21. Belotserkovsky, O. M., Numerical modeling in continuum mechanics. *Phisico-matematicheskaya literatura*, 1994, p. 443.
22. Bear, J., *Dynamics of Fluids in Porous Media*, Elsevier, New York, 1972, p. 712.
23. Gosmen, A. D., Pan, V. M., Ranchel, A. K., Spalding, D. B. and Wolshtein, M., *Numerical Methods for the Viscous Liquid Flow Study*, Nedra, 1972, p. 324.
24. Saad, Y., Iterative methods for sparse linear systems. SIAM, 2003, p. 528.
25. Saad, Y., *Iterative Methods for Sparse Linear Systems*, Society for Industrial and Applied Mathematics, 2nd edn, 2003, p. 547.
26. http://www.imperial.ac.uk/engineering/departments/earth-science/research/research-groups/perm/research/pore-scale-modelling/micro-ct-images-and-networks/
27. Zhang, D., Zhang, R., Chen, S. and Soll, W. E., Pore scale study of flow in porous media: scale dependency, REV, statistical REV. *Geophys. Res. Lett.*, 2000, **27**, 1195–1198.
28. Al-Raoush, R. and Papadopoulos, A., Representative elementary volume analysis of porous media using X-ray computed tomography. *Powder Technol.*, 2010, **200**, 69–77.

ACKNOWLEDGEMENT. This work was funded by grant (RSCF) 15-11-10015.

Received 26 June 2015; revised accepted 16 December 2015

doi: 10.18520/cs/v110/i11/2142-2148

Variations in soil alter availability of carlinoside: an anti-hepatitic compound from *Cajanus cajan* (Linn.) leaves

Subhasish Das[1], Sandip Mukherjee[2], Rakesh Kundu[2], Pradip Bhattacharyya[3], Buddhadeb Duary[4] and Satya Sundar Bhattacharya[1,*]

[1]Department of Environmental Science, Tezpur University, Tezpur 784 028, India
[2]Department of Zoology, Visva Bharati, Santiniketan 731 235, India
[3]Indian Statistical Institute, North East Centre, Tezpur 784 028, India
[4]Palli Siksha Bhavana, Visva Bharati, Sriniketan 731 236, India

Agro-climatic factors largely regulate expression of phenolic compounds in plants. Carlinoside, a flavone glycoside, is known to eliminate bilirubin accumulation in rat liver. We cultivated *Cajanus cajan* uniformly in two different soil types – alluvial (AL) and laterite (LA). The climatic attributes and soil physico-chemical properties of the two localities were significantly different from each other. Carlinoside, phenol and flavonoid concentrations were higher in plants grown in AL than in LA soils. We recorded higher UGT1A1 expression in liver hepatoma cell line HepG2 and rats treated with plant extracts from AL compared to LA.

Keywords: *Cajanus cajan*, carlinoside, plant phenollics, soil and climate.

*For correspondence. (e-mail: satya72@tezu.ernet.in)

CAJANUS CAJAN L. (Leguminosae) is an important legume crop extensively grown in India[1]. The crop contributes significantly to the total pulse production of the country. However, apart from its high nutritive value, *C. cajan* leaf extract significantly heals alcohol-induced liver dysfunction in rats[2]. The leaf extract contains a flavone glycoside, carlinoside that can convert insoluble bilirubin to soluble fram[3]. The major problem in jaundice is the accumulation of free insoluble bilirubin, which is potentially toxic and may even cause death of the patient. In general, bilirubin–UGT enzyme (UGT1A1) converts insoluble bilirubin to a soluble innocuous form, which is eliminated through urine and faeces. Suppression of UGT1A1 enzyme by certain pathogens leads to the condition of jaundice, and no drug is available that can restore UGT1A1 activity[2,3].

The phenolic or polyphenol compounds account for approximately 40% of organic carbon circulating in the biosphere. Plants need these compounds for growth, reproduction, pigmentation and disease resistance. Several thousand phenols and polyphenols have been identified having a broad range of monomeric, dimeric and polymeric structures, amongst which 8150 or more are flavonoids and found in the epidermis of leaves and fruit skin of various plants[4].

Climatic factors like solar radiation, temperature and humidity influence the concentration and quality of secondary metabolites in plants. Nutrient balance and solubility in soil is thought to play a major role in the production of secondary compounds in plants[5]. Various workers have documented considerable influence of environmental factors on the expression and concentration of bioactive compounds in plants[6]. Since plants cannot avoid environmental influence, nor can they migrate to regions with favourable habitat, they evolve highly complex mechanisms of adaptation. It has been demonstrated that expression of secondary metabolites is often altered due to these adaptive mechanisms[7].

Carlinoside from the leaf extract of *C. cajan* is highly bioavailable and therefore has a potential in the treatment of jaundice[3]. However, expression and yield of carlinoside are expected to vary in different areas depending on specific agro-climatic conditions. This is the basic reason for non-uniform success of herbal medicine prepared from plants grown at different localities under varied environmental conditions. Since carlinoside concentration in the leaf is related to the site of growth of *C. cajan*, we conducted a study on the expression of total phenol, total flavonoids and carlinoside in *C. cajan* leaves collected

from two diverse soil groups from two distinct agroclimatic regions of India.

