic advance for grain yield was reported by Solanki *et al*. (2002).Days to 50 % flowering and days to maturity showed moderate heritability with low genetic advance. Such a situation may be caused by non-additive gene action *i.e.* dominance and epistasis. Hence considering the GCV, PCV, heritability and genetic advance as percent of mean, plant height and grain yield per plant were found to be better performing traits in both F_3 and F_4 generation with less environmental influence and selection based on these traits would be effective for future pearl millet breeding.

In association study grain yield showed significant and positive association with plant height (0.476 and 0.254), number of productive tillers per plant (0.568 and 0.480), ear head length (0.195 and 0.234), ear head girth (0.759 and 0.707), single ear head weight (0.740 and 0.682), single ear head grain weight (0.854 and 0.812) and 1000 seed weight (0.570 and 0.458) in both the F_3 and F_4 generations (Table 2). This confirmed that these characters were mostly responsible for determining the yield. Hence, selection based on these characters will help in improving the yield in pearl millet.The present findings were in conformity with the results of Govindaraj and Selvi (2012) for plant height, number of productive tillers per plant.

Association of yield and its components alone are not adequate in any selection programme. The inter relationship existing between the individual characters which ultimately influence the yield component showed the following relationship. Days to 50 per cent flowering showed highly significant positive correlation with days to maturity in F_3 and F_4 generations.

Ear head length and ear head girth was positively associated with single ear head grain weight, single ear head weight and 1000 grain weight in F_3 and F_4 generations. Positive correlation of ear head length was reported by Yadav *et al*. (2012) for single ear head weight. Single ear head weight, single ear head grain weight and 1000 grain weight was positive and significantly inter correlated with each other. Similar results were reported by Vagadiya *et al*. (2010) for single ear head grain weight with single ear head weight, Meenakumari and Nagarajan (2008) for single ear head grain weight with 1000 grain weight. Number of productive tillers per plant showed significant positive association with single ear head grain weight in F_3 generation, whereas, it showed negative association with single ear head weight F_4 generation.

Path coefficient analysis was worked out to determine the direct and indirect contributions of different traits towards grain yield per plant. Hence, path coefficient analysis is the best method to evaluate the cause and effect relationship between the yield and yield components. Path coefficient provides a clear cut picture about the direct and indirect effect of component character on yield.

Positive direct effect on grain yield per plant was observed by the traits, days to 50 % flowering, plant height, productive tillers, ear head girth and single ear head weight and single ear head grain weight in F_3 and F_4 generations. The trait, single ear head grain weight (0.761 and 0.687) recorded highest positive direct effect on grain yield per plant followed by number of productive tillers per plant (0.400 and 0.401) in F_3 and F_4 generations (Table 3).This was in corroborate of the findings of Govindaraj and Selvi (2012) for number of productive tillers per plant; Vagadiya*et al*. (2010) for ear head girth; Kale *et al*. (2011) for single ear head weight Ibrahim *et al*. (2012) for single ear head grain weight.

Single ear head grain weight showed positive indirect effect towards grain yield per plant through days to maturity, plant height, number of productive tillers, ear head girth, single ear head weight and single ear head grain weight in F_3 and F_4 generations. Similar results were reported by Meenakumari and Nagarajan (2008) for ear head length, ear head girth, 1000 grain weight and plant height.

The residual effect of 0.284 and 0.400 was observed in F_3 and F_4 generations respectively. This minimum value of residual effect indicates that most of the characters contributing towards grain yield per plant are reliable and realistic. Though, about 28.4 percent and 40 percent of variability was uncounted and there might be a few more componential characters other than those studied in the present investigation, which might have been accountable for manipulating the grain yield of pearl millet. Association studies revealed that, selection based on the traits, single ear head grain weight, ear head girth, single ear head weight, number of productive tillers per plant, plant height and grain yield per plant will be effective. Therefore selection on the basis of direct and indirect effects is much more useful than selection for yield *per se* alone.

