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A simple and rapid screening technique for grain β carotene content in pearl millet through spectrophotometric method

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Pearl millet ranks sixth in annual world cereal production. Vitamin A deficiency is a global health problem affecting 140-250 million children and accounts for increased childhood mortality and diseases. Humans and animals are unable to synthesize Vitamin 'A' requirement as plant-derived-carotene are metabolized to produce Vitamin 'A'. Pearl millet grains are rich nutritionally and contain sufficient amount of β carotene which is the precursor of Vitamin A. In order to satisfy the recommended dietary allowance, the targeted level of beta carotene in pearl millet (20 µg/ g of grain), screening of large number of pearl millet genotypes is a prerequisite. Among the various available techniques, high performance liquid chromatography (HPLC) can be accurate for β carotene estimation; however it is laborious, time consuming and requires skilled labour and use of highly toxic solvents. The aim of this work is to develop a simple and rapid screening method for determination of β-carotene in pearl millet by spectrophotometry. Two hundred recombinant inbred lines developed from the cross between agronomically superior inbred line (PT 6029) and high beta carotene golden millet line (PT 6129) were evaluated at Department Vitamin A of Millets, Tamil Nadu Agricultural University, Coimbatore. The range of β carotene varied between 0.46 and 2.83 µg/g of grain. Eighty recominant inbred lines (RILs) exceeded the general mean of 1.7 µg/g of grain and 17 transgressive recombinants were obtained. These transgressive recombinants could be used in conventional plant breeding programme for development of inbred lines with high beta carotene and yield in order to meet requirement in the diet.

Key words: Pearl millet, β carotene, Vitamin A, recominant inbred lines (RILs), spectrophotometer, transgressive segregates.

INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] known as bulrush or cattail millet, is the most important among a number of unrelated millet species grown for food worldwide (Angarawai et al., 2008). In India pearl millet is fifth most important grain crop next to rice, wheat, maize and sorghum. It is grown in more than 8.39 million hectares with current grain production of 9.5 million tonnes and productivity of 1091 kg/ha (Directorate of Economics and Statistics, 2011-2012). Carotenoids are C40 isoprenoid polyene compounds that form lipid soluble yellow, orange and red pigments. One of the most important physiological functions of carotenoids in human nutrition is to act as pro-Vitamin A (Vitamin A precursors like alpha carotene, beta carotene and beta cryptoxanthin).

Many studies show strong correlations between

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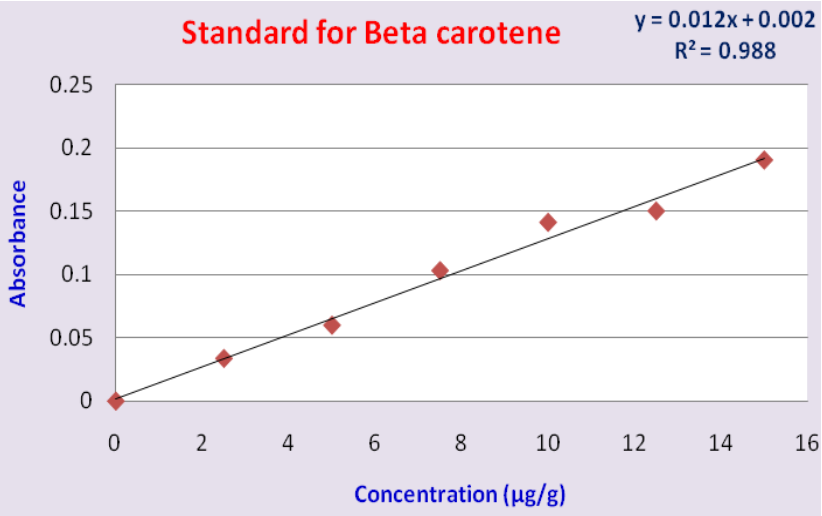

Figure 1. β Carotene standard graph.

carotenoids intake and a reduced risk of some diseases, such as cancer, atherogenesis bone calcification, eye degeneration, immune function and neuronal damage. Among the carotenoids, β-carotene is popular to consumers because bioconversion of beta carotene into Vitamin A is high as compare to other carotene (Karnjanawipagul et al., 2010). Vitamin A deficiency causes hundreds of thousands of cases of irreversible blindness every year, especially among children in developing countries. Humans and animals are unable to synthesize their own Vitamin 'A' requirement. Plant derived carotene are metabolized to produce Vitamin 'A'. Pearl millet grain is very rich nutritionally and contains higher protein content than many other cereals. Besides protein, pearl millet grains also contain sufficient amount of beta-carotene which is the precursor of Vitamin A. Pearl millet is a good source of fat soluble Vitamins, though not much information is available on Vitamins, the beta carotene content was reported to be 0.1 µg/g (Khalil and Sawaya, 1984), and thus, it can serve as an additional source of Vitamin A.

