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Pigmented radish (*Raphanus sativus*): Genetic variability, heritability and inter-relationships of total phenolics, anthocyanins and antioxidant activity

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ABSTRACT

Radish (*Raphanus sativus* L.) is an important salad vegetable grown and consumed throughout the world for fleshy roots which has numerous categories– varying in root colour, size, shape and flavour. The uses of coloured radishes in the salads and their anthocyanins as colourants are gaining popularity because of the colour characteristics, health benefits as well as antioxidant activities. However, information on the genetic variability, heritability and inter-relationship of total phenolics, anthocyanins and antioxidant activities in pigmented radish is very limited, but pre-requisite to initiate breeding programme; and therefore investigated in the present study. Radish genotypes were significantly diverse for all the antioxidants; differed by 4.98-fold for total phenolics, 36.16-fold for anthocyanins content, 4.96-fold for FRAP activity and 4.03-fold for CUPRAC activity; and the genotypes accounted for >97% of total variations. The meager differences between phenotypic and genotypic coefficient of variation reveals the greater role of genotypes and lesser influence of the environment on the biosynthesis and accumulation of antioxidants. Significantly positive correlations along with higher magnitude for anthocyanins content, total phenolics, FRAP activity and CUPRAC activity (r= 0.823 to 0.964) could be used as indirect selection criteria for improving levels of antioxidant compounds. The estimates of heritability and genetic advance indicate the role of additive and non-additive genes for biosynthesis of antioxidants and root development, respectively; therefore, recurrent selection would be the best breeding approach to improve both the traits simultaneously in coloured radish.

Key words: Anthocyanins, Antioxidants, Correlation, Inheritance, Radish, *Raphanus sativus*, Variability

Radish (*Raphanus sativus* L., 2n=2x=18), Brassicaceae family, is an important vegetable grown and consumed throughout the world for fleshy edible roots (hypocotyls) which are eaten as crunchy salad, cooked or preserved by salting, pickling, canning and drying. Also, the soft leaves are cooked and used as a leafy vegetable. Radish is an ancient crop, native to the eastern Mediterranean and the Middle East. Moreover, central China, central Asia and India appear to have been secondary centers where differing forms were evolved during the course of domestication. The first record about radish consumption in human nutrition date back to about 2700 BC in the ancient Egypt, however its cultivation started in China and Korea about 400 BC (George and Evans 1981, Kaneko and Matsuzawa 1993). Radish has numerous categories, varying in root colour, size, shape, flavour and period of maturity; moreover differences in leaf morphology were also observed. The ancient varieties were long and tapering rather than cylindric, apically bulbous, elliptic or spherical. The different forms of radishes arose in the various time sequences, i.e. black radishes were the earliest in cultivation, white radishes were being cultivated in Europe by the 1500's, and red and round radishes were developed in the 1700's. In the West, commonly, the radish is seen as a small-rooted, short-season vegetable; while in the Far East and Asian countries, a diverse, large-rooted and long-season radish is widely grown. In India, it is grown in one or the other parts of the country almost throughout the year because of the varied climatic conditions, and its stable and productive cultivars, economic importance and increasing demand.

The black to red colour of radish is due to anthocyanins, the most versatile polyphenols and a class of pigments responsible for the red, purple and blue colours of many vegetable, fruits and cereals. They have nutraceutical, colourant and anti-oxidative properties (Delgado-Vargas and Paredes-Lopez 2003, Giusti and Wrolstad 2003, Horbowicz *et al.* 2008, Pojer *et al.* 2013, Jing *et al.* 2014). Within the plant, they play a role as key antioxidants and pigments contributing to the colouration of various plant parts. Given their antioxidant properties, i.e. acting as free radical scavengers, anthocyanins in general have ability to

