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Quantification of β-carotene, aucubin content, their associations and contribution to other economic traits in *Plantago* germplasm

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ABSTRACT

Plant secondary metabolites have innumerable benefits in regard to human health. From the available 106 divergent lines of five *Plantago* species, a screening program was conducted to explore putative lines having best resources of aucubin and β-carotene. HPLC results indicated that seeds of selection LP3 of *P. ovata* contained the highest (9.53%) and *P. arenaria* contained the least amounts (7.79%) of β-carotene. An investigation was also conducted to assess the genetic variability, correlations and character contribution of β-carotene and aucubin content with other traits towards seed yield in a set of ten selected accessions in relation to seven traits in *Plantago* species. Results indicated that none of the accessions of any other species apart from *P. lanceolata* contained aucubin. PL-61 accession was detected to have maximum concentration (0.23%) of aucubin while the lowest (0.02%) was detected in PL-66. This opened the possibilities for nutritional value addition of β-carotene in seeds of isabgol.

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1. Introduction

The genus *Plantago* (Family-Plantaginaceae) has cosmopolitan distribution (Taskova et al., 2002). Medicinally, the species of *Plantago* (Gonçalves and Romano, 2016) are astringents, demulcents, emollients, expectorants, diuretics, antibacterials and antivirals (Marchesan et al., 1998). The name of the genus comes from the latin word 'planta' which means 'sole of foot' referring to the basal rosette of the broad leaves touching the ground in most of the species (Pilger, 1937). For centuries, species of *Plantago* have been used in folk medicine for their diverse properties (Wichtl, 1994; Samuelsen, 2000). Some of them are specifically valuable for the nutraceutical and pharmaceutical industries due to the mucilaginous product (psyllium) derived from the seed husk, which is used as a functional food (salads, soups or baking) as well as a dietary supplement to improve intestinal health in man (Samuelsen, 2000; Lutterodt and Cheng, 2008). The studies by Decaisne (1852), Harms and Reiche (1895) describe the macro morphological features of the species. Chemical compounds as chemotaxonomic markers have also been studied using sugars (Gorenflot and Bourdu, 1962); phenolcarboxylic acids (Andrzejewska-Golec and Swiatek, 1986); phenylethanoid glycosides (Andary et al., 1988; Ronsted et al., 2000); flavonoid glycosides (Tomas-Barberan et al., 1988; Kawashty et al., 1994) and iridoid glucosides (Kuzmanov et al., 1984; Andrzejewska-Golec and Swiatek, 1984; Andrzejewska-Golec et al., 1993; Andrzejewska-Golec, 1997).

Although *Plantago* species are widely considered as weeds, they have been used as medicinal plants for centuries. Some *Plantago* species are listed as safe herbs in the pharmacopoeias of numerous countries (Blumenthal and Busse, 1998) while others are used as food and animal feed. *Plantago major* has been used as a traditional medicinal plant for centuries in several cultures and is one of the most commonly used medicinal herbs in the world. The leaves promote

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wound healing and are still used in traditional medicine. An ethnopharmacological research has shown that it is still used for the treatment of infectious diseases, digestive and respiratory disorders, circulation and reproduction, for pain and fever relief and to prevent cancer (Samuelsen, 2000). The aerial parts of *Plantago lanceolata* possess wound healing, anti-inflammatory, antibacterial, diuretic and anti-asthmatic properties (Fons et al., 1998). The intake of *P. lanceolata* juice with wine or honey relieves gout and its crushed leaves mixed with salt have been used to treat arthritis (Adams et al., 2009). *P. lanceolata* is listed as a safe herb in the pharmacopoeias of several countries (Blumenthal and Busse, 1998). *P. asiatica* is reported to have antipyretic, antitussive, diuretic and wound healing properties (Lin and Kan, 1990). This species, along with *P. afra*, *P. ovata*, *P. indica* and *P. major* are also used in traditional medicine worldwide because the soluble fibers in the seeds promote intestinal functions. *P. ovata* has been used as a traditional remedy for intestinal ill health.

The phytochemistry of *Plantago* species has shown the presence of phenylpropanoid glycosides, iridoids, triterpenes, flavonoids and phenolic acids as the main bioactive compounds present in the aerial parts as well as the medically and industrially valuable polysaccharides in the seeds (Gonçalves and Romano, 2016). Some *Plantago* species also produce unusual fatty acids in their seeds and these lipids may be useful in the oleochemical industry (Smith et al., 2014). A considerable number of different iridoids have been isolated from *Plantago* in the recent past decades and these include aucubin, catalpol and other biosynthetically related compounds (Handjieva et al., 1993; Damtoft et al., 1994; Jensen et al., 1996; Ronsted et al., 2000). Iridoids are naturally occurring monoterpenic products of plants that can be divided into four groups: iridoid glycosides, aglycone or non-glycosidic iridoids, secoiridoids and bisiridoids (Suomi et al., 2000). The occurrence of iridoids has been reported in detail in the following families-Acanthaceae, Lamiaceae, Plantaginaceae, Rubiaceae and Scrophulariaceae (Inouye et al., 1988; Jensen et al., 1988; Junior, 1990; Andrzejewska-Golec, 1995). Considerable interest in iridoids has been generated by their relatively wide spectrum of biological activities (Andrzejewska-Golec, 1995), as well as their use as markers in plant taxonomy (Andrzejewska-Golec et al., 1993; Andrzejewska-Golec, 1997; Müller et al., 1999; Taskova et al., 2002). Among the complex mixture of biologically active compounds in the plant extracts, the iridoid glycosides aucubin and catalpol can be used as analytical markers to determine the quality of extracts from different sources (Rischer et al., 1998). Ronsted et al. (2000), observed the distribution pattern of iridoids in thirty four species of *Plantago*. "Aucubin" is an iridoid glycoside isolated from *Aucuba japonica* or *Plantago asiatica* (Chang et al., 1982). It was found to be typical for the entire genus of *Plantago*, while others like bartsioside and plantarenaloside occur almost exclusively in subgenus *Psyllium*, as also pointed out by Andrzejewska-Golec et al. (1993). An attempt was also made for the quantitative determination of aucubin in seven *Plantago* species using HPLC, HPTLC and LC-ESI-MS methods by Janković et al. (2010).

