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Changes in total phenolics and antioxidant activities in the developing fruits of mango

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ABSTRACT

The present study aimed to determine the changes in total phenolic contents and antioxidant activities during the course of fruit development in mango. Initially, 40 genotypes were assessed at ripe stage for total phenolic contents (TPC). TPC in peel was 4.87-fold higher compared to pulp. In peel and pulp tissues, the maximum total phenolic contents was in Langra (4.68 and 1.46 mg/g FW, respectively), while, minimum was in Ellaichi peel and Hybrid 1-5 pulp. Based on this, 21 mango genotypes having variable total phenols were selected to study the changes during fruit developmental stages, *viz*., marble, 15 days after marble, egg and ripe stage. Marble stage registered the highest total phenolics (28.88 mg/g FW) compared to 15-days after marble (25.18 mg/g FW), egg (21.72 mg/g FW) and ripe (2.25 mg/g FW) stages. The higher total phenols in peel was in concurrence with higher antioxidant activity (7.97 µmol trolox/g FW) compared to pulp (4.11 µmol trolox/g FW). The mean total phenols in mango genotypes showed positive correlation with total antioxidant activities in peel, pulp and mean of peel and pulp (R^2 = 0.75, 0.89 and 0.87, respectively). Langra and Bombay Green had higher phenol contents at young stage of fruit development, which progressively declined towards the approach of ripe stage.

Key words: Biochemical changes, *Mangifera indica*, fruit development, fruit tissue, genotypes.

INTRODUCTION

Mango (*Mangifera indica* L.) occupies about 2.26 million ha area with an annual production of 21.82 million tonnes, which accounts for 34.7% of area and 22.41% of total production of all fruits in the country (NHB, 11). India is the largest producer of mango accounting for almost 40% of the world production. Mango is a rich source of bioactive compounds such as ascorbic acid, polyphenols, carotenoids *etc*., and receiving more attention because of its higher antioxidant activity. Consumption of such antioxidants offers several health benefits including protection against cardiovascular diseases and cancer.

At present, global population is facing the dual challenges of nutritional insecurity and dietary disorders, leading to health problems such as obesity, cancer and cardio-vascular diseases. Phenolic compounds in mango are biologically active and responsible for health promoting effects. Compounds such as gallotanins, gallic acid and its derivatives, mangiferin, flavonoids, catechin and phenolic acids are the main bioactives (Alanona *et al*., 4). The quantity of polyphenolic compounds in mango varies in different parts of the fruit. The polyphenolic content of peel ranges from 55 to 110 mg GA equivalents per g dry weight, and is higher in unripe than in ripe fruit (Ajila *et al*., 3). Similarly, bound polyphenolic are reported in the range of 8.1-29.5 mg/g (Ajila and Rao, 2). There are pronounced differences in the concentrations of bioactive compounds in pulp, peel and seed. Therefore, the pulp, peel, and stone of mango fruit may also be of interest from an industrial point of view (Masibo and He, 10). Phenolic composition of mango fruit is influenced by several factors such as genotype (Lopez-Cobo *et al*., 9), storage conditions (Vithana *et al*., 15) and spray of chemicals (Vithana *et al*., 16). However, the most impacting factor is fruit developmental stage since this involves various physiological, biochemical and molecular changes responsible for degradation or synthesis of phenolics (Tiwari and Cummins, 14). The changes in total phenolics in fruits at different developmental stages in different mango varieties may also provide clue for their involvement in taste, flavour and colour development. Mangoes are consumed as fresh fruit or processed into value-added products. In all these cases, only the pulp is used, while the stones and peels are discarded, which results in a substantial waste of organic material. However, there is immense scope to exploit all mango derived materials in order to avoid wastage and to ensure improved nutritional security

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and health. Keeping in view the above facts, present study was carried out to track the changes in total phenolic contents in fruit peel and pulp at different developmental stages.

MATERIALS AND METHODS

The experiment was conducted at the Division of Fruits & Horticultural Technology, ICAR-IARI, New Delhi during 2016-18. The institute is located at an altitude of 228.16 m above mean sea level with 28°40' N latitude and 77°10' E longitude and located in the trans-Gangetic plains of India.