In order to overcome the malnutrition problem and satisfy the recommended dietary allowance the targeted level of beta carotene content in pearl millet 20 µg/g of grain (Bouis et al., 2011), screening of large number of pearl millet genotypes is a prerequisite. In order to achieve the target, our aim to screen the large number of pearl millet genotypes. Different methods have been proposed for the analysis of carotenoids including β-carotene. Among the various available techniques, high performance liquid chromatography (HPLC) can be accurate, however it is laborious, time consuming and requires skilled labour and use of highly toxic solvents (Karnjanawipagul et al., 2010). The aim of this work is to develop a simple and rapid screening method for determination of β-carotene in pearl millet by spectrophotometry.

MATERIALS AND METHODS

The experimental material consisted of a recombinant inbred lines (RILs) derived from the cross between PT 6029 an agronomically superior inbred line and PT 6129 a high beta carotene golden millet line. The RIL population which consist of 200 individuals was developed through ear to row method. The experiment was laid out in homogeneous block following randomized block design replicated twice with 200 RILs and parents during summer, 2013 at the Department of Millets, Tamil Nadu Agricultural University, Coimbatore. Sefled seeds from 200 RILs were used for beta carotene estimation through spectrophotometric method. The estimation of beta carotene in this study was followed as described by Sandra et al. (1955) with minor modifications. This modified protocol has been standardized in pearl millet for beta carotene estimation.

Standard and sample preparation

In a 100 ml volumetric flask, 25 mg of β carotene was weighed and it was dissolved and made up to the mark with water saturated n-butanol (WSB). 8 ml of the above homogeneous solution was then pipetted into a 100 ml volumetric flask which was made up to the mark with water saturated n-butanol. Twenty-five millimeter of this solution was taken and placed in a 100 ml volumetric flask and made up with water saturated n-butanol. With the suitable dilution of the standard solution with water saturated n-butanol in 10 ml volumetric flasks (e.g from 0.5 ml to 3 ml of standard solution in 10 ml) the calibration curve was prepared (The standard solution has the concentration of 5 µg of β carotene/ml). The absorbance of each dilution was measured and the calibration curve was established (Figure 1).

The pearl millet grain from each of the 200 RILs was collected and a small quantity of it was thoroughly grinded to make fine flour to estimate the β carotene content. 10 gram of the flour from each of the RILs was taken into 100 ml of conical flask and 40 ml of water saturated n-butanol was added. The conical flask was covered with aluminium foil in order to maintain dark condition. The content of the flask was mixed vigorously for 1 min and kept for overnight at room temperature for complete extraction of β carotene. Next day the content was shaken well and filtered completely through Whatman No 40 filter paper. The optical density

Table 1.β carotene content (mean of two replication) of Parents and RILs in Pearl millet.

