Comparative effect of environment on morphological, biochemical and phytochemical analysis of onion cultivated at high and low altitudes

Shardulya Shukla
Lovely Professional University Phagwara
Monisha Rawat
Lovely Professional University Phagwara
Nitish Kumar
Defence Institute of High Altitude Research (DIHAR), Ministry of Defence
Manoj K. Patel
Defence Institute of High Altitude Research (DIHAR), Ministry of Defence
Rakesh K. Behera
Defence Institute of High Altitude Research (DIHAR), Ministry of Defence
Mohan S. Thakur
Defence Institute of High Altitude Research (DIHAR), Ministry of Defence
Raj Kumar
Defence Institute of High Altitude Research (DIHAR), Ministry of Defence
Om P. Chaurasia
Defence Institute of High Altitude Research (DIHAR), Ministry of Defence
Shweta Saxena (drshwetasaxena.dihar@gov.in)
Defence Institute of High Altitude Research (DIHAR), Ministry of Defence

Research Article

Keywords: Ecoligical effect. Allium cepa L. Nutrition. Antioxidant. Bioactive phytochemicals

Posted Date: August 10th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3237170/v1

Abstract

Long harsh winter at the high-altitude reduces the cropping season to few months. So availability of fresh food is a major challenge at the high-altitude. Moreover vegetables imported from other regions (*i.e.*, low-altitudes) may degrade in the nutritional quality, due to the long-transportation and logistic constrains. Keeping a view on the health-promoting qualities of onion (*Allium cepa* L.); the morphological, biochemical, and phytochemical profiling was studied in onion grown at high-altitude (3340 metres above mean sea level, Leh-Ladakh, India) with the lower-altitude (321 metres above mean sea level, Chandigarh, India). Higher-altitude cultivation resulted in better yield (32.55 ± 1.33 t/ha). Total soluble solid (10.62 ± 0.08 ^{0}B), crude protein (6.86 ± 0.10 g/100g), crude fat (0.17 ± 0.01 g/100g), total carbohydrate (93.78 ± 0.67 µg/g), nitrate (180.54 ± 7.77 mg/kg), sulphate (202.77 ± 2.95 mg/kg), nitrogen (1098.03 ± 15.26 mg/100g), sodium (97.05 ± 2.63 mg/100) and manganese (3.91 ± 0.05 mg/100g) contents were found higher at high-altitude whereas, phosphate (1058.27 ± 17.6 mg/kg), magnesium (150.68 ± 0.84 mg/100g), zinc (2.63 ± 0.04 mg/100g)), copper (2.32 ± 0.13 mg/100g)) and iron (16.56 ± 0.24 mg/100g) contents were found higher at low-altitude. Total phenolic (TPC) (5.93 ± 0.06 µg/mg), total flavonoid (TFC) (10.52 ± 0.13 µg/mg), quercetin (0.43 ± 0.01 µg/mg), anti-oxidant potential as indicated by ferric-reducing antioxidant power (FRAP) (42.27 ± 0.10 µg/mg) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) (43.22 ± 0.14%) content were higher in high-altitude grown onion bulbs. The high-altitude grown bulbs were found more effective in vegetative growth, yield and health promoting biochemical and phytochemical compound in comparison with low-altitude grown bulbs.

Introduction

Globally three distinct varieties of onions (*Allium cepa* L.) *i.e.*, red, yellow, and white, are commonly cultivated and consumed. As a food commodity, onions are used as an irreplaceable ingredient for warm and comforting food preparation and also consumed uncooked as salads with other vegetables, juice, pickled, *etc* (Petropoulos et al. 2017). It contains bioactive phytocompounds *viz* organosulfur (OSCs), thiosulfinates, fructo oligosaccharides, phenolics, polysaccharides, and saponins (Marrelli et al. 2019). Therefore, onions are valued for their health promoting and disease preventive activities against cardiovascular diseases, cancer, inflammation, diabetes, digestive disorders, infectious diseases, asthma and immune dysregulation (Khajah et al. 2020).

These biological activities of onions are mainly an outcome of their unique bioactive compounds enrichment. It has been narrated that onions are an abundant source of flavonoids compared to other bioactive compounds. Also, flavonoids (*i.e.*, anthocyanins and quercetin) that play a major role in deciding onion skin colours ranging from yellow to purple (Benkeblia et al. 2007). Sagar and co-workers (2020) have enlisted major flavonoids in onion as quercetin aglycone, quercetin diglucoside, quercetin 4'-glucoside, and kaempferol. Further, Galdón's and co-workers (2009) demonstrated role of various factors especially, environment, type of cultivar, agronomic practices, stage of maturation, and storage conditions in affecting the bioactive compound enrichment of onions. In addition, studies have also suggested that post-harvesting processes *i.e.*, processing and storage, can also alter the bioactive compounds

concentration in onions consequently, impacting their bioavailability and bioefficacy (Sans et al. 2019). Furthermore, abiotic stressors *i.e.*, atmosphere, chemical elements, sunlight/temperature, wind and water also affect the bioactive compounds, pigments and secondary metabolites; subsequently, influencing the overall nutritional profile of onions (Toscano et al. 2019).

Leh-Ladakh is a high altitude region in India and owns extreme climatic conditions due to hypobaric hypoxia, high wind velocity, severe cold, high UV radiation, and very challenging rough terrain (Dame et al. 2011). The, arid climatic conditions make agriculture practices extremely difficult leading to a huge unmet demand for the availability of fresh vegetables at high altitude regions. So far, most of the research in this region is directed to improve the agriculture practices especially *via* implementation of greenhouse technology (Stobdan et al. 2018). However, there are limited studies focusing on the bioactive compound profiling of vegetables grown in this region such as onion. Keeping a view on the health promoting qualities of onions; the morphological, biochemical and bioactive compounds profiling of onions was studied in bulb grown at high altitude (Leh, India) comparison with the onions grown at the low altitude (Chandigarh, India). The output of this study has given a new insight to understand the concentration and major bioactive compounds present in the high-altitude grown onions and the impact of high altitude related abiotic stresses on the bioactive compounds enrichment.