The experiment was conducted at two sites: site 1 – Birbhum district, West Bengal, with lateritic soil (LA; Typic Haplustal) and site 2 – Sonitpur district, Assam, with alluvial soil (AL; Typic Endoaquepts).

LA occurs in the red laterite belt of the Gangetic plains in West Bengal (23°91′N and 87°53′E). The region receives an annual rainfall of 1196.1–1572.9 mm. The summers are marked by high (35–42°C) temperatures and the winters by low (10–12°C) temperatures.

AL soils are in the north bank plain zone of Assam (26°14′N and 92°50′E). This region experiences a subtropical climate and heavy rainfall (1355–2348 mm). The average temperature in the summer ranges between 18°C and 36°C, and in the winter between 7°C and 22°C. The dominant soil type is the new alluvium with a sandy loam texture.

C. cajan variety UPAS 120 was grown uniformly during monsoon of 2012–2014 according to the recommended package of practice for eastern India. In both the agroclimatic regions, treated seeds were sown during the last week of June for both seasons and the crop was mostly grown rainfed. However, light irrigation was applied once between flowering and pod filling stage. We uniformly adopted minimal plant nutrition schedules in both the agroclimatic regions by applying organic manure @ 5 tonne ha^{-1}. Weed management and other plant protection measures were undertaken on a need-based manner.

We collected leaf samples on 45th, 90th and 135th days after sowing (DAS) of the crop. Soil samples were also collected while collecting the plant materials. Plant (leaf) and soil (subsurface soil) samples were randomly collected from both the sites. The collected leaf and soil samples were air-dried, powdered and sieved (mesh = 0.5 and 2 mm) prior to analysis.

Physico-chemical and microbial analyses of soil samples were done following the authentic protocols[8]. We analysed DTPA extractable Fe, Cu, Mn, Zn, Ni and Cd by following the method of Lindsay and Norvell[9].

Climate data were acquired from the weather stations of the respective locations. The principal parameters selected for this study are: temperature (T), precipitation (P), relative humidity (H) and insolation (I). All the values were averaged monthly and the mean values were used for statistical analysis.

For preparation of the extract, 5 g air-dried leaves and 5 g powdered seeds were pulverized separately in 20 ml of chilled methanol using mortar and pestle. The resultant suspension was kept standing for 6 h in sealed plastic containers in a cool, dark place and then filtered through 0.22 µm membrane filters (RanDisk™, Rankem, India) for further biochemical analysis.

Leaf samples collected 90 DAS were utilized for enumerating the total phenol and total flavonoid contents. Total phenolic content was determined spectrophotometrically by applying Folin Ciocalteu reagent assay at 760 nm and expressed as gallic acid equivalent (GAE) kg^{-1} leaf (ref. 10). Standard $AlCl_3$ method was used to measure the total flavonoid content in the leaf samples. Colorimetric determination was made at 415 nm and finally expressed as mg quercetin equivalent (QE) kg^{-1} leaf (ref. 11).

We focussed on growth stage-wise variation of carlinoside content in *C. cajan* leaves grown in the two soil types. Leaves collected at 45, 90 and 135 DAS from AL or LA soil were separately subjected to alcohol extraction. Finally, carlinoside was separated and quantified using high performance liquid chromatography (HPLC; Waters Corp, USA), fitted with Symmetry™ C_{18} column utilizing 55 : 45 (methanol : water) mobile phase at 254 nm[3]. The carlinoside concentration in seeds was determined by running the methanolic seed extracts under similar chromatographic conditions. The alcohol was then removed from extracted solutions of leaf by rotary evaporator, and dried leaf extract with enriched carlinoside from each group was weighed and dissolved in 10% DMSO.

Adult male albino rats of the Sprague-Dawley strain weighing 180–220 g were maintained under standard laboratory conditions (2–3 animals/cage) with food and water made available *ad libitum*, under 12 h light/dark cycle at 25–28°C. Animals were acclimatized about one week prior to use. For analysis effect of alcohol on ethanol-induced liver damage, five rats per group were fed with ethanol at 3.7 g/kg body wt for 20 days. From the 20th day for the next 5 days the ethanol-treated rats were administered through oral gavage, carlinoside-enriched leaf extract from plants grown in different regions (carlinoside) at 6 mg/kg body wt, twice a day. On termination of the experiments, blood samples from rats were collected for estimation of serum bilirubin and liver function marker enzymes, GPT and GOT. Liver was perfused and tissue was processed for immunoblot analysis using anti-UGT1A1 and α-tubulin antibodies. The animal model study was performed in accordance with the guidelines prescribed by the Animal Ethics Committee at Visva-Bharati University, Santiniketan, India.

The human hepatoma HepG2 cell line was a kind gift from National Centre for Cell Science, Pune, India. HepG2 cells were cultured in MEM (minimum essential medium) containing penicillin (100 U/ml) and streptomycin (100 mg/ml) in a humidified 95% O_2/5% CO_2 atmosphere at 37°C. For treatments, 10 mg/ml of the carlinoside-enriched fractions from each group was added to HepG2 cells (1×10^6/well) for incubation. In the controls, the respective amounts of DMSO were added in the media.