β carotene estimation in F_4 progenies: Pearl millet grains containing a substantial amount of pro-vitamin A would be acceptable to farmers if this higher nutritional value can be delivered to locally adapted, pest and disease resistant cultivars that have reasonable yield potential. This emanated the thought that enhancing the β carotene content in pearl millet is feasible (Kimura *et al.*, 2007). So, distinct levels of β carotene having genotypes were used as parents in this study. The results exhibited considerable variations was observed in total carotenoid (1.706-9.013 µg/g) and β carotene content (0.120-1.698 µg/g) among the parents and the selected progenies (Fig.1a and 1b). The parent PT 6129 showed high total carotenoid (9.013 µg/g) and β carotene content (1.639 µg/g), whereas, PT 6029 showed low total carotenoid (2.986 µg/g) and β carotene content (0.712 µg/g) (Table 4).

The parent PT 6129 was recorded as yellow grain colour, while, the parent PT 6029 recorded grey colour grain. It indicated that yellow colour grain may contribute to β carotene content. The selected progenies in F_4 generation showed variations for grain colour *viz.*, grey brown, cream, yellow and yellow brown grain colour. The progeny, TNBG-06-81-9-13 registered high total carotenoids (8.106 µg/g) and β carotene (1.698 µg/g) which showed yellow brown grain colour followed by TNBG-06-81-10-9 (yellow grain colour), TNBG-06-127-3-3(yellow brown grain colour), TNBG-06-53-6-6 9 (yellow brown grain colour), TNBG-06-81-7-10 and β carotene content was ranged between 0.120 to 1.698µg/g among these progenies. Sathya *et al.*, 2014 reported the β carotene range from 0.46 to 2.83 µg/g in pearl millet and Tura *et al.*, 2010 in maize the range of 0.122 to 4.74µg/g. These variations provide a valuable basis for increasing β carotene through conventional breeding. The progenies in F_4 generation *viz.*, TNBG-06-81-9-13 (1.698 µg/g), TNBG-06-81-10-9 (1.624 µg/g), TNBG-06-127-3-3 (1.585 µg/g), TNBG-06-53-6-6 (1.460 µg/g) and TNBG-06-81-7-10 (1.420 µg/g) had high β carotene and good agronomic value. Hence these lines can be utilized after attained the stability in β carotene biofortification programme to develop β carotene rich variety/ hybrids.

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Table 1. Genetic Variability parameters for ten biometrical traits in F_3 and F_4 generations of pearl millet

Characters	Generation	Mean	PV	GV	PCV (%)	GCV (%)	Heritability (%)	GA	GA as % of Mean
Days to 50% flowering	F_3	48.56	9.36	4.86	6.30	4.54	51.94	3.26	6.71
	F_4	47.24	10.98	6.48	7.01	5.39	59.01	4.01	8.48
Days to maturity	F_3	87.53	10.50	6.00	3.70	2.79	57.15	3.80	4.34
	F_4	87.34	9.52	5.02	3.53	2.57	52.74	3.34	3.82
Plant height	F_3	163.23	563.83	383.33	14.55	11.99	67.99	33.09	20.27
	F_4	169.47	485.39	304.89	13.00	10.30	62.81	28.37	16.74
No of productive tillers / plant	F_3	2.99	0.32	0.19	19.05	14.71	59.59	0.70	23.27
	F_4	3.26	0.29	0.16	16.54	12.30	55.30	0.61	18.75
Ear Head Length	F_3	25.31	9.84	5.80	12.40	9.52	59.01	3.79	15.00
	F_4	24.07	8.90	4.86	12.39	9.16	54.67	3.34	13.89
Ear Head Girth	F_3	8.95	2.30	1.51	16.95	13.73	65.55	2.04	22.78
	F_4	9.04	2.14	1.35	16.18	12.84	62.95	1.89	20.88
Single ear head weight	F_3	39.52	42.18	23.51	16.43	12.27	55.74	7.42	18.78
	F_4	41.33	41.45	22.78	15.58	11.55	54.97	7.25	17.55
Single ear head grain weight	F_3	20.53	37.52	21.02	29.84	22.34	56.04	7.04	34.28
	F_4	21.78	35.18	18.69	27.24	19.85	53.13	6.46	29.66
1000 grain weight	F_3	9.14	2.37	1.42	16.84	13.04	59.98	1.89	20.70
	F_4	9.32	2.18	1.23	15.86	11.92	56.54	1.71	18.38
Grain yield per plant	F_3	66.30	699.37	417.51	39.89	30.82	59.70	32.36	48.82
	F_4	75.50	624.28	342.41	33.09	24.51	54.85	28.09	37.21