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lower the LDL cholesterol and the risk of cardiovascular disease (Ross and Kasum 2002, Prior and Wu 2006, Castilla *et al.* 2008), to prevent obesity (Prior and Wu 2006, Peng *et al.* 2011), to inhibit the formation and progression of atherosclerosis (Estruch *et al.* 2004, Iwasaki-Kurashige *et al.* 2006), diabetes (Iwasaki-Kurashige *et al.* 2006), certain cancers (Ross and Kasum 2002, Prior and Wu 2006, Wang and Stoner 2008), to improve visual function (Kramer 2004, Ghosh and Konishi 2007), oxidative stresses and age-related diseases (Tsuda 2012, Pojer *et al.* 2013). Within the plant, they play a role as pigments contributing to the colouration of various plant parts. Moreover, they act as antioxidants protecting cells against environmental stresses such as ultraviolet and high intensity light, wounding, cold temperature and water stress (Mo *et al.* 1992, Koes *et al.* 1993, Li *et al.* 1993, Dixon and Palva 1995, Holton and Cornish 1995, Shirley 1996, Chalker-Scott 1999, Gould *et al.* 2002, Misyura 2014). Furthermore, uses of anthocyanins as colourants have gained prominence as a result of legislative action and consumers' concerns over the use of synthetic additives in foods. Among the various anthocyanins, pelargonidine (acylated pelargonidin-3-sophoroside-5-glucoside) and cyanidine (acylated cyaniding-3-sophoroside-5-glucoside) are responsible for red/pink and purple/violet colour of the roots in radish, respectively (Giusti and Wrolstad 1996, Tatsuzawa *et al.* 2010). The absence of anthocyanins resulted in a white colour. The uses of coloured radish in the salads or as garnish gaining popularity in India as it makes salad more nutritious, healthier and attractive appearance. Radish anthocyanins have been applied as natural colourants due to their tinctorial power (i.e. colour intensity), stability, colour characteristics, health benefits as well as antioxidant activities (Giusti and Wrolstad 1996, Giusti *et al.* 1998, Matsufuji *et al.* 2007, Rahman *et al.* 2006). Most of the *Brassica* vegetables are good source of antioxidants, ultimately benefiting the human and plant health (Singh 2007, Soengas *et al.* 2011, Singh *et al.* 2010, Cartea *et al.* 2011, Kapusta-Duch *et al.* 2012). Generally, the pigmented radish contains higher amounts of anthocyanins, phenolic compounds and antioxidant activity than non-pigmented radish. The authors have estimated 20-250% higher antioxidants in coloured-rooted radishes as compared to white-rooted commercial/ national check cultivars, namely Japanese White and Kashi Shweta (unpublished data). The daily intake of anthocyanins in the USA diet is estimated to be as much as 180–255 mg/day (McGhie and Walton 2007). Genetically, the presence or absence of anthocyanins in grape skin is qualitative in nature, i.e. controlled by oligogenes, while anthocyanins content is quantitative character and controlled by polygenes (Liang *et al.* 2009). The antioxidants and yield contributing traits are genetically regulated and can be improved simultaneously through various breeding approaches. Hence, the opportunity to develop coloured radish genotypes/varieties with higher antioxidants is great. To the best of our knowledge and available literatures, very fragmented information is available on genetic variability, heritability and inter-relationship of total phenolics, anthocyanins and antioxidant activities in radish. Therefore, the present study was assumed with the objective to estimate antioxidants' variability, their heritability and inter-relationship for possible exploitation to breed the genotypes/varieties having higher antioxidant content/activity as well as better yield potential.

MATERIALS AND METHODS

Twenty-four lines, including eleven of pink-rooted and thirteen of purple colour, comprised the basic experimental materials, were evaluated for this study. All the coloured-rooted genotypes are developed and maintained at ICAR-Indian Institute of Vegetable Research (ICAR-IIVR), Shahanshahpur, Varanasi, Uttar Pradesh. The details of morphological traits of basic experimental materials used are given in Table 1.

Crop was raised during 2014-2015 at the Research Farm, ICAR-IIVR, Varanasi, Uttar Pradesh. The Farm is located at 25°10'55'' N latitude and 82°52'36'' E longitude

Table 1 Details of basic experimental materials

Genotype	Root colour-Exterior	Root shape	Leaf division-Incision (LDI)	Petiole colour
VRRAD 88	Pink*	Iciclical	Lyrate	Green
VRRAD 125	Pink	Iciclical	Lyrate	Pink
VRRAD 126	Light pink	Iciclical	Lyrate	Green
VRRAD 127	Pink	Elliptical	Sinuate	Pink
VRRAD 127-1	Pink	Obtriangular	Sinuate	Pink
VRRAD 128	Purple	Iciclical	Lyrate	Purple
VRRAD 128-1	Purplish white	Iciclical	Sinuate	Purple
VRRAD 128-2	Light purple	Iciclical	Sinuate	Purple
VRRAD 129	Purple*	Iciclical	Lyrate	Purple
VRRAD 130	Pink	Iciclical	Lyrate	Pink
VRRAD 130-1	Light pink	Iciclical	Lyrate	Green
VRRAD 130-2	Purple	Iciclical	Lyrate	Purple
VRRAD 130-3	Dark purple	Iciclical	Lyrate	Purple
VRRAD 130-4	Dark purple	Iciclical	Lyrate	Purple
VRRAD 131	Purple	Iciclical	Lyrate	Purple
VRRAD 131-1	Purplish white	Iciclical	Lyrate	Purple
VRRAD 131-2	Pink	Iciclical	Sinuate	Pink
VRRAD 132	Purple	Iciclical	Lyrate	Purple
VRRAD 134	Light purple	Obtriangular	Lyrate	Purple
VRRAD 135	Purple	Iciclical	Lyrate	Purple
VRRAD 136	Pink	Obtriangular	Lyrate	Pink
VRRAD 143	Pink	Iciclical	Lyrate	Pink
VRRAD 143-1	Pink	Iciclical	Sinuate	Green
VRRAD 143-2	Purple	Iciclical	Sinuate	Purple