On termination of incubation at 24 h, 70 µg protein from control or treated HepG2 cell and rat hepatocyte lysates were resolved on 10% SDS-PAGE and transferred to PVDF membranes (Millipore, MA, USA) with the help of semi-dry trans blot apparatus (Bio-Rad Trans-Blot[1] SD Cell, USA) using transfer buffer (25 mM *Tris*, 193 mM

glycine, 20% methanol, pH 8.5). The membranes were first incubated with primary antibody for UGT1A1 (goat polyclonal, 1 : 1000) followed by corresponding ALP-conjugated secondary antibody at 1 : 1000 dilution using SNAP i.d. (Millipore, MA). The protein bands were detected using 5-bromro 4-chloro 3-indolyl phosphate/ nitroblue tetrazolium (BCIP/NBT).

Total RNA was extracted from different incubations using TRI-reagent, according to the manufacturer's instructions. RT-PCR was performed with First strand cDNA synthesis kit, (Fermentas Life Sciences, Revert Aid^{TM}, MD, USA). Then 2 µl of cDNA was subjected to RT-PCR along with the following primer sets and conditions; Primers – human UGT1A1 forward: 5′-AACAAGGAGCTCATGGCCTCC-3′; reverse: 5′-GTTCGCAAGATTCGATGGTCG-3′; *gapdh* forward: 5′-GCCATCAACGACCCCTTC-3′; reverse: 5′-AGCCCCAGCCTTCTCCA-3′) and the cycling conditions were 1 min at 95°C followed by 45 sec at 55°C for annealing and finally 45 sec at 72°C for extension for 30 cycles. The *gapdh* was simultaneously amplified in separate reactions.

The statistical analysis was done with the help of SPSS (v.20 for Windows). ANOVA was performed to find out the significance of the experiments. The climatic data for the two soil regions were analysed through PCA using factor extraction (eigen value > 1). Regression analysis was also carried out to find the probable relation between the studied compounds with their environment, irrespective of the geographic locations.

Figure 1 *a* presents total phenol and flavonoid content recorded in *C. cajan* leaves. Table 1 represents the soil and climatic attributes recorded during the growth period of the crop. Interestingly, both groups of compounds significantly differed in concentration ($P = 0.000$) with respect to their growing locations. The carlinoside concentration in leaves was found to be significantly more in the AL-grown ($P < 0.05$) than the LA-grown plants (Figure 1 *b*). We also enumerated the carlinoside concentration in the edible part (seeds) of this crop and observed that the leaves significantly ($P < 0.05$) outnumber the seeds with regard to carlinoside content (Figure 1 *c*). Figure 2 shows the typical HPLC chromatograms illustrating carlinoside peaks. We collected the leaves uniformly during the same growth stage of the crop from the two localities grown using the same management practices. The two agroclimates significantly differ with respect to precipitation and air temperature (precipitation: $P = 0.03$; temperature: $P = 0.01$). Low precipitation with high solar insolation resulted in moisture stress in the Birbhum soil. This may influence the biosynthesis of phenolics in general and carlinoside, in particular, in *C. cajan*. Our results are in good agreement with previous findings[12–14].

LA soils exhibited significantly higher acidity (pH = 5.17) compared to AL soils (pH = 6.07). AL exhibited higher conductivity compared to LA. Water holding capacity (WHC), total organic carbon (TOC), total nitrogen (TKN), available N (av N) and available P (av P) were significantly higher in AL compared to LA. High TOC content indicates high soil C storage in AL. Therefore, this soil recorded lower acidity and higher nutrient content compared to LA. Significantly higher share of organic matter in the form of microbial biomass C (MBC) probably led to enhanced WHC of the Sonitpur AL soil. High MBC resulted in significantly higher rate of CO_2 respiration from AL compared to LA. This may be due to high abundance of organic matter in AL. Furthermore, significantly higher Ca, Mg and Mn and lower Fe levels