Materials and methods

Experimental field and Condition

The field trials were coordinated during the two consecutive years *i.e.* 2019–2020 and 2020-21 in the open-field at low altitude location, Defence Institute of High Altitude Research, base lab Chandigarh, India (30º41 31 N and 76º47 10 E at 321 meters above MSL) and at high altitude location, Agriculture Research Unit (ARU), Defence Institute of High Altitude Research (Leh), India (elevation 3340 m, $34^0$08.2′N; $77^0$34.3′E). With the help of hygro-thermometer (445,702, Extech Instruments), temperature and relative humidity were noted daily at both locations. The average mean minimum and maximum temperatures at high altitude, during cropping season in 2019-20 and 2020-21 (May-August) was 4.80 ± 1.66 and 25.6 ± 4.06 ºC, respectively, Whereas at low altitude average mean minimum and maximum temperatures during the cropping season (October-January) in 2019-20 and 2020-21 in the experimental field was 10.62 ± 1.21 and 33.45 ± 1.31 ºC sequentially, where as the mean maximum and minimum relative humidity was 87.87 ± 3.68% and 62.6 ± 1.09% respectively. The soil samples were collected from the depth (0–20 cm) of the experimental field prior to compost application for soil chemical analysis at both locations. Furthermore, the chemical characteristics of the soil at both sites were measured according to AOAC (2006). The field experimental soil of low and high altitude was pH (8.63 ± 0.49 and 7.76 ± 0.17), ECe (0.37 ± 0.02 and 1.36 ± 0.13 dS/m), organic carbon (0.28 ± 0.01 and 0.84 ± 0.01%) available N, (9.41 ± 0.63 and 37.63 ± 1.26 kg/ha), available P (23.54 ± 0.65 and 18.50 ± 0.26 kg/ha), available K (461.19 ± 6.07and 346.25 ± 7.41 kg/ha), available S (80.21 ± 3.61 and 138.18 ± 4.74 mg/kg), available Zn (8.77 ± 0.65 and 5.03 ± 0.65 mg/kg), available Fe (34.17 ± 1.76 and 8.33 ± 0.32 mg/kg),

available Cu (34.23 ± 1.84 and 22.46 ± 1.00 mg/kg) and available Mn (10.29 ± 0.23 and 16.95 ± 1.02 mg/kg) respectively.

Chemicals and Reagent

HPLC grade methanol, acetonitrile, acetone, sodium nitrite, sodium hydroxide and gallic acid were procured from Merck (India). Hydrogen sulphate, DPPH (1,1-diphenyl-2-picrilhydrazyl), potassium persulfate (PPS), Folin–Ciocalteu (FC) reagent, aluminium chloride, Trolox, quercitin and anion multi-element standard were purchased from the Sigma Aldrich Pvt. Ltd (Switzerland). Sodium bicarbonate, sodium chloride, boric acid, rutintrihydrate and sodium carbonate were purchased from Himedia (India). The water from the water purification instrument [Merck Millipore Academic, United States of America (USA)] was used for various analyses.

Plant material and experimental design

Experimentation of Onion *(Allium cepa* L.*)* cv. Liberty was conducted at both high and low altitude fields. The experimental plot size was 2.0 m × 1.0 m and was prepared in at least three replicates. Farmyard manure, (at a dose of 1.5 kg per m^2) was mixed to the soil at field preparation stage at both the locations. Earlier study suggested that the size of seedling at transplanting stage influences the size of plant at maturity (Mettananda and Fordham 1999). A uniform distance of 20 cm × 30 cm was kept amongst plant to plant and line to line in all the experimental plots. The field was irrigated by flooding at an interval of three days at high altitude and 6–7 days interval at low altitude during early stage of plant establishment pursued by one week interval (high altitude) and two weeks interval (low altitude) at later stages. At both locations, there were no uses of synthetic fertilizer and pesticides. Weed was removed manually.

Vegetative growth and yield attributes

There was random selection of five representative plants from every plot and tagging of the selected plants was done for further measurement. Morphological parameters were recorded at 30, 45, 60, 75, 90, 105 and 120 days after transplantation (DAT). The height of the plant (cm) was estimated from the surface level to the apex of the highest leaves. Per plant, leaves number was measured by manual counting method. Neck thickness (mm) was measured by vernier calipers. Leaf chlorophyll content (CCI) was determined by portable chlorophyll meter (CCM-200 plus, ADC Bioscientific, UK). Leaf anthocyanin content (ACI) was determined by portable anthocyanin meter (ACM-200 plus, ADC Bioscientific, UK). The maturity index was confirmed with the sign of drying leaves and true shape of the onion bulbs. Equatorial diameter (mm) and polar diameter (mm) were measured by vernier calipers. Yield (q/ha) was recorded by bulb weight per plot and it was converted to per ha to express the result as total yield (q/ha). After harvesting, the dried onion samples were stored at a temperature of -20°C for future analysis.

Biochemical parameters

Estimation of Total soluble solids (TSS) and Titrable acidity (TA)

About 10 g fresh bulb was blended and the juice was extracted to estimate the TSS by using Hand refractometer (ATAGO, Tokyo). Titrable acidity (TA) was determined by titrating fresh bulb juice with 0.1 N NaOH up to pH 8.2 and was indicated as percentage of malic acid (Ranganna 1986).

