Indian Journal of Agricultural Sciences **86** (11): 1471–6, November 2016/Article
https://doi.org/10.56093/ijas.v86i11.62931

Genetic analysis of bioactive compounds and antioxidant properties in lettuce (*Lactuca sativa*)

SWATI SAHA[1], PRITAM KALIA[2], A K SUREJA[3], ARPITA SRIVASTAVA[4] and S K SARKAR[5]

Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi 110 012

Received: 14 September 2015; Accepted: 27 July 2016

ABSTRACT

Leaves of lettuce (*Lactuca sativa* L.) are the store house of various phytonutrients which have protective properties. Being an important dietary leafy vegetable, it is primarily consumed fresh as salad and in sandwiches, burgers etc. Its beneficial effects are primarily due to the presence of different phytochemicals such as ascorbic acid, carotenoids, polyphenols and fibre which helps in protecting key biological constituents such as lipoproteins, membranes and DNA. However, systematic biochemical nutrient analysis has not been carried out in this important salad vegetable so far. In the present investigation, 36 genotypes were analysed for phytochemicals such as total carotenoids, lycopene, ascorbic acid, total phenolic content, Cupric ion Reducing Antioxidant Capacity (CUPRAC) and Ferric Reducing Antioxidant Power (FRAP). The CUPRAC ranged from 0.05 to 1.98 µmol trolox/g with the highest content in Stem lettuce Angustana, whereas FRAP ranged from 0.06 to 4.70 µmol trolox/g showing, thereby, a considerable variation amongst genotypes. Total phenolics ranged from 41.94 to 501.88 µg gallic acid/g fresh weight. Total carotenoids were found in appreciable amount in Wo Suen (46.13 mg/100g fresh weight), whereas lycopene in New Chicken (17.01 mg/100g fresh weight). Ascorbic content ranged from 1.14 to 3.75 mg/100g fresh weight, whereas per cent moisture ranged from 86.50 (NVRS 10:001818) to 97.32 (Sheetal). Positive correlation was observed between total carotenoids and lycopene, chlorophyll b with chlorophyll a, total chlorophyll with both chlorophyll a and b, FRAP with CUPRAC and phenols with total chlorophyll, chlorophyll a and b. Maximum phenotypic and genotypic coefficients of variance were calculated for FRAP (165.98, 165.98) followed by CUPRAC (122.10,122.10) and lycopene content (83.33, 80.84), respectively. These genotypes can be further utilized for development of multinutrient rich varieties. Regular consumption of lettuce can go a long way in tackling osteoporosis, anemia, and cardiovascular related problems.

Key words: Antioxidant, Carotenoids, Chlorophyll, CUPRAC, FRAP, *Lactuca sativa*, Lettuce, Lycopene, Phenol, Phytochemicals

Lettuce (*Lactuca sativa* L.; 2n=2x=18) is herbaceous self-pollinated crop belonging to Compositae family. For its nutritional value, it is the most widely used salad crop in North America, Australia and Europe. Of late, salad crops have also started gaining popularity in India, because of nutritional importance (Sharma 2002). Lettuce is almost exclusively used as a fresh salad crop; however some of its forms are also cooked (Lebeda *et al*. 2007). Its use is also increasing in soup, sandwiches, burgers etc. It is a good source of vitamin A, vitamin K and potassium besides dietary fiber, carbohydrates, protein and some fat. With the exception of the iceberg type, lettuce also provides some amount of vitamin C, calcium, iron and copper, with vitamins and minerals largely found in the leaf. The beneficial health effects are mainly attributed due to the presence of diverse antioxidant compounds such as anthocyanins, total carotenoids etc. Natural phytochemicals with antioxidant properties are multifunctional, and a reliable antioxidant protocol should estimate more than one relevant property (Kang and Saltveit 2002). It contains significantly high levels of antioxidants and phenolic components (Heimler *et al.* 2007) varying with the varieties (Lee and Kader 2000). In view of the importance of the crop, develop acceptable nutritionally rich varieties for cultivation, the present study was undertaken for nutritional profiling of lettuce germplasm and knowing the estimates of genetic parameters for developing high yielding and nutritionally rich good quality lettuce varieties for consumption by Indian population.

