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The genetic variability, inheritance and inter-relationships of ascorbic acid, β-carotene, phenol and anthocyanin content in strawberry (*Fragaria* × *ananassa* Duch.)

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ABSTRACT

Strawberry (*Fragaria* × *ananassa* Duch.) is rich source of dietary antioxidants, minerals and nutrients. Dietary antioxidants have been known as beneficial for enhancing the fitness, preventing certain diseases and even mitigating the effects of ageing. The objectives of the present study were to determine variability and inheritance of antioxidants, to identify antioxidant rich and productive genotypes, and to suggest suitable breeding approaches. The genotypes, namely Ofra, Chandler, Festival and Camarosa showed higher concentrations of dietary antioxidants and therefore could be useful in future breeding. Results indicate that the effect of the genotypes on antioxidant contents is stronger than that of the environment. The high heritability (>80%) and low genetic advance as percentage of mean (<40%) for ascorbic acid and β-carotene contents could be improved by heterosis breeding. However, selection and hybridization would be effective tools to enhance the phenols and anthocyanin content, and yield potential as these traits showed high heritability (>80%) and high genetic advance as percentage of mean (>40%). Positive direct effect on fruit yield was highest for phenol content (0.609) which is also fairly close to its correlation coefficient (0.765) indicating that a direct selection based on phenol content would be most effective and that the phenol content could be used as a reliable biochemical marker to identify the productive genotypes having higher amounts of dietary antioxidants. The information could also be used for developing antioxidant rich cultivars, i.e. 'Breeding Strawberry for High Antioxidants'.

1. Introduction

The strawberry (*Fragaria* × *ananassa* Duch.), one of the most delicious and refreshing fruits, is rich source of dietary antioxidants and nutrients. Antioxidants are the phyto-chemicals including vitamins, pigments, phenols, flavonoids, enzymes and minerals that have the ability to protect our body against oxidative damage. Most of the metabolic abnormalities in living organisms are caused through the production of deleterious active oxygen species (AOS) such as singlet oxygen, superoxide radical, hydrogen peroxide, hydroxyl ion and free hydroxyl radical ($^1O_2$, $^\bullet O^-_2$, H_2O_2, OH^-, and $^\bullet OH$, respectively) which are invariably produced during normal metabolism and exposure to stresses (Singh et al., 2009a).

Since AOS are highly reactive and toxic, aerobic organisms cannot survive without antioxidant systems that counteract the detrimental effects of AOS. The system includes vitamins like β-carotene (a vitamin-A precursor), ascorbic acid and vitamin-E; phyto-chemicals such as phenols, flavonoids (anthocyanin, resveratrol, quercitin and catechin) and phenylopropanoid; enzymes such as superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT); and minerals like selenium and zinc (Mckersie, 1996). In humans, the AOS, if not neutralized, damage various body cells (cell membrane, lipids, proteins, DNA and other cell structures) causing many degenerative diseases associated with ageing, cardiovascular disorder, cancer, loss of memory and paralysis (Byers and Perry, 1992; Sies and Stahl, 1995; Cao et al., 1998; Adams and Best, 2002; Dorge, 2005).

Humans have no enzymatic capability to synthesize ascorbic acid and its deficiency causes scurvy disease. Ascorbic acid is also a highly effective antioxidant and a substrate for ascorbate peroxidase as well as an enzyme cofactor for the biosynthesis of several other important biochemicals. It helps in maintaining a healthy immune system, neutralizing the pollutants and production of antibody. Carotenoids are secondary plant compounds that form lipid soluble yellow, orange and red pigments. Lutein, an oxygenated xanthophylls, and β-carotene (a hydrocarbon carotene and precursor of vitamin-A) are two nutritionally important plant derived carotenoids (Zaripheh and Erdman, 2002). Diets containing

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β-carotene rich fruits and vegetables are associated with decreased risk of chronic diseases related to vision, skin, infection and reproduction besides being AOS scavenger. Epidemiological studies have shown that consumption of food rich in phenol content can reduce the risk of heart disorder by slowing the progression of atherosclerosis (Kinsella et al., 1993). Anthocyanin is water-soluble vacuolar pigment that may appear red, purple or blue color, and belongs to flavonoids. The diets rich in anthocyanin are very effective against cancer, ageing, heart problem, inflammation, diabetes and infections.