Determination of Anions (Nitrate, Phosphate and Sulphate)

Anions (Nitrate, Phosphate and Sulphate) of fresh onion samples were determined by using ion exchange chromatography (Cataldi et al. 2003). For this, fresh samples (1000 mg) of bulb was homogenized with a homogenizer at 12000 rpm using deionized water for two min respectively, followed by sonication in an ultrasonic bath (Ultrasonic cleaner YJ5120-1, India) at 45°Cfor 35 minute. The supernatant was diluted in distilled water and filtered by using a syringe filter (0.22 µm). In a column (Metrosep A Supp 5- 250/4.0) an injection volume of 20 µL with a flow rate of 0.6 mL/min and mobile phase, containing 3.2 mM sodium carbonate and 1 mM sodium bicarbonate was used (930 Compact IC flex Metrom; Switzerland). Detection was performed by conductivity detector and outcomes were indicated in mg/100g of fresh weight (FW).

Estimation of crude fat

Crude fat in dried samples was estimated with the help of Soxhlet system (AOAC, 2006). The dried onion powder (5 g) was taken in three soxhlet extractor with continuous usage of petroleum ether by maintaining flow rate, 2–3 drops per second succeeded by sample drying at 95 ± 4°C. The crude fat (g/100g DW) was evaluated by following equation:

$$\mathrm{Crudefat}\,(\backslash\%) = \frac{\mathrm{Flaskweightwithfat}\,(\mathrm{g}) - \mathrm{Flaskweightwithoutfat}\,(\mathrm{g})}{\mathrm{Sampleweight}\,(\mathrm{g})} \times 100$$

Estimation of Total Kjeldahl Nitrogen and crude protein (CP)

The total kjeldahl nitrogen and CP in onion samples was examined in accordance with modified method of Kjeldahl instrument (K-355, BuchiLabortechnik, Switzerland) (AOAC, 2006). For this, oven dried onion sample (0.2 g) was digested through concentrated H_2SO_4 and digestion tablets until light greenish color. Distillation was done with 32% NaOH after digestion. The released ammonium gas was captured in 4% boric acid solution consisting of methyl red and bromo cresol green (indicator), generating ammonium borate that indicates nitrogen content. At last, the distillate was titrated with 0.25 M H_2SO_4 till light pinkish color and the volume consumed was noted and outcomes were demonstrated in g/100 g of dry weight. To calculate crude protein in dried sample, nitrogen was multiplied by correction factor (*i.e.* 6.25) (Wang et al. 2016).

Determination of macro and micro elements

Potassium and sodium contents were analyzed by flame photometer (Jenway PFP7, Bibby scientific Ltd, UK) (Yoldas et al. 2011) while Zn, Cu, Fe, Mn and Mg were evaluated by an atomic absorption spectrophotometer (AAS ZEEnit 700 plus, analytik Jena AG, Germany) (AOAC, 2006; Lee, et al. 2010). For this, dry bulb powder (200 mg) was digested with a micro digester (Analytik Jena AG, Germany) using nitric acid and hydrochloric acid at 3:1 ratio. The supernatant was diluted in distilled water to make up to volume of 50 mL and was filtered by using a Whatman filter paper grade 1. The outcomes were indicated in mg/100 g of dry weight.

Phytochemical analysis

Sample extraction

Isolation of key compounds from plants largely depends on different factors *viz*. extraction method, time, temperature, solvent, moisture content and particle size. Hence, to have a better yield a suitable extraction technique is needed (Bhardwaj et al. 2019). Therefore, maceration was used in this study. Thirty grams pulverized sample was extracted thrice by using 100 ml (each time) of solvent (80% methanol and 20% distilled water) for 24 hours. The extraction was performed under dark conditions at room temperature. Further, filtration of all these extracts was done by Whatman filter paper grade 1. In order to concentrate filtered extract, rotavapor (Buchi R-215, Switzerland) was used at a temperature of 45°C and lyophilized (Esquire biotech Freeze dryer 18N, India) at -80°C and 0.050 mbar pressures and stored in air tight plastic containers at − 20 ^{0}C for future analysis.

Evaluation of total carbohydrate content

The estimation of the total carbohydrate contentof extracts was done by anthrone method which was given by Arguello et al. 2006. In 100 mL of concentrated sulphuric acid, anthrone (200 mg) was dissolved and cooled by ice cooling. 400 µL of different concentrations of standard solution (Glucose; 3.9-1.000 µg/mL) and extracts were mixed with 2000 µL of anthrone reagent respectively, succeeded by placing in a water bath at 95°C for 17 minute followed by cooling at room temperature. Using spectrophotometer (Molecular devices UV-Visible SpectraMax i3x Spectrophotometer, USA), absorbance of the standard and samples was calculated at 620 nm. Expression of the results was in µg of glucose equivalent per gram of Dry Powder Extract (DPE).

Estimation of total phenolic content

Total phenolic content (TPC) was evaluated in sample extracts by of Folin– Ciocalteu reagent with minor modifications (Cozzolino et al. 2021). Seventy micro liters of different concentrations of standard solution (Gallic acid; 2.000–0.332 µg/mL) and extracts were mixed up in 630 µL of deionized water respectively, pursued by addition of FC reagent (70 µL) and incubation at room temperature for 5 minute. Further, in each reaction mixture, 140 µL of sodium carbonate solution (20%) was mixed up and incubated in dark conditions for 60 minute at ambient temperature. After process of incubation,

absorbance of the samples and standard was estimated spectrophotometrically at 750 nm. Findings were indicated in µg of Gallic Acid Equivalent (GAE)/ g of Dry Powder Extract (DPE).

Estimation of total flavonoids content (TFC)

TFC was evaluated by aluminium chloride method with few changes (Benitez et al. 2011). One hundred seventy micro liters of different concentrations of standard solution (Rutintrihydrate; 1.46-3.000 µg/mL) and two extracts were mixed with 680 µL of deionized water respectively, succeeded by addition of 51 µL of sodium nitrite solution (0.724 M) and incubation for 5 min. Successively, in each reaction mixture, 51 µL of aluminium chloride (0.75 M) was added then incubated for 6 minute. Further, 340 µL of sodium hydroxide (1.0 M) was added to each reaction mixture. Total reaction volume was made to up 1700 µL by addition of 408 µL deionized water. Finally, the absorbance was recorded at 510 nm using spectrophotometer. The outcomes were presented in µg of rutintrihydrate equivalent (RE)/ g of DPE.

