Indian Journal of Agricultural Sciences **86** (3): 347–54, March 2016/Article
https://doi.org/10.56093/ijas.v86i3.57013

Antioxidant and phytochemical levels and their interrelation in stem and leaf extract of water spinach (*Ipomea aquatica*)

P K SINGH[1], S K TIWARI[2], N RAI[3], KRISHNA RAI[4] and MAJOR SINGH[5]

Indian Institute of Vegetable Reserch, Jakhini (Sahanshahpur), Varanasi, Uttar Pradesh 221 305

Received: 12 October 2015; Accepted: 6 November 2015

ABSTRACT

An investigation was carried out to evaluate nutritional and anti-oxidant properties of leaf and stem extracts from 10 genotypes of water spinach (*Ipomea aquatica* Forsk). The extracts were analyzed in terms for protein, sugar, chlorophyll, carotenoid, phenol, proline, flavonoids and ascorbic acid contents. The analysis revealed a significant variation in the level of protein (10.4-151.6 mg/g FW), sugar (0.33-2.98 g/100g FW), chlorophyll (2.50-4.98 mg/g FW), phenol (0.75-2.11 mg/g FW), proline (0.14-36.14 µg/g FW), carotenoid (0.85-0.1.59 mg/g FW), flavonoid (0.1-0.431 mg/g FW) and ascorbic acid (0.21-1.03 g/100g FW) in leaf extract and (protein 13.1-144.8 mg/g FW, sugar 0.43-3.55 g/100g FW, chlorophyll 0.32-0.76 mg/g FW, phenol 0.54-4.76 mg/g FW, proline 0.09-29.46 µg/g FW, carotenoid 0.12-0.29 mg/g FW, flavonoid 0.05-0.231 mg/g FW and ascorbic acid 0.28-0.62 g/100g FW) in stem extract of the genotypes. The results indicate that total sugar, proline and protein in both stem and leaf extracts expressed high heritability coupled with high genetic advance indicating that these traits are mainly controlled by additive genes and progeny selection will be rewarding for improvement of these traits. High heritability values in these traits indicate that expression of characters under study is less influenced by environment. Plant breeders on such basis may make the safe selection on the basis of phenotype of the plant by adopting simple selection schemes. For most of the nutritional and anti-nutritional qualities the genotypic correlation coefficient was recorded higher than phenotypic correlation coefficient. The study indicates significantly less influence of environment on the expression of these nutritional and antioxidant traits.

Key words: Antioxidants, Kong Kong, Phytochemicals, Under-utilized vegetable, Water spinach

Water spinach (*Ipomoea aquatic* Forsk) is an underutilized vegetable, which is supposed to be originated in China (Edie and Ho 1969). It is distributed throughout India, Sri Lanka, Tropical Asia, Africa and Australia (Kirtikar and Basu 1952). The plant grows wildly as weed in India and USA (Reed 1977) while in South East Asia like Malaysia, China, Hong Kong, Singapore and Indonesia, the crop is commercially cultivated (Candlish *et al.* 1987). The crop is propagated mostly by fragmentation (Edie and Ho 1969) and vigorous growth of the plants quickly covers expanses of water and multiplies rapidly with pieces of the stems covering a large place in a short time (Heyne 1927).

The young terminal shoots and leaves of water spinach are eaten as green leafy vegetable, in salads (Ismail *et al.* 2004) and also used as fodder (Phimmasan *et al.* 2004). Its leaves are very rich source of proteins (Ngamsaeng *et al.* 2004), carotenes (Chen and Chen 1992), amino acids like aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, leucine, tyrosine, lysine, histidine and arginine (Rao and Vijay 2002), minerals like sodium, potassium, calcium, iron, magnesium and zinc (Due *et al.* 1999), sugars like glucose, fructose, sucrose (Wills *et al.* 1984), fiber, lipids and fats (Imbs and Pham 1995), organic acids like malic acid, citric acid, oxalic acid (Wills *et al.* 1984), vitamins (Due *et al.* 1999), starch (Candlish *et al.* 1987), polyphenols like myricetin, quercetin, luteolin, apigenin, kaempferol (Chu *et al.* 2000, Daniel 1989, Koo and Suhaila 2001), dihydroquercetin glycoside (Prasad *et al.* 2005a) and ash (Ogle *et al.* 2001). In the ancient science of Indian medicine (Ayurveda) and homeopathy, extracts of water spinach leaves are administered orally to alleviate antioxidant related disorders. The plant extract is also use in curing nose bleeding and high blood pressure. Further, leaf extract can be used to reduce blood sugar levels and as antibiotics against *Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis*. The floral buds are used as an anthelminthic.