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Table 2. Simple correlation coefficient between yield and yield components in $F_3$and F_4 generations

Characters	Generation	Days to 50% flowering	Days to maturity	Plant height	No of productive tillers / plant	Ear head length	Ear head girth	Single ear head weight	Single ear head grain weight	1000 grain weight	Grain yield / plant
Days to 50% flowering	F_3	1.000	0.936**	-0.058	0.073	-0.118	-0.100	-0.030	-0.044	0.014	-0.016
	F_4	1.000	0.922**	-0.009	0.007	-0.027	-0.116	-0.117	-0.087	-0.060	-0.054
Days to maturity	F_3		1.000	-0.043	0.033	-0.101	-0.088	-0.023	-0.038	0.026	-0.033
	F_4		1.000	-0.008	-0.028	-0.026	-0.100	-0.072	-0.051	0.007	-0.053
Plant height	F_3			1.000	0.505**	0.438**	0.243**	0.181*	0.269**	0.231**	0.476**
	F_4			1.000	0.153	0.346**	0.248**	0.192*	0.216**	0.109	0.254**
No of productive tillers/ plant	F_3				1.000	0.152	0.113	0.099	0.167*	0.075	0.568**
	F_4				1.000	0.010	0.057	0.224**	0.077	-0.027	0.480**
Ear head length	F_3					1.000	0.122	0.179*	0.183*	0.242**	0.195*
	F_4					1.000	0.254**	0.238**	0.248**	0.235**	0.234**
Ear head girth	F_3						1.000	0.804**	0.899**	0.590**	0.759**
	F_4						1.000	0.604**	0.859**	0.556**	0.707**
Single ear head weight	F_3							1.000	0.893**	0.606**	0.740**
	F_4							1.000	0.705**	0.515**	0.682**
Single ear head grain weight	F_3								1.000	0.707**	0.854**
	F_4								1.000	0.598**	0.812**
1000 grain weight	F_3									1.000	0.570**
	F_4									1.000	0.458**
Grain yield / plant	F_3										1.000
	F_4										1.000

*, ** Significance at 5 and 1 per cent level, respectively

Table 3. Path coefficient analysis showing direct and indirect effects on grain yield per plant in F_3 and F_4 generations