Strawberry is very good source of antioxidants and has the potential to scavenge the detrimental AOS (Wang and Jiao, 2000; Kahkonen et al., 2001; Meyers et al., 2003; Tsao et al., 2005). An inherent antioxidant system has the ability to neutralize the AOS and contributes towards healthy life style. There is meager information available on genetic variability, heritability and interrelationships of antioxidants (ascorbic acid, β-carotene, phenols and anthocyanin) in strawberry. The knowledge of dietary antioxidants in the fruit-berries is of significance not only for humans, in terms of better nutrition and health, but also for growers, in terms of fetching a better price of their produces. The present study aimed to estimate variation in dietary antioxidants, its inheritance and correlations, to identify antioxidant rich and productive genotypes and also to suggest suitable breeding approaches to be followed to harness genetic potential of strawberry.

2. Materials and methods

2.1. Basic experimental materials

Twenty-one diverse genotypes of strawberry (*Fragaria* × *ananassa* Duch.) including cultivars and germplasm comprised basic experimental materials. The runners were collected from three sources: the Indian Agricultural Research Institute (IARI) Regional station, Shimla, Himachal Pradesh; Dr. Y.S. Parmar University of Horticulture and Forestry Solan, Himachal Pradesh; and the Strawberry Growers Association, Panchagani, Mahabaleshwar, Maharastra.

2.2. Field experiments

The runners were transplanted at the Horticulture Research Farm, ICAR Research complex for NEH Region Umiam, Meghalaya, India during two consecutive seasons (2006–2007 and 2007–2008). The experimental Farm is situated at an altitude of 900 m above the mean sea level, and at 25.30°N and 90.55°E latitude and longitude, respectively. Minimum and maximum temperature of sub-temperate climate ranged from 6 to 29 °C and receives an average annual rainfall of 280–300 cm. The runners were transplanted on 20 cm raised beds under double row hill system in open field conditions during first week of October each year. Thirty plants were accommodated in each replication having inter- and intra-row spacing of 30 cm × 45 cm, respectively. The beds were mulched with black polythene sheet (50 μm) and triplicated in randomized block design. The crops were raised and fed for optimum morphological and phenotypic traits. The ripe berries were harvested from February to April next year and analyzed for antioxidant contents from blend of 10 randomly selected fruits at peak harvesting time.

2.3. Estimation of ascorbic acid content

Ascorbic acid was determined by the direct colorimetric method as mentioned by Rangana (1979). This method involves measuring the extent to which 2,6-dichlorophenol indo-phenol solution (dye) is decolorized by addition of ascorbic acid in the sample extract. The blended sample (2 g) was extracted with 20 ml of 2% m-phosphoric acid. Homogenate was centrifuged at 6000 × *g* and 4 °C, and supernatant was used for estimation. Later on 1 ml of the extract was mixed with 4 ml of 2% m-phosphoric acid followed by addition of 10 ml of dye with rapid delivery pipette. The mixture was shaken well and reading was taken in a spectrophotometer (UV 1601 Shimadzu double beam spectrophotometer) within 15–20 s. The concentration of ascorbic acid was calculated from a standard curve plotted with known concentration of ascorbic acid and expressed as mg 100 g^{-1} fresh weight (FW).