The present study was undertaken to investigate antioxidants and photosynthetic pigments of water spinach fractions and to evaluate antioxidant activity by using different laboratory techniques. Different fractions of water spinach, namely leaf and stem fractions, were analysed to make systematic comparisons among their antioxidants and photosynthetic pigment contents and to identify the fractions with high antioxidants for further studies. In addition,

[1]Senior Scientist (Vegetable crops) (e mail: pksingh128@gmail.com)

Table 1 List of *Ipomea aquatica* genotypes used in the study

Accession no.	Place of collection	Flower color	Stem color	Leaf color
VRSWS-1	Kushinagar	White with purple tinge	Purplish green green	Green
VRSWS-2	Basti	White with purple tinge	Purplish green	Green
VRSWS-3	Gorakhpur	White with purple tinge	Purplish green	Green
VRSWS-4	Deoria	White with purple tinge	Purplish green	Green
VRSWS-5	Kalimpong	Pure White	Green	Green
VRS WS-6	Varanasi	White with purple tinge	Purplish green	Green
VRWS-7	Lucknow	White with purple tinge	Purplish green	Green
VR WS-8	Hardoi	White with purple tinge	Purplish green	Green
VRWS-9	Farukhabad	White with purple tinge	Purplish green	Green
VRWS-10	Sahjahanpur	White with purple tinge	Purplish green	Green

correlations between total phenol content and antioxidants, assessed by different methods, were also evaluated.

MATERIALS AND METHODS

Ten water spinach genotypes were collected from different sources and were maintained at Indian Institute of Vegetable Research, Seed Production Center, Sargatia, Seorahi, Kushi Nagar, India (Table 1). The experiment was conducted in complete randomized block design with three replications during August to March of 2013-2014. The soil of experimental field was sandy loam, non-saline and neutral (pH 7.2) in reaction. Seeds were sown at row to-row spacing of 3.0 m and plant-to-plant spacing of 1.5 m and all the recommended cultural operations were performed to raise a good crop.

The analysis of biochemical parameters were carried out in the Division of Crop Improvement at Indian Institute of Vegetable Research (IIVR), Varanasi. The leaf and stem samples of 10 genotypes harvested between 60-65 days after sowing were collected and stored at -80°C for further analysis in three replicates.

The chlorophyll and carotenoids were extracted in 80% acetone (Porra *et al.* 1989). The absorption of the extracts at wavelengths of 663 nm, 645 nm for chlorophyll and 480nm, 510nm for carotenoid were measured with a Elico SL-159 UV/VIS spectrophotometer (Perkin elmer, elico Ltd. China). The concentrations of chlorophyll a (Chl-a), chlorophyll b (Chl-b), were then calculated using the equations as follow (Arnon DI 1949).

$$\text{Chl-a} = 12.72A663\text{-}2.59A645$$
$$\text{Chl-b} = 22.9A645\ \text{-}4.67A663$$

The total phenolic contents were determined by the method described by Imeh and Khokhar (2002). Aliquots of the extracts were taken in a 10 ml glass tube and made up to a volume of 1.58 ml with distilled water. Then 0.1 ml folin-ciocalteau reagent and 0.3 ml Na_2CO_3 (20%) were added sequentially in each tube. A blue color developed in each tube due to complex redox reaction of phenols with phosphomolibdic acid in folinciocalteau reagent in alkaline medium, resulting in a blue colored complex, molybdenum blue. The test solutions were warmed for 30 min at 40ºC cooled and absorbance was measured at 650 nm. A standard calibration plot was generated (Fig 1) at 650 nm using known concentrations of catechol. The concentration of phenols in the test samples was calculated from the calibration plot and expressed as mg catechol equivalent of phenol/g of sample.

The ascorbic acid content of samples was estimated by the AOAC method (1990) using the Metrohm 670 titroprocessor (MetrohmHerisau, Switzerland). The fresh samples were homogenized in a blender and 1 g of this sample were mixed with 40mL of buffer (1g/L oxalic acid plus 4g/L anhydrous sodium acetate. This was titrated against a solution containing 295mg/L DPIP (phenolindo-2-6-dichlorophenol) and 100mg/L sodium bicarbonate. The auto-titrator was calibrated using standard L-ascorbic acid (Analar, BDH, UK) and the results were expressed as mg/100g FW.

The aluminum chloride method (Chang *et al.* 2002) was used for the determination of total flavonoid content of the sample extracts. Dry tissue was powdered and extracted with 25 ml of 95% of ethanol. Then 2 ml of extract was mixed with 0.1ml $AlCl_3$ (10%), 0.1ml potassium acetate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance was recorded at 415 nm after 30 minutes of incubation at 37ºC and standard calibration plot was generated at 415 nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent/g of sample.

The estimation of protein was done by Lowry method (Lowry *et al.* 1951), 0.5 g of sample was crushed in 5ml of (0.1M) tris buffer and centrifuged and supernatant was taken and 10% TCA was added and again centrifuged. Finally the pellet was dried and dissolves in NaOH (0.1N) for use in estimation of total protein. A standard calibration plot was generated at 650 nm using known concentrations of BSA (Bovine serum albumin). The concentrations of protein in the test samples were calculated from the calibration plot and expressed as mg protein equivalent/g of sample.