Aucubin possesses diverse biological activities such as antimicrobial, hepatoprotective (Chang, 1998), antitumoral (Ishiguro et al., 1983), hemodynamic (Circosta et al., 1984), collagen synthesis (Li et al., 2000) and anti-inflammatory (Miyagoshi et al., 1988). Based on the iridoid patterns of the species, some hypothetical evolutionary lines in genus *Plantago* were outlined: (i) species in which the iridoid biosynthesis is limited to earlier stages, containing mainly aucubin (*P. major*, *P. cornuti* and *P. gentianoides*), (ii) species containing aucubin and derivatives of aucubin such as monomelittoside (*P. subulata* and *P. media*), (iii) species containing aucubin and catalpol (*P. lanceolata*, *P. altissima*, *P. argentea* and *P. lagopus*) noting that the three former species belonging to Rahn's section Lanceifolia Arnoglossum Decne. (Rahn, 1996) have more advanced biosynthetic pathways containing derivatives of catalpol, and (iv) species containing aucubin and plantarenaloside (*P. afra* and *P. scabra* (=*P. arenaria*)).

On the other aspect, carotenoids are organic pigments that are produced predominantly by photosynthetic organisms. In plants, carotenoids fulfill two essential functions during photosynthesis, i.e. light harvesting and protecting the photosynthetic apparatus from photo-oxidation (Demmig-Adams and Adams III, 1996). As precursors of signaling molecules that influence development and biotic/abiotic stress responses, they facilitate photo morphogenesis, non-photochemical quenching and lipid peroxidation and attracting pollinators (Pogson et al., 1998; Havaux and Niyogi, 1999; Park et al., 2002; Calucci et al., 2004; Franco et al., 2007; McNulty et al., 2007). Particularly four carotenoids (β-carotene, α-carotene, γ-carotene and β-cryptoxanthin) have vitamin A activity in humans, which means they are convertable into the visual pigment retinal and are therefore essential nutrients. β-carotene (pro-vitamin A) is a precursor of vitamin A in the human body. It is present in a wide variety of yellow-orange colored fruits, dark green and yellow vegetables such as broccoli, spinach, turnip greens, carrots, squash, sweet potatoes, and pumpkin (Harrison, 2005; Othman et al., 2017). Liver, milk, butter, cheese, and whole eggs are direct sources of vitamin A. Vitamin A plays an important role in the human body for normal growth and tissue repair (Melse-Boonstra et al., 2017). The visual and immune systems are particularly dependent on this vitamin for normal function (FAO, 2003).

It was observed during the field studies that some particular plants of *P. ovata* having longer panicle length had distinguishable orange colour seeds compared to the normal pink seeds (Fig. 1). According to Ronsted et al. (2000), although the most notable secondary metabolites of *Plantago* sp are the

a) Seeds of Cultivar Mayuri (*Plantago ovata*) having pink seeds

b) Seeds of selected line of *Plantago ovata* having orange seeds

Fig. 1. Morphological difference in the colour of seed coats between (a) Cultivar Mayuri and (b) selected line (LP3) of *Plantago ovata* having highest content of β carotene.

representative iridoid glycosides and phenylethanoids, yet researchers interrogated chemotaxonomic alliance between different *Plantago* species (*P.major*, *P. australis*, *P. lanceolata* & *P. catharinea*) with other metabolites also principally flavonoids (De Souza Mesquita et al., 2017) present in those species which are commercially marketed under the name "Plantain". The surprising presence of carotenoids in the *P. ovata* of longer panicle length prompted our survey to precisely quantitate this high end nutritional plant in terms of this tetraterpenoid (β-carotene) content for the first time of our currently studied species. Keeping in view about the importance of aucubin and β-carotene, an experiment was planned (i) to screen out those putative lines from our germplasm collection containing the best resource of aucubin and β-carotene present in the seeds and more specifically in the seed husks in isabgol (ii) to study the character contribution and genetic association with β-carotene and seed yield and other developmental traits.

2. Experimental

2.1. Plant material

The seeds of six selected plants of *P. ovata* germplasm bearing orange seeds (named as LP-1, LP-2, LP-3, LP-4, LP-5 and LP-6), two cultivars of *P. ovata* Forsk-Mayuri and Niharika released by CSIR-CIMAP for commercial cultivation in India (Lal et al., 1998; Lal et al., 2004) and two other species-*P. arenaria* Waldst and *P. major* L. were subjected to β-carotene analysis as well as character contribution and genetic association with β-carotene and seed yield and other developmental traits.

A total of twenty two accessions comprising five species of *Plantago*, namely *P. ovata* Forsk. (ten accessions-PO-1, 18, 22, 34, 46, 52, 53, 55, 57 and 78), *P. lanceolata* L. (nine accessions-PL-61, 63, 64, 65, 66, 68, 69, 70 and 71), *P. arenaria* Waldst (one accession-PA-102), *Plantago major* L. (one accession-PM-32) and *P. psyllium* L (one accession-PP-03) were subjected to aucubin analysis.

2.2. Chemicals and standards

HPLC grade methanol, ethyl acetate, isopropanol and acetone were purchased from Sigma-Aldrich, India. The working analytical standards of aucubin and β-carotene of purity (99%) were provided from Sigma-Aldrich, India.