S/N	RILs	Content (µg/g)	Colour of the grain	S/N	RILs	Content (µg/g)	Colour of the grain	S/N	RILs	Content (µg/g)	Colour of the grain	S/N	RILs	Content (µg/g)	Colour of the grain
1	PT 6029	0.46 (0.43)	G	26	RIL24	1.39	YB	51	RIL49	0.73	YB	76	RIL74	1.99	YB
2	PT 6129	2.53	Y	27	RIL25	1.70	C	52	RIL50	1.00	YB	77	RIL75	1.48	Y
3	RIL 1	1.14	Y	28	RIL26	1.14	YB	53	RIL51	1.59	C	78	RIL76	1.50	Y
4	RIL2	1.27	GB	29	RIL27	0.51	YB	54	RIL52	2.25	C	79	RIL77	2.72 (2.60)	Y
5	RIL3	1.21	GB	30	RIL28	0.85	DG	55	RIL53	2.83	YB	80	RIL78	2.53	Y
6	RIL4	1.47	G	31	RIL29	1.33	Y	56	RIL54	1.43	YB	81	RIL79	1.45	YB
7	RIL5	1.14	GB	32	RIL30	2.11(2.06)	YB	57	RIL55	1.59	YB	82	RIL80	1.41	Y
8	RIL6	1.30	Y	33	RIL31	1.64	YB	58	RIL56	1.16	Y	83	RIL81	2.72	YB
9	RIL7	1.24	GB	34	RIL32	1.52	Y	59	RIL57	1.06 (0.97)	C	84	RIL82	2.66 (2.54)	C
10	RIL8	1.66	Y	35	RIL33	0.89	Y	60	RIL58	1.62	C	85	RIL83	1.71	C
11	RIL9	0.87	Y	36	RIL34	1.25	Y	61	RIL59	1.28	G	86	RIL84	1.62	C
12	RIL10	2.28 (2.18)	C	37	RIL35	1.26	GB	62	RIL60	1.88	YB	87	RIL85	1.57	C
13	RIL11	2.71	Y	38	RIL36	1.39	Y	63	RIL61	1.46	Y	88	RIL86	1.69	Y
14	RIL12	2.83	G	39	RIL37	1.70	YB	64	RIL62	1.97	YB	89	RIL87	0.81	C
15	RIL13	1.48	Y	40	RIL38	1.97(1.86)	YB	65	RIL63	1.45	G	90	RIL88	1.64	Y
16	RIL14	1.63	G	41	RIL39	2.43	DG	66	RIL64	1.10	Y	91	RIL89	1.63 (1.58)	Y
17	RIL15	1.01	C	42	RIL40	2.68	G	67	RIL65	1.61	Y	92	RIL90	1.36	C
18	RIL16	1.01	YB	43	RIL41	1.64	C	68	RIL66	1.68	Y	93	RIL91	1.59	YB
19	RIL17	1.40	YB	44	RIL42	1.67	G	69	RIL67	2.21	G	94	RIL92	1.26	Y
20	RIL18	2.05	YB	45	RIL43	1.81	Y	70	RIL68	2.08	G	95	RIL93	1.96	Y
21	RIL19	0.77(0.68)	YB	46	RIL44	2.10	Y	71	RIL69	1.67	G	96	RIL94	1.42	YB
22	RIL20	2.11	Y	47	RIL45	2.74	YB	72	RIL70	1.64	GY	97	RIL95	2.23	G
23	RIL21	2.46	YB	48	RIL46	1.71	YB	73	RIL71	1.23(1.20)	C	98	RIL96	1.47	YB
24	RIL22	1.75	Y	49	RIL47	0.98	Y	74	RIL72	2.62	YB	99	RIL97	1.75 (1.68)	YB
25	RIL23	1.01	C	50	RIL48	2.31(2.20)	Y	75	RIL73	2.54	Y	100	RIL98	1.75	YB
S/N	**RILs**	**Content (µg/g)**	**Colour of the grain**	**S/N**	**RILs**	**Content (µg/g)**	**Colour of the grain**	**S/N**	**RILs**	**Content (µg/g)**	**Colour of the grain**	**S/N**	**RILs**	**Content (µg/g)**	**Colour of the grain**
101	RIL99	1.14	Y	127	RIL125	1.11	YB	153	RIL151	1.60	Y	179	RIL177	1.42 (1.26)	Y
102	RIL100	1.68	C	128	RIL126	1.00	Y	154	RIL152	1.41	Y	180	RIL178	1.66	Y
103	RIL101	0.85	Y	129	RIL127	1.18	G	155	RIL153	1.57	YB	181	RIL179	1.73	YB
104	RIL102	0.56	YB	130	RIL128	1.92	C	156	RIL154	2.06	DG	182	RIL180	1.66	YB
105	RIL103	0.46	Y	131	RIL129	1.58	Y	157	RIL155	1.41	Y	183	RIL181	0.96	Y
106	RIL104	1.07	Y	132	RIL130	1.97	G	158	RIL156	1.56	Y	184	RIL182	1.66	YB

Table 1. Contd.

107	RIL105	0.76	C	133	RIL131	1.44	YB	159	RIL157	1.49	Y	185	RIL183	1.20	G
108	RIL106	0.83	YB	134	RIL132	2.21	Y	160	RIL158	1.57 (1.50)	Y	186	RIL184	1.45	G
109	RIL107	0.95	C	135	RIL133	1.91	YB	161	RIL159	1.70	Y	187	RIL185	1.45	G
110	RIL108	1.90 (1.86)	YB	136	RIL134	2.70	YB	162	RIL160	1.43	Y	188	RIL186	1.84 (1.78)	YB
111	RIL109	1.78	C	137	RIL135	2.57 (2.52)	YB	163	RIL161	1.57	Y	189	RIL187	2.04	YB
112	RIL110	1.81	Y	138	RIL136	2.47	YB	164	RIL162	1.55	Y	190	RIL188	1.28	YB
113	RIL111	2.25	Y	139	RIL137	2.45	YB	165	RIL163	2.31	Y	191	RIL189	2.45	Y
114	RIL112	1.02	C	140	RIL138	1.94	Y	166	RIL164	1.76	Y	192	RIL190	2.45	Y
115	RIL113	1.67	G	141	RIL139	2.66	YB	167	RIL165	1.28 (1.20)	G	193	RIL191	2.33	YB
116	RIL114	1.58	YB	142	RIL140	1.92	Y	168	RIL166	1.58	Y	194	RIL192	1.73	YB
117	RIL115	0.86	G	143	RIL141	1.39	Y	169	RIL167	1.65	G	195	RIL193	1.75	G
118	RIL116	0.55	YB	144	RIL142	1.49	Y	170	RIL168	1.88	Y	196	RIL194	2.70	YB
119	RIL117	0.62	C	145	RIL143	1.58	Y	171	RIL169	1.59	YB	197	RIL195	2.32	YB
120	RIL118	0.83	C	146	RIL144	1.73 (1.58)	G	172	RIL170	1.51	YB	198	RIL196	1.94 (1.92)	YB
121	RIL119	1.91 (1.88)	YB	147	RIL 145	1.66	G	173	RIL171	2.12	YB	199	RIL197	2.15	YB
122	RIL120	0.62	C	148	RIL146	1.68 (1.55)	Y	174	RIL172	1.82 (1.65)	Y	200	RIL198	2.61	G
123	RIL121	1.42	C	149	RIL147	1.33	Y	175	RIL173	2.59	YB	201	RIL199	1.91	Y
124	RIL122	1.47	YB	150	RIL148	1.41	Y	176	RIL174	2.68	G	202	RIL200	1.75	Y
125	RIL123	1.72	C	151	RIL149	2.00	Y	177	RIL175	1.42	YB				
126	RIL124	2.06	G	152	RIL150	1.91	Y	178	RIL176	1.74	YB				