Determination of Antioxidant capacity (DPPH radical scavenging activity)

The DPPH radical scavenging activity of extracts was estimated by the technique mentioned by Bhardwaj et al. (2019) with minute corrections. DPPH (0.135 millimolar) in methanol was formulated and methanol extract of onion bulb (30.000 mg/mL) / standard (0.480–1.500 µg/mL) were mixed in the ratio of 1:15 using vortex and kept for 30 min in the dark at ambient temperature. After incubation, absorbance was computed by spectrophotometer at 517 nm. As a reference standard Quercetin (QR) was used. The potential to scavenge radicals was determined by given formula:

$$\mathrm{Radicalscavengingactivity}\,(\backslash\%) = \frac{\mathrm{X_{control}} - \mathrm{X_{sample}}}{\mathrm{X_{control}}}$$

where, $X_{control}$ = DPPH radical absorbance in methanol; X_{sample} = DPPH radical absorbance in sample/standard.

Estimation of ferric reducing antioxidant power (FRAP)

The FRAPassay was accomplished as per the techniqe suggested by Sagar et al. (2020)with minute changes.Acetate buffer (pH 3.6) 300 mM, TPTZ solution (20 mM in 40 mM HCl) and 20 mM $FeCl_3$(dissolved in water) were mixed together in the ratio of 10:1:1 to make FRAP solution and this FRAP solution was reacted with methanol extract of onion bulb (10.000 mg/mL) in ratio of 1:30 followed by incubation in dark (30 minute, 37°C temp.). The blue colored product (Ferrous tripyridyltriazine complex) was obtained and absorbance was recorded at 593 nm spectrophotometrically. Trolox (0.976-250.00 µg/mL) was used as an assay standard and outcomes were indicated in µg of trolox equivalent (TE)/ g of DPE.

RP-HPLC analysis of Quercetin

The determination of quercetin was measured by using HPLC with diode array detector (DAD) method suggested by Kwak et al. (2017) with some modifications in flow rate of the mobile phase. An Agilent 1200 Series HPLC system (Agilent Technologies, Palo Alto, CA, USA) was operated in present study. Samples were segregated on a Phenomenex C18 column (5µm 100A, 250 X 4.6 mm), temperature was maintained at 25 ^{0}C with flow rate (0.6 mL/min). An isocratic solvent system was deputed using 50% formic acid (0.1%, v/v) and 50% acetonitrile for 18 minutes. The absorbance was observed at 254 nm. Quercetin standards were used for identification and quantification by making a comparison between RT (retention times) of unspecified peaks with specified standard, and outcomes were presented as µg per gram of DPE.

Statistical analysis

All experimental data were repeated thrice and indicated as mean ± standard deviation (SD). One-way ANOVA (Analysis of variance) and post hoc analysis with Duncan's tests $p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.05$ level and independent t-test was used in SPSS 16.0 (Statistical Program for Social Sciences, SPSS Corporation, Chicago, IL) to determine the significance of the results.

Results

Growth attributes

A comparative study was undertaken to inspect the effect of altitude on growth and quality attributes of onion bulb such as plant height and number of leaves, neck thickness, total chlorophyll content and total anthocyanin content *etc*. The data showed in Table 1 exposed that all the variables varied significantly. At both locations (*i.e.* high and low altitude experimental fields) there was a steady increase in plant height with maturity stages. However, it was noted that plant height was higher at low altitude than at high altitude during the initial stages of plant growth. Interestingly, in subsequent stages of development, *i.e.* after 75 days, plant height of high altitude grown plants was higher than those grown at low altitude. Further, after 120 days the maximum plant height (60.66 ± 0.20 cm) was recorded at high altitude in compared to low altitude (56.42 ± 0.59 cm), respectively.

Table 1
Comparative vegetative growth of onion cv. Liberty at high altitude and low altitude

ALT	DAT	Plant height (cm)	No of leaves	Neck thickness (mm)	Leaf chlorophyll content	Leaf anthocyanin content
HA	30	19.40 ± 0.50$^{a\$}$	3.96 ± 0.07$^{a\$}$	4.09 ± 0.02$^{a\$}$	3.19 ± 0.07$^{a\#}$	1.91 ± 0.07$^{a@}$
	45	27.11 ± 0.90$^{b\$}$	5.25 ± 0.13$^{b\#}$	6.35 ± 0.07$^{b\$}$	13.34 ± 0.68$^{b@}$	3.63 ± 0.02$^{b\$}$
	60	41.99 ± 1.32$^{c\#}$	7.34 ± 0.19$^{c@}$	9.53 ± 0.02$^{c\$}$	14.71 ± 0.06$^{c\$}$	7.88 ± 0.02$^{d\$}$
	75	52.78 ± 1.20$^{d\#}$	8.50 ± 0.25$^{d\$}$	12.42 ± 0.03$^{d\$}$	19.72 ± 0.38$^{e\$}$	8.56 ± 0.06$^{e\$}$
	90	54.21 ± 0.31$^{e\$}$	9.50 ± 0.00$^{f\$}$	14.70 ± 0.28$^{f\$}$	22.73 ± 0.28$^{f\$}$	9.03 ± 0.05$^{f\$}$
	105	55.63 ± 0.25$^{f\$}$	9.67 ± 0.07$^{f\$}$	15.47 ± 0.06$^{g\#}$	20.15 ± 0.26$^{e\$}$	9.44 ± 0.06$^{g\$}$
	120	60.66 ± 0.20$^{g\$}$	9.04 ± 0.26$^{e\#}$	12.74 ± 0.13$^{e\#}$	17.16 ± 0.21$^{d\$}$	6.80 ± 0.09$^{c\$}$
LA	30	30.04 ± 1.08^{a}	5.06 ± 0.10^{a}	6.14 ± 0.06^{a}	2.87 ± 0.06^{a}	2.23 ± 0.14^{a}
	45	41.07 ± 0.58^{b}	6.11 ± 0.19^{b}	7.04 ± 0.03^{b}	11.74 ± 0.05^{b}	3.02 ± 0.05^{b}
	60	46.37 ± 0.49^{c}	6.78 ± 0.19^{c}	9.23 ± 0.06^{c}	12.60 ± 0.19^{c}	6.78 ± 0.02^{d}
	75	48.97 ± 0.70^{d}	7.00 ± 0.00^{c}	12.09 ± 0.03^{d}	14.45 ± 0.11^{e}	7.51 ± 0.16^{e}
	90	51.82 ± 0.40^{e}	7.94 ± 0.20^{d}	12.52 ± 0.07^{e}	18.66 ± 0.16^{g}	7.99 ± 0.08^{f}
	105	53.58 ± 0.20^{f}	8.50 ± 0.17^{e}	14.46 ± 0.31^{f}	16.10 ± 0.09^{f}	8.29 ± 0.10^{g}