2.3. Chromatographic analysis

HPLC analysis was performed using a Shimadzu LC-10AD liquid chromatograph equipped with two LC-10A pumps controlled by a CBM-10 interface module, SPD-M10A VP diode array detector and SIL-10ADVP auto injector. Data were collected and analyzed using a class LC-10 Work Station. The samples were analyzed by using reverse phase chromatography on waters spherisorb ODS2 (250×4.6 mm i.d., 10 µm).

2.3.1. Extraction of β-carotene from respective *Plantago* seeds and sample preparation

1.0g dried and milled seed samples were extracted with methanol (3×10 mL) by shaking at room temperature for 30 min and centrifuged. The combined supernatant were diluted to 10 mL with the same solvent filtered, concentrated and made up to 1.0 mL for HPLC analysis.

2.3.2. Quantification of β-carotene

Chromatographic separation was achieved on

above-mentioned column in an isocratic mobile phase composition of acetonitrile: ethyl acetate: isopropyl alcohol (propane-2-ol) 80:10:10 (v/v/v) at a flow-rate of 0.8 mL/min and UV detection at λ 260 nm under ambient conditions (25 ºC). 10 µL of standard β-carotene in acetone (1.0 mg/mL) and sample solution (1.0 g seed extract/mL) in acetone were injected separately and the % content of β-carotene was estimated by area count of β-carotene in standard and sample tracks. The percentage data was finally converted into mg/g.

2.3.3. Extraction of aucubin from respective *Plantago* seeds and sample preparation

1.0 g of dried and milled seed and leaf samples were extracted with methanol (3×10 mL) by shaking at room temperature for 30 min and centrifuged. The combined supernatants were diluted to 10 mL with the same solvent filtered, concentrated and made up to 1.0 mL for the HPLC analysis.

2.3.4. Quantification of aucubin

Chromatographic separation was achieved on above-mentioned column in an isocratic elution mode in a mobile phase composition of methanol and water in the ratio of (17:83) (v/v) at a flow-rate of 0.6 mL/min, and subsequent UV detection at λ 210 nm under ambient conditions (25 ºC). 10 µL of standard aucubin in methanol (1mg/mL) and sample solution (1.0 g seed extract/mL) in methanol were injected separately and the % content of aucubin was estimated by area count of aucubin in standard and sample tracks. The percentage data was finally converted into mg/g.

2.4. Field preparation and allied practices

All the genetic stocks were evaluated by growing at the research farm of CSIR-Central Institute of Medicinal and Aromatic Plants, P.O.-CIMAP, Lucknow, (U.P.) 226 015 (India) located at 26.5º N latitude and 80.50º E longitude, and 120 m above mean sea level. The climate was semiarid to subtropical in nature. Minimum and maximum night and day temperatures ranged 8-11 ºC to 15-17 ºC, respectively during growth period and from 25-30 ºC to 35-40 ºC, during harvesting time, respectively. The average rainfall during the growing season was 5-7 mm according to weather data of the Metrological Laboratory of CSIR-CIMAP, Lucknow in randomized complete block design, in three replications with rows 3 m long and 40 cm apart having plant to plant distance 20 cm. Standard cultural practices were followed throughout the crop season for having a good crop. The crop received normal intercultural operations, irrigations and fertilizer applications (80 kg N, 40 kg P_2O_5 and 40 kg K_2O per hectare).

2.5. Statistical analysis

The morpho-metric observations were recorded for statistical analysis on five randomly chosen plants in a line from ten accessions with respect to seven important characters as following: Plant height (cm), days to flower (50%), days to maturity (days), panicle length (cm), seed yield (g/plot), husk yield (g/plot) and β-carotene (mg/g). The mean data were collected and offered to statistical analysis for associations, co-heritability and path coefficient analysis as suggested by Dewey and Lu (1959) using statistical software ver. 0.3 based on Singh and Chaudhary (1979) and Panse and Sukhatme (1967) available at the Genetics and Plant Breeding Department of our Institute for variances, co-variances and variability parameters as per standard procedure including heritability and genetic advance.

The genetic advance over mean in (%) is expressed as per the formula (Eqn. 1):

$$\text{G.A. over mean (\%)} = (\text{G.A.} / \text{Mean of treatment}) \times 100 \quad \text{(Eqn. 1)}$$

Where G.A. represents genetic advance.

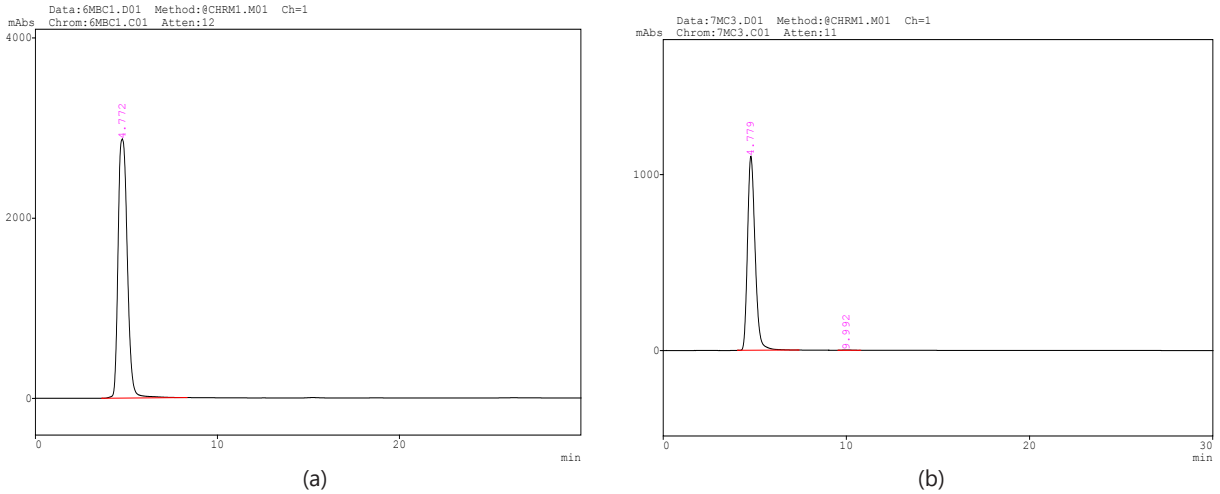


Fig. 2. HPLC chromatogram showing (a) β carotene standard peak and (b) peak of sample LP3.