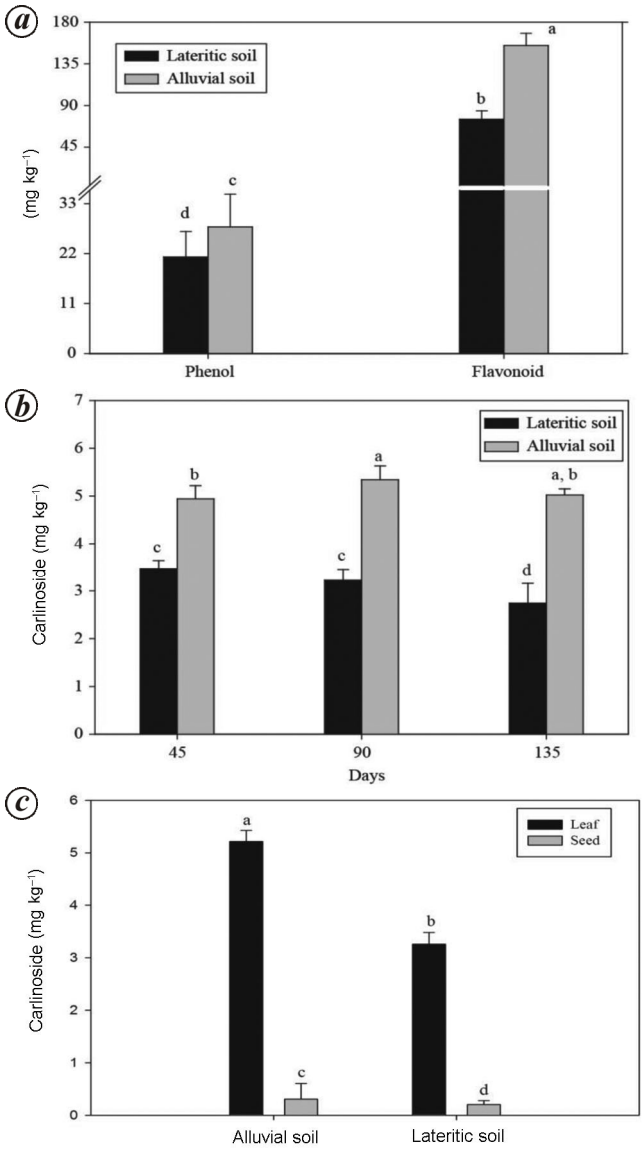

Figure 1. ***a***, Concentrations of total phenol and total flavonoids in *Cajanus cajan*. Error bars represent standard deviation. ***b***, Temporal variation in carlinoside content from leaf extracts of *C. cajan* grown in alluvial and lateritic soil zones. ***c***, Observed carlinoside concentration in leaves and seeds of *C. cajan* from alluvial and lateritic soil zones. In (***a***–***c***) arithmetic means with the same letter are not significantly different from each other at 5% probability level.

Table 1. Soil and climatic variables recorded in the selected sites during the period of *Cajanus cajan* cultivation

Environmental parameters	Birbhum	Sonitpur	*P*-value
Temperature (°C)	29.97 ± 3.05	25.72 ± 3.15	0.010
Insolation (h day^{-1})	5.49 ± 1.81	5.07 ± 0.58	0.872
Precipitation (mm)	38.94 ± 25.71	193.00 ± 215.38	0.033
Humidity (%)	81.58 ± 11.05	86.56 ± 19.02	0.134
pH	5.17 ± 1.06	6.03 ± 1.21	0.000
Electrical conductivity (μS cm^{-1})	62.23 ± 6.11	81.33 ± 2.52	0.000
Cation exchange capacity (meq 100 g^{-1})	52.49 ± 0.85	70.60 ± 0.40	0.000
Bulk density (g cm^3)	0.72 ± 0.08	0.77 ± 0.04	0.000
Water holding capacity (%)	52.49 ± 0.85	70.60 ± 0.40	0.000
Total Kjeldahl N (%)	1.73 ± 0.29	2.29 ± 1.3	0.000
Total organic C (%)	2.07 ± 0.10	4.16 ± 0.44	0.000
Microbial biomass C (μg C g^{-1})	5.34 ± 0.89	12.26 ± 1.1	0.000
Soil respiration (μg CO_2 g^{-1} h^{-1})	4.58 ± 0.26	9.17 ± 0.93	0.000
Av. N (mg kg^{-1})	111.94 ± 11.20	168.00 ± 11.20	0.000
Av. P (mg kg^{-1})	70.70 ± 5.61	84.81 ± 18.88	0.004
Av. K (mg kg^{-1})	347.60 ± 4.11	108.80 ± 3.80	0.000
Av. S (mg kg^{-1})	5.00 ± 0.12	2.18 ± 0.01	0.000
DTPA extractable Ca (mg kg^{-1})	244.97 ± 1.12	323.87 ± 41.46	0.000
DTPA extractable Mg (mg kg^{-1})	32.46 ± 3.01	45.37 ± 0.12	0.000
DTPA extractable Mn (mg kg^{-1})	3.86 ± 0.93	22.28 ± 0.14	0.000
DTPA extractable Fe (mg kg^{-1})	79.25 ± 0.64	57.41 ± 6.07	0.000
DTPA extractable Ni (mg kg^{-1})	0.21 ± 0.05	0.29 ± 0.00	0.000
DTPA extractable Cd (mg kg^{-1})	0.03 ± 0.01	0.01 ± 0.00	0.104
DTPA extractable Cu (mg kg^{-1})	1.18 ± 0.19	0.98 ± 0.00	0.000
DTPA extractable Zn (mg kg^{-1})	0.36 ± 0.05	0.52 ± 0.00	0.000

in AL possibly resulted in stress in *C. cajan* that triggered production of phenolic compounds, including carlinoside compared to LA.

To examine whether the results obtained with different soil conditions may influence chemical properties of *C. cajan* plants, we collected the leaves of this plant grown in two widely different areas, i.e. AL and LA, at various time intervals. It would be evident from the results in Figure 1 *b*, that there is significant ($P < 0.01$) variation in relation to the quantity of desired chemical compound produced, i.e. carlinoside from leaves obtained from the above-mentioned regions. Excess accumulation of bilirubin causes serious liver injury or jaundice due to impaired activity of UGT1A1, a key enzyme converting insoluble bilirubin to the soluble glucoronidated form that is ultimately secreted out from the body[3]. In the present study, we analysed whether variation in carlinoside content in plants grown in these two regions could also influence the level of UGT1A1. For this, leaves obtained from *C. cajan* plants grown in two different areas at varied periods (45, 90 and 135 DAS) were subjected to solvent extraction. With the help of thin layer chromatography we selected only that solvent which showed highest carlinoside availability, and alcohol extraction was found to be most potent. We performed both *in vitro* and *in vivo* experiments to determine whether the variations in carlinoside from *C. cajan* leaf alcoholic extract could be reflected in the biological activity of this medicinal plant. For *in vitro* observations, we selected human hepatoma cell line (HepG2, liver cell line) and for *in vivo* observations we used alcohol-induced liver damaged rat model. These two types of experiments are expected to provide information on the effect of carlinoside in the reversal of liver damage, which would enable us to indicate the potentiality of soils in endowing medicinal property (i.e. carlinoside) to the leaf of this plant.

