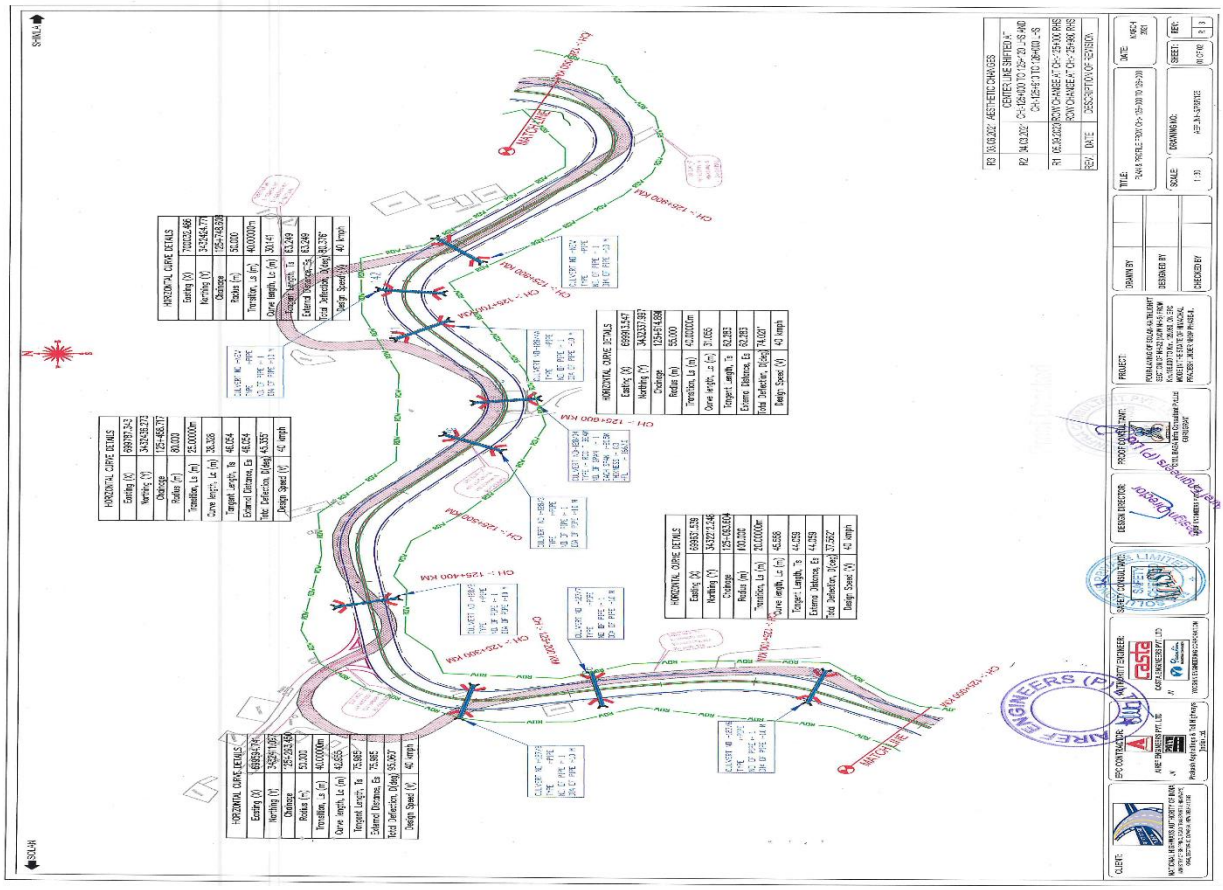
Department of Civil Engineering
Jaypee University of Information Technology,
Waknaghat (H P) - 173234

Ref. No. JUIT/CED/2021-22/018 17-11-2021

Site Survey Report for NHAI

With reference to letter 11008/2/2015/S-K/LA/PIU-SML/1252 dated 25th August 2021 following suggestions are given for remediation of landslide prone slides at Eutopia (Chainage 125+750 to 125+880) and Bahra University (122+410 to 122+450) on the basis of preliminary investigation report provided by NHAI and field visits done (on 26th August 2021 and 08th November 2021) by the expert committee comprising Prof. Sandeep Singh (Department of Geoscience, IITR), Prof. Ashish Kumar, Dr. Tanmay Gupta and Mr. Niraj Singh Parihar (CE Department, JUIT Waknaghat).