The total sugar was measured by estimation of reducing and non-reducing sugar using the dinitro-salicylic acid (Miller 1972). One gram sample was extracted in 80% ethanol and centrifuged at 10000xg for 20 min and the supernatant was saved. Supernatant was evaporated to remove ethanol and then the volume was made up to 10 ml using distilled water. Further, to 0.5 ml sample, 3.0ml DNS (dinitro-salicylic acid) was added, and after heating for 5

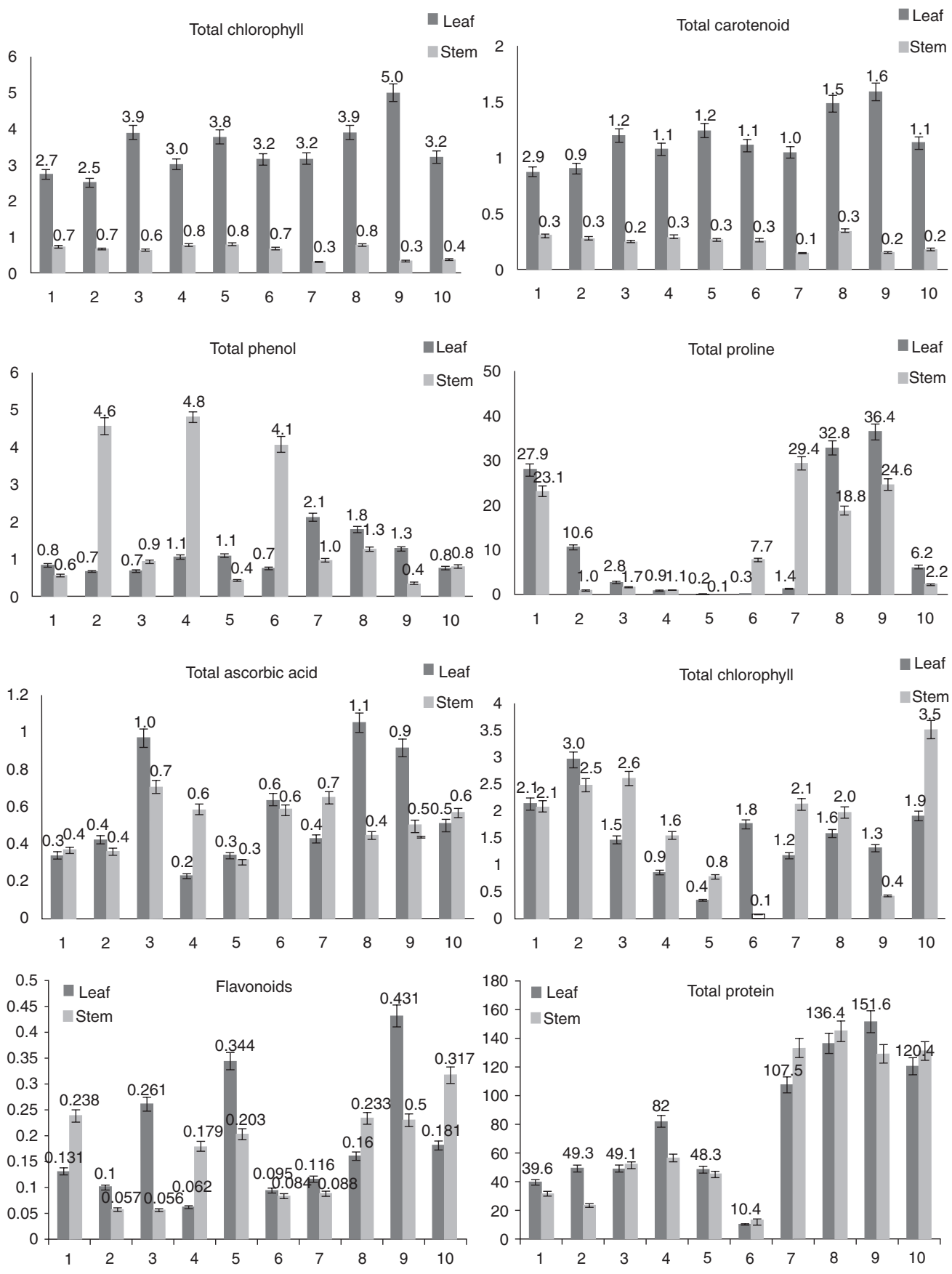

Fig 1 Graphical representation of variation in nutritional component of leaf and stem extract of *Ipomea aquatica* Forsk. Each value represents the mean of three replicates ± standard error were significantly different at the 0.05 level.

min, 1.0 ml Rochelle salt was added, and finally, the absorbance of solution was recorded at 520 nm. In case of non-reducing sugar, the method of Malhotra and Sarkar (1979) was followed, where all the procedure was same as for reducing sugar, except the samples were neutralized with 1ml 1N NaOH. Total sugar was expressed as g/100 g of fresh weight.

Proline was estimated according to Bates *et al.* (1973) and for that A 200 mg fresh leaf sample was homogenized in 5 ml of 3% aqueous sulphosalycylic acid and centrifuged at 22 000 g for 5 min. After that, 2 ml of supernatant and 2 ml of acid ninhydrin was added. Further, 2 ml of glacial acetic acid was added to the mixture and boiled in a water bath at 100ºC for 1 hr. The mixture was further extracted with 4 ml of toluene by mixing the two thoroughly in a test tube with vigorous stirring. Absorbance of chromophore was read at 520 nm against toluene as blank in an Elico SL-159 spectrophotometer (India). L-Proline (Merck) was used for the preparation of the standard curve. The amount of proline in the samples was calculated in µg proline/g fresh weight.