To assess whether the same amount of alcoholic extract (AE) from AL and LA leaves could give better or less activity, we incubated HepG2 cells with ethanolic extracts of leaves and found that UGT1A1 gene and protein expressions with 10 mg/ml of AL produced greater effect in comparison to LA, particularly on 45 and 90 day samples (Figure 3 *a* and *b*). However, similar effect may not appear in *in vivo* condition, because availability of chemical compound to different tissues and cells significantly varies during *in vivo* condition. Therefore, we treated rats with alcohol (ethanol) to induce impairment in the liver. Alcohol-induced animal is an accepted model, and it is well known that excess intake of alcohol severely damages the liver. We observed that in alcohol-fed rats (3.7 g/kg body wt), serum SGPT and SGOT levels sharply enhanced, while administration of AE from both AL and LA leaves effectively reversed the SGOT and SGPT profile (Figure 3 *c*). Bilirubin also showed remarkable improvement due to AL and LA extracts. Figure 3 *d* shows that alcohol treatment greatly increases unconjugated bilirubin, which is remarkably decreased by the administration of carlinoside-enriched leaf extracts

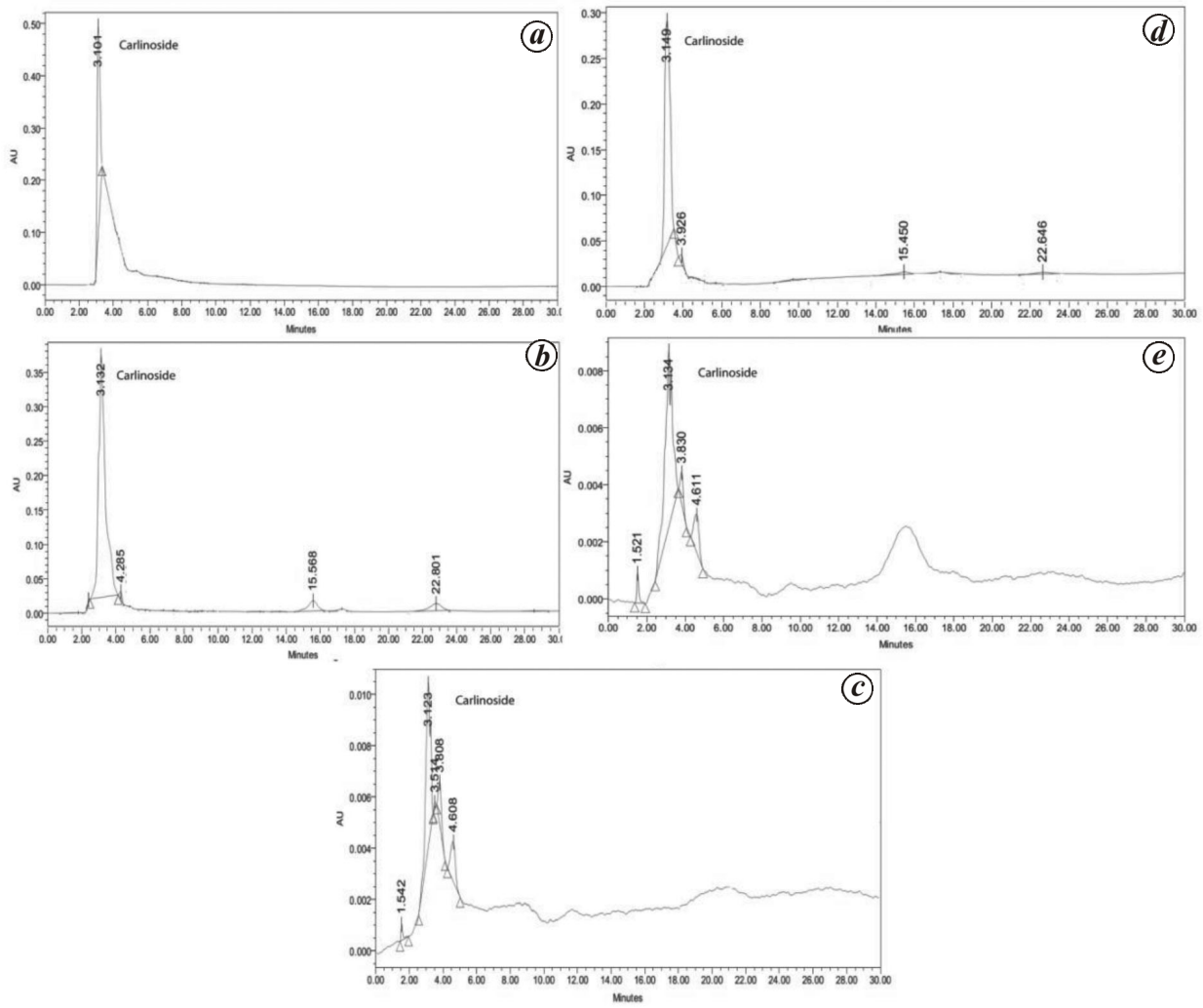

Figure 2. HPLC chromatograms of (***a***) standard carlinoside and (***b***–***e***) carlinoside from (***b***) leaf extract of alluvial zone, (***c***) seed extract of alluvial zone, (***d***) leaf extract of lateritic zone and (***e***) seed extract of lateritic zone.