HA- High altitude; LA- Low altitude; DAT = Days after transplanting; ALT: Altitude; ALT x TRE - interaction of altitude and treatment.

Values in columns followed by the same letter (small alphabet) are not significantly different, $P < 0.05$, Duncan's multiple range test between days after transplanting. Mean values in each column with different sign $, # and @ (between group) is showed significantly different by independent t-test. Level of significance: $,*** $p \leq 0.001$; #,** $p \leq 0.01$ and @,* $p \leq 0.05$.

ALT	DAT	Plant height (cm)	No of leaves	Neck thickness (mm)	Leaf chlorophyll content	Leaf anthocyanin content
	120	56.42 ± 0.59^{g}	7.67 ± 0.17^{d}	12.17 ± 0.10^{d}	14.21 ± 0.14^{d}	6.00 ± 0.06^{c}
ALT		***	***	***	***	***
DAT		***	***	***	***	***
ALT×DAT		***	***	***	***	***

HA- High altitude; LA- Low altitude; DAT = Days after transplanting; ALT: Altitude; ALT x TRE - interaction of altitude and treatment.

Values in columns followed by the same letter (small alphabet) are not significantly different, $P < 0.05$, Duncan's multiple range test between days after transplanting. Mean values in each column with different sign $, # and @ (between group) is showed significantly different by independent t-test. Level of significance: $,*** $p \leq 0.001$; #,** $p \leq 0.01$ and @,* $p \leq 0.05$.

Significant difference in number of leaves and neck thickness was recorded throughout the growing period at both locations. However, during 30–45 DAT, it was observed that number of leaves and neck thickness was higher at low altitude compared to high altitude while this pattern was reversed following 60 DAT. At 105 DAT, at high altitude experimental fields showed maximum number of leaves (9.67 ± 0.07) and neck thickness (9.67 ± 0.07 mm) as compared to low altitude experimental fields (8.50 ± 0.17) and (14.46 ± 0.31 mm), respectively.

In the present study, high altitude conditions significantly increased the chlorophyll content and anthocyanin content at different days after transplanting. At 90 DAT, maximum chlorophyll content (22.73 ± 0.28 cci) was recorded at HA as compared to LA (18.66 ± 0.16 cci), respectively, whereas at 105 DAT, maximum anthocyanin content was observed (9.44 ± 0.06 aci) at HA as compared to LA (8.29 ± 0.10 aci), respectively.

Yield attributes

Differential altitudinal exposure revealed a significant effect on onion yield attributes (Table 2) including polar and equatorial diameter of bulb (mm), average bulb weight (g) and yield ($t.ha^{-1}$). The maximum polar diameter (80.90 ± 2.16 mm) and equatorial diameter (78.46 ± 2.31 mm) was recorded in high altitude grown plants in comparison with low altitude grown plant (63.97 ± 1.30 mm) and (59.14 ± 1.63mm), respectively. The higher average bulb weight (589.50 ± 17.77 g) and yield (32.55 ± 1.33 t/ha) were recorded at high altitude in comparison with low altitude (157.25 ± 6.01 g) and (17.00 ± 0.08 t//ha), respectively.

Table 2
Comparative Yield and quality attributes of onion cv. Liberty at high altitude and low altitude

Parameters	HA	LA
Polar diameter of bulb (mm)	80.90 ± 2.16***	63.97 ± 1.30
Equatorial diameter of bulb (mm)	78.46 ± 2.31***	59.14 ± 1.63
Average bulb weight (g)	589.50 ± 17.77***	157.25 ± 6.01
Yield (t/ha)	32.55 ± 1.33***	17.00 ± 0.08
Total Soluble Solid (^{0}B)	10.62 ± 0.08**	10.15 ± 0.10
Titrable Acidity (%)	0.38 ± 0.03	0.37 ± 0.00
Crude fat (g/100 g)	0.17 ± 0.01***	0.13 ± 0.01
Crude Protein (g/100 g)	6.86 ± 0.10***	4.73 ± 0.17
Total carbohydrate content (µg /g of DPE)	93.78 ± 0.67**	91.36 ± 0.54
Nitrate (mg/kg)	180.54 ± 7.77***	64.16 ± 1.68
Phosphate (mg/kg)	713.29 ± 13.99***	1058.27 ± 17.6
Sulphate (mg/kg)	202.77 ± 2.95***	157.28 ± 7.74
HA- High altitude; LA- Low altitude		
Values in rows were significantly different between HA and LA at *** $p \leq 0.001$, ** $p \leq 0.01$ and * $p \leq 0.05$, *via.*, Independent t-test analysis.		

Total soluble solid (^{0}B) and titratable acidity (%)

Significant difference was observed in the total soluble solid (Table 2) while the difference in the titratable acidity was no significant in samples of onion bulbs grown at high *vs.* low altitude. Maximum soluble solid content was observed in onion grown at high altitude (10.62 ± 0.08 ^{0}B) as compared to those grown at low altitude (10.15 ± 0.10 ^{0}B), respectively.