Initially, 40 mango genotypes (Table 1) of diverse origin were used in the present study to investigate the level of phenols in mango peel and pulp at ripe stage. Based on the results, 21 mango genotypes, namely, Mallika, Amrapali, Pusa Shreshth, Pusa Lalima, Pusa Surya, Pusa Peetamber, Pusa Arunima, Pusa Pratibha, Dashehari, Maya, Langra, Lucknow Safeda, Neelum, H-11-2, Bombay Green, Bhadauran, Extreema, Sensation, Totapuri, Tommy Atkins and Kesar representing high to low total phenols types were selected for determination of phenols in peel and pulp at fruit developmental stages, *i.e.*, marble, 15 days after marble, egg and ripestage. Fruits free from injuries and bruises were randomly harvested along with 1-2 cm pedicel in the morning hours. Fruits were washed twice using double-distilled water and kept on blotting paper for removal of adhered water and then labeled pouches having the samples were transferred to freezer at -80°C (Thermo Scientific, USA). Freeze-dried powder (2 g) from peel and pulp tissues was dissolved in 10 ml methanol/water (80:20, v/v). The mixture was sonicated in an ultrasonic bath for 15 min. After the extraction process, mixture was centrifuged for 15 min at 10000 × g at 4°C. The supernatant was collected and the extraction step was repeated twice (Gomez-Caravaca *et al*., 7). This supernatant was further used for estimation of total phenolic contents and antioxidant activities.

Total phenolics was measured using Folin-Ciocalteu reagent using gallic acid as a standard (Singleton *et al*., 13). Absorbance was measured at 760 nm with the help of double beam UV-VIS Spectrophotometer (UVD3200, Labomed, USA). The amount of phenolics was expressed in mg as gallic acid equivalents (GAE)/100 g FW). Total antioxidant activity was measured as per the method suggested by Brand-Williams *et al*. (5) and absorbance was measured at 515 nm using double beam UV-VIS spectrophotometer (Labomed, USA). Results were expressed in terms of Trolox equivalents per g FW.

Table 1. Mango genotypes along with place of origin used in the present study.

Sl. No.	Genotype	Origin	Sl. No.	Genotype	Origin
1	Alphan	North India	21	Lucknow Safeda	North India
2	Ametista	Brazil	22	Mallika	North India
3	Amrapali	North India	23	Maya	Israel
4	Bhadauran	North India	24	Neelum	South India
5	Bombay Green	North India	25	Olour	South India
6	Chausa	North India	26	Primor de Amoriera	Brazil
7	Dashehari	North India	27	Pusa Arunima	North India
8	Edward	Florida	28	Pusa Lalima	North India
9	Ellaichi	North India	29	Pusa Peetamber	North India
10	Exteema	Brazil	30	Pusa Pratibha	North India
11	H-3-2	North India	31	Pusa Shreshth	North India
12	H-1-11	North India	32	Pusa Surya	Florida
13	H-11-2	North India	33	Ratna	West India
14	H-12-5	North India	34	Sensation	Florida
15	H-1-5	North India	35	Smith	Florida
16	H-165	North India	36	St. Alexandrina	Brazil
17	Kerala-1	South India	37	Suvarnarekha	South India
18	Kesar	West India	38	Tommy Atkins	Florida
19	Kurukkan	South India	39	Totapuri	South India
20	Langra	North India	40	Willard	Sri Lanka

The present experiment was laid out in Factorial Randomised Block Design and replicated thrice. Statistical analysis was performed using SAS 9.3 (SAS Institute, Cary, NC, USA) and valid conclusions were drawn only on significant differences between the treatment mean at 0.05 level of probability. In order to compare treatment means, Least Significant Difference (LSD) was calculated.