[1]e mail: swatisaha1980@gmail.com, ICAR–Indian Agricultural Research Institute, Regional Station, Pune, Maharashtra. [5]ICAR–Indian Agricultural Statistics Research Institute, New Delhi 110 012.

MATERIALS AND METHODS

Freshly harvested leaves from each of the 36 genotypes from each replication were washed and wiped with tissue paper to remove the water droplets. The leaves were weighed as per different analytical requirements.

For determining moisture content, small pieces of lettuce leaves were dried in Petri plates. Drying was done to a constant weight, at a temperature not exceeding 70º C

in hot oven. Then the samples were allowed to cool and based on dry weight, sample moisture percentage was calculated as per the standard formula. Total carotene and lycopene was determined by using acetone and petroleum ether as extracting solvents (Ranganna 1986). The 5g of sample was extracted with acetone using pestle and mortar repeatedly until the residue was colourless. The extract was pooled, filtered and transferred in the separating funnel containing 20 ml of petroleum ether and gently mixed. The 20 ml of 5% sodium sulphate was added and shaken gently. Again 20 ml of petroleum ether was added. The upper layer contained petroleum ether. The two phases were separated and the lower aqueous phase was re-extracted with additional petroleum ether till it became colourless. The extract was pooled and washed with distilled water. The volume was made up in 50 ml volumetric flask and the absorbance was measured in UV-VIS spectrophotometer at 452 and 503 nm using petroleum ether as a blank. The chlorophyll content (chlorophyll a, b and total chlorophyll) of the leaves were estimated following Barnes *et al*. (1992). Accurately weighed 2g of leaf sample was immersed in 10 ml of DMSO (AR grade). The samples were incubated at 70^0C for 4 hr in incubator. Then it was read on a UV-VIS spectrophotometer at 645 and 663nm wavelengths using pure DMSO as blank. Cupric ion reducing antioxidant capacity (CUPRAC) was a widely applicable antioxidant activity, utilizing the copper (II)-neocuproine [Cu(II)-Nc] reagent as the chromogenic oxidizing agent. Reducing ability of polyphenols to copper(II) (or cupric) ion was measured. The method was named by a research group (Apax *et al*. 2004) "cupric reducing antioxidant capacity" abbreviated as the CUPRAC method. Ethanol extract was prepared by homogenized 5 g of leaf sample in 15 ml ethanol (100%) with pestle and mortar. Then it was centrifuged at 10,000 rpm for 15 min at 4^0C. The supernatant was stored at -20ºC.100 µl ethanolic extract of respective sample was taken, 1 ml neucuproine, 1 ml ammonium acetate, 1 ml $CuCl_2$ and 1 ml double distilled water were added and absorbance was recorded at 450 nm by using UV-VIS double beam PC 8 scanning Auto Cell Spectrophotometer. Ferric ion Reducing Antioxidant Power (FRAP) assay was measured according to Benzie and Strain (1999). FRAP assay uses antioxidants as reductance in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess. For that, FRAP reagent was prepared by adding acetate buffer (300 mM, pH 3.6), tripyridyl triazine (TPTZ 10 mM) and $FeCl_3$ (20 mM) in the ratio of 10:1:1. Ethanol extract was prepared by homogenized 5 g of leaf sample in 15 ml ethanol (100%) with pestle mortar. Then it was centrifuged at 10000 rpm for 15 min at 4^0C. The supernatant was stored at -20ºC. Then 100 µl ethanolic extract of respective genotype was mixed with 3 ml FRAP reagent and kept for 30 min. Absorbance was recorded at 593 nm by using UV-VIS double beam PC 8 scanning Auto Cell Spectrophotometer. The calibration curve was prepared with ascorbic acid. The total polyphenolic content were determined in ethanol extracts with Folin-Ciocalteau reagent using gallic acid as a standard (Singleton and Rossi 1965). Ascorbic acid was estimated titrimetrically using 2, 6-dichlorophenol indophenols (Ranganna 1986). 10g sample was crushed with 3% HPO_3 solution and volume made up to 100 ml. The sample was filtered using Whatmann filter paper no.1 and filtrate was used for analysis. 10 ml of sample was titrated against the dye till the faint pink colour appeared which remains for at least 15 seconds (Volume of sample should be taken as the dye titre value should be 3-5 ml).