*Shoulder colour

with an altitude of 85 m above the mean sea level, and receives an annual rainfall of 1050-1100 mm. Soil preparation, sowing and other agronomic practices were carried out uniformly to get better morphological expression (Singh and Karmakar 2015). The seeds were sown at 1.0-1.5 cm interval in double row of 7-8 cm apart and 25-28 cm wide ridge with the spacing of about 80 cm between each pair of ridges. Each genotype comprises three ridges of 5.50 m long and triplicated in a randomized block design. The crop was unvaryingly fertilized with optimum doses of chemical fertilizers, i.e. 80 kg N, 40 kg P_2O_5 and 30 kg K_2O per hectare which were supplied as urea, single super phosphate and muriate of potash, correspondingly. Half of the N, and full P_2O_5 and K_2O were applied as basal dressing at time of ridges preparation, while remaining half dose of N was furrow-dressed at 30 days after sowing. After seed germination, thinning was done 10-12 days after sowing keeping the distance in between the plants about 5-6 cm apart for proper root development.

Each genotype in replicated trial was harvested at marketable stage (horticultural maturity) i.e. 50 days after sowing. Ten roots of each randomly selected and leaves were cut manually. The roots were washed thoroughly with normal tap water to remove adhering soil and other extraneous matters. A representative of the edible root part was taken for subsequent assay and estimation on fresh weight (FW) basis.

Radish was minced with stainless steel knife and homogenized using a warring blander. An aliquot of 25 g the commuted radish was extracted with 100 ml of methanol/0.1% HCl (v/v) for 2 h under dark condition. After centrifugation at 13000 rpm for 15 min, the supernatant was recovered and the extract residues were re-extracted with the same method. The combined supernatant were evapourated to dryness and re-dissolved in distilled water. The total monomeric anthocyanins content (anthocyanins) was determined through measuring absorbance at 520 nm against the blank on a UV–Visible spectrophotometer by the pH-differential method (Wrolstad *et al.* 2005). Pigment content was expressed as total monomeric anthocyanins equivalents (µg/g FW).

Water soluble phytochemicals of radish were extracted according to the method reported previously (Chu *et al.* 2002) with slight modification. For the extraction of soluble nutraceuticals, 5 g of the edible part of radish was weighed and homogenized with 80% ethanol (1:2 w/v) using a chilled warring blender for 5 min. The sample was then further homogenized using a polytron homogenizer for an additional 3 min to obtain a thoroughly homogenized sample. The homogenates were centrifuge at 13000 rpm for 15 min. The supernatant were collected and stored at -20 °C until analysis of total phenolics, ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) activity.

Total phenol was estimated spectrophotometrically using Folin–Ciocalteu reagent (Singleton *et al.* 1999). Aliquots (100 µl) of hydrophilic extract were mixed with 2.9 ml of deionized water, 0.5 ml of Folin–Ciocalteu reagent and 2.0 ml of 20% Na_2CO_3 solution. The mixture was allowed to stand for 90 min and absorption was measured at 760 nm against a reagent blank in UV–Visible spectrophotometer. Results were expressed as gallic acid equivalent (mg GAE/100 g FW).

The FRAP activity was performed according to the procedure described by Benzie and Strain (1996). FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mmol HCl and 20 mmol $FeCl_3$ in the ratio 10:1:1 (v/v/v). Three milliliter (3 ml) of the FRAP reagent was mixed with 100 µl aliquot of hydrophilic extract in a test tube and vortexed in the incubator at 37°C for 30 min in a water bath. Reduction of ferric-tripyridyltriazine to the ferrous complex formed an intense blue colour which was measured on a UV–Visible spectrophotometer at 593 nm. Results were expressed in terms of Trolox equivalent (µmol TE/g FW).

The cupric ion reducing antioxidant capacity of root was determined according to the method of Apak *et al.* (2008). Briefly, according to the protocol 100 µl of hydrophilic extract was mixed with 1 ml each of $CuCl_2$ solution (1.0×10^{-2} mol/l), neocuproine alcoholic solution (7.5×10^{-3} mol/l), and NH_4Ac (1 mol/l, pH 7.0) buffer solution and 1 ml of water to make the final volume 4.1 ml. After 30 min, the absorbance was recorded at 450 nm against the reagent blank. Standard curve was prepared using different concentration of Trolox and results were expressed in terms of µmol TE/g FW.

The data were analysed statistically for analysis of variance (Panse and Sukhatme 1967), estimation of variability (Burton and DeVane 1953) and correlation (Searle 1961). The standard error for genotypes was calculated as per Singh and Chaudhary (1977). The variability estimates (genotypic and phenotypic variance; and genotypic and phenotypic coefficient of variation) were worked out through analysis of variance, while correlation coefficients were determined by covariance and variance between traits. Broad-sense heritability (h^2b) for each trait was calculated by multiplying the ratio of genotypic variance (Vg) to phenotypic variance (Vp) with 100, i.e. $h^2b=(Vg/Vp)*100$. Moreover, genetic advance (GA) as percentage of mean was computed through dividing the factor of square root of phenotypic variance ($Vp^{½}$), h^2b and selection differential constant (k) at 5% (i.e. 2.06) by its mean, i.e. GA as % of mean=($Vp^{½}* h^2b*k$)/mean.