Analysis of crude protein, crude fat and total carbohydrate content

In this investigation, significant differences were found in crude protein, crude fat and total carbohydrate content among the onion bulbs grown at high and low altitude experimental fields (Table 2). The maximum content of crude protein (6.86 ± 0.10 g/100 DW), Crude fat (0.17 ± 0.01 g/100 DW) and total carbohydrate (93.78 ± 0.67 µg/g DPE) were observed at high altitude grown onion samples as compared

with low altitude grown sample (4.73 ± 0.17 g/100g DW), (0.13 ± 0.01 g/100g DW) and (91.36 ± 0.54 µg/g DPE), successively.

Analysis of anions content

The contents of important anions (*viz*. nitrate, phosphate and sulphate) were estimated with the help of ion exchange chromatography. The nitrate and sulphate contents were significantly higher in high altitude grown onion samples *i.e.* 180.54 ± 7.77 mg/kg FW and 202.77 ± 2.95 mg/kg FW as compared to samples grown at low altitude (64.16 ± 1.68 and 157.28 ± 7.74 mg/kg FW), respectively, whereas, maximum phosphate content (1058.27 ± 17.6 mg/kg FW) was recorded at low altitude as compared with high altitude grown samples (713.29 ± 13.99 mg/kg FW), respectively (Table 2).

Estimation of macro and micro elements

The results of the macro and micro elements explored in the onion bulbs grown at high and low altitude experimental fields have been presented in Table 3. The maximum of nitrogen (N) content of bulb was grown at high altitude was strikingly higher (1098.03 ± 15.26 mg/100g DW) than the bulbs grown at low altitude (756.46 ± 27.13 mg/100g DW). Whereas, higher potassium (K) content (1225.00 ± 25.00 mg/100g DW) was recorded at LA grown samples in comparison with HA sample (1041.3 ± 6.82 mg/100g DW), respectively (Fig. 4b). The maximum content of magnesium (Mg) was analyzed at low altitude grown samples (150.68 ± 0.84 mg/100g DW) as compared with high altitude sample (62.16 ± 2.53 mg/100g DW). Whereas, higher content of sodium (Na) was recorded at HA (97.05 ± 2.63 mg/100g DW) as compared to LA grown samples (72.92 ± 0.72 mg/100g DW), respectively. The maximum manganese (Mn) was observed in high altitude grown samples (3.91 ± 0.05 mg/100g DW) than low altitude grown samples (2.81 ± 0.07 mg/100g DW), respectively. Whereas, higher zinc (Zn), copper (Cu) and iron (Fe) was recorded in samples from low altitude experimental fields (2.63 ± 0.04, 2.32 ± 0.13 and 16.56 ± 0.24 mg/100g DW) as compared to high altitude experimental fields (2.34 ± 0.06, 1.65 ± 0.03 and 11.32 ± 0.20 mg/100g DW), respectively.

Table 3
Comparative macro and micro elements content of onion cv. Liberty at high altitude and low altitude

Parameters	HA	LA
N (mg/100 g)	1098.03 ± 15.26***	756.46 ± 27.13
K (mg/100 g)	1041.3 ± 6.82***	1225.00 ± 25.00
Mg (mg/100 g)	62.16 ± 2.53***	150.68 ± 0.84
Na (mg/100 g)	97.05 ± 2.63***	72.92 ± 0.72
Zn (mg/100 g)	2.34 ± 0.06**	2.63 ± 0.04
Cu (mg/100 g)	1.65 ± 0.03***	2.32 ± 0.13
Fe (mg/100 g)	11.32 ± 0.20***	16.56 ± 0.24
Mn (mg/100 g)	3.91 ± 0.05***	2.81 ± 0.07
HA- High altitude and LA- Low altitude		
Values in rows were significantly different between HA and LA at *** $p \leq 0.001$, ** $p \leq 0.01$ and * $p \leq 0.05$, *via.*, Independent t-test analysis.		

Total phenolics and flavonoid contents

The phenolics content of onion bulbs grown at high altitude (5.93 ± 0.06 µg GAE/mg DPE) was significantly higher as compared to those grown at low altitude (4.93 ± 0.06 µg GAE/mg DPE) (Table 4). Similarly, TFC of onion bulb was significantly higher in samples from high altitude field (10.52 ± 0.13 µg RE/mg DPE) as compared to those from low altitude field (9.31 ± 0.19 µg RE/mg DPE).

Table 4
Comparative phenols, flavanoids, antioxidant activity and quercetin content of onion cv. Liberty at high altitude and low altitude

Parameters	HA	LA
TPC(µg of GAE /mg of DPE)	5.93 ± 0.06***	4.93 ± 0.06
TFC (µg of RE/mg of DPE)	10.52 ± 0.13***	9.31 ± 0.19
FRAP (µg of TE/mg of DPE)	13.53 ± 0.07***	12.93 ± 0.09
DPPH (% inhibition)	43.22 ± 0.14***	42.27 ± 0.10
Quercetin (µg/mg)	0.43 ± 0.01***	0.15 ± 0.01
HA- High altitude; LA- Low altitude		
TPC- Total phenolic content; GAE- Gallic acid equivalent; DPE- Dry powder extract; TFC- Total flavonoids content; RE- Rutin trihydrate equivalent; FRAP- Ferric reducing antioxidant power; TE- Ttrolox equivalent; DPPH = 1,1-diphenyl-2-picrylhydrazyl.		
Values in rows were significantly different between HA and LA at *** $p \leq 0.001$, ** $p \leq 0.01$ and * $p \leq 0.05$, *via*., Independent t-test analysis.		

Antioxidant activity (DPPH and FRAP assay)

Free radical scavenging capacity of 80% methanolic extract of *Allium cepa* was evaluated by testing their potential to scavenge radicals produced by DPPH. The DPPH radical scavenging capacity of onion bulb grown at high altitude (43.22 ± 0.14%) was significantly higher compared to at low altitude (42.27 ± 0.10%). Similarly, the higher FRAP content (42.27 ± 0.10 µg TE/mg DPE) was recorded at HA as compared with LA sample (12.93 ± 0.09 µg TE/mg DPE), respectively (Table 4).