RESULTS AND DISCUSSION

The mean effect of mango genotype, fruit tissue and their interaction significantly ($P \leq 0.05$) affected the total phenolics. For the 40 genotypes studied, among the two fruit tissues taken, peel had higher total phenolic contents (3.16 mg/ g FW) than pulp (0.66 mg/ g FW) at ripe stage, regardless of genotype. It was also evident that phenol content in peel was 4.87-fold higher compared to pulp tissue. In peel tissue, maximum total phenols was observed in Langra (4.68 mg/ g FW) and the minimum was in Ellaichi (1.95 mg/ g FW). Similarly, for pulp too, Langra had the maximum total phenols (1.46 mg/ g FW) but the minimum was recorded in Hybrid 1-5 (0.25 mg/ g FW) (Fig. 1). The mean effect of mango genotypes indicated significantly ($P \leq 0.05$) higher total phenols in Langra which was 2.51-fold higher than the Ellaichi (Fig. 1). Plant phenolics are natural metabolites arising from shikimate/ phenylpropanoid pathway or the acetate/malonate pathway which can produce both monomeric and polymeric polyphenols. Higher plants synthesize several thousand different phenolics compounds. The ability to synthesize phenolic compounds has been selected throughout the course of evolution in different plant lineages, thus permitting plants to cope with the constantly changing environmental challenges over evolutionary time (Lattanzio, 8). The differences in total phenolic contents in mango genotypes may be attributed to the genotypic effect and its interaction with the environment. Present results are in conformity with the findings of Alanona *et al.* (4).

Total phenols in peel and pulp tissues of 21 mango genotypes was assessed at fruit developmental stages, *viz*., marble, 15 days after marble, egg and ripe stages. Amongst the four developmental stages, fruits at marble stage registered the highest total phenolics (28.88 mg/ g FW) and the lowest at ripe stage (2.25 mg/ g FW). Irrespective of mango genotypes, the maximum total phenols in peel was observed at marble stage (34.89 mg/ g FW), which progressively declined at 15 days after marble (29.17 mg/ g FW), egg (27.09 mg/ g FW) and ripe (3.59 mg / g FW) stages. Similar trend was observed within the total phenolic contents of pulp (Table 2; Fig. 2a,b). We observed a linear relationship (R^2 = 0.98) between peel and pulp mean total phenolic content in 21 mango genotypes at different fruit developmental stages (Fig. 2d). At 15 days after marble stage, again peel of genotype Langra had highest total phenols (40.71 mg/ g FW), while peel of genotype Maya registered the lowest total phenols (19.56 mg/ g FW). For pulp tissue, Bombay Green registered highest total phenolics (27.11 mg/ g FW) followed by Totapuri (26.73 mg/ g FW), and lowest