Statistical analyses were conducted using SPSS (Statistical Program for Social Sciences, SPSS Corporation, Chicago, IL) version 12.0 and SPAR (Statistical Package for Agricultural Research, IARI New Delhi) Version 2.0 for Windows. Analysis of variance (ANOVA) in a completely randomized design and Duncan's multiple range test were performed to compare the data. All determinations were done at least in triplicate and all were averaged. The confidence limits used in this study were based on 95% ($P < 0.05$). The correlation between antioxidants and photochemical levels and variability among genotypes was also studied.

RESULTS AND DISCUSSION

Variability

A significant and wide variation was recorded for nutritional and anti-oxidants in leaf and stem extract of 10 genotypes of water spinach (Table 1). The biochemical estimation revealed that the leaf extract of VRS WS-9 contained the highest total chlorophyll (4.98 mg/g) followed by VRS WS-3 and VRS WS-8 (3.89 and 3.88mg/g); the lowest was exhibited by the VRS WS- 2 (2.50 mg/g). Stem extract of VRS WS-8 and VRS WS-4 (0.77 mg/g) showed highest chlorophyll content and lowest was recorded in VRS WS-7 (0.23 mg/g).The amount of Chlorophyll a (2.79mg/g) was about three folds higher than chlorophyll b (0.092 mg/g). Lin (2002) reported that in higher plants, chlorophyll a was the major pigment and chlorophyll b was an accessory pigment, and a/b ratio was generally around 3:1 and that the chlorophyll a: chlorophyll b ratio was 3:2 while Comar (1942) reported that green tissues of higher plants contain 67-78% more chlorophyll and formed much more rapidly compared to chlorophyll b. Leafy vegetables contain several other types of photosynthetic pigments that are grouped in chlorophylls and carotenoids (Kimura and Rodriguez-Amaya 2002). The composition of these pigments produces specific color of the food, which is one of the assessed visual quality attributes.

Total sugar was observed maximum in leaf extracts as compared to stem extract. In leaf extract, VRSWS-2 (2.98 g/100g) and VRS WS-1 (2.11 g/100g) recorded highest total sugar content and VRSWS-5 recorded lowest (0.33 g/100g), whereas in stem extract highest total sugar was recorded in VRSWS-10 (3.55g/100g) and VRSWS-2 (2.51g/100g) and lowest was recorded in VRSWS-6 (0.07g/100g). The reducing sugar showed similar trend in all accessions as compared to total sugar. The reducing sugar was also recorded highest for leaf extract as compared to stem extract. In leaf extract VRSWS- 2 (0.88g/100g) and VRSWS-1(0.71g/100g) and lowest was recorded in VRSWS-5 (0.14g/100g), whereas in stem extract, VRSWS-2 and VRSWS-8 recorded highest reducing sugar content. Polysaccharides serve for the storage of energy (e.g. starch and glycogen), and as structural components (e.g. cellulose in plants and chitin in arthropods) 1-5. The 5-carbon monosaccharide ribose is an important component of coenzymes (e.g. ATP, FAD, and NAD) and the backbone of the genetic molecule known as RNA (Langenhoven *et al.* 1991). Sugar production begins with the initiation photosynthesis, the product of which translocate to developing fruits. Carbohydrates act as the primary source of energy which is converted into glucose to generate energy essential for metabolism in all cells of the body. Though there is no absolute requirement of carbohydrates, they are essential to ensure that energy is available to the body to perform its normal function.

The biochemical estimations revealed the maximum total protein concentration was recorded in the leaf extract of VRSWS-9 (151.6 mg/g edible pod) and stem extract of VRS WS-8 (144.8 mg/g) followed by the leaf extract of VRS WS-8 (136.4 mg/g) and stem extract of VRS WS-7 (133.1 mg/g), it was the lowest in leaf extract and stem extract of VRS WS- 6. The protein content of the leafy vegetables in this study is higher than the protein content of commercial vegetables with the exception of certain legumes, as observed by Langenhoven *et al.* (1991).The ascorbic acid content was also recorded highest in leaf extract as compared to stem extract. In leaf extract, highest content was recorded in VRSWS- 8 (1.03 g/100g) and VRSWS-3 (0.97 g/100g) and lowest was recorded in VRSWS- 4 (0.21 g/100g), whereas in stem extract highest content was recorded in VRSWS- 3 (0.69g/100g) and VRSWS-7 (0.62g/100g) and lowest was recorded in VRSWS-5 (0.28 g/100g).

The distribution of carotenoids in plants appears to be shaped by the changes in their physiological, biochemical and genetic factors (Goldman *et al.* 1999, Grusak *et al.* 1999). Leafy vegetables generally contain high (~10 folds) carotenoids (Tee and Lim 1991b). In present study, carotenoid content ranged between 0.04 mg and 0.231 mg/g. Siong (1995) recorded significant differences in carotenoids content in vegetables. Among the antioxidants, the total carotenoid ranged from 0.045 to 1.59 mg/g. The

Table 2 PCV, GCV and heritability of nutritional component in leaf and stem of *Ipomea aquatica*