RESULTS AND DISCUSSION

Nowadays, the breeders are giving due consideration for the improvement of nutritional qualities as well as yield potential. Like yield, nutritional improvement in crops also requires sufficient genetic variation for phytochemical in the gene pool. The mean square (Table 2) showed that total phenolics, anthocyanins, FRAP value and CUPRAC value, and root weight of radish varied significantly ($P < 0.01$) among the 24 genotypes of coloured radish. Highly significant mean squares for antioxidants and root weight

Table 2 Mean squares for total phenolics, anthocyanins, FRAP activity, CUPRAC activity and root weight in coloured radish genotypes

Source of variation	df	Mean square				
		Total phenolics	Anthocy-anins	FRAP activity	CUPRAC activity	Root weight
Replication	2	5.7	32.8	0.02	0.06	184.3
Genotype	23	403.9**	4803.2**	3.73**	8.61**	511.6**
Error	46	2.6	20.8	0.04	0.12	75.8

**Significant at $P< 0.01$

indicate the presence of sufficient natural variation among radish genotypes that could be effectively harnessed through various breeding approaches. The mean performance, standard error and range (Table 3) also showed large variation for various antioxidants; while narrow range of variation for root weight. Total phenolics differed by 4.98-fold (12.4-61.7 mg/100 g FW), anthocyanins content by 36.16-fold (4.6-166.3 µg/g FW), FRAP value by 4.96-fold (1.14-5.66 µmol/g FW), CUPRAC value by 4.03-fold (2.73-11.01 µmol/g FW) and root weight by 1.37-fold (120.1-164.8 g) among various genotypes. Previous study has reported that the anthocyanins content in different radish cultivars varied from 47-530 µg/g FW (Guisti *et al.* 1998). Furthermore, Karmakar *et al.* (2013) also reported sufficient variation for antioxidant content and activity (total phenolics, ascorbic acid, total carotenoids, DPPH-RSA, ABTS-RSA and CUPRAC assay) in the parents and hybrids of ridge gourd.

There was large contribution of radish genotypes to total variations for total phenolics (97.98%), anthocyanins (98.90%), FRAP activity (98.44%) and CUPRAC activity (97.91%); while medium contribution for root weight (66.29%). These results reveal that the genotypes are main source of variations for total phenolics, anthocyanins and antioxidant activity in radish; hence, it would be possible to improve the content/activity of antioxidants by selection. In corn and strawberry too, the researchers have reported higher contribution of genotypes to the total variations for anthocyanin, phenols, antioxidant activity and other phytochemicals (Chander *et al.* 2008, Singh *et al.* 2011, Mahan *et al.* 2013, Harakotr *et al.* 2015). The magnitudes of Vp and PCV were slightly higher than their corresponding Vg and GCV for antioxidants, but the magnitude was wider for root weight. The lower differences between Vp and Vg, and PCV and GCV for total phenolics, anthocyanins, FRAP and CUPRAC activity indicate the higher role of genotypes and lesser influence of the environment on the biosynthesis and accumulation of antioxidants because the anthocyanins' presence and content is governed by dominant polygenes (Liang *et al.* 2009). While, there is considerable impact of environments on root yield of radish indicated by comparatively higher differences between Vp and Vg, and PCV and GCV. The root yield is a complex trait that depends on several growth and component traits, and various quantitative trait loci. The respective PCV and GCV were high for total phenolics (40.4% and 40.8%), anthocyanins (96.5% and 97.1%), FRAP value (35.3% and 35.8%) and CUPRAC value (30.3% and 30.9%); while it was low for root weight (8.5% and 10.5%). The trait having greater GCV possesses a higher magnitude of variability and thus, presents a better possibility of exploitation for improvement in radish through various breeding approaches. Singh *et al.* (2010), Singh *et al.* (2011) and Harakotr *et al.* (2015) also reported stronger effects of genotypes for antioxidant enzymes in cabbage, phenols and anthocyanins in strawberry, and antioxidant content and activity in corn, respectively. Kumar *et al.* (2014) also reported higher degree of Vg and GCV for root length and root weight in radish.

Heritable portion of variation can be deduced by computing the heritability in broad-sense and genetic advance as percentage of mean (Table 4). High heritability (>80%) was estimated for total phenolics (98.1%), anthocyanins (98.7%), FRAP value (96.9%) and CUPRAC value (95.8%). High heritability for a trait indicates that a large portion of phenotypic variance is contributed through genotypic variance and therefore, a reliable selection can be made for these traits. Moreover, moderate heritability (50-80%) was estimated for root weight (65.7%) which indicates a considerable influence of environment on root development. These findings are getting support from estimates of

Table 3 Estimates of variance and coefficient of variation for total phenolics, anthocyanins, FRAP activity, CUPRAC activity and root weight in coloured radish genotypes