2.4. Estimation of carotenoid content

The analysis of β-carotene is based on the extraction of crude pigment mixture in lipid solvent as described by Rangana (1979). The blended sample (2 g) was taken and mixed with acetone. The extract was decanted into a conical flask. The extraction was continued till the residue became colorless. All the extracts were pooled and transferred into a separating funnel. Then 10 ml of petroleum ether (BP 60–80 °C) was added and stirred thoroughly. The pigments were transferred into petroleum ether phase by diluting the acetone with water containing 5% sodium sulphate (Na_2SO_4) to remove excess water. Finally, uniform volume was made up with petroleum ether and color intensity was measured in a spectrophotometer [Evolution 300 UV–VIS, Thermo-electron Corp., Waltham, Massachusetts (MA), USA] using 3% acetone in petroleum ether as blank. The absorbance was measured at 452 nm by a spectrophotometer. The β-carotene content (mg 100 g^{-1} FW) was calculated using a calibration curve prepared against a high purity β-carotene obtained from Sigma Chemical Co., USA.

2.5. Estimation of phenol content

Total phenolics were determined using the Folin-Ciocalteau reagent (Singleton and Rossi, 1965). The blended samples (2 g) were mixed with 80% aqueous ethanol at room temperature, centrifuged at 10,000 × *g* for 15 min and the supernatant was taken out. The residue was re-extracted twice and supernatants were pooled followed by evaporated at room temperature. The remainder was dissolved in 5 ml of double distilled water (DDW). The 100 μl of this extract was diluted to 3 ml DDW and 0.5 ml of Folin-Ciocalteau reagent was added. After 3 min, 2 ml of 20% sodium carbonate (Na_2CO_3) was added and shaken thoroughly. Having waited for 60 min, the absorbance was measured at 650 nm in spectrophotometer using catcheol as a standard. The concentration was expressed as mg of catcheol equivalents per 100 g FW.

2.6. Estimation of anthocyanin content

The blended sample (10 g) was mixed with 10 ml of ethanol–hydrochloric acid mixture (95% C_2H_5OH and 1.5 N HCl in the ratio of 85:15), transferred into a 100 ml volumetric flask, kept overnight at 4 °C, filtered through Whatman No. 1 and eventually optical density of filtrate was measured at 535 nm in a spectrophotometer. Total anthocyanin content was expressed as mg 100 g^{-1} FW (Harborne, 1973).

2.7. Statistical analyses

The data were analyzed statistically for analysis of variance and standard error (Singh and Chaudhary, 1977); estimation of variability, heritability and genetic advance (Burton and DeVane, 1953), correlation (Searle, 1961) and path coefficient analysis (Dewey and Lu, 1959).

Table 1
Mean square for dietary antioxidants and fruit yield in 21 strawberry genotypes.

Source of variation	d. f.	Mean square				
		Ascorbic acid	β-Carotene	Phenols	Anthocyanin	Fruit yield
Replication	2	24.72	0.27	15.98	11.46	88.26
Environment	1	86.80**	0.66	46.08*	704.04**	1309.51*
G × E interaction	2	0.82	0.063	1.03	0.99	56.42
Genotype	20	678.16**	10.17**	2214.97**	3585.53**	22850.68**
Error	100	15.25	0.41	18.10	21.66	65.38

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.

3. Results

The mean square (Table 1) showed that the contents of ascorbic acid, β-carotene, phenols and anthocyanin, and fruit yield varied highly significantly among strawberry genotypes. The significant mean square of environment showed the role of environment in synthesis of all dietary antioxidants except β-carotene. The mean performance, range, coefficient of variation (CV) and standard errors (Table 2) also showed sufficient amount of variation for dietary antioxidant contents and yield potential.

Ascorbic acid contents (mg 100 g^{-1} FW) differed by 1.57-fold and varied from 68.32 to 107.50 with a mean value of 81.76 ± 1.59. The genotypes Ofra, Chandler, Shasta, Larsan, Sweet Charlie and Red Coat showed high ascorbic acid contents in their fruits; while low content was analyzed for lines Horsella, Belrubi, Camarosa, Fern, Douglas and Selva. Moreover, β-carotene contents (mg 100 g^{-1} FW) varied 1.78-fold among cultivars and germplasm and ranged from 5.63 to 10.02 with a general mean value of 7.75 ± 0.26. The concentration was highest in Festival followed by Blakemore, Selva, Ofra, Sweet Charlie and Elista; while lowest content was estimated in Horsella followed by Gorella, Etna, Fern, Douglas and Dana.