RP-HPLC analysis of quercetin

In the present investigation, RP-HPLC was used to examine presence of quercetin in 80% methanolic extract of *Allium cepa* which were grown at high and low altitude experimental fields (Table 4). Using a quercetin standard curve method it was observed that a significant difference in quercetin contents of high altitude *vs.* low altitude grown onion bulbs. High altitude grown onion samples possessed the maximum amount of quercetin (0.43 ± 0.01 mg QR/g DPE) in comparison with low altitude grown onion samples (0.15 ± 0.01 mg QR/g DPE), respectively.

Discussion

The current work explored the impact of altitudinal variation on the morphological, biochemical and bioactive phytocompound profiles of onions grown at high altitude (Leh) and low altitude (Chandigarh). The study included intensive recording of plant attributes such as plant height, number of leaves, neck

thickness, chlorophyll and anthocyanin contents in onion plants grown at various altitudinal experimental locations (Table 1). It was observed that during the initial stage of plant growth, the plant height, neck thickness and number of leaves was relatively suppressed at high altitude than at lower altitude. This could be due to the effects of abiotic stressors such as frost, cold, salinity, drought, high wind velocity, intense UV radiations and low oxygen, among others, at high altitude (Kumar, 2020). It was further observed that during the later stages of growth the attributes like, number of leaves, plant height and neck thickness were significantly increased at high altitude as compared to low altitude location. In general, the total chlorophyll and anthocyanin contents were significantly higher at high altitude as high mountainous region of Leh Ladakh receives high light intensity and high ultra-violet radiation which might have increased the chlorophyll content in onion leaves (Stobdan et al., 2018). Gao et al 2020 also reported that the photosynthetic efficiency as well as chlorophyll content of Welsh onions was significantly improved by blue and white light. Additionally, high levels of ultraviolet radiation may have contributed to the maximum anthocyanin content in onions grown at high altitudes. These results are consistent with those of (Mahdavian et al., 2008), who observed that UV-B and UV-C increased the concentration of anthocyanin in *Capsicum annum* leaves.

In the present study, maximum bulb yield was obtained at high altitude than at low altitude (Table 2) which depends on the polar and equatorial diameter. Longer photoperiod at high altitude is one of the prime factors for relatively better bulb development (Caruso et al. 2014). Furthermore, better physical properties and high yield of the onion bulb may be due to superior plant growth attributes at high altitude, which may have resulted in increased photosynthetic rate and assimilation of biosynthesis products in storage plant tissues such as onion bulbs, leading to much better physical indices of onion bulbs grown at high altitude compared to those grown at low altitude (Aisha et al. 2007).

The TSS content of white onion bulbs cultivated at high altitude was higher than that of onion bulbs grown at low altitude, which may be related to the high rates and high efficiency of photosynthesis at high altitude. Naryal et al (2020) also reported high TSS content in mandarin and apricot fruit at higher altitude or increasing elevation.

The titrable acidity content didn't show altitude variation and was comparable in onion bulbs grown at High and low altitude regions. Further, higher crude fat content was found in samples at high altitude experimental fields. The crude fat are a vital part in biosynthesis of organic substances, plant development and its content is known to be directly affected by macro and microelements composition of the soil in which plant is grown (Yassen et al. 2009). Similarly, another macro-nutrient *i.e.* carbohydrates are also required for various biochemical reactions with energy metabolism (Bhattacharjee et al. 2013) and have an important role in abiotic stress (Cataldi et al. 2000). In present investigation, higher content of carbohydrate was observed at high altitude as compared to low altitude.

The nutritional composition of bulb largely depends upon the physiochemical properties of the soil along with several other physiological factors (Agegnehu & Amede 2017). In current findings, nutritional composition of bulb was fluctuating in relation to chemical properties of soil at high and low altitude

experimental fields. The nitrate and phosphate contents of bulbs were found to be higher at high altitude whereas, sulphate content was higher at low altitude while, nitrogen, sodium and manganese were found to be higher in high altitude grown samples whereas, potassium, magnesium, iron, copper and zinc were higher in low altitude grown samples.

Apart from macro and micro-nutrients, phenolic compounds have many essential nutraceutical properties, potential health benefits and biological activities (Kapoor et al., 2018). The biosynthesis of flavanoids, which are largest group of biological phenolic compound, is usually influenced by various cultivation conditions, like plant location, weather conditions and harvest period (Lu et al., 2011). The TPC and TFC were estimated in the present study across all the onion bulb samples harvested from high and low altitude fields. In present investigation, high TPC and TFC was observed in onion bulb samples obtained from high altitude experimental fields as compared to those obtained from low altitude field (Table 4). This could be due to abiotic stress of extreme environmental conditions at high altitude which resulted in higher TPC and TFC values. Our results are similar to previous study on plant where an extensive range of related phenolic compounds and flavonoids were found to be produced against the stressful conditions (Rozema et al., 1997). In general, higher UV-B radiation activates phenolic and flavonoids synthesis (Angmo et al., 2021).

The antioxidant potential of bulb extracts were estimated using stable free radicals of DPPH in addition to FRAP (Lu et al., 2011; Sager et al., 2020). In this investigation DPPH and FRAP were found higher in samples collected from high altitude as compared to those collected from low altitude.

The presence of quercetin in onion bulb was estimated by HPLC profiling which is presented in Fig. 1. It was found that high altitude grown onion bulbs showed much higher content of quercetin than those grown at lower altitude and which could be due to stressful conditions of high altitude (Sulastri et al. 2018).