Data analysis was carried out on observations taken on 36 genotypes in each of the 3 replications using SAS, Version 9.3 (SAS 2010). Analysis of variance (ANOVA) was used to compare genotypic mean value of each character at 5% level of significance. The data were subjected to analysis of variance as per the design using standard statistical procedure (Panse and Sukhatme 1967). The structure of ANOVA for Randomized Complete Block Design has been given and the replication and treatment mean sum of squares (MSS) were tested against error mean sum of square by F-test.Various parameters of genetic variability such as mean, range, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), broad sense heritability and genetic advance as a percentage of mean were worked out.

Mean was calculated using following conventional formula given below:

$X = \sum_{i=1}^{n} xi/n$, where, X = General mean of a character; xi = Value of i^{th} observation; n = Total number of observations; $\sum$ = Summation notation. Range, indicated the lowest and the highest values present in the observations included in a sample. Genotypic and phenotypic coefficient of variation were computed for each trait using the formula given by Burton (1952) and expressed in terms of percentage.

$$\text{Genotypic coefficient of variation (GCV)} = \frac{\sqrt{\sigma 2g} \times 100}{X}$$

where, $\sigma 2g = \frac{\sigma 2p - \sigma 2e}{r}$; $\sigma 2g$ = genotypic variance; $\sigma 2p$ = phenotypic variance; $\sigma 2e$ = environment variance; r = number of replication.

$$PCV = \frac{\sqrt{\sigma 2p}}{X} * 100$$; where, $\sigma 2p$ = phenotypic variance; X = general mean of the character under consideration. Heritability in broad sense (Hb) was estimated according to the formula given by Hanson *et al*. (1956).

$$Hb = \frac{\sqrt{\sigma 2g} \times 100}{X}$$ where, $\sigma 2g$ = genotypic variance; $\sigma 2p$ = phenotypic variance.

Genetic advance (GA) = Kσp. Hb

The genetic advance was calculated as suggested by Lush (1949) and Johnson *et al*. (1955) using following formula where, K = constant, 2.06 at 5 % selection intensity,

σp = Phenotypic standard deviation; Hb = Broad sense heritability. Genetic advance as a percentage of mean was calculated as GA(% of mean)

$$= \frac{GA}{X} \times 100$$

where, GA = Genetic advance; X = general mean of the character under consideration.