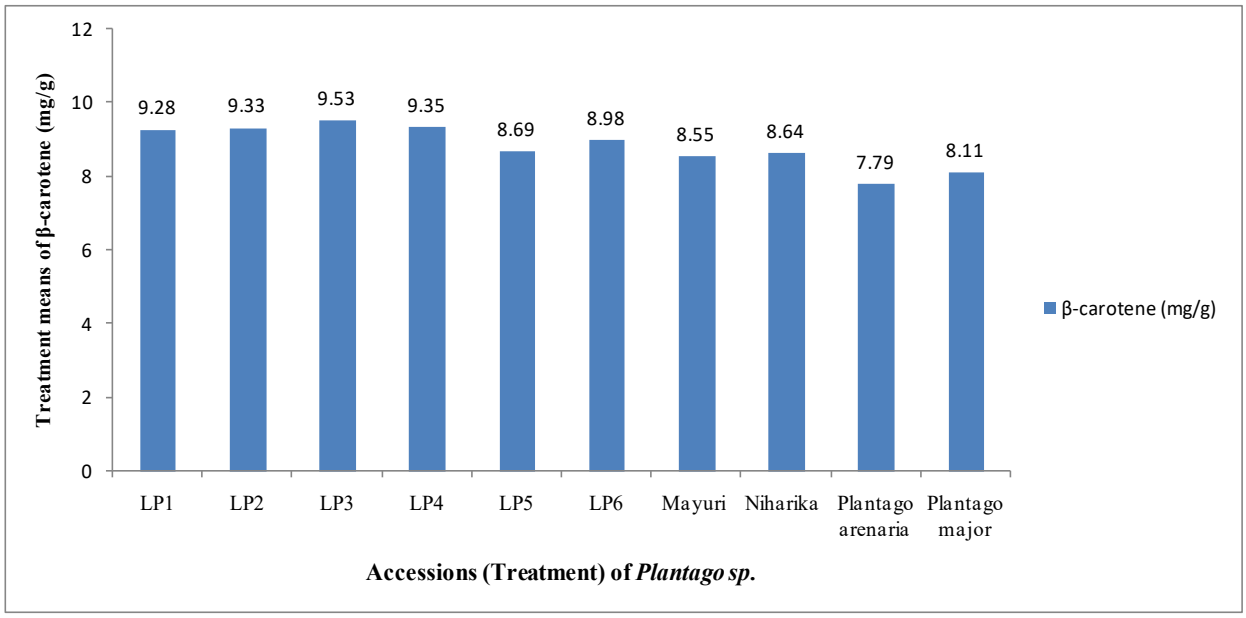


Fig. 3. Quantification of β carotene in six selections, two cultivars of *Plantago ovata*, *Plantago arenaria* and *Plantago major*.

Table 1
Mean performance and other allied parameters in *Plantago* sp.

	Plant height (cm)	Days to 50% flowering	Days to maturity	Panicle length (cm)	Seed yield/plot (g)	Husk yield/plot (g)	β-carotene (mg/g)
LP1	36.00	85.67	100.67	5.50	58	24.00	9.28
LP2	38.00	88.67	100.67	6.00	35	12.33	9.33
LP3	38.50	87.67	100.33	6.17	78	30.00	9.53
LP4	38.40	86.33	102.67	5.33	50	16.33	9.35
LP5	42.15	77.67	103.33	5.50	35	14.00	8.69
LP6	42.27	86.67	102.33	5.17	52	27.67	8.98
Mayuri	30.20	84.67	93.67	4.67	15	7.67	8.55
Niharika	39.43	84.33	97.67	4.83	38	14.00	8.64
Plantago arenaria	75.50	106.00	93.67	3.67	65	22.67	7.79
Plantago major	41.17	187.67	195.00	37.00	40	12.00	8.11
Mean	41.90	97.57	109.00	8.83	21.00	18.07	8.83
Range	30.20-75.50	77.67-187.67	93.67-195.00	3.67-37.00	15-78	7.67-30.00	7.79-9.53
F values	140.16**	198.32**	307.22**	1038.47**	21.48**	15.84**	337.80**
$CD_{5\%}$	3.07	6.85	5.15	0.93	11.50	5.59	0.0093
$CD_{1\%}$	4.21	9.39	7.07	1.27	15.77	7.67	0.0127
CV%	4.24	4.09	2.76	6.46	14.37	18.04	0.6117

*p<0.05, **p<0.01; CD = Critical difference, CV=Critical variance.

Co-heritability value of a character contribution suggests that the increase in one of the characters of those contributions would be coupled in increasing trend in its co-heritable character. Where co-heritability (trait 1, trait 2) is genotypic covariance/phenotypic covariance of trait 1 and trait 2 (Singh, 1988; Lal et al., 2014).

3. Results and Discussion

3.1. Quantification of β-carotene in seeds of six selections, two cultivars and two species of *Plantago*

Detection of some unique lines in *P. ovata* accessions containing β-carotene as an additional value added active principle can become a very useful selection from nutraceutical point of view. Intake of psyllum husk containing β-carotene (as pro-vitamin A) in it can surely act as a supplement/add to the health benefits for patients suffering from diarrhea/constipation/uneasy bowel movement. Thus, the whole seeds of *P. ovata* (LP-1, LP-2, LP-3, LP-4, LP-5, LP-6, Mayuri and Niharika), *P. arenaria* and *P. major* were subjected to estimation of β-carotene by HPLC method in which the highest concentration was detected in selection LP3 with 9.53 mg/g of β-carotene (Fig. 2) while the lowest was detected in selection LP5 with 8.69 mg/g of β-carotene. The lowest amount of β-carotene was recorded in *Plantago arenaria* with 7.79 mg/g while *Plantago major* had a concentration of 8.11 mg/g of β-carotene.