Site1: Near Eutopia resort (Chainage 125+750 to 125+880)



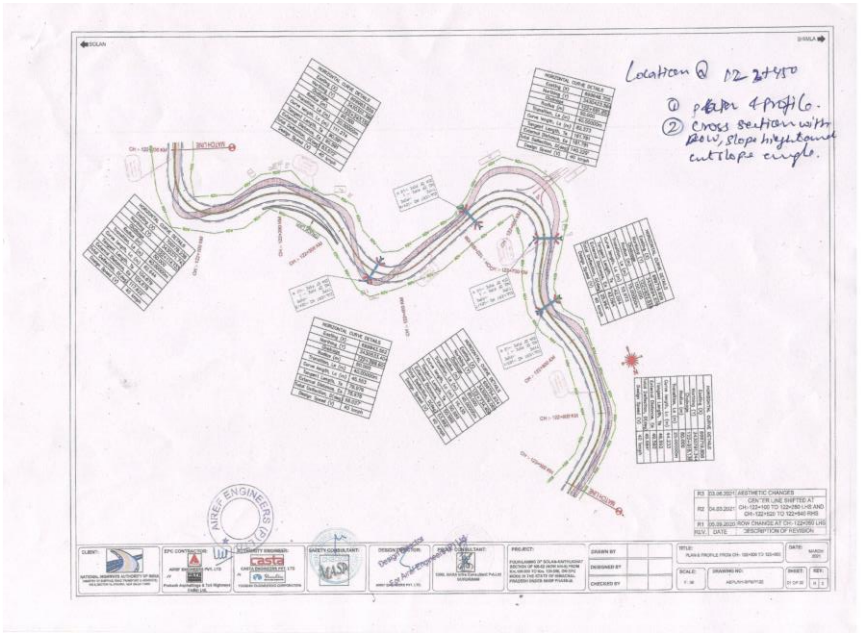
1. The top stratum (2 to 4 m depth as per previous seismic refraction reports) of the slope is a loose mass which is mainly responsible for debris flows (Seismic Refraction profile elaborates top layer comprises of unconsolidated loose soil mixed with boulder with

seismic velocity of 270m/s) and the same needs to be scrapped and cleared in stepwise manner initiating from the top towards bottom. The scrapped material should be parallelly cleared from the bottom at the level of road with the help of loaders. The scrapper or JCB can go uphill from the right end of the slope (right picture). In order to cross-check the seismic refraction profile data it is further recommended to have 3 bore logs to be done at the worksite under-supervision of a proficient consultant.

2. The rocky outcrops (consisting of shale and sandstone mainly and based on the evidence from seismic refraction profile where second layer was found of highly withered siltstone having high seismic velocity of 1394m/s and third layer was found by moderately weathered siltstone with seismic velocity of 2800m/s) suggest the presence of good bearing material with sufficient stability underneath the top layer. However, the depth of embedment of rocky strata cannot be judged, it is advisable to use the steel netting (wire mesh) with bolted anchors at least 15 m in length at 45° angle inclining downwards after clearing the loose strata and for more critical masses use of inclined helical soil nails along with shotcreting of the slope is recommended. The design of which shall be proof checked by proficient consultant prior to construction.

3. It was also observed that the retaining wall constructed foothill at the extreme end of the slope (towards Shoghi side) is inefficiently constructed. There is absence of any weep holes in the wall and a geosynthetic layer is provided at the hill side which could escalate the pore pressure. It is recommended to provide the weepholes in the wall, with base drainage towards the valley side and extend the wall to further height. The deign of retaining wall with sufficient drainage and setback space shall be done under a proficient consultant. Use of stone concrete blocks as per IS 12240 (already being made at Jaypee University of Information Technology) for design of retaining wall is highly recommended in this situation.

Site2: Bahra University (Chainage 122+410 to 122+450)



1. As there are no geological investigations available for the site, at least 3 bore holes are recommended for obtaining the soil/rock profile. There is evident seepage at the bottom of the slope from unknown source which needs to be controlled and drained away from the source itself.

2. The folding of the slope mass is towards the reverse direction. It is recommended to imply the use of steel netting (wire mesh) with bolted anchors of at least 15 m length at 60° angle to the horizontal to contain the moving mass, and for more critical masses use of inclined helical soil nails along with shotcreting of the slope is recommended. The design of which shall be proof checked by proficient consultant prior to construction.

3. There is presence of organic rocks with greenish tinge having low strength bedding material which should be avoided for anchoring.

4. The retaining wall height is insufficient to hold the moving mass and could be escalated to at least 20 ft, use of stone concrete blocks as per IS 12240 (already being made at Jaypee University of Information Technology) for design of retaining wall is highly recommended in this situation.

Note: Above are the observations and suggestions from the concerned team from Civil Engineering Department, JUIT which is on the basis of reports made available by NHAI and site visits done by the team. Hydrological and geological testing data for sites was not available. It is suggested that before implementation of the suggestions, it may be further verified from other agencies also.