(AE), suggesting marked conversion of insoluble bilirubin to its soluble form due to enhanced glucoronidation reaction which is catalysed by bilirubin-UGT enzyme (UGT1A1). We therefore observed the effect of AE of AL and LA on UGT1A1 expression in the liver of control and alcohol-treated rats. Alcohol treatment significantly reduced UGT1A1 expression, while administration of both carlinoside-enriched leaf extracts markedly enhanced UGT1A1 by both AL and LA; here also, AL showed greater effect than LA (Figure 3 *e*). Taken together, these findings demonstrate that alcohol-induced liver damage in rats is effectively cured with administration of carlinoside-enriched leaf extracts; the extracts obtained from plants grown in alluvial soil were found to show greater yield of carlinoside. This indicates that AL soils are more productive compared to LA soils with regard to carlinoside yield. Hence, soil quality plays a significant role in contributing to the yield of metabolites, for which the plant is marked for its medicinal value.

Figure 4 *a* and *b* presents PCA and factor analysis respectively. We know that several environmental factors, including soil health strongly influence expression and concentration of bioactive compounds in plants[15,16]. We were interested in identifying these factors for *C. cajan* grown under the given environmental conditions.

We observed strong positive loading of pH, WHC, TKN, TOC, av N and av P in principal component (PC) 1 and equally strong negative loading of Fe, av K and S in PC 2 (Figure 4 *a*). Rich organic matter, low pH with high levels of exchangeable and total nitrogen were found to be essential to enhance production of some secondary metabolites in plants[17,18]. Interestingly, positive loading of precipitation, humidity and solar insolation was also noteworthy. Duration, wavelength and quantity of solar radiation strongly influence photosynthetic carbon fixation and biomass accumulation[12,16]. Positive loading of some trace elements like Mn, Mg, Ca and Zn indicates significant influence of bioavailability of these elements