Antioxidant	Mean±SEm	Range	Difference in fold	CGTV (%)	Vg	Vp	GCV (%)	PCV (%)
Total phenolics (mg/100 g FW)	28.6±0.9	12.4-61.7	4.98	97.98	133.8	136.4	40.4	40.8
Anthocyanins (µg/g FW)	41.4±2.6	4.6-166.3	36.16	98.90	1594.1	1615.0	96.5	97.1
FRAP activity (µmol/g FW)	3.15±0.11	1.14-5.66	4.96	98.44	1.23	1.27	35.3	35.8
CUPRAC activity (µmol/g FW)	5.55±0.20	2.73-11.01	4.03	97.91	2.83	2.95	30.3	30.9
Root weight (g)	141.6±5.0	120.1-164.8	1.37	66.29	145.2	221.1	8.5	10.5

FW, Fresh weight; SEm, Standard error of mean; CGTV, Contribution of genotypes to the total variation; Vg, Genotypic variance; Vp, Phenotypic variance; GCV, Genotypic coefficient of variation; PCV, Phenotypic coefficient of variation

Table 4 Estimates of heritability, genetic advance (GA) and GA as percentage of mean for total phenolics, anthocyanins, FRAP activity, CUPRAC activity and root weight in coloured radish genotypes

Antioxidant	Heritability (%)	GA	GA as percentage of mean (%)
Total phenolics	98.1	23.6	82.5
Anthocyanins	98.7	81.7	197.4
FRAP activity	96.9	2.2	71.5
CUPRAC activity	95.8	3.4	61.1
Root weight	65.7	20.1	14.2

GA: Genetic advance

coefficient of variation (GCV and PCV) reported in Table 3. Mahan *et al.* (2013) also estimated high heritability (broad and narrow sense) for total phenolic content in corn. The efficacy 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. Genetic advance as percentage of mean ranged from 14.2-197.4% for antioxidants and root weight in radish. It was high for anthocyanins content (197.4%) followed by total phenolics (82.5%), FRAP activity (65.35%), CUPRAC activity (61.1%), and low for root weight (14.2%). In the present study, a high heritability accompanied with a high genetic advance for total phenolics, anthocyanins, FRAP value and CUPRAC value clearly reflect the role of additive genes, and thus, a high genetic gain is expected from selection and hybridization for these traits. This finding is in accordance to Singh *et al.* (2011) for phenol and anthocyanins content in strawberry. In ridge gourd too, Karmakar *et al.* (2013) found the role of additive genes for various phytonutrients and antioxidants (total phenolics, ascorbic acid, total carotenoids, DPPH-RSA, ABTS-RSA and CUPRAC assay). Furthermore, Mahan *et al.* (2013) also observed role of additive genes for phenolic content in corn through the estimates of narrow sense heritability and combining ability. Moreover, root weight showed a low genetic advance along with moderate heritability and consequently, reflected the regulation of aforesaid trait through non-additive genes that could be harnessed effectively through heterosis, synthetics and hybridization in coloured radish. Present result is in concurrence with the findings of Kutty and Sirohi (2003), and Kumar *et al.* (2012) for root weight. Realizing the importance of additive and non-additive genes for biosynthesis of antioxidants and root development, respectively; recurrent selection would be the best breeding approach to improve both traits antioxidants and root yield simultaneously.

The inter-relationship among antioxidants and root weight was analyzed to determine the direction and magnitude of association at the genotypic and phenotypic levels (Table 5). Genetic associations provide basic criteria for selection. The correlation coefficients at genotypic level were higher in magnitude than of the corresponding

Table 5 Correlation coefficient between total phenolics, anthocyanins, FRAP activity, CUPRAC activity and root weight in coloured radish genotypes

Antioxidant		Total phenolics	Antho-cyanin	FRAP activity	CUPRAC activity	Root weight
Total phe-nolics	g		0.964**	0.918**	0.940**	0.165
	p		0.948**	0.899**	0.908**	0.125
Anthocya-nins	g			0.859**	0.908**	0.083
	p			0.833**	0.882**	0.078
FRAP activity	g				0.862**	0.195
	p				0.823**	0.163
CUPRAC activity	g					0.132
	p					0.123
Root weight	g					
	p					