Dr. Tanmay Gupta Assistant Prof. CED, JUIT	Mr. Niraj Singh Parihar Assistant Prof. CED, JUIT	Dr. Ashish Kumar Professor & Head

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY
(Established by H.P. State Legislature vide Act No. 14 of 2002)
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Website : www.juit.ac.in
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Bridge Inspection report for 40.0 m Span PSC Bridge over Katli khud on Kupvi to Mashot road at RD 6/395

District Name	Zone	Circle	Division	Block Name	Package No.	Sanctioned Year	Work Type	Sanction Cost	Bridge Length (Mtrs)
Shimla	Shimla	Shimla	Chopal	Chopal	HP09636	2017 - 2018	LSB	165.34	40.000

Inspection Date: 04-07-2021

Inspector's Name and affiliation: Dr. Tanmay Gupta, Assistant Professor Civil Engineering Department, JUIT

Official start date of project: 28-08-2018

Completion date as per agreement: 31-08-2021

Work Progress:

Land disputes have halted all work progress. Since the beginning of the project at both ends (Kupvi and Mashot) landowners have raised their disputes due to which even excavation for substructure was not done. Recently in June 2021 land dispute on Kupvi side has been resolved, however Mashot side land is still under debate.

Monitored developments on worksite:

Main Information board and citizen information board available.

Benchmark for proposed elevation of the bridge available.

High flood level benchmark available.

No excavation or material procurement is done yet.

All drawings duly vetted by NIT Hamirpur are available.

Observations:

Although sufficient staff (A.E + J.E + intern) is available at the work site and contractor is also willing to start the work, due to dispute of lands at both ends no progress in the work was observed while the completion date as per the agreement is approaching soon.

Site photographs and duly signed copy of work progress is attached here with.

Dr. Tanmay Gupta

Dr. Ashok Kumar Gupta

Main Information and Citizen Information Board at Worksite

General Overview of the Work Site

General Overview of the Work Site from Mashot side

General Overview of Kupvi end

Benchmark for Proposed Elevation at Kupvi end (986.5m)

Benchmark for Proposed Elevation at Mashot end (986.5m)

HFL mark of Katli Khad (980.5m)

BRIDGE INSPECTION PMGSY

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY

District Name	Programme Implementation Unit	Zone	Circle	Division	Block Name	Package No.	Sanctioned Year	Work Type	Road Name / Bridge Name	Stage Construction	Sanction Cost	Road Length (Kms)	Bridge Length (Mtrs)
Shimla	DPIU Of Shimla	Shimla	Shimla	Chopal	Chopal	HP09636	2017 - 2018	LSB	katli khad	Bridge	165.34	0.000	40.000

PMGSY Batch - 2

Present status of the bridge construction: Land dispute Mashot side.
Land dispute Kupvi side resolved in June 2021.
once other side Land dispute is resolved work will begin.
Drawing vetted by Dr. Amrit Kumar Roy, NIT Hamirpur. on 01/04/2021
No materials have been procured.
No excavation done yet.
office staff Kupvi → A.E + J.E + internship

Inspector's Signature
Dr. Tanmay Gupta

Bridge Supervisor's Signature
(AJAY GAZTA)
Assistant Engineer
HPPWD Sub-div. Kupvi

Duly Signed Work progress report

SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the Ph.D thesis entitled **"Fruit quality, phytochemical and diversity studies of apricot (*Prunus armeniaca* L.) along an altitudinal gradient in trans-Himalayan Ladakh, India"** submitted by **Mr. Avilekh** at **Jaypee University of Information Technology, Waknaghat, India,** is the record of candidate's own work carried out by him under our supervision. This work has not been submitted elsewhere for any other degree or diploma.

(Dr. Anil Kant)
Associate Professor
Department of Biotechnology Bioinformatics
Jaypee University of Information Technology
Waknaghat, Solan- 173215
Date:

(Dr. Tsering Stobdan)
Scientist 'E'
Defense Institute of High Altitude Research
Defense Research & Development Organisation
Leh Ladakh-194101
Date:

To

The Vice Chancellor
Jaypee University of Information Technology
Waknaghat Solan

Subject: Request to approve inclusion of Dr Sundresha Siddapa as Co-guide in the DPMC of Ms Suhani Bhagta (196552) PhD

Sir

(A) It is requested to approve the inclusion of name of Dr Sundresha Siddapa as Co-supervisor of my PhD student Ms Suhani Bhagta (196552). Ms Suhani has been assigned research topic "RNAi meditated spray-induced silencing of multiple genes of *Venturia inaequalis* for apple scab management". Dr Sundresha is working of RNAi since long and we will be able to utilize the lab facilities and expertise of Dr Sundresha by doing so, at CPRI, Shimla. We already have an MoU with CPRI Shimla and outcome of this research work would be shared by both the institutes.

Dr Anil Kahl 12/02/2020

(A) Dr. Sundresha may be approved as Co-PI of Ms. Suhani Bhagta (196552) (Ph.D. student)

12/02/2020.