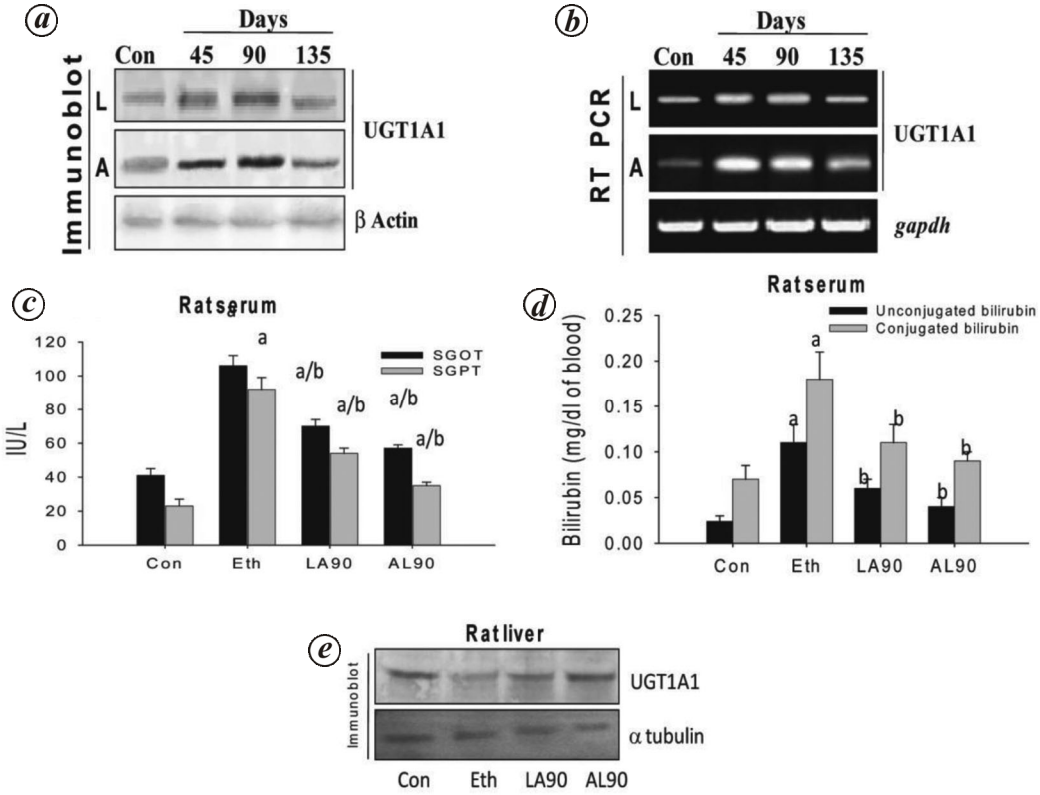

Figure 3. UGT1A1 expression by carlinoside-enriched fractions obtained from *C. cajan* leaves grown in two different soil types (L – lateritic, A – alluvial), and collected at the indicated days. ***a***, HepG2 cells were incubated with or without carlinoside-enriched fractions (10 mg/ml) for 24 h. After incubation, the cells were lysed and microsomal sample was immunoblotted with anti-UGT1A1 antibody. *β*-Actin served as loading control. ***b***, In a similar experiment, RNA was extracted for RT-PCR analysis using UGT1A1 gene-specific primers, where *gapdh* served as an internal control. ***c***, Changes in SGOT and SGPT enzyme levels in rat serum after treatment [Con – contol, Eth – Ethanol (3.7 g/kg body wt), LA90 – ethanol (3.7 g/kg body wt) + Cln extracted from plant leaf of lateritic soil, A90 – ethanol (3.7 g/kg body wt) + Cln extracted from plant leaf of alluvial soil]. ***d***, Variation in conjugated and unconjugated bilirubin in serum of rats under different treatments [Con – Contol, Eth – ethanol (3.7 g/kg body wt), LA90 – ethanol (3.7 g/kg body wt) + Cln extracted from plant leaf of lateritic soil, A90 – ethanol (3.7 g/kg body wt) + Cln extracted from plant leaf of alluvial soil], ***e***, Rat primary hepatocytes incubated with or without carlinoside-enriched fractions (10 mg/ml) for 24 h. After incubation, the cells were lysed and microsomal sample was immunoblotted with anti-UGT1A1 antibody. *α*-Tubulin served as loading control. Arithmetic means with the same letter are not significantly different from each other at 5% probability level.

on expression of phenolic compounds in *C. cajan*. Furthermore, factor analysis (Figure 4 *b*) reveals that the two sites are distinctly different from each other with respect to physico-chemical properties. Generally, reduced water availability and high temperature trigger the production of phenolics in plants[12,19]. Interestingly, PCA suggests that temperature and water content have a positive influence on carlinoside content in *C. cajan.*

R-square values for regression analysis ranged from 0.901 to 0.958. This indicates the significance of the analysis. The regression models derived for total phenol, total flavonoids and carlinoside are given below

$$\text{Total phenol} = 3.08 - 0.84\,\text{WHC} + 3.28\,\text{TKN} - 0.21\,\text{TOC} + 0.24\,\text{Ca} - 0.93\,\text{Mn} + 0.83\,\text{Fe} + 0.14\,\text{T} - 0.05\,\text{P},$$

$$\text{Total flavonoids} = 54.26 + 14.66\,\text{WHC} - 57.74\,\text{TKN} + 7.17\,\text{TOC} + 1.42\,\text{Mn} - 25.09\,\text{Fe} - 23.68\,\text{T} + 9.24\,\text{P},$$

$$\text{Carlinoside} = 6.54 + 0.88\,\text{WHC} + 5.09\,\text{TKN} - 0.17\,\text{TOC} - 0.17\,\text{Mn} + 0.97\,\text{Fe} + 0.33\,\text{T} + 0.04\,\text{P}.$$

Total phenol content in *C. cajan* leaves was positively correlated to TKN, Ca, Fe and air temperature, while precipitation, soil WHC, TOC and Mn content had negative correlation with total phenol. Interestingly, total flavonoids and carlinoside contents correlated positively with WHC and TOC of soil. Carlinoside had a positive correlation with air temperature and precipitation. The regression analysis suggests that C/N balance in soil, along with soil moisture and some metal ions (Ca, Fe and Mn), strongly influence production of secondary metabolites in *C. cajan*. Hence, the differences observed in the present study are directly related to environmental attributes, as

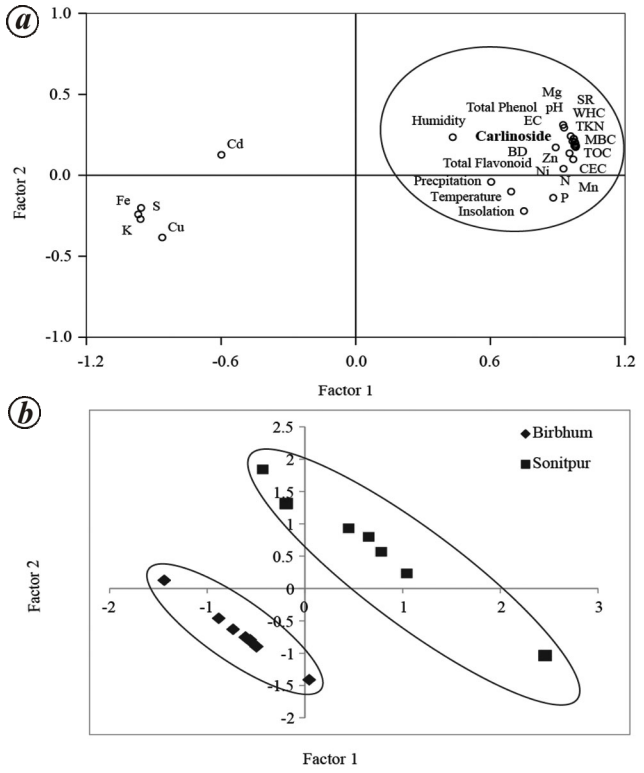

Figure 4. Interactions of environmental attributes and total phenol, flavonoids and carlinoside concentrations in *C. cajan*: ***a***, Loading plots of PCA for the variables under study. ***b***, Factor score analysis for Birbhum and Sonitpur.

noted by Ncube *et al.*[16] that plants evolve physiological mechanisms to acclimatize with respect to their ambience.