Characters	Generation	Days to 50% flowering	Days to maturity	Plant height (cm)	No of tillers / plant	Ear head length (cm)	Ear head girth (cm)	Single ear head weight (g)	Single ear head grain weight (g)	1000 grain weight (g)	Grain yield / plant (g)
Days to 50% flowering	F_3	**0.045**	-0.053	-0.005	0.030	0.005	-0.002	-0.0005	-0.034	-0.0004	-0.016
	F_4	**0.070**	-0.072	0.0001	0.003	-0.0008	-0.005	-0.010	-0.060	0.0006	-0.054
Days to maturity	F_3	0.042	**-0.057**	-0.004	0.013	0.004	-0.002	-0.0004	-0.030	-0.0007	-0.033
	F_4	0.083	**-0.078**	-0.0001	-0.011	-0.0008	-0.004	-0.006	-0.035	-0.0001	-0.053
Plant height	F_3	-0.002	0.002	**0.087**	0.202	-0.019	0.004	0.003	0.205	-0.006	0.476**
	F_4	0.0009	0.0006	**0.006**	0.062	0.010	0.010	0.016	0.149	-0.001	0.254**
No of productive tillers/ plant	F_3	0.003	-0.002	0.0440	**0.400**	-0.006	0.002	0.002	0.128	-0.002	0.568**
	F_4	0.0007	0.002	0.001	**0.401**	0.0003	0.002	0.019	0.053	0.0003	0.480**
Ear head length	F_3	-0.005	0.006	0.038	0.061	**-0.042**	0.002	0.003	0.139	-0.007	0.195*
	F_4	-0.003	0.002	0.002	0.004	**0.029**	0.011	0.020	0.171	-0.003	0.234**
Ear head girth	F_3	-0.005	0.005	0.021	0.045	-0.005	**0.017**	0.013	0.684	-0.016	0.759**
	F_4	-0.010	0.008	0.002	0.023	0.007	**0.0423**	0.051	0.590	-0.006	0.707**
Single ear head weight	F_3	-0.001	0.001	0.016	0.040	-0.008	0.013	**0.016**	0.680	-0.017	0.740**
	F_4	-0.011	0.006	0.001	0.090	0.007	0.026	**0.084**	0.485	-0.006	0.682**
Single ear head grain weight	F_3	-0.002	0.002	0.023	0.067	-0.008	0.015	0.015	**0.761**	-0.020	0.854**
	F_4	-0.008	0.004	0.001	0.031	0.007	0.036	0.059	**0.687**	-0.006	0.812**
1000 grain weight	F_3	0.0007	-0.002	0.020	0.030	-0.010	0.010	0.010	0.539	**-0.028**	0.570**
	F_4	-0.005	-0.0006	0.0007	-0.011	0.007	0.024	0.043	0.411	**-0.011**	0.458**

Residual effect (F_3) = 0.2839, Residual Effect (F_4) = 0.400

Table 4.Total carotenoid and β carotene content (μg/g) of parents and selected progenies in F_4 generation

Entry	Colour	Grain colour score	Total carotenoids (μg/g)	β Carotene content (μg/g)
PT 6129 (P)	Yellow	3	9.013	1.639
PT 6029 (P)	Grey	4	2.986	0.712
TNBG-06-3-3-4	Grey brown	6	1.706	0.360
TNBG-06-3-12-2	Grey brown	6	1.866	0.120
TNBG-06-53-6-1	Cream	2	4.000	0.440
TNBG-06-53-6-6	Yellow brown	7	5.653	1.460
TNBG-06-53-8-2	Cream	2	4.298	0.663
TNBG-06-81-7-10	Yellow	3	5.226	1.420
TNBG-06-81-9-13	Yellow brown	7	8.106	1.698
TNBG-06-81-10-9	Yellow	3	5.653	1.624
TNBG-06-127-1-9	Cream	2	4.949	0.760
TNBG-06-127-3-3	Yellow brown	7	4.56	1.585

(P) - Parents; Grain colour score: 1- Whitish 2- Cream 3- Yellow 4- Gray 5- Deep gray 6- Gray brown 7- Yellow brown 8- Purple 9- Purplish black