(B)

Dean Academic

12/02/2020

R | AR

Agbd 13/02/2020

(B) may be approved subject to systemic support towards our students & scholars being benefitted for academic gain by use of CPRI infra-structure

VC

13/02/2020

To

Head Plant Protection
Central Potato Research institute
Shimla HP

Subject: Proposal of research collaboration on Phytophathora infestance

Sir

It is matter of great pride that scientists of Division of plant protection CPRI Shimla have identified and validated dsDNA target genes of Phytophathora infestance, detrimental for its establishment on potato. We want to test and validate these targets in case of tomato as host via a small project to be executed through BTech /MSc project at JUIT Waknaghat. Sir due credit will be given to the scientists and institute in case of publication any other positive development out of this. We already have MoU regarding research and development work. In this context I request you to allow following

1. Sharing culture of appropriate culture of Phytophathora infestance
2. Sharing the amplified potential dsDNAs
3. 2 days orientation cum training of students at Plant pathology lab at CPRI to have hands on detached leaf assay.

17/08/2019

Dr Anil Kant Associate Professor

Deptt. of Biotechnology, and BI Jaypee University Waknaghat Solan HP

To

Vice-Chancellor
Jaypee University of Information Technology
Waknaghat

Subject: Permission to visit CPRI Shimla on 03.05.2019 regarding discussion and request to facilitate training cum hands on experience on genetic analyzer for our 4 personals (Deptt of BT and BI 1, faculty 01 technician and 2 research scholars)

Sir

I am to state that we at the Deptt. of Biotechnology and Bioinformatics, JUIT have Genetic analyzer API 3500xl, however due to lack of trained human resources we are not able to use this machine. We are interested to increase its usage for research as well as for exposure to our B.Tech. students. This will increase their prospectus for getting jobs in genomics based companies. Towards this end we are looking for opportunity of training cum hands on experience to few of our personal on this equipment. Similar Genetic analyzer is being operated routinely at CPRI Shimla, and we would like to request Director CPRI to facilitate training cum hands on experience to few of our personal on this equipment. In this endeavour I am seeking your permission to visit CPRI Shimla, to have discussion and request to facilitate training cum hands on experience on genetic analyzer. You are also requested to grant permission for transport from JUIT Wakanghat and back, in JUIT Vehicle.

Thanking You

Your Faithfully

01/5/2019

Dr Anil Kant
Associate Professor Deptt. of Biotechnology, and BI
Jaypee University Waknaghat
Solan HP

(A) may be approved.
(B) Dr. Viney Bhardwaj - member BOS-BT & BI Dept will help us for this training. He is HoD- Crop Improvement Division, CPRI Shimla.

01/05/19.

Hony SM Sh Negi ji
FWA

Appd (A) Visit is on 3/5/19

1.5.19

भा.कृ.अ.प - केंद्रीय आलू अनुसंधान संस्थान
शिमला-171001, हि.प्र. (भारत)
ICAR-Central Potato Research Institute
(Indian Council of Agricultural Research)
SHIMLA 171 001, HP

ISO 9001:2015

Dr Brajesh Singh
Incharge, PME Cell

Phone: 0177-2625073 (O), Fax: 0177-2624460
E-mail- directorcpri@gmail.com Website : http//cpri.icar.gov.in

No.F.PME/04-27/2019 1159
May 03, 2019

To

Dr VU Patil,
Scientist,
Division of Crop Improvement,
ICAR-CPRI, Shimla 171 001

Thro' Head, Div. of Crop Improvement, CPRI, Shimla.

Sub : Request to conduct training-cum- hands on experience on genetic Analyser for 3 Researchers of Department of BT & BI, Jaypee University of IT Waknaghat.

Sir,

This is in reference to letter dated 1st May, 2019 from and Prof. Sudhir Kumar, HOD and Dr. Anil Kant, Associate Professors, Department of Biotechnology and BI Jaypee University, Waknaghat, District Solan, HP on the subject cited above. In this connection, I am pleased to inform you that your name has been nominated by the Competent Authority to allow and facilitate an exposure cum hands on experience to three (3) personnel's of Jaypee University on Genetic Analyzer 3500 for at least 3-4 days on the dates as per convenience during the month of May-June, 2019.

This is for your information and necessary action.

Yours faithfully,

Sd/-
Brajesh Singh
Incharge, PME Cell

Copy for information to:

1. Prof. Sudhir Kumar, HOD and Associate Professor, Department of Biotechnology and BI Jaypee University, Waknaghat, District Solan, HP.
2. Dr. Anil Kant, Department of Biotechnology and BI Jaypee University, Waknaghat, District Solan, HP.

Incharge, PME Cell

SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled **"Gender differences in antioxidant properties, phenotypic plasticity and freeze tolerance in Seabuckthorn (*Hippophae rhamnoides* L.) along an altitudinal gradient in trans-Himalayan Ladakh, India"** at **Jaypee University of Information Technology, Waknaghat, India,** is a bonafide record of her original work carried out under our supervision. This work has not been submitted elsewhere for any other degree or diploma.