**Significant at $P< 0.01$; g, Genotypic level; p, Phenotypic level.

phenotypic correlation coefficients. Highly significant positive correlations, ranged from 0.823-0.964, were observed for all the antioxidants, namely total phenolics, anthocyanins, FRAP value and CUPRAC value. Previous studies also confirmed a significant positive correlation among phenols, anthocyanins and antioxidants in corn (Hu and Xu 2011, Zilic *et al.* 2012, Rodriguez *et al.* 2013, Harakotr *et al.* 2015), strawberry (Singh *et al.* 2011) and carrot (Koley *et al.* 2014). In contrast to these findings, Kallithraka *et al.* (2005) reported statistically insignificant correlation between total anthocyanins content and antioxidant capacity in Greek grape cultivars at harvest stage. However, there were no correlations between radish root weight and antioxidants (0.078-0.195) in the present study. Nevertheless, negative correlations between fruit weight and antioxidants were reported by Hanson *et al.* (2004), Connor *et al.* (2005) and Karmakar *et al.* (2013) in tomato, raspberry and ridge gourd, respectively. Phenotypic correlation coefficients, in general, were slightly lower in magnitude than of the corresponding genotypic correlation coefficients especially for antioxidants which indicate the lesser influence of environmental interactions on the genotypic expression. In this study, there was strongest positive association between total phenolics and anthocyanins content (0.964) because anthocyanins are the phenolic subgroup with same biosynthetic pathway. As anthocyanins content is directly related to total phenolics and antioxidant activity in coloured radish, opportunity to breed varieties/genotypes with high antioxidants is great.

In conclusion, sufficient amount of genotypic variation were estimated for total phenolics, anthocyanins, FRAP activity and CUPRAC activity among coloured radish genotypes. High heritability was estimated for all the antioxidant compounds indicated that a large portion of

variance is contributed through genotypic variance and a reliable selection could be made for antioxidant compounds. The significant positive correlations among total phenolics, anthocyanins content, FRAP value and CUPRAC value could be used as indirect selection criteria for improving the levels of antioxidants. Examining the involvement of additive and non-additive genes for biosynthesis of antioxidants and root development, respectively; recurrent selection would be the best breeding approach to improve the both traits antioxidant concentration and root yield simultaneously. The information, therefore, gained from this study could be used in future breeding programmes to improve the yield and antioxidant levels of radish.

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REFERENCES

Apak R, Guclu K, Ozyurek M and Celik S E. 2008. Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. *Microchimica Acta* **160**: 413–9.

Benzie I F F and Strain J J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry* **239**: 70–6.

Burton G W and DeVane D H. 1953. Estimating heritability in fall fescue from replicated clonal material. *Agronomy Journal* **4**: 78–81.

Cartea M E, Francisco M, Soengas P and Velasco P. 2011. Phenolic compounds in *Brassica* vegetables. *Molecules* **16**: 251–80.

Castilla P, Davalos A, Teruel J L, Cerrato F, Fernandez-Lucas M, Merino J L, Sanchez-Martin C C, Ortuno J and Lasuncion M A. 2008. Comparative effects of dietary supplementation with red grape juice and vitamin E on production of superoxide by circulating neutrophil NADPH oxidase in hemodialysis patients. *American Journal of Clinical Nutrition* **87**: 1053–61.

Chalker-Scott L. 1999. Environmental significance of anthocyanins in plant stress responses. *Photochemistry and Photobiology* **70**: 1–9.

Chander S, Meng Y, Zhang Y, Yan J and Li J. 2008. Comparison of nutritional traits variability in selected eighty-seven inbreds from Chinese maize (*Zea mays* L.) germplasm. *Journal of Agricultural and Food Chemistry* **56**: 6506–11.

Chu Y F, Sun J, Wu X and Liu R H. 2002. Antioxidant and anti-proliferative activities of common vegetables. *Journal of Agricultural and Food Chemistry* **50**: 6910–6.

Connor A M, Stephens M J, Hall H K and Alspach P A. 2005. Variation and heritabilities of antioxidant activity and total phenolic content estimated from a red raspberry factorial experiment. *Journal of the American Society for Horticultural Science* **130**: 403–11.

Delgado-Vargas F and Paredes-Lopez O. 2003. *Natural Colorants for Food and Nutraceutical Uses*. CRC Press, Boca Raton, Florida, USA.

Dixon R A and Palva N L. 1995. Stress-induced phenylpropanoid metabolism. *Plant Cell* **7**: 1085–97.

Estruch R, Sacanella E, Badia E, Antunez E, Nicolas J M, Fernandez-Sola J, Rotiliob D, Gaetanoc G D, Rubind E and Urbano-Márqueza A. 2004. Different effects of red wine and gin consumption on inflammatory biomarkers of atherosclerosis: a prospective randomized crossover trial: effects of wine on inflammatory markers. *Atherosclerosis* **175**: 117–23.

George R A T and Evans D R. 1981. A classification of winter radish cultivars. *Euphytica* **30**: 483–92.

Ghosh D and Konishi T. 2007. Anthocyanins and anthocyanin-rich extracts: role in diabetes and eye function. *Asia Pacific Journal of Clinical Nutrition* **16**(2): 200–208.

Giusti M M, Rodriguez-Saona L E, Baggett J R, Reed G L, Durst R W and Wrolstad R E. 1998. Anthocyanin pigment composition of red radish cultivars as potential food colorants. *Journal of Food Science* **63**(2): 219–24.

Giusti M M and Wrolstad R E. 1996. Characterization of radish anthocyanins. *Journal of Food Science* **61**: 322–6.

Giusti M M and Wrolstad R E. 2003. Acylated anthocyanins from edible sources and their applications in food systems. *Biochemical Engineering Journal* **14**: 217–25.