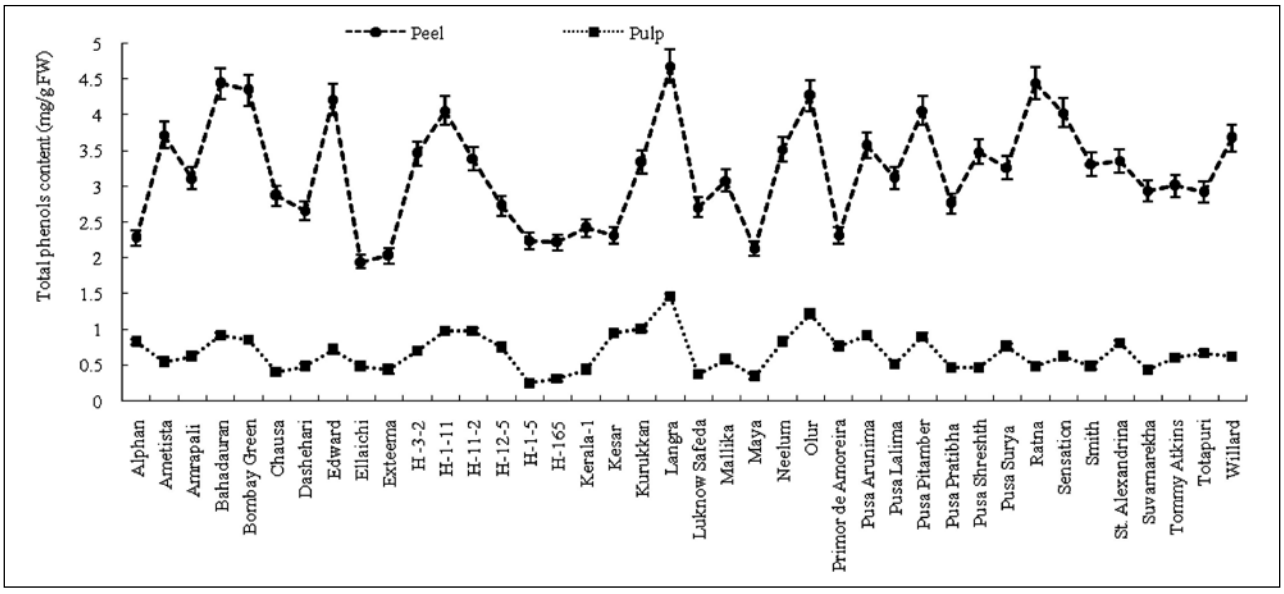

Fig. 1. Total phenols in peel and pulp of 40 mango genotypes at ripe stage. Vertical bars indicate ± SE of means. LSDs ($P \leq 0.05$) were: mango genotype (G)= 0.63; fruit tissue (T) = 0.17; G × T = 1.15, n = 3.

was in Maya (14.61 mg/ g FW). At egg stage, Bombay Green peel showed highest phenol contents (36.68 mg/ g FW) and had non-significant differences with Langra peel (35.70 mg/ g FW) while Maya registered the lowest phenol contents (19.30 mg/ g FW) and had non-significant difference with Dashehari peel (19.35 mg/ g FW). For pulp tissue, almost similar trend was observed and Langra had the highest total phenolic contents (23.21 mg/ g FW) which was on par with content of total phenols in Bombay Green pulp (22.65 mg/ g FW). Pulp of Maya recorded the lowest total phenolics (11.68 mg/ g FW). At ripe stage, total phenols decreased sharply and it was highest in peel and pulp of genotype Langra (4.88 and 1.68 mg/ g FW, respectively). However, it was minimum in peel of Maya (2.63 mg/ g FW) and pulp of Pusa Pratibha (0.47 mg/ g FW).

Considering all stages and fruit tissues together, significant variation (LSD = 3.58; $P \leq 0.05$) in total phenolic contents was observed among 21 mango genotypes studied which ranged from 13.43 to 25.43 mg/ g FW. Langra had maximum total phenols non-significantly followed by Bombay Green (25.09 mg/ g FW). The minimum total phenol contents was recorded in Maya (Table 2; Fig. 2c). It was also evident that peel fraction had significantly (LSD = 1.18; $P \leq 0.05$) higher total phenols (23.68 mg/ g FW) compared to pulp (15.33 mg/ g FW) fraction, regardless of genotype and fruit development stage (Table 2; Fig. 2a,b). The interaction effect of mango genotype and fruit development stages on total phenolic contents was also significant (LSD = 7.65; $P \leq 0.05$). The maximum cumulative total phenols in both fractions was recorded in Langra at marble stage (38.27 mg/ g FW). Minimum cumulative total phenols was noted at ripe stage in Maya (0.58 mg/ g FW) (Fig. 2c). Similarly, the interaction effect of mango genotype and fruit tissue on total phenolic

Table 2. Total phenol contents (mg/ g FW) in mango genotypes at different fruit developmental stages.