(Dr. Tsering Stobdan)
Defence Institute of High Altitude Research
Defence Research and Development Organisation
Leh, Ladakh-194101
Date: 19 May 2018

(Dr. Anil Kant)
Department of Biotechnology & Bioinformatics
Jaypee University of Information Technology
Waknaghat, Solan- 173215
Date: 19 May 2018

To,

Date : Sept.1st;17

The Vice Chancellor,

Prof.(Dr.) Vinod Kumar

JUIT,Waknaghat

Distt. Solan

Respected Sir,

Subject : Student exchange program consent and permission for condense semester.

With all due respect , the following students of B.Tech and M.Tech Biotechnology would like to go for the final semester project under the student exchange program as per our MOU with South Dakota Schools of Mines and Technology,USA from Feb.2018 to May/June 2018:

S.No	Roll No.	Name	B.Tech/M.Tech	SGPA*	CGPA
1.	141828	Wageesha Sharma	B.Tech	9.7	8.5
2.	141807	Pratha Sood	B.Tech	7.4	7.4
3.	141818	Pallavi Soni	B.Tech	8.2	7.1
4.	141847	Rishabh Nautiyal	B.Tech	7.7	6.7
5.	141850	Anandita Govil	B.Tech	7.2	6.5
6.	133808	Shagun Choudhary	M.Tech	9.2	8.0
7.	133815	Chetansee Khanna	M.Tech	9.2	7.8
8.	131573	Mehul Salaria	M.Tech	9.2	6.8
9.	131579	Gorky	M.Tech	10.0	6.5

Total (9)

01/9/17

* *Previous semester SGPA*

We will be bearing travel ,accommodation and food expenses by ourselves . No charges will be incurred for our final semester project (B.Tech 10 credits and M.Tech 8 credits) in SDSMT.Guidlines for the same is attached with the application.

Condensed semester for courses (theory) of 8th semester for 2nd B.Tech (BT) & M.Tech (BT) to undertake project as marked (A) may be approved

04/09/2017

VC

Pl give a copy of MOU to Dy Cdr

04/09/2017

Kindly, allow us condense semester Dec-Jan in campus so that we could clear all our final semester theory subjects before going for project. We shall be highly grateful to you.

Thank You !

Yours sincerely

141828

141807

141818

141847

141850

133808

133815

131573

131579

(1) may be allowed for the final (sem.) Yr. project at SDSMT, USA.

(2) JUIT and SDSMT are having MOU for student exchange.

(3) Condensed semester (Dec-Jan 2018) may be allowed at JUIT.

(4) Consent of Dr. Sani / Dr. Robb winter of SDSMT is attached.

01/09/17

Consent letters of the parents attached.

To

The Head of Department,

Department of Biotechnology and Bioinformatics,

Jaypee University of Information Technology

October 26th, 2021

Subject: Consent for 6 months internship program at SDSMT

Respected Sir,

We, the students of Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, as undersigned, provide our consent to extend our Internship program duration to 6 months instead of the speculated 5 months 10 days, such that our program requires us to stay there up to August 10th, 2022. We understand that doing so shall delay the reception of our degrees and the national competitive exams which are scheduled in that duration.

Regards,

Yash Sharma 207802 2nd Year M.Sc. Biotechnology	
Jasmeet Kaur 207804 2nd Year M.Sc. Biotechnology	
Sarita Vatwani 207812 2nd Year M.Sc. Biotechnology	
Smriti Gaba 207825 2nd Year M.Sc. Biotechnology	
Aditya Tiku 181809 4th Year B.Tech Biotechnology	

A maybe allowed.

26/10/2021

(Prof. Sudhir Kumar)

26/10/21

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY

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Fax : +91-01792-245362

August 5, 2020

To,
Suzi Aadland, Director
Ivanhoe International Center
South Dakota Mines
501 E. Saint Joseph St., Rapid City, SD 57701
1.605.394.6884

This is to certify that **Mr. Vaibhav Handa**, Enrolment No.161813, S/o. Mr. Vikas Handa is a bonafide student of this University. He is studying Bachelor of Technology, a 4 years undergraduate degree programme, from this University in the branch of Biotechnology. Upto the end of the 7th semester his CGPA is 7.4 on scale of 10. The CGPA of 7.4 equates to 74% as per JUIT Conversion table 2013, approved by Academic Council.

Mr. Vaibhav Handa went to South Dakota School of Mines and Technology, USA to complete the 4th year project as per the collaboration between Jaypee University of Information Technology, Waknaghat and the said University.

The result of the project in respect of Mr. Vaibhav Handa from South Dakota School of Mines and Technology, USA has not been received by Jaypee University of Information Technology, Waknaghat as yet and hence the final result of Mr. Vaibhav Handa cannot be published as on date.

The result from South Dakota School of Mines and Technology, USA is expected by end August, 2020.