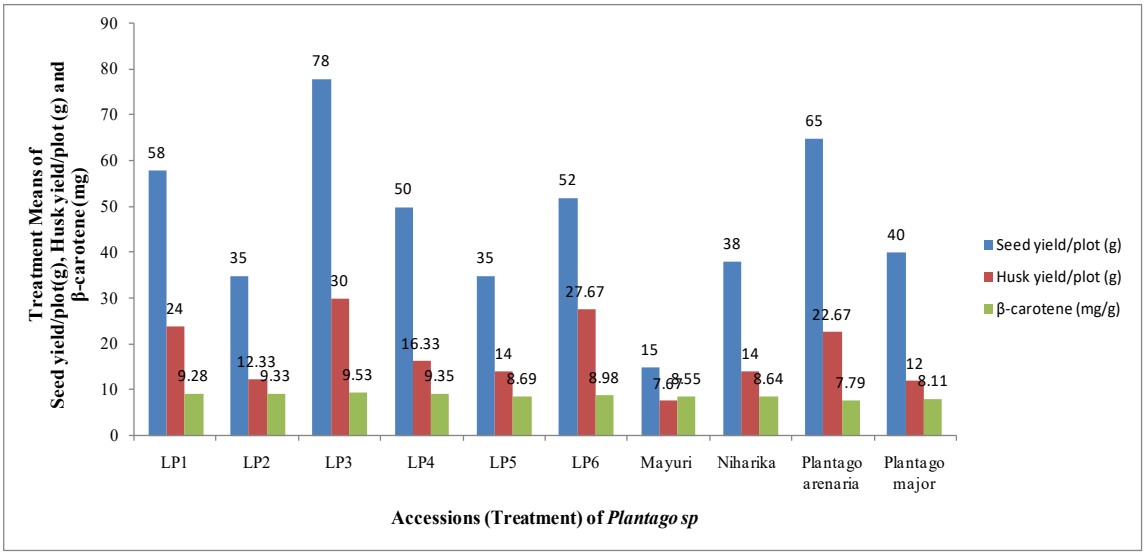


Fig. 4. Relation between seed yield/plot, husk yield/plot and β-carotene in ten accessions in *Plantago* sp.

Table 2
Estimates of genetic parameters in *Plantago* sp. for different economic traits.

Genetic parameters	Plant height (cm)	Days to flowering (50%)	Days to maturity	Panicle length (cm)	Seed yield/plot (g)	Husk yield/plot (g)	β-carotene (mg/g)
Genotypic variance ($\hat{\sigma}^2\ g$)	148.468	1049.182	922.026	101.507	52.552	0.003	307.223
Phenotypic variance ($\hat{\sigma}^2\ p$)	151.668	1065.133	931.059	101.801	63.178	0.003	352.222
Genotypic coefficient of variation (GCV) (%)	28.900	33.210	27.858	120.180	40.125	6.481	37.559
Phenotypic coefficient of variation (PCV) (%)	29.209	33.462	27.994	120.354	43.995	6.510	40.216
Heritability $_{BS}$ % ($\hat{h}^2\ (BS)$ %)	97.889	98.502	99.029	99.712	83.181	99.117	87.224
Genetic advance (GA)	24.571	65.726	61.945	20.695	12.422	0.116	31.494
Genetic advance over mean (%)	58.64	67.36	56.83	234.37	59.55	0.642	-

- = spurious

However, Mayuri and Niharika exhibited almost similar concentration of β-carotene being 8.55 mg/g and 8.64 mg/g, respectively (Table 1, Fig. 3).

3.2. Associations and contribution of β-carotene affecting other economic traits in *P. ovata*

It was observed in all the cases that these special orange color seeds bearing plants of *P. ovata* had longer panicles compared to the other accessions. Now, it was a matter of further study to explore whether the length of panicle and orange coloured seeds (exhibited due to presence of β-carotene) in *P. ovata* go hand to hand. Considering this to be true, it is then quite possible to unveil a strain depicting some very desirable features like-longer panicle length which leads to production of more seeds. More seeds will lead to the production of more husks. Eventually, these seeds will contain more β-carotene. The relation between seed yield/plot (g), husk yield/plot (g) and β-carotene (mg/g) is exhibited in Fig. 4.

Means of the ten treatments (ten accessions) over seven traits along with their range, critical difference (CD) at both 5% and 1% levels and critical variance percentage (CV%) is represented in Table 1. CD at both the levels was highest in seed yield/plot (11.50, 15.77) followed by days to 50% flowering (6.85, 9.39), husk yield/plot (5.59, 7.67) and lowest in β-carotene (0.0093, 0.0127). In addition, CV was highest in husk yield/plot (18.04%) followed by seed yield/plot (14.37%), panicle length (6.46%) and was lowest in β-carotene (0.6117%).

Panicle length exhibited maximum (120.18%) genotypic coefficient of variation (GCV). Moderate GCV was recorded in seed yield/plot (40.1251%) and β-carotene (37.5594%) suggesting the scope of effective selection in these traits for further genetic improvement. The lowest estimation of GCV (6.48124%) was displayed by husk yield/plot. For all the traits phenotypic coefficient of variation (PCV) was higher than their corresponding GCV (Table 2), which reflects that the apparent variation was due to genotypes and environment both. The maximum amount of PCV (120.3536%) was displayed by panicle length; moderate was seed yield/plot (43.99%) followed by β-carotene (40.21%), days to 50% flowering (33.46%), plant height (29.20%) and days to maturity (27.994%). The lowest

Table 3

Genotypic (r_g), phenotypic (r_p), environmental (r_e) correlation and coheritability in broad sense (Co-Her(B)) for seven traits in *Plantago* germplasm.