G - Gray, Y - Yellow, C - Cream, YB - yellow Brown, GB - Gray Brown and DG - Deep Gray.

of clear filtrate of the sample from 200 RILs was measured at 440 nm using SL 150 UV VIS Spectrophotometer. Pure water saturated n-butanol was used as the blank.

RESULTS AND DISCUSSION

Plant carotenoids are the primary source of provitamin A with β carotene as the most well known source of it. A lack of β carotene is a major cause of Vitamin A deficiency (Julie et al., 2009). Vitamin A deficiency (VAD) causes about 70% of childhood deaths worldwide and blindness in 0.25 to 0.5 million children every year (Vignesh et al., 2012). Vitamin A as a key factor in our health plays an important role in vision, bone growth, reproduction and cell division. It also regulates the immune system. Association of β carotene in staple crops shows lower risk to Vitamin A deficiency and cancer (Yan et al., 2010). It was reported earlier that in pearl millet flour, the amount of beta carotene was found to be less than 0.01 mg/100 g (0.1 ppm) (Khalil and Sawaya, 1984). Highest beta carotene content was reported in a high yielding hybrid which recorded a beta carotene content of 36.7 µg/100 g (0.36 ppm) (Khangura et al., 1980). Comparative studies between the traditional landraces versus improved varieties revealed that the concentration of beta carotene was highest in the improved varieties with a beta carotene content of 0.13 µmol kg^{-1} (Buerkert et al., 2001).

Though, pearl millet contains beta-carotene, it is very less compared to other cereal crops especially maize. This emanated the thought that enhancing the beta-carotene content in pearl millet is feasible. Pearl millet has a vast reservoir of genetic variability for various qualitative and quantitative traits. By considering above fact, the present investigation was carried out to assess the genetic variability for β carotene content among inbred lines of pearl millet. This could be the first report for the estimation of β carotene content through spectrophometric method. β carotene content in parents and RILs were presented in Table 1. The β-carotene content of

the inbreds such as PT 6129 was found to be comparable to that of β-carotene content in maize as reported by Kimura et al. (2007).

Range of beta carotene in this study was recorded from 0.46 to 2.83 µg/g of grain. Santra et al. (1955) reported β carotene content in wheat (2.25 - 5.82 µg/g). Eighty RILs exceeded the general mean of 1.7 µg/g. Beta carotene estimation was repeated twice for all the samples and the result was almost similar as that of previous estimation as the value given in parenthesis (Table 1). There is no correlation between the grain colour and beta carotene content.

The value that fall outside the parental range are said to be transgressive recombinant and the number of transgressive recombinants were obtained by comparing *per se* performance of RILs to that of parents. The number of positive transgressive recombinants obtained for beta carotene are RIL 12 and RIL 53 (2.83 µg/g), followed by RIL 47 (2.74 µg/g), RIL 77 and RIL 81 (2.72 µg/g), RIL 11 (2071 µg/g), RIL 194 and RIL 134 (2.70 µg/g), RIL 174 and RIL 40 (2.68 µg/g), RIL 139 and RIL 82 (2.66 µg/g), RIL 72 (2.62 µg/g), RIL 198 (2.61 µg/g), RIL 173 (2.59 µg/g), RIL 135 (2.57 µg/g) and RIL 73 with 2.54 µg/g, these lines could be used as donor in crossing programme.

Based on the present study, the RILs with better mean values for beta carotene will be helpful to developing beta carotene rich inbred lines which can be used as pre breeding material for further improvement of pearl millet. Further, the mapping population could be used for mapping of genes /QTLs for beta carotene.

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