Maj Gen Rakesh Bassi (Retd)
Registrar & Dean of Students
REGISTRAR,
Jaypee University of Information Technology
Waknaghat, Distt. Solan (H.P.)

JUIT

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY

(Established by H.P. State Legislature vide Act No. 14 of 2002)
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Fax : +91-01792-245362

Ref. No. JUIT/WKG/REGR/2018-19/090 September 20, 2018

E-mail Transmission: rajesh.saini@sdsmt.edu

Prof. Rajesh Sani
Department of Chemical and Biological Engineering & Chemistry and Applied Biological Sciences
South Dakota School of Mines and Technology
501 East St. Joseph Street
Rapid City, SD 57701-3995, USA

Ref:- E-mail dated September 13, 2018 from Dr. Sudhir Kumar and your subsequent E-mail dated September 14, 2018.

Sub:- Nomination of students for final semester research project under students exchange program at South Dakota School of Mines and Technology (February 2019 to August 2019).

Sir,

Reference is invited towards E-mail dated September 13, 2018 from Dr. Sudhir Kumar, Professor and Head, BT / BI Deptt., Jaypee University of Information Technology, Waknaghat, Solan, Himachal Pradesh (India) and your subsequent confirmation E-mail dated September 14, 2018 on the subject.

As desired, I am hereby forwarding the details of the students who have shown their interest and given their consent for their final semester research project under students exchange program at South Dakota School of Mines and Technology from February 2019 to August 2019 for consideration at your end. The details of the students are as under:-

S.No.	Name	Enrolment No.
1.	PRIYA SAXENA	151806
2.	TANYA KUTHIALA	151807
3.	KUNJAN GOVIL	151839
4.	SAVEENA SOLANKI	151842
5.	VAISHALI SHARMA	151850
6.	PAYAL THAKUR	151853

Brief resume and copy of the transcript in respect above six (06) students is attached herewith your kind perusal and consideration. Hope you will find the same in order as per your requirement. Kindly acknowledge the same and accord your kind approval for the same.

Thanking you

Yours sincerely,

Maj. Gen. Rakesh Bassi, SM (Retd.)
Registrar & Dean of Students

Encl: as above

Cc to:- Dr. Sudhir Kumar, Professor & Head, BT/BI Deptt., {for his information and records}

Defence Life Science Journal, Vol. 5, No. 1, January 2020, pp. 18-24, DOI : 10.14429/dlsj.5.14656

Effect of Altitude on the Phenology and Fruit Quality Attributes of Apricot (*Prunus armeniaca* L.) Fruits

Avilekh Naryal[#], Diskit Dolkar[#], Ashwani Kumar Bhardwaj[#], Anil Kant[@], O.P. Chaurasia[#], and Tsering Stobdan[#,*]

[#]*DRDO-Defence Institute of High Altitude Research, Leh-Ladakh - 194 101, India*
[@]*Jaypee University of Information Technology, Wakhnaghat, Solan - 173 215, India*
[*]*E-mail: stobdan@dihar.drdo.in*

ABSTRACT

Consumer concern about poor taste of fresh apricots is increasing and knowledge about the more suitable production requirement is essential. Genetic component influencing apricots quality is well known. However, there is limited information on environmental effect on fruit quality. This study aims to evaluate influence of altitude on phenological and fruit quality characters of apricot genotypes. Fruits from 162 genotype were collected from nine locations from 3006-3346 m asl in trans-Himalaya. The altitude had a marked influence on date of flowering, fruit weight, moisture and TSS content. For every 100 m increase in altitude, flowering and fruit ripening delayed by 3.3 and 7.1 day, respectively. Inverse relationship between altitude and fruit weight (R^2=0.310) was observed. For every 100 m increase in altitude the fruit weight decrease by 0.5 g. Fruit moisture content decreased significantly with increase in elevation (R^2=0.585). Decrease in moisture content was 1.9% for every 100 m increase in elevation. Altitude showed linear relationship with fruit TSS content (R^2=0.726). For every 100 m increase in altitude, the fruit TSS increased by 1.2ºBrix. Knowledge from the present study on the impact of altitude on fruit quality characters provides a useful guide for selecting orchard location towards improving fruit quality.

Keywords: Elevation; Flowering; Ladakh; Quality improvement; Sweetness

1. INTRODUCTION

Apricot (*Prunus armeniaca* L.) is an important temperate fruit tree species. While consumers cherish the aromatic flavor and beauty of high-quality apricot fruits, there are often complaints about the suboptimal fruit quality in the marketplace[1]. A lack of sweetness or sugar in purchased apricot fruit is among the most common of the consumer complaints[2]. Growing concern of the consumers for poor quality apricots needs serious consideration. Accordingly, breeding programs based on up-to-date scientific approaches are being taken to develop cultivars with high level of product quality[3]. However, fruit quality depends on two main components, genotype and environment. A great deal of research is carried out on the genetic component influencing apricots quality character[4-8]. However, there is limited information on environmental effect on fruit quality.