Traits	Correlations	Plant height (cm)	Days to 50% flowering	Days to maturity	Panicle length (cm)	Seed yield/plot (g)	Husk yield/plot (g)	β-carotene (mg/g)
Plant height	r_g	-	0.162	-0.071	-0.076	0.313	-0.623*	0.461
	r_p		0.155	-0.075	-0.076	0.274	-0.619*	0.400
Days to 50% flowering	r_e	-0.180	-	0.957**	0.966**	-0.226	-0.532	-0.029
	Co-Her(B).	1.021		0.948**	0.958**	-0.185	-0.527	-0.019
Days to maturity	r_e	-0.355	0.232	-	0.998**	-0.266	-0.366	-0.120
	Co-Her(B).	0.932	0.997		0.995**	-0.234	-0.359	-0.089
Panicle length	r_e	-0.076	0.214	0.543	-	-0.283	-0.380	-0.127
	Co-Her(B).	0.992	0.999	0.997		-0.257	-0.377	-0.114
Seed yield/plot	r_e	-0.145	0.385	0.182	0.044	-	0.337	0.943**
	Co-Her(B).	1.032	1.104	1.031	1.004		0.304	0.833**
Husk yield/plot	r_e	-0.398	-0.091	0.293	0.137	-0.064	-	0.244
	Co-Her(B).	0.991	0.998	1.008	1.002	1.008		0.227
β-carotene	r_e	-0.489	0.167	0.630	0.234	0.200	0.003	-
	Co-Her(B).	1.063	1.371	1.248	1.039	0.965	0.999	

*-p<0.05; **-p<0.01; r_g=genotypic correlation, r_p=phenotypic correlation, r_e=environmental correlation, Co-Her(B)=Coheritability in broad sense, correlation due to genotype=number at the top, correlation due to phenotype=number below the top in above diagonal area, correlation due to environment=number at the top, coheritability in broad sense=number below the top in below diagonal area.

Table 4

Direct (in bold and above diagonal) and indirect (below diagonal) effect of seven traits on seed yield in *Plantago* sp. Residual effect=0.273; r_g=genotypic correlation.

	Plant height (cm)	Days to 50% flowering	Days to maturity	Panicle length (cm)	Husk yield/ plot (g)	*β*-carotene (mg/g)	r_g (g)
Plant height	**-0.755**	0.730	-0.431	0.787	0.259	-0.129	0.461
Days to 50% flowering	-0.122	**4.520**	5.826	-9.956	-0.187	-0.110	-0.029
Days to maturity	0.053	4.327	**6.086**	-10.29	-0.220	-0.076	-0.120
Panicle length	0.058	4.364	6.074	**-10.310**	-0.235	-7.884	-0.127
Husk yield/plot	-0.236	-1.019	-1.619	2.920	**0.828**	0.069	0.943
β-carotene	0.471	-2.405	-2.227	3.919	0.279	**0.207**	0.244

estimate of PCV (6.51004%) was exhibited by husk yield/ plot. The minimum difference between PCV and GCV was observed in husk yield/plot (0.029%) followed by days to maturity (0.136%) and panicle length (0.174%); which suggests that these traits were least effected by environment. This in turn was also supported by higher values of heritability in broad sense for these traits. Wide differences between PCV and GCV were recorded in seed yield/plot (3.87%) and β-carotene (2.657%). This was further supported by least values of heritability in broad sense for these traits (Table 2). These are an indicative sign of the fact that the characters were much influenced by environmental fluctuation and hence, they are not easy traits for selection.

Genotypic correlation coefficients were higher in magnitude than phenotypic correlation coefficients among all traits involved except days to maturity (Table 3) which implies that environment had a little role to play in the expression of characters, suggesting inherent associations between these traits at their genotypic level. It is evident from the results that plant height depicted significant and positive genotypic and phenotypic correlation with husk yield/plot (r_g=0.623, r_p=0.619) whereas, days to 50% flowering showed the same with days to maturity (r_g=0.957, r_p=0.948) and panicle length (r_g=0.966, r_p=0.958). Panicle length depicted the same response with days to maturity (r_g=0.998, r_p=0.995). It is interesting to note that seed yield/plot and β-carotene were positively correlated to each other (r_g=0.943, r_p=0.833). This indicates that the selection of the above mentioned characters directly affect the seed yield positively and would simultaneously lead to its improvement. Maximum co-heritability was exhibited by days to maturity and β-carotene (1.317) followed by panicle length and β-carotene (1.248), days to 50% flowering and seed yield/plot (1.104) whereas, the minimum co-heritability was exhibited by plant height and days to maturity (0.932).