Sl. No.	Genotype	Marble stage			15 Days after marble stage			Egg stage			Ripe stage		
		Peel	Pulp	Mean	Peel	Pulp	Mean	Peel	Pulp	Mean	Peel	Pulp	Mean
1	Amrapali	33.78	20.65	27.22	27.61	20.58	24.10	20.73	10.63	15.68	3.21	0.83	2.02
2	Bhadauran	30.50	18.08	24.29	26.75	18.58	22.67	33.18	18.26	25.72	4.68	0.99	2.84
3	Bombay Green	45.25	22.65	33.95	40.58	27.11	33.85	36.68	22.65	29.67	4.35	1.43	2.89
4	Dashehari	29.46	25.61	27.54	23.81	21.33	22.57	19.35	12.26	15.81	3.16	0.68	1.92
5	Exteema	42.48	22.05	32.27	37.93	16.26	27.10	33.25	19.76	26.51	4.03	0.93	2.48
6	H-11-2	31.18	21.08	26.13	26.08	25.61	25.85	24.66	15.70	20.18	3.39	0.98	2.19
7	Kesar	38.21	21.68	29.95	26.05	26.21	26.13	23.25	21.61	22.43	2.82	0.65	1.74
8	Langra	45.73	30.80	38.27	40.71	20.75	30.73	35.70	23.21	29.46	4.88	1.63	3.26
9	Lucknow Safeda	38.06	19.51	28.79	31.31	14.73	23.02	28.15	12.13	20.14	3.71	0.78	2.25
10	Mallika	35.25	29.41	32.33	31.10	24.53	27.82	30.43	19.78	25.11	3.18	0.67	1.93
11	Maya	24.45	14.60	19.53	19.56	14.61	17.09	19.30	11.68	15.49	2.63	0.58	1.61
12	Neelum	30.91	20.60	25.76	28.30	22.15	25.23	25.26	12.61	18.94	3.52	1.04	2.28
13	Pusa Arunima	28.90	15.38	22.14	22.61	16.40	19.51	20.15	16.16	18.16	3.57	0.92	2.25
14	Pusa Lalima	38.43	24.56	31.50	32.96	24.71	28.84	31.23	18.33	24.78	3.12	0.62	1.87
15	Pusa Peetamber	30.36	20.23	25.30	26.28	15.63	20.96	25.11	11.98	18.55	4.06	0.93	2.50
16	Pusa Pratibha	34.43	24.15	29.29	26.91	18.71	22.81	25.30	16.91	21.11	3.18	0.47	1.83
17	Pusa Shreshth	40.75	25.56	33.16	30.96	20.30	25.63	31.13	15.51	23.32	3.49	0.67	2.08
18	Pusa Surya	35.51	31.01	33.26	30.75	26.71	28.73	28.88	18.68	23.78	3.27	0.86	2.07
19	Sensation	37.33	21.28	29.31	21.76	22.63	22.20	20.16	15.75	17.96	4.03	1.12	2.58
20	Tommy Atkins	34.01	25.80	29.91	28.56	20.66	24.61	28.16	13.16	20.66	3.68	1.20	2.44
21	Totapuri	27.71	25.71	26.71	31.90	26.73	29.32	28.75	16.68	22.72	3.52	0.87	2.20
	Mean	34.89	22.88	28.88	29.17	21.19	25.18	27.09	16.35	21.72	3.59	0.90	2.25
	Factor	Stage (S)		Tissue (T)		Genotype (G)		SxT		SxG		GxT	SxGxT
	LSD ($P \leq 0.05$)	3.12		4.01		2.63		4.21		7.12		5.44	10.88

content was also found significant (LSD = 54.41; *P* ≤0.05). The maximum total phenols was found in peel of Langra and minimum was in pulp of Maya (Table 2). The interaction effect of fruit development stage and type of fruit tissue was significant (P ≤0.05) and maximum mean content of total phenols was recorded in peel at marble stage (34.89 mg/ g FW). However, the minimum mean total phenols was noted in ripe pulp (0.90 mg/ g FW), regardless of genotypes (Table 2). The interaction effect of genotype, fruit developmental stage and fruit tissue was significant (LSD = 10.83; *P* ≤0.05) for total phenolic contents. Maximum total phenols was observed in peel of Langra at marble stage (45.73 mg/ 100 g FW), which