Characters		Range	Mean ± S.E	GCV (%)	PCV (%)	Heritability (%)	Genetic advance
Protein (mg/GFW)	Leaf	10.4 - 151.6	79.7 + 0.32	58.19	59.07	99.0	0.94
	Stem	13.6 – 144.8	76.1 + 0.27	67.05	68.48	98.0	0.92
Phenol (mg/GFW)	Leaf	0.67 - 2.11	1.10 + 0.07	96.21	97.05	89.0	0.85
	Stem	0.32 – 4.76	1.88 + 0.08	96.21	97.05	96.0	0.85
Chlorophyll(mg/GFW)	Leaf	1.71 - 2.70	2.12 + 0.08	12.92	13.32	92.0	0.91
	Stem	0.21 – 0.57	0.44 + 0.07	30.26	31.66	99.0	0.91
Carotenoid(mg/GFW)	Leaf	0.85 - 1.59	1.16 + 0.07	18.57	19.87	96.0	0.92
	Stem	0.12 – 0.32	0.25 + 0.06	26.83	27.41	95.0	0.93
Proline (µg/GFW)	Leaf	0.14 - 36.14	11.9 + 0.11	120.2	122.1	99.0	1.06
	Stem	0.09 – 29.46	10.9 + 0.12	106.10	106.40	86.0	1.06
Total sugar (g/100GFW)	Leaf	0.33 – 2.98	1.55 + 0.07	45.12	46.08	95.0	0.89
	Stem	0.07 – 3.55	1.77 + 0.09	60.12	60.36	94.0	0.79
Ascorbic acid(mg/100GFW)	Leaf	0.21 – 1.03	0.59 + 0.07	48.29	50.29	97.0	0.92
	Stem	0.28 – 0.71	0.51 + 0.05	25.99	26.41	99.0	0.92

Table 3 Pair-wise Correlation among nutritional and antioxidants contents in leaves and stem of *Ipomea aquatica* genotypes

	T. chlorophyll	T. carotenoid	Phenol	Flavonoid	Proline	Ascorbic acid	Protein	Reducing sugar	Total sugar
T. chlorophyll	1	0.96	0.381	-0.121	-0.458	-0.468	-0.611	-0.008	-0.191
T. carotenoid	0.96	1	0.406	-0.049	-0.371	-0.541	-0.497	0.206	-0.038
Phenol	0.381	0.406	1	-0.476	-0.456	0.044	-0.518	0.185	-0.133
Flavonoid	-0.121	-0.049	-0.476	1	0.14	-0.309	0.49	-0.062	0.181
Proline	0.458	-0.371	-0.456	0.14	1	0.03	0.51	0.212	-0.181
Ascorbic acid	-0.468	-0.541	0.044	-0.309	0.03	1	0.253	-0.188	0.169
Protein	-0.611	-0.497	-0.518	0.49	0.51	0.253	1	-0.008	0.257
Reducing sugar	-0.008	0.206	0.185	0.062	0.212	-0.188	-0.008	1	0.408
Total sugar	-0.191	0.038	-0.133	0.181	-0.181	0.169	0.257	0.408	1

highest carotenoid content was recorded in the leaf extract of VRSWS- 9 (1.59mg/g) followed by VRSWS- 8 (1.44 mg/g), in contrast, VRSWS-1 showed the lowest (0.44mg/g) carotenoid content. Among the stem extract, a wide variation was recorded for carotenoid content, it was recorded the highest (0.32 mg/g) in VRSWS- 8, and the lowest (0.12 mg/g) in VRSWS- 7 (Table 4).

Phenols are produced in response to certain pathogen and are considered essential for the growth and reproduction of plants. The highest amount of phenol was observed in leaf extract of VRS WS-7 (2.11 mg catechol/g), whereas minimum was recorded in VRSWS-2 about 0.67 mg catechol/g). In stem extract, highest phenol was recorded in VRSWS-4 (4.76 mg catechol/g) and minimum was recorded in VRSWS-9 (0.32 mg catechol/g). The level of phenolic compounds is generally influenced by genotype, agronomic practices, and maturity at harvest, post-harvest storage and climatic conditions (Dixon and Paiva 1995, Ninfali and Bacchiocca 2003, Hakkinen and Torronen 2000).

Proline is a compatible solute which accumulates in many plant species under abiotic and biotic stress conditions such as moisture-deficit, salinity, high temperatures and light intensity (Delauney and Verna 1993, Hare *et al.* 1999). However, it is best recognized as stress marker and also protects folded protein structures against denaturation, stabilises cell membranes by interacting with phospholipids, acts as a hydroxyl radical scavenger. Great variation in proline content was observed in both leaf and stem extract of all 10 accessions which ranged between 0.09-36.14 µg. Proline was maximum in VRSWS-9 (36.14µg/g) of leaf extracts and lowest in VRS WS-5 (0.14µg/g) whereas in stem extract highest was recorded in VRS WS-7 (29.46µg/g) and lowest was recorded in VRS WS-5 (0.09µg/g).

Polyphenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity.The antioxidants have different functional properties, such as reactive oxygen species scavenging, eg. quercetin and catechin, inhibition of the generation of free radicals and chain-breaking activity, e.g. p-coumaric acids (Mansour 2000). These compounds are normally phenolic compounds, which are effective proton donors, and include tocopherols, flavonoids, and other organic acids. The highest flavonoid was observed in VRSWS-9 (0.431 mg/g) and lowest recorded in VRSWS-2 (0.1 mg/g), whereas in stem extract maximum was observed in VRSWS-10 (0.317 mg/g) and minimum was recorded in VRS WS-3 (0.056 mg/g). In our findings, flavonoids content was observed less in stem extracts than leaves extracts, this could possibly be due to the synthesis of these flavonoids in leaves and their slow translocation thorugh stems.