Genetic component determining the apricot fruit quality characters is well established. However, little is known about the influence of environmental factor on fruit quality. Few studies on limited number of cultivars are available on the variation of fruit quality characters with reference to environmental factors. Güleryüz[9], *et al*. found that when Hasenbey and Şekerpare cultivars were grown at two different altitudes (850-1200; 1150-1600 m asl), the fruit TSS increases and fruit size decreases at higher elevation. Olmez[10], *et al*. evaluated three apricot cultivars at 731, 855 and 1115 m asl. No linear relation was observed between increasing altitude and fruit quality traits such as TSS and fruit weight. Recently we have demonstrated the importance of environmental factors for determining sugar content and sugar profile in dried apricots[11]. The geographical elevation had no influence on kernel amygdalin content[12]. In view of the contrasting results from limited studies, it is felt that more studies involving larger number of samples across different altitudes are needed to better understand the altitude effects on apricot fruit quality characters. While most of the studied were conducted below 1500 m asl, there is little information from regions above 3000 m asl. The knowledge on altitudinal effect on fruit quality is vital since it guide us in selecting orchard location towards improving fruit quality.

Altitudinal gradients are among the most powerful 'natural environments' for testing effect of environmental factors on fruit quality characters. Steep changes in temperature, moisture, atmospheric pressure, ultraviolet radiation, hours of sunshine, wind, season length and geology occurs along altitudinal gradient[13]. Accordingly, to obtain a better understanding of the effect of climatic variables, this study was conducted to study the effect of growing locations with different altitudes on fruit quality characters of fresh apricots. In particular, great attention was given to phenology, sugar content and fruit size on account of large variation in date of flowering (25-114

Received : 12 June 2019, Revised : 14 January 2020
Accepted : 04 February 2020, Online published : 19 February 2020

Julian days), total soluble solids (TSS) content (9.3-37.9ºBrix) and fruit weight (5.6-105.3 g) reported from different apricot growing regions around the world.

2. MATERIALS AND METHODS

2.1 Study Sites and Materials

Apricot fruits were collected from nine villages spread across trans-Himalayan Ladakh region. Apricots are being grown in the region either as individual trees or small groups of trees in traditional way without the use of chemical fertilizer and pesticides. Majority of the apricots in Ladakh are raised from seedlings. The altitude of collection sites ranged from 3006 to 3346 m above sea level (asl) (Table 1). The mean minimum and maximum temperature of the region recorded daily during cropping season (April-October) in 2015 at an experimental orchard (elevation 3340 m) was 5.8±5.2 ºC and 18.8±5.4 ºC, respectively, while the mean minimum and maximum relative humidity was 22.1±2.0% and 28.3±2.7%, respectively. The light intensity at noon in open field was 131194±43574 lux[8]. The annual precipitation of the region is less than 200 mm of which more than 70% is in the form of snowfall[14]. From each site equal numbers of genotypes differing in seed stone colour (i.e brown and white) and kernel taste (i.e sweet and bitter) were selected as previously described[8]. From each site six samples each with brown stone with a bitter kernel, brown stone with a sweet kernel, and white stone with a sweet kernel were selected.

2.2 Phenological, Pomological and Quality Traits

Flowering date was evaluated when 50% of the floral buds reached the full bloom stage and expressed in Julian days (JD) (natural days from 1st January). Date of fruit harvesting from each genotype was determined by a panel of four assessors who identified the best ripening stage for fresh consumption based on colour, taste and fruit firmness. Fruit samples (50 fruits per tree) were randomly handpicked at eating maturity stage. Harvested fruits were immediately transported to the laboratory and pomological traits and standard fruit quality parameters (Table 2) were determined on the day of harvest. Stone and fruit weight were measured with an electronic balance to an accuracy of 0.001 g. Dimensional properties were measured with a digimatic calliper (CD-6"CS, Mitutoyo, Japan) to an accuracy of 0.01 mm. TSS were measured with the refractometer (ATAGO, Tokyo, Japan) and values were corrected at 20ºC. Fruit moisture content was determined using oven drying method at 50°C till constant weight and expressed as percentage of fresh weight. The perimeter of the blush area was drawn on a tracing paper and used to determine fruit blush area using graph paper[15].

2.3 Statistical Analysis

Pomological and quality traits were performed in triplicate. Each replicate consisted of three fruits. The experimental results were expressed as the mean ± standard deviation (SD) using statistical analysis with SPSS 16 (Statistical Program for Social Sciences, SPSS Corporation, Chicago, Illinois, USA) and MS Excel 2007. One way analysis of variance (ANOVA) and post hoc analysis with 2-sided Tukey's HSD at $p \leq 0.05$ level were performed. Pearson's correlation analysis was performed to find correlation between the variables.