Gould K S, McKelvie J and Markham K R. 2002. Do anthocyanins function as antioxidants in leaves? Imaging of H_2O_2 in red and green leaves after mechanical injury. *Plant, Cell and Environment* **25**: 1261–9.

Hanson P M, Yang R, Wu J, Chen J, Ledesma D and Tsou S C S. 2004. Variation for antioxidant activity and antioxidants in tomato. *Journal of the American Society for Horticultural Science* **129**(5): 704–11.

Harakotr B, Suriharn B, Scott M P and Lertrat K. 2015. Genotypic variability in anthocyanins, total phenolics, and antioxidant activity among diverse waxy corn germplasm. *Euphytica* **203**: 237–48.

Holton T A and Cornish E C. 1995. Genetics and biochemistry of anthocyanin biosynthesis. *Plant Cell* **7**: 1071–83.

Horbowicz M, Kosson R, Grzesiuk A and Debski H. 2008. Anthocyanins of fruits and vegetables- their occurrence, analysis and role in human nutrition. *Vegetable Crops Research Bulletin* **68**: 5–22.

Hu Q P and Xu J G. 2011. Profiles of carotenoids, anthocyanins, phenolics, and antioxidant activity of selected color waxy corn grains during maturation. *Journal of Agricultural and Food Chemistry* **59**: 2026–33.

Iwasaki-Kurashige K, Loyaga-Rendon Y, Matsumoto H, Tokunaga T and Azuma H. 2006. Possible mediators involved in decreasing peripheral vascular resistance with blackcurrant concentrate (BC) in hind-limb perfusion model of the rat. *Vascular Pharmacology* **44**: 215–23.

Jing P, Zhao S, Ruan S, Sui Z, Chen L, Jiang L and Qian B. 2014. Quantitative studies on structure-ORAC relationships of anthocyanins from eggplant and radish using 3D-QSAR. *Food Chemistry* **145**: 365–71.

Kallithraka S, Mohdaly A A, Makris D P and Kefalas P. 2005. Determination of major anthocyanin pigments in Hellenic native grape varieties (*Vitis vinifera*): association with antiradical activity. *Journal of Food Composition and Analysis* **18**: 375–86.

Kaneko Y and Matsuzawa Y. 1993. Radish (*Raphanus sativus* L.). (*In*): *Genetic Improvement of Vegetable Crops*, pp 487–505. Kalloo G and Bergh B O (Eds). Pergamon Press Ltd., Oxford, England.

Kapusta-Duch J, Kopec A, Piatkowska E, Borczak B and Leszczynska T. 2012. The beneficial effects of Brassica vegetables on human health. *Roczniki Państwowego Zakładu*

Higieny **63**(4): 389–95.

Karmakar P, Munshi A D, Behera T K, Kumar R, Kaur C and Singh B K. 2013. Hermaphrodite inbreds with better combining ability to improve antioxidant properties in ridge gourd [*Luffa acutangula* (Roxb.) L.]. *Euphytica* **191**(1): 75–84.

Koes R E, Quattrocchio F and Mol J N M. 1993. The flavonoid biosynthetic pathway in plants: function and evolution. *Bioessays* **16**: 123–32.

Koley T K, Singh S, Khemariya P, Sarkar A, Kaur C, Chaurasia S N S and Naik P S. 2014. Evaluation of bioactive properties of Indian carrot (*Daucus carota* L.): A chemometric approach. *Food Research International* **60**: 76–85.

Kramer J H. 2004. Anthocyanosides of *Vaccinium myrtillus* (bilberry) for night vision—a systematic review of placebo-controlled trials. *Survey of Ophthalmology* **49**: 618.

Kumar R, Sharma R, Gupta R K and Singh M. 2014. Determination of genetic variability and divergence for root yield and quality characters in temperate radishes. *International Journal of Vegetable Science* **18**(4): 307–18.

Kutty C N and Sirohi P S. 2003. Combining ability studies in radish (*Raphanus sativus* L.). *Vegetable Science* **30**(2): 120–3.

Li J, Ou-Lee T M, Raba R, Amundson R G and Last R L. 1993. Arabidopsis flavonoid mutants are hypersensitive to UV-B irradiation. *Plant Cell* **5**: 171–9.

Liang Z, Yang C, Yang J, Wu B, Wang L, Cheng J and Li S. 2009. Inheritance of anthocyanins in berries of *Vitis vinifera* grapes. *Euphytica* **167**: 113–25.

Mahan A L, Murray S C, Rooney L W and Crosby K M. 2013. Combining ability for total phenols and secondary traits in a diverse set of coloured (red, blue and purple) maize. *Crop Science* **53**: 1–8.

Matsufuji H, Kido H, Misawa H, Yaguchi J, Otsuki T, Chino M, Takeda M and Yamagata K. 2007. Stability to light, heat, and hydrogen peroxide at different pH values and DPPH radical scavenging activity of acylated anthocyanins from red radish extract. *Journal of Agricultural and Food Chemistry* **55**: 3692–3701.