Table 4 Level of different nutritional/anti-nutritional factors among genotypes of *Ipomea aquatica*

Genotype	PR	PH	CH A	CH B	CA	RS	TS	PL	AsA	FLA
VRSWS-1 L	39.6±0.2	0.83±0.05	1.82±0.08	0.91±0.30	0.85±0.05	0.71±0.23	2.11±0.09	27.8±0.03	0.34±0.02	0.13±0.02
VRSWS-1 S	231.7±0.22	0.54±0.05	0.50±0.08	0.21±0.22	0.28±0.22	0.68±0.08	2.04±0.09	23.0±0.05	0.34±0.05	0.23±0.05
VRSWS-2 L	49.3±0.30	0.67±0.04	1.71±0.10	0.79±0.22	0.88±0.03	0.88±0.17	2.98±0.06	10.5±0.04	0.41±0.06	0.11±0.05
VRSWS-2 S	23.4±0.22	4.51±0.04	0.46±0.10	0.19±0.10	0.28±0.03	0.82±0.08	2.51±0.06	0.92±0.05	0.34±0.05	0.57±0.03
VRSWS-3 L	49.1±0.15	0.99±0.02	2.18±0.47	1.70±0.47	1.18±0.08	0.46±0.04	1.46±0.05	2.76±0.03	0.97±0.03	0.26±0.02
VRSWS-3 S	51.6±0.04	0.91±0.02	0.44±0.47	0.18±0.47	0.22±0.10	0.42±0.08	2.51±0.05	1.66±0.05	0.69±0.05	0.05±0.05
VRSWS-4	82.1±0.21	1.06±0.01	1.98±0.09	1.02±0.09	1.05±0.03	0.28±0.05	0.83±0.04	0.87±0.03	0.21±0.04	0.06±0.03
LVRSWS-4 S	56.6±0.21	4.76±0.04	0.55±0.04	0.21±0.15	0.29±0.09	0.26±0.05	1.52±0.05	1.06±0.05	0.55±0.05	0.17±0.05
VRSWS-5 L	48.3±0.25	1.07±0.05	2.21±0.37	1.54±0.05	1.22±0.04	0.14±0.07	0.33±0.03	0.14±0.01	0.32±0.05	0.34±0.05
VRSWS-5 S	45.1±0.21	0.41±0.05	0.57±0.05	0.21±0.05	0.27±0.05	0.13±0.07	0.79±0.05	0.09±0.05	0.28±0.05	0.20±0.03
VRSWS-6 L	10.1±0.19	0.75±0.09	2.01±0.34	1.14±0.09	1.09±0.08	0.55±0.03	1.76±0.01	0.27±0.08	0..62±0.01	0.09±0.02
VRSWS-6 S	13.6±0.21	4.03±0.09	0.48±0.09	0.18±0.09	0.23±0.09	0.54±0.03	0.07±0.05	7.73±0.05	0.55±0.05	0.08±0.05
VRSWS-7 L	107.1±0.21	2.11±0.07	2.01±0.43	1.15±0.43	1.03±0.09	0.44±0.09	1.15±0.08	1.38±0.01	0.42±0.03	0.11±0.01
VRSWS-7 S	133.1±0.21	0.94±0.07	0.21±0.07	0.08±0.07	0.12±0.05	0.43±0.09	2.01±0.05	29.4±0.05	0.62±0.05	0.08±0.05
VRSWS-8 L	136.4±0.18	1.79±0.05	2.36±0.32	1.52±0.07	1.46±0.04	0.59±0.09	1.57±0.07	32.7±0.03	1.03±0.07	0.16±0.03
VRSWS-8 S	144.8±0.21	1.25±0.07	0.54±0.21	0.22±0.21	0.32±0.05	0.71±0.04	1.99±0.05	18.9±0.05	0.41±0.05	0.23±0.05
VRSWS-9 L	151.6±0.21	1.27±0.07	2.70±0.21	2.28±0.18	1.59±0.21	0.42±0.05	1.30±0.05	36.1±0.05	0.90±0.05	0.43±0.02
VRSWS-9 S	129.1±0.21	0.32±0.07	0.23±0.18	0.09±0.19	0.13±0.19	0.41±0.21	0.43±0.09	24.6±0.05	0.48±0.05	0.23±0.05
VRSWS-10 L	120.4±0.21	0.76±0.07	2.02±0.21	1.18±0.04	1.13±0.04	0.57±0.04	1.89±0.05	6.2±0.05	0.48±0.05	0.18±0.03
VRSWS-10 S	131.1±0.21	0.78±0.07	0.25±0.19	0.10±0.04	0.15±0.05	0.55±0.05	3.55±0.09	2.1±0.05	0.55±0.05	0.31±0.02

PR: Protein, PH: phenol, CH A: chlorophyll A, CH B: chlorophyll B, CA: carotenoid, RS: reducing sugar, TS: total sugar, PL: proline, FLA: flavonoid, AsA: ascorbic acid