RESULTS AND DISCUSSION

Phytochemicals in lettuce

The mean values of quality traits presented in Table 1 revealed significant variability among 36 genotypes for different biochemical constituents. The per cent moisture content in leaves varied from 88.53 to 97.32%, with most of the genotypes possessing more than 90%. Among 36 different genotypes, maximum lycopene content were observed in New Chicken (17.01 mg). Variation in the lycopene content ranged from 1.32 to 17.01 mg. Most of the genotypes were within the range of 1 to 10 mg/100g fresh weight. The total carotenoid content in leaf tissue varied considerably among the accessions. The breeding material, Wo Suen, contained almost 46.13 mg carotenoids per 100 g raw tissue, twice the content of other carotenoid-rich accessions. Excluding this accession, carotenoid content varied from almost 8.35 mg/100 g in Iceberg Dublin F_1 to 42.17 mg/100 g in Criolla Verde cultivar. Chlorophyll a, Chlorophyll b and total chlorophyll content were highest in stem lettuce (NVRS10:001818). Antioxidant activities of different lettuce genotypes studied by CUPRAC and FRAP methods showed significant differences. Antioxidant activity estimated by CUPRAC method was high in Stem lettuce Angustana (1.98 µmol trolox/ g). This genotype also recorded highest antioxidant activity from FRAP (4.70 µmol trolox/ g). In this study, several genotypes of lettuce were found to have high antioxidants, which could be suitable for making further improvement. Hence, it can be said that the antioxidant activity can be elucidated by different biochemical methods which can investigate the type of phenolic compounds involved in antioxidant activity. The biochemical parameters studied showed significant differences among different genotypes. Total phenolic content ranged from 91.44 (Yellow Lettuce) to 501.88 µg gallic acid equivalent (GAE)/g fresh weight (IHRGRU10-006614). These findings are in line with those of Shyamla *et al*. (2005) who also reported wide variation in total phenolic content in leafy vegetables. The phenolic compounds have contributed significantly to the antioxidant activity in lettuce. The phenolic compounds have been found to be efficient radical scavengers which inactivate the damaging species and prevent the deleterious consequences of their reactions. Moreover, these beneficial effects are believed to be due to the presence of different phytochemicals such as ascorbic acid, carotenoids, polyphenols and fibre helps to protect key biological constituents such as lipoproteins, membranes, and DNA (Szeto *et al.* 2004). Vitamin C was highest in Parris Island Cos, Arctic Kwig, HRI 10: 006780, Iceberg, Iceberg Dublin F1 Hybrid, Costa Verde, Red Revolution F1 Hybrid, Curled Lettuce and Yellow Lettuce genotypes with 3.75mg/100g fw. In this context, the recommended intake of Vitamin C reported by FAO is between 25 and 70 mg per day, thus, although, the vitamin C content of lettuce is modest, a serving of these lettuce genotypes could provide around 26% of this daily intake. Fresh lettuce leaves has good amounts of vitamin C and its intake will help in developing resistance against infectious agents and scavenge harmful, pro-inflammatory free radicals. The loose leaf lettuce is regarded as better food because of high iron, vitamin A and C content than stem varieties. It is rich source of mineral salts, especially the alkaline elements. There are some purple lettuces which are rich in anthocyanin. Red and dark green lettuces are generally higher in antioxidants, vitamin B6 and other nutrients than light coloured greens (Bunning and Kendal 2012). Romaine or the Cos lettuce is the most nutrient-dense of all the lettuce varieties and is an excellent source of vitamins A, B1, B2 and C, folic acid, manganese and chromium. Many compounds found in lettuce have antioxidant and other health promoting properties and leaf type lettuce has abundance of health promoting phytochemicals (Liu *et al*. 2007). The high water content in lettuce provides little health benefits beyond its nutrient content and nutritional value varies greatly with the type of lettuce. The synthesis and absorption of many nutrients are light dependent; the lower nutrient content of crisphead lettuce is largely due to the enclosure of its leaves in the head structure (Mou and Ryder 2004). Most natural phytochemicals with antioxidant properties are multifunctional, and a reliable antioxidant protocol should measure more than one relevant property. Two effective methods to measure antioxidant capacity in foods is the Cupric reducing antioxidant capacity (CUPRAC) and the ferric reducing antioxidant power (FRAP). The relationship between antioxidant capacity, as determined by the FRAP assay, and total phenols was linear (r = 0.96) (Deighton *et al*. 2000). These data are important because the polyphenols act as natural antioxidants and may be these compounds are part of the reason for the protective effect against degenerative diseases when this type of food is a significant part of the diet (Dupont *et al*. 2000). The phenolic content of plants depends both qualitatively and quantitatively on their genetic information (variety). In addition to genotype, growing conditions can significantly affect the content of many phenolic compounds which have a positive impact on its health promoting qualities (Oh *et al*. 2011). Therefore, the variability in the compositions and quantities of compounds in different foods indicate the importance of eating a variety of foods, especially the coloured ones. However, different agronomic or environmental conditions and tissue type could affect the phenolic content present in vegetables (Dupont *et al*. 2000, Manach *et al*. 2004, Nicolle *et al*. 2004, Tomas-Barberan *et al*. 2000). Nevertheless, the vitamin C content not only

depends on variety, other pre- and postharvest factors can influence the vitamin C content (Lee and Kader 2000). Thus, the ascorbic acid levels could increase in response to high light intensities (Nicolle *et al*. 2004). However, the selection of the genotype with the highest vitamin C content in any commodity is an important factor (Lee and Kader 2000).

Cluster analysis of 36 genotypes based on 10 quantitative traits was performed by UPGMA method. The genotypes were grouped into two clusters based on quantitative traits. Cluster I was the largest comprising 30 genotypes followed by cluster II (6 genotypes). In cluster II, Valmaine Cos (3) was diverse from rest of the five genotypes HRI10:001730 (9), Curled lettuce (32), NVRS 10:001818 (8), Sun Mandela (15) and IHRGRU10.006614 (2). Hierarchical cluster analysis allowed the assessment of similarity and clarified relationships among different lettuce genotypes.