3.3. Path analysis and character contribution towards

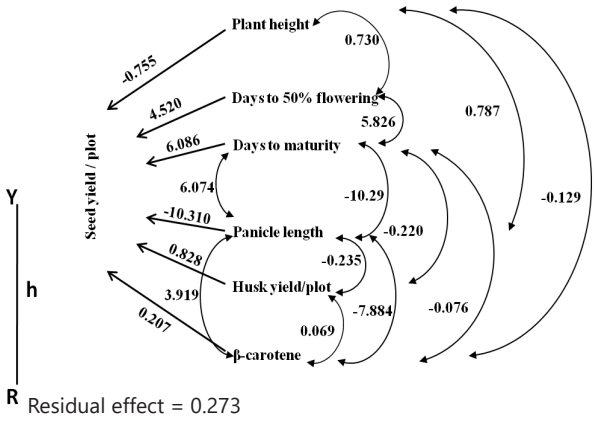
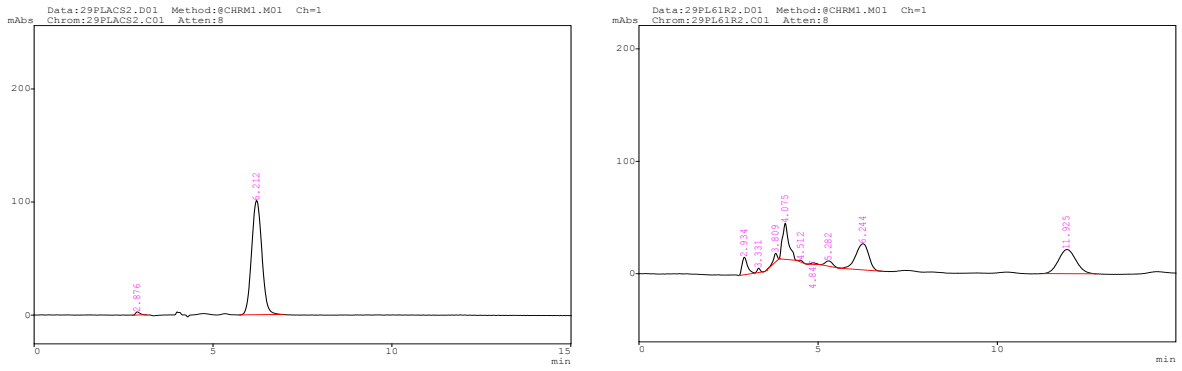


Fig. 5. Path diagram from 10 accessions of *Plantago* species.



(a) (b)

Fig. 6. The HPLC chromatogram of (a) aucubin standard (b) sample PL-61.

Table 5

Estimation of aucubin in some distinct lines in five *Plantago* species.

No	Accession/ Sample I.D.	Botanical Name	Origin	Differentiating characters	Plant part used		Aucubin content (mg/g)
1	PO-1	*P. ovata*	Bulgaria	Normal panicle	Seeds	Leaves	Not detected
2	PO-18	*P. ovata*	Uttar Pradesh (Lucknow), India.	Dispersed flowers in panicle	Seeds	Leaves	Not detected
3	PO-22	*P. ovata*	Uttar Pradesh (Lucknow), India.	Feathery branched panicle	Seeds	Leaves	Not detected
4	PO-34	*P. ovata*	Uttar Pradesh (Lucknow), India.	Two branched panicle	Seeds	Leaves	Not detected
5	PO-46	*P. ovata*	Uttar Pradesh (Lucknow), India.	Three branched panicle	Seeds	Leaves	Not detected
6	PO-52	*P. ovata*	Uttar Pradesh (Lucknow), India.	Cultivar Mayuri	Seeds	Leaves	Not detected
7	PO-53	*P. ovata*	Uttar Pradesh (Lucknow), India.	Seven branched panicle	Seeds	Leaves	Not detected
8	PO-55	*P. ovata*	Uttar Pradesh (Lucknow), India.	Long panicle	Seeds	Leaves	Not detected
9	PO-57	*P. ovata*	Uttar Pradesh (Lucknow), India.	Feathery panicle	Seeds	Leaves	Not detected
10	PO-78	*P. ovata*	Uttar Pradesh (Lucknow), India.	Club shaped inflorescence	Seeds	Leaves	Not detected
11	PP-03	*P. psyllium*	Uttar Pradesh (Lucknow), India.	Normal small panicle	Seeds	Leaves	Not detected
12	PM-32	*P. major*	Slovak Republic	Normal long panicle	Seeds	Leaves	Not detected
13	PA-102	*P. arenaria*	Italy	Normal medium panicle	Seeds	Leaves	Not detected
14	PL-61	*P. lanceolata*	Malayasia	Normal long panicle	Seeds	-	0.233
15	PL-63	*P. lanceolata*	Malayasia	Normal long panicle	Seeds	-	0.059
16	PL-64	*P. lanceolata*	Hungary	Normal long panicle	Seeds	-	0.117
17	PL-65	*P. lanceolata*	Poland	Normal long panicle	Seeds	-	0.212
18	PL-66	*P. lanceolata*	France	Normal long panicle	Seeds	-	0.022
19	PL-68	*P. lanceolata*	Uttar Pradesh (Lucknow), India.	Normal long panicle	Seeds	-	0.025
20	PL-69	*P. lanceolata*	Uttar Pradesh (Lucknow), India.	Normal long panicle	Seeds	-	0.053
21	PL-70	*P. lanceolata*	Uttar Pradesh (Lucknow), India.	Normal long panicle	Seeds	-	0.041
22	PL-71	*P. lanceolata*	Uttar Pradesh (Lucknow), India.	Normal long panicle	Seeds	-	0.065

- = leaf not used

seed yield

A critical perusal of path coefficient and character contributions study revealed that the largest direct positive contributors to seed yield (Table 4, Fig. 5) were days to maturity (6.086) followed by days to 50% flowering (4.520), husk yield/plot (0.828), β-carotene (0.207) whereas, plant height (-0.755) and panicle length (-10.310) were negative direct contributors. Indirect contributions were large via days to maturity (5.826) followed by panicle length (0.787), days to 50% flowering (0.730), husk yield/plot (0.2599) and β-carotene (0.069) though the residual effect was a little higher (R=0.273). The indirect contributors that contributed to direct contributors (diagonal values) through upper indirect contributors was also taken into account. Panicle length (6.074) was the highest indirect contributor to seed yield through days to maturity and β-carotene (-2.405) was the lowest indirect contributor to seed yield through days to 50% flowering.

Heritability and genetic advance parameters also played a major role in unison with character association and path analysis in the genetic improvement program in *Plantago* crop (Table 2). The highest heritability percent in broad sense was observed for panicle length (99.71%) followed by husk yield/plot (99.12%), days to maturity (99.03%), days to 50% flowering (98.50%) and plant height (97.89%). Hence, selections of these above mentioned traits are easy in isabgol crop. Moderate heritability was observed for β-carotene (87.22%) and

Table 6
Quantification of β-carotene in ten elite lines/cultivars of three *Plantago* species by HPLC.