Heritability and genetic advance

In variability studies, genetic advance is useful indicator of the progress that can be expected as a result of performing selection on the relevant population. Heritability coupled with genetic advance would give a more reliable index of selection value and effectiveness of selection depends not only on heritability but also on genetic advance (Johnson *et al.* 1955). Data presented in Table 1 showed that the characters namely total sugar, proline and protein in both stem and leaf extracts expressed high heritability coupled with high genetic advance indicating that these traits are mainly controlled by additive genes and progeny selection will be rewarding for improvement of these traits. However, moderate heritability coupled with low genetic advance was observed for chlorophyll, carotenoid and ascorbic acid in both stem and leaf extracts (Table 2) indicating that the character is controlled by non-additive genes (dominance and epistasis). High heritability values indicate that expression of characters under study is less influenced by environment. Plant breeders on such basis may make the safe selection on the basis of phenotype of the plant by adopting simple selection schemes. Therefore, judicious application of pure line selection may be effective for improving the characters with moderate or high heritability and with low genetic advance. On the other hand, difference in GCV and PCV was observed for the characters like proline, ascorbic acid and protein indicating significant influence of environment over these characters (Table 2). High GCV was observed for character like proline indicating the scope of selection to improve the genotypes.

Stem correlation

Phenotypic and genotypic correlations of eight biochemical traits were analyzed to decipher their inter-relationships and identification of trait for selection program (Johnson *et al.* 1955). Chlorophyll a showed the maximum positive and significant association with carotenoid both genotypic and phenotypic levels (Table 3). Proline showed a positive and significant association with reducing sugar both at phenotypic and genotypic levels. Phenol showed a positive and significant correlation with chlorophyll and reducing sugar. Total chlorophyll showed positive and significant correlations with phenol. Reducing sugar also showed positive association with carotenoid, phenol, flavonoid and proline. Total sugar also showed positive correlation with carotenoid, ascorbic acid, and protein. Similar results have also been reported in the case of cowpea (Saha *et al.* 2009).

Leaves correlation

The correlation values for leaves and antioxidants are presented in Table 3. Reducing sugar, total sugar and phenol showed significant positive correlation with leaves. On the other hand, proline and non-reducing sugar exhibited a significant negative correlation with number of pods per plant (Table 3). Protein showed positive correlations with flavonoid, proline and ascorbic acid. Phenol was also in positive association with chlorophyll, carotenoid, ascorbic acid and reducing sugar. Ascorbic acid showed positive association with phenol, proline, protein and total sugar. This indicates that leaves play an important role on both antioxidant and phytochemicals in

Ipomea aquatica. These findings clearly indicate that both antioxidant and phytochemicals factors of *Ipomea aquatica* are significantly affected by leaves as compared to stems.

REFERENCES

AOAC. 1995. *Official Methods of Analysis,* 16th Ed. Association of Official Analytical Chemists, Washington DC.

Arnon D I. 1949. Copper enzymes in isolated chloroplasts: Polyphenol oxidase in *Beta vulgaris*. *Plant Physiology* **24**: 1–15.

Bates L S, Waldren R P and Teare I D. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil* **39**: 205–7.

Candlish I K, Gourley L and Lee H P. 1987. Dietary fiber and starch contents of some Southeast Asian vegetables. *Journal of Agriculture and Food Chemistry* **35**: 319–21.

Chang C, Yang M, Wen H and Chern J. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analaysis* **10**: 178–82.

Chen B H and Chen Y Y. 1992. Determination of carotenoids and chlorophylls in water convolvulus (*Ipomoea aquatica*) by liquid chromatography. *Food Chemistry* **45**: 129–34.

Chu Y H, Chang C R and Hsu H F. 2000. Flavonoid content of several vegetables and their antioxidant activity. *Journal of the Science of Food and Agriculture* **80**: 561–6.

Comar C L. 1942. Analysis of plant extracts for chlorophylls a and b using a commercial spectrophotometer. *Industrial and Engineering Chemistry Analytical Ed* **14**: 877–9.

Daniel M. 1989. Polyphenols of some Indian vegetables. *Current Science* **58**: 1 332–3.

Delauney A J and Verma D P S. 1993. Proline biosynthesis and osmoregulation in plants. *Plant Journal* **4**: 215–23.

Dixon R A and Paiva N L. 1995. Stress-induced phenylpropanoid metabolism. *Plant Cell* **7**: 1 085–97.

Due B M, Humphries D, Mai L T B, Dao A H, Co T M, Nga H H and Kim P T. 1999. Iron and vitamin C content of commonly consumed foods in Vietnam. *Asia-Pacific Journal of Clinical Nutrition* **8**: 36–8.

Edie E E and Ho B W C. 1969. *Ipomoea aquatica* as a vegetable crop in Hong Kong. *Economic Botany* **23**:32–6.

Goldman I L, Kader A A and Heintz C H. 1999. Influence of production handling and storage on phytonutrient content of foods. *Nutrition Reviews* **57(9)**: S46–S52.

Grusak M A, Della Penna D and Welch R M. 1999. Physiological process affecting the content and distribution of phytonutrients in plants. *Nutrition Reviews* **57(9)**: S27–S33.

Hakkinen S and Torronen R. 2000. Content of ûavonols and selected phenolic acids in strawberries vaccinium species: Inûuence of cultivar, cultivation site and technique. *Food Reviews International* **33**: 517–24.