Analysis of variance

The analysis of variance was carried out for 10 quality traits such as moisture per cent, lycopene content, total carotenoids, chlorophyll a, chlorophyll b, total chlorophyll, Cupric ion reducing antioxidant capacity (CUPRAC), Ferric ion Reducing Antioxidant Power (FRAP), phenol, and Vitamin C and results are presented in Table 2. The analysis of variance revealed that the mean sum of square due to genotypes/accessions were highly significant for all the characters at 5 % probability level. There was significant variation among the genotypes providing thereby a gateway for genetic improvement in the material. Phenotypic and genotypic coefficients of variation for most of the traits were similar except for the lycopene content.

Estimates of genetic parameters

Highest phenotypic and genotypic coefficients of variance were recorded for FRAP (165.98, 165.98), followed by CUPRAC (122.10, 122.10), lycopene content (83.33 and 80.84), total chlorophyll (62.47, 62.47), Chlorophyll b (59.94 and 59.85), phenolics (58.45, 58.45), Chlorophyll a (53.81 and 53.60), vitamin C (44.26 and 44.16), total carotenoids (38.86,38.86), and moisture per cent (2.52, 2.52), respectively (Table 3). High estimates of heritability were recorded in moisture percent, total carotenoids and total chlorophyll (1.00), whereas high genetic advance as percentage of mean was highest in CUPRAC (125.3) followed by FRAP (96.25), total chlorophyll (84.25) and lycopene content (82.53).

Correlation matrix and principal components of lettuce genotypes

In the present study, there was negative correlation between chlorophyll a and chlorophyll b and total chlorophyll was also negatively correlated with the per cent moisture (Table 4). Vitamin C has also negative correlation with the chlorophyll. Positive correlation was observed between total carotenoids and lycopene, chlorophyll b with chlorophyll a, total chlorophyll with both chlorophyll a and b, FRAP with CUPRAC and phenols with total chlorophyll, chlorophyll a and b.

However, in multivariate situation correlation analysis alone could not give a complete picture of interrelations because it considers only two components at a time, regardless of the interrelationship with other components in the set of data. Hence, PCA was carried out to understand the underlying interrelationships in the whole set of data and to select the best linear combination of quality traits which explains the largest proportion of the variation in the data set. PCA revealed that the first five principal components (PCs) together governed 88.41% of the total variability (Table 5). In these principal components, the coefficients corresponding to chlorophyll-a, chlorophyll-b and total chlorophyll were consistent, they were the most appropriate to use in the preliminary grouping of the 36 lettuce genotypes evaluated in this study. Here, it was worth to describe scree plot and variance component plot, as in the scree plot it is customary to see elbow, although it is not very prominent here but first 5 principal components seems to form elbow. It is also evident from the proportion of variance explained by these principal components. The perfect variance component plot looks like a horse shoe; the obtained plot is following it. On the basis of these dominant factors cluster analysis has been performed to observe the closeness of genotypes. Thirty six genotypes were broadly classified into two clusters based on phytochemical analysis and in this Group I consisted of six genotypes and the remaining were in group II. In group I, Valmaine cos (3) was found to be most diverse from the rest five genotypes.

Association of characters

Correlation coefficients (r) estimated among nutritional and antioxidant properties to know the relationships among them are presented in Table 6. Moisture per cent recorded positive phenotypic correlation with vitamin C (0.13) only which was negative with rest of the traits. Lycopene content showed positive correlation with total carotenoids (0.45) followed by Vitamin C (0.16) and phenol (0.07). Total carotenoids showed positive correlation with most of the characters except FRAP (-0.17) and Vitamin C (-0.02). CUPRAC showed positive correlation with FRAP (0.71), Vitamin C (0.15) and phenol (0.09). Relationship of these character pairs at genotypic level were of similar sign and magnitude. However, in most of the cases, the values of genotypic correlation also were higher than those of phenotypic correlation, which suggested that the character pairs with positive and significant correlation may be used for bringing improvement in these traits simultaneously. Positive and significant correlation coefficient of lycopene with phenol and vitamin C was observed while it was negative with Chlorophyll a and b, total chlorophyll. Positive and negative significant correlations among different characters have also been reported by Dolma and Gupta (2008) in lettuce, Liu and Coa (1993) in Chinese cabbage, Varalakshmi and Reddy (1997) in amaranthus and Yuan *et al*. (2001) in Savoy Chinese cabbage cultivars.