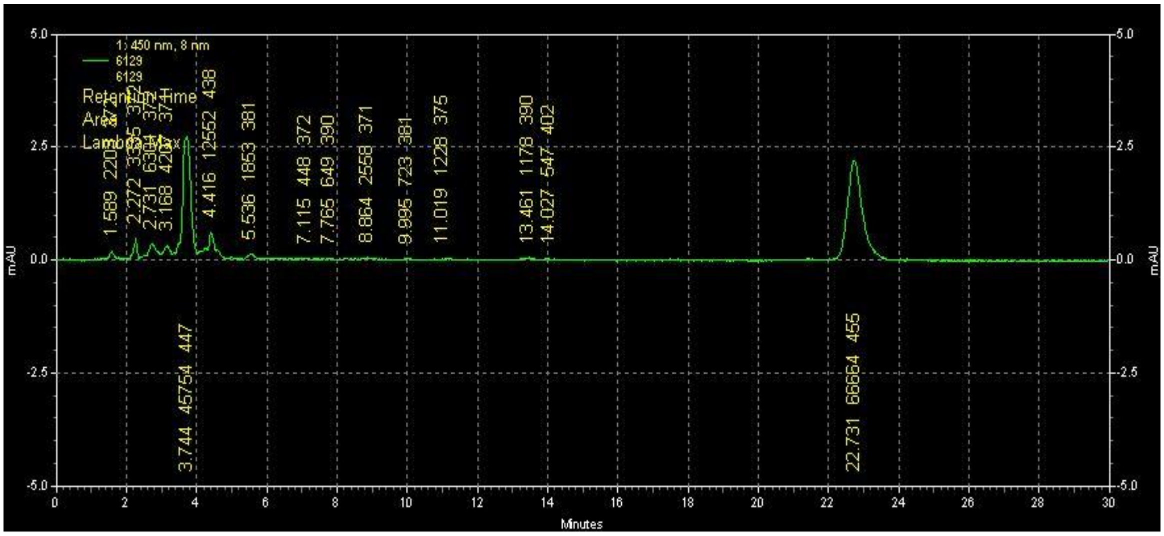

Fig. 1a HPLC chromatogram for β carotene content in parent PT 6129 (yellow colour grain and rich β carotene content)

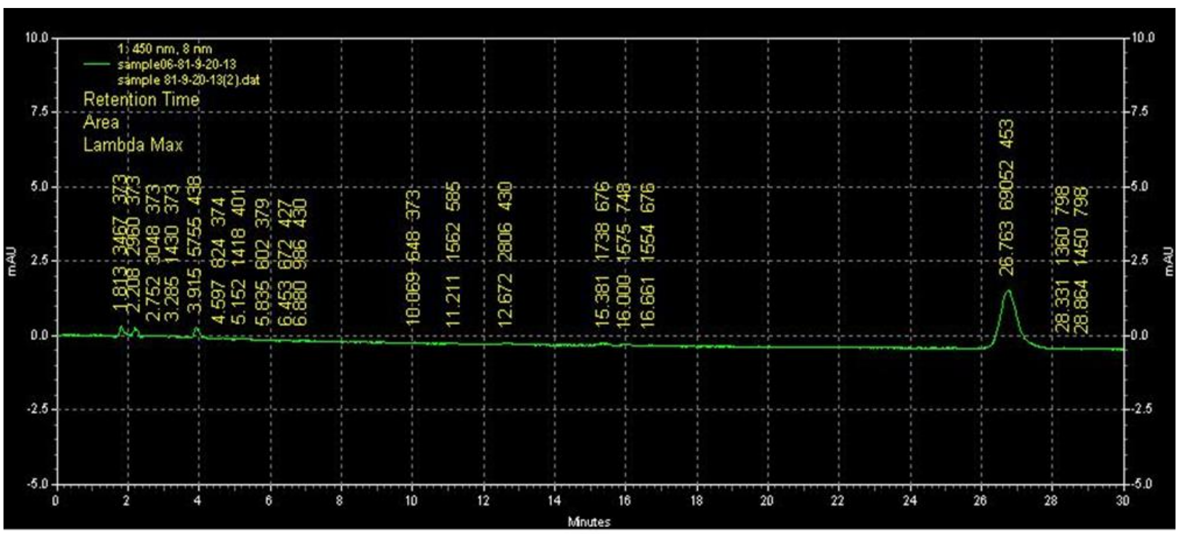

Fig. 1b HPLC chromatogram for β carotene content in TNBG-06-81-9-13 line