No.	Accession/Sample I.D.	Botanical Name	Origin	Sample features	β-carotene (mg/g)
1	LP1	*P. ovata*	Uttar Pradesh (Lucknow), India.	Whole seeds	9.28
2	LP2	*P. ovata*	Uttar Pradesh (Lucknow), India.	Whole seeds	9.33
3	LP3	*P. ovata*	Uttar Pradesh (Lucknow), India.	Whole seeds	9.53
4	LP4	*P. ovata*	Uttar Pradesh (Lucknow), India.	Whole seeds	9.35
5	LP5	*P. ovata*	Uttar Pradesh (Lucknow), India.	Whole seeds	8.69
6	LP6	*P. ovata*	Uttar Pradesh (Lucknow), India.	Whole seeds	8.98
7	Cultivar Mayuri	*P. ovata*	Uttar Pradesh (Lucknow), India.	Whole seeds	8.55
8	Cultivar Niharika	*P. ovata*	Uttar Pradesh (Lucknow), India.	Whole seeds	8.64
9	PA	*P. arenaria*	Italy	Whole seeds	7.79
10	PM	*P. major*	Slovak Republic	Whole seeds	8.11

seed yield/plot (83.18%). The genetic advance (GA) was observed to be highest in days to 50% flowering (65.73) followed by days to maturity (61.95). It was found to be medium for β-carotene (31.49) followed by plant height (24.57), panicle length (20.70). Genetic advance was found to be low in seed yield/plot (12.42) and lowest in husk yield/plot (0.12). High heritability and high genetic advance were observed for the traits days to maturity (99.03%, 61.95) and days to 50% flowering (98.50%, 65.73). It was possibly governed due to additive gene action (Khanna and Shukla, 1989) and therefore, selection maybe easy for these traits. However, moderate heritability and moderate genetic advance were exhibited by β-carotene (87.22%, 31.49). It is evident from the results that genetic advance (%) over mean was highest for panicle length (234.37%) followed by days to 50% flowering (67.36%), seed yield/plot (59.55%), plant height (58.64%) and days to maturity (56.83%), whereas it was lowest (0.642) in husk yield/plot (Table 2). Seed yield is also highly significant and positively correlated with β-carotene with high to moderated heritability in broad sense and genetic advance which means selection of seed yield will automatically affect β-carotene.

3.4. Quantification of aucubin in *Plantago* species

Leaves and whole seeds of some selected lines of five species of *Plantago* were also subjected to aucubin analysis by HPLC methods. It is interesting to note that the presence of aucubin was only detected in seeds of *P. lanceolata* whereas; aucubin was not detected in none of the other lines in any species. The highest amount of aucubin derived from whole seeds was detected in PL-61 accession of *P. lanceolata* with 0.233 mg/g aucubin (Fig. 6), followed by 0.212 mg/g in PL-65, 0.1 mg/g in PL-64. The least concentration of aucubin was detected to be 0.022 mg/g in PL-66 accession of *P. lanceolata* (Table 5, Fig. 7).

The concentration of β-carotene among all the ten accessions was highest in *P. ovata* (Table 6, Fig. 3) selection-LP3 (9.53 mg/g) followed by in selection LP4 (9.35 mg/g) and selection LP2 (9.33 mg/g). Cultivar Niharika (8.64 mg/g) and Mayuri (8.55 mg/g) both of the varieties were released by CSIR-CIMAP estimated β-carotene almost equally whereas *P. major* (8.11 mg/g) estimated higher values than *P. arenaria* (8.11 mg/g). None of the species of *Plantago* apart from the nine accession of *P. lanceolata* were positive in the determination of aucubin. The highest concentration of aucubin was found in the whole seeds of accession PL-61 and lowest in accession PL-66 of *P. lanceolata*.

According to the overall performance of mean, correlations, character contributions, heritability, genetic advance, role of β-carotene concentration and aucubin concentration, accessions LP3, LP4 and LP2 of *P. ovata* were selected as high β-carotene producing lines whereas, accessions PL-65 and PL-61 of *P. lanceolata* were selected as high aucubin producing lines in whole seeds respectively and these lines could be exploited commercially.

4. Concluding remarks

In this this study, a tremendous variability among all the traits was noted. β-carotene had highly significant and positive correlation to seed yield which means selection for seed yield will automatically affect β-carotene. Study of path coefficient revealed that the highest positive direct contributor to seed yield was days to maturity whereas the least was related to β-carotene. Indirect effect was highest for days to maturity and lowest for β-carotene while other traits exhibited negative values. Indirect effect of panicle length through days to maturity was highest; similarly β-carotene through days to 50% flowering was lowest in relation to seed yield/plot, although residual effect was a little high. The maximum heritability was observed for panicle length and the least was detected for seed yield/plot. High heritability and high genetic advance were observed for days to maturity mainly. Thus, on the basis of the overall performance accessions LP3, LP4, LP2 of *P. ovata* and PL-65 and PL-61 accessions of *P. lanceolata* were found to be more suitable for specific value addition purpose of β-carotene and seed yield for commercial cultivation. The article represents a unique observation of the presence of a tetraterpenoid secondary metabolite (β-carotene) that has been

reported for the first time in the seed coats of different accessions of *P. ovata* and other species *P. major* and *P. arenaria*. In addition, the idea to estimate their content based on the chemotaxonomic marker aucubin along with the other metabolite β-carotene multiplies the nutritional aspects of this genus in a new dimension. The concept of carotene as a marker based quantification also earmark a new field of study in chemotaxonomy where possibly apart from iridoids, phenylethanoids, flavones the tetraterpenoids could now be considered or can be further studied for chemotaxonomic based classification and other generic traits.

Conflict of interest

The authors declare that there is no conflict of interest.

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