Conclusion

Altitudinal variation resulted in a range of positive responses on morphological parameters, biochemical and major bioactive compound composition of onion bulbs cv. “Liberty”, grown at high altitude (Leh) and low altitude (Chandigarh). The results showed that cultivation at high altitude resulted in 1.9 times increase in the yield as compared to low altitude. Bulb quality in respect of crude fat, TSS, crude protein, carbohydrate and content of most mineral nutrients was also observed to be significantly superior at high altitude as compared with grown at low altitude. The phytochemical analysis of hydro-methanol extract of bulbs obtained from *Allium cepa* revealed presence of higher concentration of compounds with antioxidant ability at high altitude in comparison with grown at low altitude. Further, HPLC analysis, it was found that onion samples of high altitude possessed higher content of quercetin as compared to low altitude onion sample.

Declarations

Acknowledgement

The authors are immensely obliged to DRDO for extending a helping hand in terms of financial assistance for carrying out the experimentation and analysis work.

Author contribution

Shardulya S. writing-Original draft preparation and carried out experiments. Monisha R. supervised the work and edited the manuscript. Nitish K. Experimentation and data analysis. Manoj K. Patel. Rakesh K. Behera. Mohan S. Thakur. Help in Collection of growth and yield data. Raj K. supervised in HPLC analysis. Om P. Chaurasia. supervision, project administration and funding acquisition. Shweta S. supervised the work and edited the manuscript. All authors have seen the draft copy and approved the final version of manuscript.

Funding

This research is funded by the Defence Institute of High Altitude Research (DIHAR)-DRDO, Ministry of Defence, C/o 56 APO Leh-Ladakh-194101, India.

Competing of Interest

There is no competing of interest amongst the authors.

Ethics approval

There is no need of any ethics approval as this investigation was not related with any animal or human subject.

Consent for publication

All authors have approved the manuscript and agree with its submission to plant ecology.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

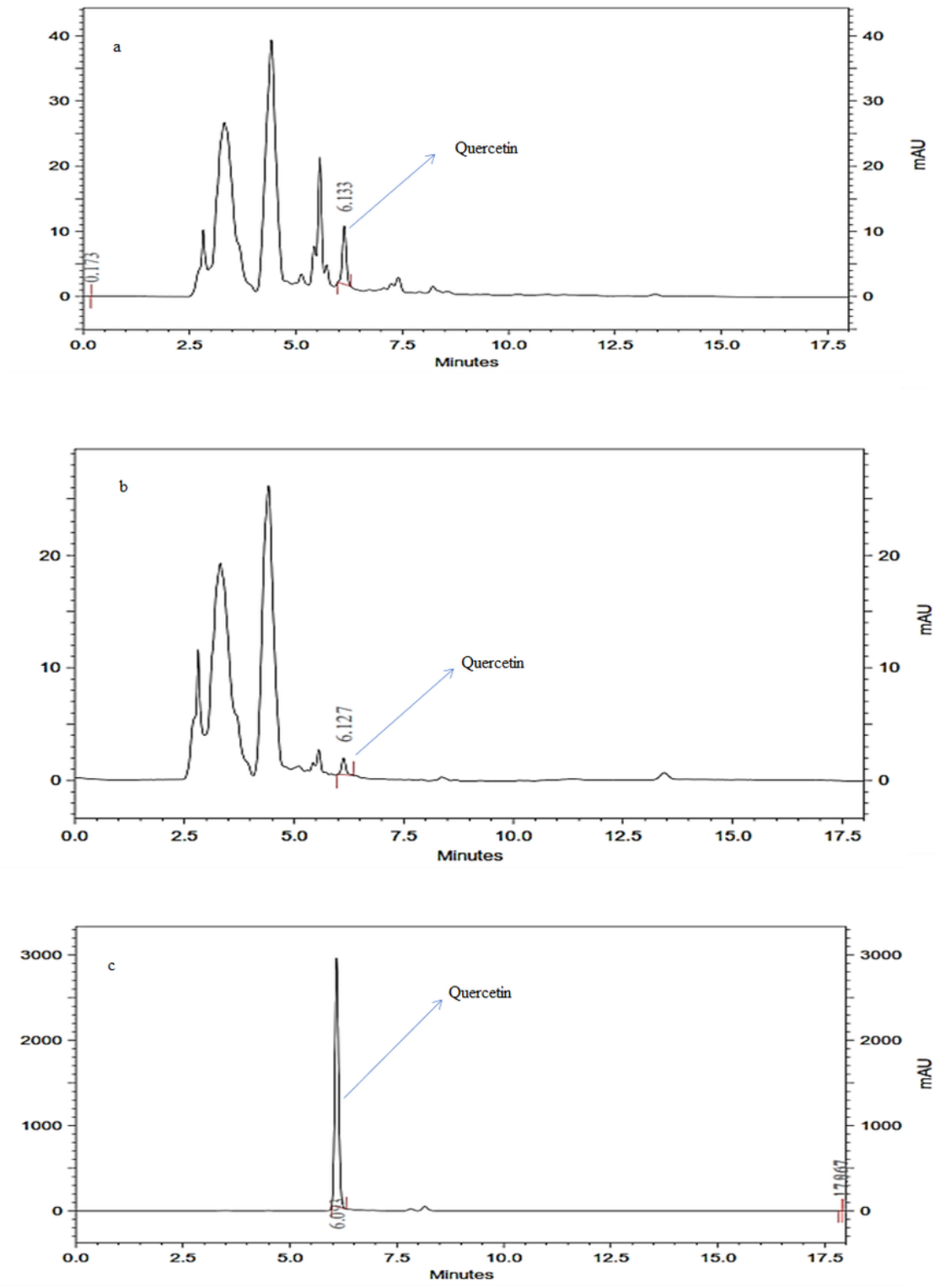

Figure 1

HPLC chromatogram profile of quercetin in 80% methanolic extract of *Allium cepa* bulb and std. (**a)** Peak of quercetin present in high altitude onion extract, (**b)**Peak of quercetin present in low altitude onion extract, and (**c)** Standard peak of quercetin.