Estimates of direct and indirect effects of causal variables were worked out using path coefficient analysis and the results are presented in Table 7. It is evident from the perusal of the table that moisture percentage had negative direct effect (-0.300), whereas the indirect effects via lycopene content, total chlorophyll, CUPRAC and vitamin C were positive and negative via total carotenoids, Chlorophyll a and b, FRAP and phenol. Path coefficient analysis on genotypic correlation revealed maximum positive effect of lycopene content in lettuce leaves followed by chlorophyll b and CUPRAC suggesting thereby that the characters with maximum direct effects should be selected for improving quality in lettuce. The residual effect (res, r) indicated that the ten characters studied showed moderate to high percentage of variation in phytochemicals among the genotypes. Since majority of the values of path coefficients are less than unity, therefore it revealed that inflation due to multicolinearity is minimal (Gravois and Helms 1992). The success or failure of a breeding programme largely depends on the extent of variability in the base population which is measured by different population parameters including genotypic and phenotypic coefficient of variation (Usmani *et al.* 2014).

The present work revealed that the phytochemical profile such as total carotenoids, lycopene, per cent moisture, ascorbic acid, total phenolic content, Cupric ion Reducing Antioxidant Capacity (CUPRAC) and Ferric Reducing Antioxidant Power (FRAP) showed a considerable variation among thirty six genotypes of lettuce . A good correlation between total carotenoids and lycopene, chlorophyll b and a, total chlorophyll and both chlorophyll a and b, FRAP and CUPRAC and phenols with total chlorophyll, chlorophyll a and b was found. Phenotypic and genotypic coefficients of variance were found to be highest for FRAP followed by CUPRAC and lycopene. Overall, the present study revealed that lettuce is an important leafy vegetable with good nutritional value and the genotypes of present study can be further utilized to develop nutritionally rich lettuce varieties.

ACKNOWLEDGEMENT

The authors acknowledge Indian Council of Agricultural Research for funding the study.

REFERENCES

Apax R, Guclu K, Ozyurek M and Karademir S E. 2004. A novel total antioxidant capacity index for dietary polyphenols, vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry* **52**: 970–81.

Barnes J D, Balaguer L, Maurigue E, Elvira S and Davidson A W. 1992. A reappraisal of the use of DMSO for the extraction and determination of chlorophyll a and b in lichens and higher plants. *Environment Experimental Botany* **32**: 87–99.

Benzie F F and Strain J J. 1999. Ferric reducing antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology* **299**: 15–23.

Bunning M and Kendall P. 2012. Salad greens: Health benefits and safe handling. *Food and Nutrition Series* Fact sheet No **9**: 373.

Burton G W. 1952. Quantitative inheritance in grasses. *Proceedings of Sixth International Grassland Congress* **7**: 277–83.

Deighton N, Brennan R, Finn C and Davies H V. 2000. Antioxidant properties of domesticated and wild *Rubus* species. *Journal of the Science of Food and Agriculture* **80**: 1 307–13.

Dolma T and Gupta A. 2008. Character association and path coefficient analysis in lettuce (*Lactuca sativa* L.) genotypes under Kashmir condition. *Vegetable Science* **35**: 185–9.

Dupont S, Mondi Z, Willamson G and Price K. 2000. Effect of variety, processing, and storage on the flavonoid glycoside and composition of lettuce and chicory. *Journal of Agricultural and Food Chemistry* **48**: 3 957–64.

Gravios K A and Helms R S. 1992. Path analysis of rice yield and yield components as affected by seedling rate. *Agronomy Journal* **84**: 1–4.

Hanson C H, Robinson H F and Comstock R E. 1956. Biometric studies of yield in segregating population of Korean Lespedezer. *Agronomy Journal* **48**: 268–72.