The contents of phenols (mg 100 g^{-1} FW) differed by 2.4-fold among genotypes and ranged from 59.9 to 143.7 with an average value of 84.5 ± 1.7. Its content was highest in Ofra followed by Chandler, Camarosa, Elista, Fairfax and Selva with lowest contents in Gorella, Etna, Larsan, Belrubi, Phenomenal and Fern. However, the anthocyanin contents (mg 100 g^{-1} FW) ranged from 40.53 to 125.41 (3.1-fold difference) with a genotype mean of 66.18 ± 3.30. The maximum concentration was assayed in Ofra followed by Chandler, Seascape, Festival and Camarosa. Genotypes that possessed low anthocyanin contents were Phenomenal, Etna, Shasta, Belrubi, Gorella and Fern. The fruit yield (g plant^{-1}) differed greatly by 9.33-fold and ranged from 26.6 to 248.2 with a mean value of 111.9 ± 3.3. The genotypes, namely Camarosa, Ofra, Chandler, Elista, Festival and Dana showed higher yield potential.

The extent of variability (Table 3) present among germplasm was estimated in terms of phenotypic, genotypic, and environmental variance (Vp, Vg and Ve); and phenotypic and genotypic coefficient of variation (PCV and GCV). The Vg was highest for fruit yield followed by anthocyanin, phenols and ascorbic acid. The magnitude of PCV was slightly higher than the corresponding GCV for all the traits under investigation. The PCV and GCV were high for yield followed by anthocyanin and phenol content, while it was found low for β-carotene and ascorbic acid content. The heritable portion of variations can be deduced by computing the heritability and genetic advance as percentage of mean (Table 4). High heri-

Table 2
Mean performance of 21 strawberry genotypes for dietary antioxidants contents and fruit yields.

S. No.	Genotype	Ascorbic acid (mg 100 g^{-1} FW)	β-Carotene (mg 100 g^{-1} FW)	Phenols (mg 100 g^{-1} FW)	Anthocyanin (mg 100 g^{-1} FW)	Fruit yield (g plant^{-1})
1.	Belrubi	68.73	7.31	69.4	45.97	98.8
2.	Blakemore	82.82	9.44	78.8	56.52	124.4
3.	Camarosa	70.72	8.52	98.5	96.90	248.2
4.	Chandler	102.22	8.03	116.3	104.54	187.7
5.	Dana	83.71	6.82	73.6	49.60	127.2
6.	Douglas	71.66	6.62	77.0	58.35	115.8
7.	Elista	79.31	9.07	97.8	53.25	155.3
8.	Etna	77.52	5.96	64.9	43.26	96.4
9.	Fairfax	78.73	7.81	96.6	70.57	126.4
10.	Fern	70.50	6.56	71.9	46.21	26.6
11.	Festival	80.26	10.02	90.8	99.89	146.8
12.	Gorella	84.60	5.73	59.9	47.58	26.7
13.	Horsella	68.32	5.63	73.2	52.21	40.6
14.	Larsan	90.22	6.84	68.7	60.25	39.6
15.	Ofra	107.50	9.21	143.7	125.41	228.2
16.	Phenomenal	79.31	7.03	69.5	40.53	91.1
17.	Redcoat	87.24	7.82	85.6	62.31	61.7
18.	Seascape	75.78	7.88	78.2	101.83	97.9
19.	Selva	74.02	9.30	91.7	64.86	80.9
20.	Shasta	95.17	8.06	80.8	45.78	63.3
21.	Sweet Charlie	88.60	9.15	88.5	63.85	165.5
	Mean	81.76	7.75	84.5	66.18	111.9
	Range	68.32–107.50	5.63–10.02	59.9–143.7	40.53–125.41	26.6–248.2
	Coefficient of variation	4.78	8.21	5.0	7.23	7.2
	Standard error	1.59	0.26	1.7	3.30	3.3
	LSD at $P < 0.05$	4.47	0.73	4.9	9.26	9.3

FW, fresh weight.