McGhie T K and Walton M C. 2007. The bioavailability and absorption of anthocyanins: towards a better understanding. *Molecular Nutrition and Food Research* **51**: 702–13.

Misyura M. 2014. 'High density stress response in plants and the role of anthocyanin biosynthesis under adverse environmental conditions.' Ph D Thesis, Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada.

Mo Y, Nagel C and Taylor L P. 1992. Biochemical complementation of chalcone synthase mutants defines a role for flavonols in functional pollen. *Proceedings of the National Academy of Sciences USA* **89**: 7213–7.

Panse V G and Sukhatme P V. 1967. *Statistical Methods for Agricultural Workers*. Indian Council of Agricultural Research (ICAR), New Delhi, India.

Peng C H, Liu L K, Chuang C M, Chyau C C, Huang C N and Wang C J. 2011. Mulberry water extracts possess an anti-obesity effect and ability to inhibit hepatic lipogenesis and promote lipolysis. *Journal of Agricultural and Food Chemistry* **59**: 2663–71.

Pojer E, Mattivi F, Johnson D and Stockley C S. 2013. The case for anthocyanin consumption to promote human health: A review. *Comprehensive Reviews in Food Science and Food Safety* **12**: 483–508.

Prior R L and Wu X. 2006. Anthocyanins: structural characteristics that result in unique metabolic patterns and biological activities. *Free Radical Research* **40**: 1014–28.

Rahman M M, Ichiyanagi T, Komiyama T, Hatano Y and Konishi T. 2006. Superoxide radical- and peroxynitrite-scavenging activity of anthocyanins; structure-activity relationship and their synergism. *Free Radical Research* **40**: 993–1002.

Rodriguez V M, Soengas P, Landa A, Ordas A and Revilla P. 2013. Effects of selection for color intensity on antioxidant capacity in maize (*Zea mays* L.). *Euphytica* **193**: 339–45.

Ross J A and Kasum C M. 2002. Dietary flavonoids: bioavailability, metabolic effects and safety. *Annual Review of Nutrition* **22**: 19–34.

Searle S R. 1961. Phenotypic, genotypic and environmental correlations. *Biometrics* **17**: 474–80.

Shirley B W. 1996. Flavonoid biosynthesis: ''new'' functions for an ''old'' pathway. *Trends in Plant Science* **1**: 377–82.

Singh A, Singh B K, Deka B C, Sanwal S K, Patel R K and Verma M R. 2011. The genetic variability, inheritance and inter-relationships of ascorbic acid, β-carotene, phenol and anthocyanin content in strawberry. *Scientia Horticulturae* **129**(1): 86–90.

Singh B K. 2007. 'Studies on vand heterosis of important economic and nutritive traits in cabbage.' Ph D Thesis. IARI, Pusa, New Delhi, India.

Singh B K and Karmakar P. 2015. Improved production technology for root crops. (*In*): *Improved Production Technologies in Vegetable Crops*, pp. 120–33. Singh N, Roy S, Karmakar P, Chaurasia S N S, Gupta S and Singh B (Eds). IIVR Training Manual No. 59, Indian Institute of Vegetable Research, Varanasi.

Singh B K, Sharma S R and Singh B. 2010. Antioxidant enzymes in cabbage: variability and inheritance of superoxide dismutase, peroxidase and catalase. *Scientia Horticulturae* **124**(1): 9–13.

Singh R K and Chaudhary B D. 1977. *Biometrical Methods in Quantitative Genetic Analysis*. Kalyani Publishers, Ludhiana, India.

Singleton V L, Orthofer R and Lamuela-Ranventos R M. 1999. Analysis of total phenols other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology* **299**: 152–78.

Soengas P, Sotelo T, Velasco P and Cartea M E. 2011. Antioxidant properties of Brassica vegetables. *Functional Plant Science and Biotechnology* **5**(2): 43–55.

Tatsuzawa F, Saito N, Toki K, Shinoda K, Shigihara A and Honda T. 2010. Acylated cyanidin 3-sophoroside-5-glucosides from the purple roots of red radish (*Raphanus sativus* L.) 'Benikanmi'. *Journal of the Japanese Society for Horticultural Science* **79**(1): 103–7.

Tsuda T. 2012. Dietary anthocyanin-rich plants: biochemical basis and recent progress in health benefits studies. *Molecular Nutrition and Food Research* **56**: 159–70.

Wang L S and Stoner G D. 2008. Anthocyanins and their role in cancer prevention. *Cancer Letter* **269**: 281–90.

Wrolstad R E, Durst R W and Lee J. 2005. Tracking color and pigment changes in anthocyanin products. *Trends in Food Science and Technology* **16**(9): 423–8.

Zilic S, Serpen A, Akıllioglu G, Gokmen V and Vancetovic J. 2012. Phenolic compounds, carotenoids, anthocyanins and antioxidant capacity of colored maize (*Zea mays* L.) kernels. *Journal of Agricultural and Food Chemistry* **5**: 1224–31.