Heimler D, Isolani L, Vignolini P, Tombelli S and Romani A. 2007. Polyphenol content and antioxidative activity in some species of freshly consumed salads. *Journal of Agricultural and Food Chemistry* **55**: 1 724–9.

Johnson H W, Robinson H F and Comstock R E. 1955. Estimation of genetic and environmental variability in soybean. *Agronomy Journal* **47**: 314–8.

Kang H M and Saltveit M E. 2002. Antioxidant capacity of lettuce leaf tissue increases after wounding. *Journal of Agriculture and Food Chemistry* **50**: 7 536–41.

Lebeda A, Ryder E J, Grube R, Dolezalova I and Kristkova E. 2007. *Lettuce. In: Genetic Resources, Chromosome Engineering and Crop Improvement, Vegetable Crops*, pp 377–82. CRC Press, Taylor and Francis, Boca Raton.

Lee K and Kader A. 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology* **20**: 207–20.

Liu S, Ardo M, Bunning J, Parry K and Zhou C. 2007. Total phenolic content and DPPH radical scavenging activity of lettuce (*Lactuca sativa* L.) grown in Colorado. *Food Science and Technology* **40**: 552–7.

Liu W X and Coa S C. 1993. A study on evaluation and related characteristic of non-heading Chinese cabbage and under summer high temperature. *Journal of Shandong Agricultural University* **24**: 176–82.

Lush J L. 1949. Heritability of quantitative characters in farm animals. *Heriditas* **35:** 256–61.

Manach C, Scalbert A, Morand C, Remesy C and Jimenez L. 2004. Polyphenols: Food sources and bioavailability. *American Journal of Clinical Nutrition* **79**: 727–47.

Mou B and Ryder E J. 2004. Relationship between the nutritional value and the head structure of lettuce. *Acta Horticulturae* **637**: 361–7.

Nicolle C, Carnat A, Fraisse D, Lamaison J L, Rock E and Michel H. *2004*. Characterization and variation of antioxidant micronutrients in lettuce (*Lactuca sativa*). *Journal of the Science of Food and Agriculture* **84**: 2 061–9.

Panse V G and Sukhatme P V. 1967. *Statistical Methods for*

Agriculture Workers, p 381. ICAR Publication, New Delhi.

Ranganna S. 1986. *Handbook of Analysis and Quality Control for Fruit and Vegetable Products*, pp 84–99.Tata Mc Graw Hill Publishing company Ltd, New Delhi.

SAS. 2010. Statistical Analysis Software System, Version 9.3. SAS Institute, Cary, NC, USA.

Sharma J P. 2002. *Crop Production Technology for Cold Arid Region*, pp 220–6. Kalyani Publisher, Ludhiana, New Delhi.

Shyamala B N, Gupta S, Lakshmi J A and Prakash J. 2005. Leafy vegetable extracts-antioxidant activity and effect on storage stability of heated oils. *Innovative Food Science and Emerging Technologies* **6**: 239–45.

Singleton V L and Rossi J A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. *American Journal of Enology Viticulture* **16**: 144–58.

Szeto Y T, Kwok T C and Benzie I F. 2004. Effects of a long term vegetarian diet on biomarkers of antioxidant status and cardiovascular disease risk. *Nutrition* **20:** 863-66.

Tomas-Barberan F A, Ferreres F and Gil M I. 2000. Antioxidant phenolic metabolites from fruit and vegetables and changes during postharvest storage and processing. (*In*) *Bioactive and Natural Products*, Part D, pp 739–95. Rahman A (Ed.). Elsevier Science, Amsterdam, The Netherlands.

Usmani G, Pravin H, Mishra Y and Mandal A K. 2014. Variability, heritability and character association analysis of chemoagronomic traits in *Rauwolfia serpentina*. *Euphytica* **200**: 259–68.

Varalakshmi B and Reddy V V P. 1997. Variability, heritability and correlation studies in vegetable amaranth. *Indian Journal of Horticulture* **54**: 167–70.

Yuan H, Sun Y, Yuan H L and Sun Y W. 2001. Correlation and path and path analysis of some agronomic characters of savoy Chinese cabbage (*Brassica chinensis* var. *rosularis*). *China Vegetables* **5**: 17–8.