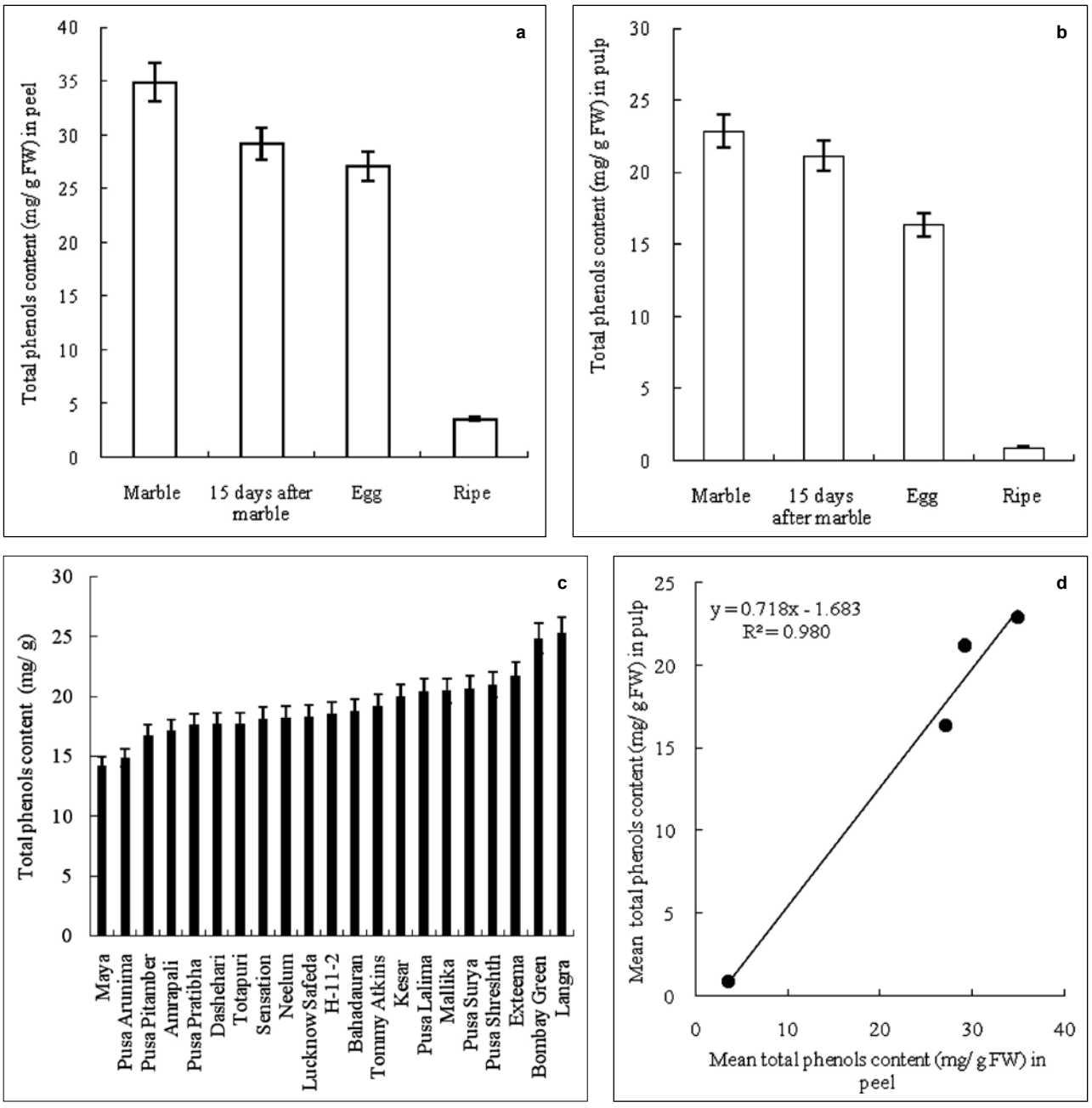

Fig. 2. a. Mean total phenols content in peel at different fruit growth stages. b. Mean total phenols content in pulp at different fruit growth stages. c. Mean total phenols in mango genotypes regardless of stage and tissue, d. Correlation between mean peel and pulp total phenols content in mango genotypes, Vertical bars indicate ±SE of means. n = 3.

did not differ significantly with the total phenol content in peel of Bombay Green at marble stage. However, the minimum total phenols was noted in ripe pulp of Pusa Pratibha (0.47 mg/ g FW), which was on par with total phenolic content in ripe pulp of Maya, Pusa Lalima, Pusa Shreshth and Kesar (Table 2).