Hare P D, Cress W A and Staden V J. 1999. Proline synthesis and degradation: a model system for elucidating stress-related signal transduction. *Journal of Experimental Botany* **50**: 413–34.

Heyne K. 1927. *De Nuttige Planten van NederlandischIndië*, Second Edition. Department van Landbouw, Nijverheid and Handel, Buitenzorg.

Imbs A B and Pham L Q. 1995. Lipid composition often edible seed species from North Vietnam. *Journal of American Oil Chemist Society* **72**: 957–61.

Imeh U and Khokhar S. 2002. Distribution of conjugated and free phenols in fruits: antioxidant activity and cultivar variations. *Journal of Agricultural Food Chemistry* **50**: 6 301–6.

Ismail A, Marjan Z M and Foong C W. 2004. Total antioxidant activity and phenolic contents in selected vegetables. *Food Chemistry* **87**: 581–6.

Johnson H W, Robinson H F and Comstock R E. 1955. Genotypic and phenotypic correlation in soybean and their implication in selection. *Agronomy Journal* **47**: 477–80.

Kimura M and Rodriguez-Amaya DB. 2002. A scheme for obtaining standards and HPLC quantification of leafy vegetable carotenoid. *Food Chemistry* **78**: 389–98.

Koo HM and Suhaila M. 2001. Flavonoid (Myricetin, quercetin, kaempferol, luteolin and apegenin) content of edible tropical plants. *Journal of Agriculture and Food Chemistry* **49**: 3 106–12.

Kirtikar K R and Basu B D. 1995. *Indian Medicinal Plants*, Vol I, 3rd Edn. Sri Satguru Publications, New Delhi.

Langenhoven M L, Kruger M, Gouws E and Faber M. 1991b. *MRC Food Composition Tables, 1991*, 3rd edn,p 245. Medical Research Council, Parow.

Lin W X, Liang Y Y, Chen F Y and Guo Y C. 2002. Effects of enhanced UV-B radiation stress on kinetics of chlorophyll fluorescence in rice. *Ecology and Agriculture* **10(1)**: 8–12.

Lowry O H, Rosebrough N J, Farr A L, Randall R J. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193 (1)**: 265–75.

Malhotra S S and Sarkar S K. 1979. Effects of sulphur dioxide on sugar and free amino acid content of pine seedlings. *Physiologia Plantarum* **47**: 223–8.

Mansour M M F. 2000. Nitrogen containing compounds and adaptation of plants to salinity stress. *Biologia Plantarum* **43**: 491–500.

Miller GL. 1972. Use of DNS reagent for determination of reducing sugar. *Analytical Chemistry* **31**: 1 012–3.

Ngamsaeng A, Thy S and Preston T R. 2004. Duckweed (*Lemna minor*) and water spinach (*Ipomoea aquatica*) as protein supplements for ducks fed broken rice as the basal diet. *Livestock Research for Rural Development* **16**: 18–24.

Ninfali P and Bacchiocca M. 2003. Polyphenols and antioxidant capacity of vegetables under fresh and frozen conditions. *Journal of Agriculture and Food Chemistry* **51**: 2 222–6.

Ogle B M, Ha-Thi A D, Mulokozi G and Hambraeus L. 2001. Micronutrient composition and nutritional importance of gathered vegetables in Vietnam. *International Journal of Food Science and Nutrition* **52**: 485–99.

Phimmasan H, Kongvongxay S, Chhayty P and Preston T R. 2004. Water spinach (*Ipomoea aquatica*) and Stylo 184 (Stylosanthesguianesis CIAT 184) as basal diets for growing rabbits. *Livestock Research for Rural Development* **16**: 46–59.

Porra R J, Klein O and Wright P E. 1983. The proof by 13C-NMR spectroscopy of the predominance of the C5 pathway over the Shemin pathway in chlorophyll biosynthesis in higher plants and of the formation of the methyl ester group of chlorophyll from glycine. *European Journal of Biochemistry* **130**: 509–16.

Prasad N K, Divakar S, Shivamurthy G R and Aradhya S M. 2005a. Isolation of a free radical scavenging antioxidant from water spinach (*Ipomoea aquatic* Forsk.). *Journal of the Science of Food and Agriculture* **85**: 1 461–8.

Rao T V R K and Vijay T. 2002. Iron, calcium, carotene, ascorbic acid and oxalic acid contents of some less common leafy vegetables consumed by the tribals of Purnia district of Bihar. *Journal of Food Science and Technology* **39**: 560–2.

Reed C F. 1977. Economically important foreign weeds: Potential

problems in the United States. Agriculture Hand book, USDA, Washington.

Saha S, Singh G, Mahajan V and Gupta H S. 2009. Variability of nutritional and cooking quality in bean (*Phaseolus vulgaris* L.) as a function of genotype. *Plant Foods and Human Nutrition* **64**: 174–80.

Siong T E, Goh A H and Khor S C. 1995. Carotenoid composition and content of legumes, tubers and starchy roots by HPLC. *Malaysian Journal of Nutrition* **1**: 63–74.

Wills R B H, Wong A W K, Scriven F M and Greenfield H. 1984. Nutrient composition of Chinese vegetables. *Journal of Agricultural and Food Chemistry* **32**: 413–6.