The selected locations differed significantly with regard to soil and climatic characteristics. These variations noticeably influenced total phenol, flavonoids and carlinoside concentrations in *C. cajan* leaves. Carlinoside extracted from *C. cajan* from both the locations showed significant variability with respect to the UGT1A1 gene expression in HepG2 cell line. The overall results suggest that *C. cajan* cultivated in alluvial soil yields more carlinoside, a potent candidate against hepatitis.

1. Odeny, D. A., The potential of pigeonpea (*Cajanus cajan* (L.) Millsp.) in Africa. *Nat. Resour. Forum*, 2007, **31**, 297–305.
2. Kundu, R. *et al.*, *Cajanus cajan* Linn. (Leguminosae) prevents alcohol-induced rat liver damage and augments cytoprotective function. *J. Ethnopharmacol.*, 2008, **118**, 440–447.
3. Kundu, R. *et al.*, Carlinoside reduces hepatic bilirubin accumulation by stimulating bilirubin-UGT activity through Nrf2 gene expression. *Biochem. Pharmacol.*, 2011, **82**, 1186–1197.
4. Harborne, J. B., General procedures and measurement of total phenolics. In *Methods in Plant Biochemistry: Plant Phenolics* (ed. Harborne, J. B.), Academic Press, London, 1989, pp. 1–28.
5. Chludil, H. D., Corbino, G. B. and Leicach, L. R., Soil quality effects on *Chenopodium album* flavonoid content and antioxidant potential. *J. Agric. Food Chem.*, 2008, **56**, 5050–5056.
6. Garruna-Hernandez, R., Monforte-Gonzalez, M., Canto-Aguilar, A., Vazquez-Flota, F. and Orellana, R., Enrichment of carbon dioxide in the atmosphere increases the capsaicinoids content in Habanero peppers (*Capsicum chinense* Jacq.). *J. Sci. Food Agric.*, 2013, **93**, 1385–1388.
7. Dong, J., Ma, X., Wei, Q., Peng, S. and Zhang, S., Effects of growing location on the contents of secondary metabolites in the leaves of four selected superior clones of *Eucommia ulmoides*. *Ind. Crop Prod.*, 2011, **34**, 1607–1614.
8. Page, A. L., Miller, R. H. and Keeney, D. R., *Methods of Soil Analysis. Part 2*, Soil Science Society of America Inc, Madison, 1982.
9. Lindsay, W. L. and Norvell, W. A., Development of a DTPA soil test for zinc, iron, manganese and copper. *J. Am. Soil Sci. Soc.*, 1978, **42**, 421–428.
10. Siddique, N. A., Mujeeb, M., Najmi, A. K. and Akram, M., Evaluation of antioxidant activity, quantitative estimation of phenols and flavonoids in different parts of *Aegle marmelos*. *Afr. J. Plant Sci.*, 2010, **4**, 1–5.
11. Ozkok, A., Darcy, B. and Sorkun, K., Total phenolic acid and total flavonoid content of Turkish Pine Honeydew Honey. *J. Apiprod. Apimed. Sci.*, 2010, **2**, 65–71.
12. Alonso-Amelot, M. E., Oliveros-Bastidas, A. and Calcagno-Pisarelli, M. P., Phenolics and condensed tannins of high altitude *Pteridium arachnoideum* in relation to sunlight exposure, elevation and rain regime. *Biochem. Syst. Ecol.*, 2007, **35**, 1–10.
13. Lommen, W. J. M., Bouwmeester, H. J., Schenk, E., Verstappen, F. W. A., Elzinga, S. and Struik, P. C., Modelling processes determining and limiting the production of secondary metabolites during crop growth: the example of antimalarial artemisinin produced in *Artemisia annua*. *Acta Hortic.*, 2008, **765**, 87–94.
14. Zhang, X. X., Li, C. J. and Nan, Z. B., Effects of salt and drought stress on alkaloid production in endophyte-infected drunken horse grass (*Achnatherum inebrians*). *Biochem. Syst. Ecol.*, 2011, **39**, 471–476.
15. Bagdonaite, E., Martonfi, P., Repcal, M. and Labokas, J., Variation in concentrations of major bioactive compounds in *Hypericum perforatum* L. from Lithuania. *Ind. Crop Prod.*, 2012, **35**, 302–308.
16. Ncube, B., Finnie, J. F. and van Staden, J., Quality from the field: the impact of environmental factors as quality determinants in medicinal plants. *S. Afr. J. Bot.*, 2012, **82**, 11–20.
17. Yan, X., Wu, S., Wang, Y., Shang, X. and Dai, S., Soil nutrient factors related to salidroside production of *Rhodiola sachalinensis* distributed in Chang Bai Mountain. *Environ. Exp. Bot.*, 2004, **52**, 267–276.
18. Simon, J., Gleadow, R. M. and Woodrow, I. E., Allocation of nitrogen to chemical defence and plant functional traits is constrained by soil N. *Tree Physiol.*, 2010, **30**, 1111–1117.
19. Glynn, C., Ronnberg-Wastljung, A. C., Julkunen-Tiitto, R. and Weih, M., Willow genotype, but not drought treatment, affects foliar phenolic concentrations and leaf-beetle resistance. *Entomol. Exp. Appl.*, 2004, **113**, 1–14.

ACKNOWLEDGEMENT. We thank the Department of Science and Technology, Government of India for INSPIRE fellowship.

Received 6 January 2015; revised accepted 1 February 2016

doi: 10.18520/cs/v110/i11/2148-2154