In the present investigation, significant variation was observed in terms of total phenolic contents in peel and pulp tissues at different fruit developmental stages of mango genotypes. Results clearly indicated a decrease in total phenols in the two different fruit tissues with the advancement of fruit developmental stages. Peel had significantly higher phenolic content compared to pulp and it was higher at marble stage compared to other advance maturation stages. The variation in phenolics among mango genotypes may be attributed to the genotypic effect and their interaction with the prevailing environmental conditions during course of study. Alanona *et al*. (4) also reported that phenolic composition of mango is influenced by multiple factors such as cultivar, storage conditions, application of chemicals and maturation stage. The present results are in conformity with the findings of Pinsirodom *et al*. (12), Agatonovic-Kustrin *et al.* (1) and Alanona *et al*. (4).

Plant phenolics are considered to have a key role in defense mechanism when environmental stresses lead to increased free radicals and other oxidative species in plants. Both biotic and abiotic stresses stimulate carbon fluxes from the primary to the secondary metabolic pathways, thus inducing a shift of the available resources in favour of the synthesis of secondary products (Lattanzio, 8). It was also observed that Langra was less infested by fruit flies and borers and had a after taste (data unpublished). These features of Langra may have some relationship with high phenolics in fruit tissues.

The data on antioxidant activities of peel and pulp in a set of 21 mango genotypes revealed that mean effect of genotype, fruit tissue and their interaction was significant ($P \leq 0.05$). The mean total antioxidant activity (7.97 µmol trolox/ g FW) was higher in peel compared to pulp (4.11 µmol trolox/ g FW), regardless of genotype. In peel, the maximum antioxidant activity (10.14 µmol trolox/ g FW) was noted in Langra and minimum was in Maya (3.68 µmol trolox/ g FW). For pulp also, Langra and Maya genotypes showed maximum and minimum total antioxidant activity (6.32 and 1.82 µmol trolox/ g FW). The interaction effect of genotype and fruit tissue on total antioxidant activity was significant (LSD = 0.55; $P \leq 0.05$). We observed that antioxidant activity of peel, pulp and mean of peel and pulp had positive correlation (R^2 = 0.75, 0.89, 0.87) with mean total phenolic content in mango genotypes, regardless of fruit development stage and fruit tissue (Fig. 3). Our results are in conformity with the findings of Ajila *et al*. (3), who reported that mango polyphenols exhibited good antioxidant activity by effectively scavenging various free radicals such as DPPH radicals, hydroxyl radicals, peroxyl radicals and reducing the ferric to ferrous ion in different antioxidant systems. The difference in antioxidant activity of the peel of different varieties at different stages of maturity may be due to variation in composition and content of antioxidants such as polyphenols, carotenoids and anthocyanins (Pinsirodom *et al*., 12; Agatonovic-Kustrin *et al*., 1; Coelho *et al*., 6).

Based on the results, it is concluded that mango genotypes vary significantly with respect to total phenolic contents at different fruit developmental stages. Among mango genotypes, Langra and Bombay Green had higher total phenols. Phenolics were higher at young stage of fruit development, which significantly reduced with the advancement of fruit maturity. Phenol compounds were more in peel than pulp fractions at all stages and antioxidant activities in both peel and pulp was positively correlated with phenolic contents. The present study clearly demonstrated that mango fruit is a rich source of phenolics, which adds additional health benefits in terms of antioxidant properties. Thus, the mango by-products such as peel, which is rich in phenolics, can be used in functional foods. From a functional point of view, unripe mango from Langra and Bombay Green seems to be an excellent natural source of bioactive compounds.

DECLARATION

The authors declare no conflict of interest.