Table 3
Estimates of variance and coefficient of variation for dietary antioxidants and fruit yield in 21 strawberry genotypes.

Dietary antioxidant	Genotypic variance	Phenotypic variance	Environmental variance	Genotypic coefficient of variation (%)	Phenotypic coefficient of variation (%)
Ascorbic acid	110.49	125.73	15.25	12.86	13.72
β-Carotene	1.63	2.03	0.41	16.46	18.39
Phenols	366.15	384.25	18.10	22.63	23.19
Anthocyanin	593.98	615.64	21.66	36.83	37.50
Fruit yield	3797.55	3862.93	98.31	55.09	55.56

Table 4
Estimates of heritability, genetic advance (GA) and GA as percentage of mean for dietary antioxidants and fruit yield in 21 strawberry genotypes.

Dietary antioxidant	Heritability (%)	Genetic advance (GA)	GA as percentage of mean (%)
Ascorbic acid	87.87	20.30	24.83
β-Carotene	80.06	2.35	30.34
Phenols	95.29	38.48	45.51
Anthocyanin	96.48	49.31	74.52
Fruit yield	98.31	125.87	112.52

tability (>80.00%) was estimated for all the traits under study such as fruit yield followed by anthocyanin, phenols, ascorbic acid and β-carotene content. The genetic advance as percentage of mean was low (<40.00%) for ascorbic acid and β-carotene, while it was high for fruit yield, anthocyanin and phenols.

The inter-relationship among antioxidants and fruit yield was analyzed to determine the direction and magnitude of association at the genotypic and phenotypic level (Table 5). The correlation coefficients at genotypic level were higher in magnitude than of the corresponding phenotypic correlation coefficients. The significant positive correlations were observed for phenol content with all the traits along with higher magnitude, nevertheless ascorbic acid content showed positive association only with phenol content. Correlation coefficients indicate only the general association between any two traits without tracing any possible causes of such association. In such situation, the path coefficient analysis at genotypic level (Table 6) was done to partition the correlation coefficient into direct and indirect effects. Fruit yield was taken as dependant variable while computing the path coefficient. The phenol content showed highest positive direct effect on fruit yield which is fairly close to its correlation coefficient.

4. Discussion

Highly significant mean squares indicate the presence of adequate natural variation for ascorbic acid, β-carotene, phenols and anthocyanin contents, and fruit yield among strawberry genotypes which could be improved by various breeding approaches. Results also indicate that the effect of the genotype on various traits of economic importance is stronger than that of the environment (cultivation conditions). Significant variation for various quality traits and antioxidants was also reported by Wang and Jiao (2000), Tsao et al. (2005), Capocasa et al. (2008) and Singh et al. (2009c). It has been well established that higher contents of antioxidants scavenge the lethal effect of AOS and thereby protect our body against cardiovascular disorder, cancer and other diseases (Cao et al., 1998; Pajk et al., 2006; Ozgen et al., 2009). The genotypes such as Ofra, Chandler, Festival and Camarosa showed higher contents of dietary antioxidants along with good yield potential. These aforementioned genotypes could be useful in breeding for developing cultivars with high antioxidant contents. Nevertheless, two genotypes, namely Fern and Gorella revealed uneconomical as well as less amounts of antioxidants in their berries.