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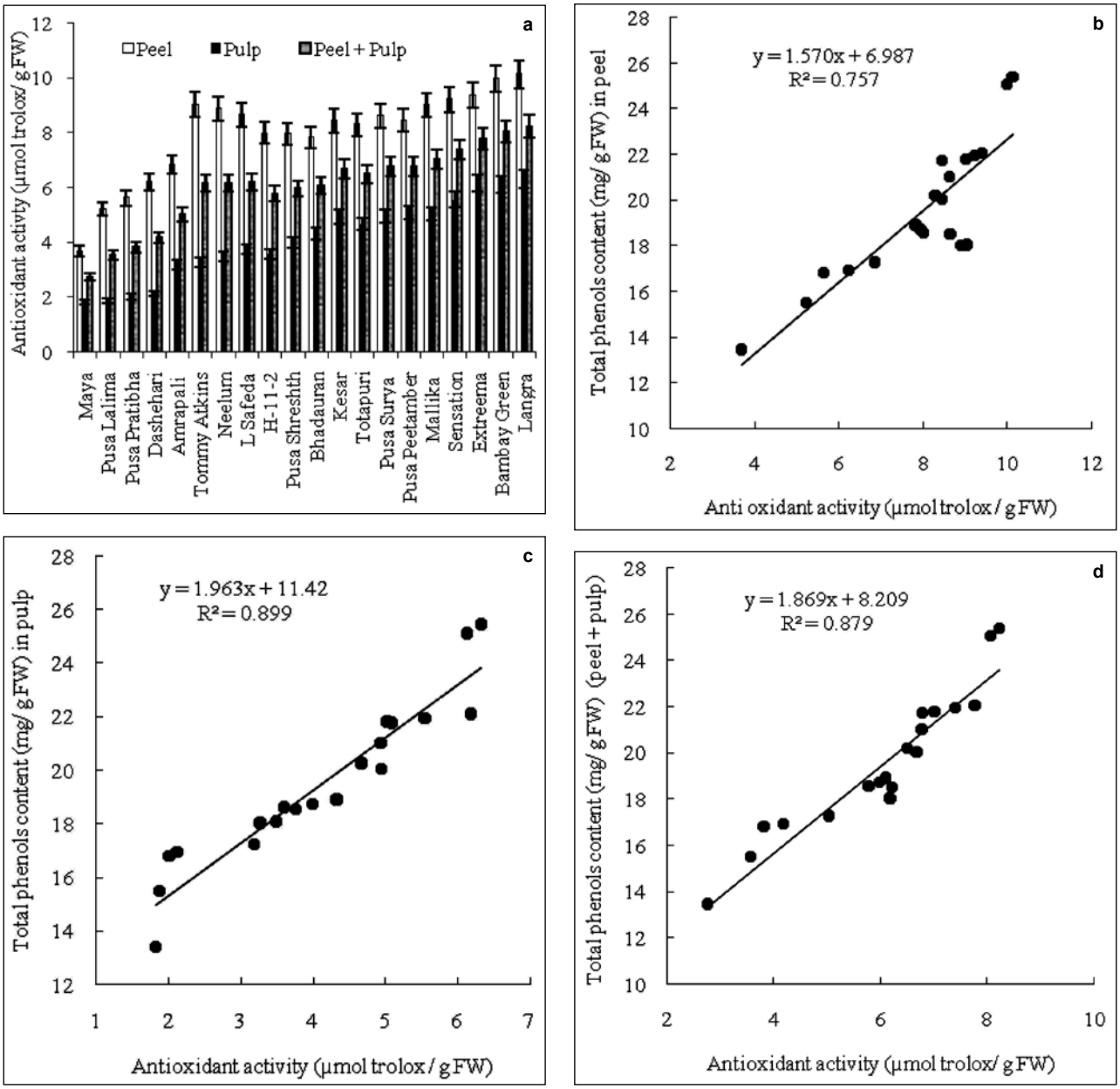

Fig. 3. a. Antioxidant activity in peel, pulp and peel+pulp, b. correlation between peel antioxidant activity and mean total phenols, c. correlation between pulp antioxidant activity and mean total phenols, d. correlation between peel + pulp antioxidant activity and mean total phenols contents. Vertical bars indicate ± SE of means. n= 3.

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