Anthocyanin, phenols and fruit yield had greater amount of Vg and GCV and therefore, present a better possibility and potential of improvement through hybridization and selection. Lower values of GCV and PCV for ascorbic acid has also been reported by Singh (2007) in cabbage head. A high heritability for all antioxidants as well as yield indicates that a large portion of phenotypic variance is contributed through genotypic variance. Therefore, a reliable selection can be made for these traits. Effectiveness and potentiality of the traits under selection could be revealed by an assessment of genetic gain. Heritability values along with genetic advance as percentage of mean, together, are more useful tools for selection than either of them alone. In the present study, the ascorbic acid and β-carotene contents showed high heritability along with low genetic advance as percentage of mean indicating the prevalence of non-additive genes and so improvement could be

Table 5
Correlation coefficients for dietary antioxidants and fruit yield in 21strawberry genotypes.

Dietary antioxidant		Ascorbic acid	β-Carotene	Phenols	Anthocyanin	Fruit yield
Ascorbic acid	g	–	0.316	0.586**	0.427	0.306
	p	–	0.254	0.543*	0.399	0.284
β-Carotene	g		–	0.653**	0.554**	0.627**
	p		–	0.564**	0.497*	0.542*
Phenols	g			–	0.802**	0.765**
	p			–	0.764**	0.741**
Anthocyanin	g				–	0.690**
	p				–	0.672**
Fruit yield	g					–
	p					–

g, genotypic level; p, phenotypic level.
* Significant at $P < 0.05$.
** Significant at $P < 0.01$.

Table 6
Genotypic path coefficients of dietary antioxidants showing the direct and indirect effects on fruit yield.

Dietary antioxidant	Ascorbic acid	β-Carotene	Phenols	Anthocyanin	'r' value with fruit yield
Ascorbic acid	−**0.187**	0.061	0.357	0.075	0.306**
β-Carotene	−0.059	**0.191**	0.398	0.097	0.627**
Phenols	−0.110	0.125	**0.609**	0.141	0.765**
Anthocyanin	−0.080	0.106	0.489	**0.175**	0.690**

Residual effect = 0.592.
The bold values indicate direct effect, while others indicate indirect effect.
** Significant at $P < 0.01$.

made through heterosis breeding. Sreekala and Raghava (2003), and Singh et al. (2009b, 2009c) have also suggested heterosis breeding for the improvement of total carotenoid in petals of marigold (*Tagetes erecta*), and ascorbic acid and antioxidant enzymes in cabbage head, respectively. Moreover, phenols, anthocyanin and fruit yield expressed high heritability coupled with high genetic advance as percentage of mean which reflect the regulation of the aforesaid traits through additive gene. Hence these traits could be harnessed through selection and hybridization.

Phenotypic correlation coefficients, in general, were slightly lower in magnitude than of the corresponding genotypic correlation coefficients which indicate the apparent association of two traits is not only due to genes but also due to influence of environmental interactions. The meager differences revealed that the effect of the genotype is stronger than that of the environment. Phenol contents expressed a significant positive correlation with all the traits including antioxidants and fruit yield. Thus, it may be inferred that the plant selection should be based on phenol content. No correlation among ascorbic acid, carotene and yield corroborates with the finding of Singh (2007) in cabbage head. Positive direct effect on fruit yield was highest for phenol content which is also fairly close to its correlation coefficient and indicates that a direct selection based on phenol content would be most effective. Therefore, phenols could be serving as a reliable biochemical marker, as justified by correlation and path coefficient values, to identify the productive genotypes/ cultivars having higher amounts of dietary antioxidants such as ascorbic acid, β-carotene, phenols and anthocyanin.

5. Conclusion

The genotypes Ofra, Chandler, Festival and Camarosa were found to be prospective as they possessed a higher content of antioxidants along with a better yield potential. The ascorbic acid and β-carotene contents could be improved by heterosis breeding, while selection and hybridization could be effective breeding approach to enhance the contents of phenols and anthocyanin, and also the yield potential. Moreover, phenol content could serve as a reliable biochemical marker to identify the productive genotypes having higher amounts of dietary antioxidants (ascorbic acid, β-carotene, phenols and anthocyanin). The information gained from present investigation could be very useful in the further breeding programmes to enhance the antioxidant content in the fruit of strawberry.

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