3. RESULTS AND DISCUSSION

3.1 Altitude Effects on Flowering Phenology and Fruit Ripening Date

The altitude of growing location had a marked influence on the flowering phenology (Table 2). Linear relationship between date of flowering and increasing elevation was observed (R^2=0.914, $p \leq 0.001$) (Fig. 1(a)). For every 100 m increase in geographical elevation, flowering delayed by 3.3 days. Flowering dates showed marked variability and ranged from averaged 104.0 at 3006 m to 116 JD at 3346 m elevation (Fig. 2). Delay in flowering in higher altitude regions may be associated with decreasing temperature. Significant differences in flowering dates in apricot have been reported from different regions. Blooming dates of apricots in Italy and Spain ranged from 25-80 JD[16], 79.9-88.7 JD in Serbia[17] and 111-114 JD in Ladakh[8]. Results of the present study suggested that differences in date of flowering are largely due to environmental factors associated with altitude. Late blossoming is an important factor in high altitude regions experiencing spring frost. It protects the flowers from damage caused by spring frost. Similarly, linear relationship between harvest date and increasing elevation was observed (R^2=0.820, $p \leq 0.001$). For every 100 m increase in elevation, fruit ripening delayed by 7.1 days. Apricots from different regions are known for marked differences in date

Table 1. Geographical locations and sampling sites of apricots in trans-Himalaya

Sampling localities	Population ID	Latitude (N)	Longitude (E)	Altitude (m) (asl)	Sample size
Takmachik	TAK	34° 23.522"	76° 45.981"	3006	18
Domkhar	DOM	34° 23.522"	76° 45.984"	3008	18
Khalsi	KLS	34° 19.166"	76° 52.564"	3011	18
Nurla	NUR	34° 17.941'	76° 59.490"	3046	18
Saspol	SPL	34° 14.251"	77° 10.194"	3116	18
Nimmu	NMU	34° 11.357"	77° 20.437"	3190	18
Tamisgam	TSG	34° 19.444"	76° 59.463"	3241	18
Tia Khaling	TIA	34° 19.979"	76° 58.685"	3311	18
Leh	LEH	34° 08.267"	77° 34.378"	3346	18

of fruit ripening. Apricots are harvested in May to June in Greece and America[18] while cultivars and selections grown in Spain attain maturity in mid-May and late June[19]. Fruits attain maturity in late June and early July in Anatolia, Turkey[20] while those from Lake Van Region, Turkey are harvested in late July to early August[21]. In trans-Himalayan Ladakh fruits are harvested between August and early September[8]. Results of the present study highlighted that difference in fruit harvesting dates from different apricot growing regions is primarily due to environmental effects.

3.2 Altitude Effects on Pomological Traits

Table 2 presents pomological attributes of 162 apricot genotypes collected from nine locations. Fruit weight ranged from 5.3-52.5 g. In comparison, fruit weight among the promising genotypes of the Lake Van region ranged from 24.2-48.3 g[21]. Drogoudi[18], *et al*. reported 36.5-105.3 g fruit in 29 cultivars/hybrids of Greek and American origin. Milošević[22], *et al*. reported fruit weight of 49.1-81.5 g in promising apricot resources in Central Serbia. The fruit weight of 21 apricot cultivars collected from Canada, Czech Republic, Ukraine and USA ranged between 28.1-77.7 g with mean weight of 42.44 g[4]. We observed inverse relationship between altitude and fruit weight (R^2=0.310, $p\leq$0.1) (Fig. 1(b)). For every 100 m increase in elevation the fruit weight decrease by 0.5 gm. Small fruit size may also be due to genotypic effect. Ledbetter[1], *et al*. observed that when Central Asian apricot germplasm were grown in California conditions the fruit remained small (9.4 g), which underline importance of genotypic effect on fruit size. Wide variability in fruit weight (5.6-105.3 g) among cultivated apricot is therefore the result of both genotypic and environmental factors.

Our data partially agree with the observations made by Olmez[10], *et al*. who reported that only one out of three apricot cultivars studied showed decreasing fruit size with increasing elevation. However, in the two other cultivars, fruit size increases with increasing from 731 to 855 m asl and then showed declining trend when grown at 1115 m asl. Our data is in contrast with studied on other fruits such as fig[23] and chestnut[24] where increase in fruit size was observed with increasing elevation. In sweet cherry and mandarin, no relation was found between fruit weight and altitude[25,26]. Contrasting results in the previous studies could be due to difference in altitude of studied areas. While most of the studied were conducted below 1500 m asl, our study focus of high altitude regions above 3000 m asl.

Fruit moisture content decreased significantly with increase in elevation (R^2=0.585, $p\leq$0.05) (Fig. 1(c)). Decrease in moisture content was 1.9% for every 100 m increase in elevation. Fruits of trans-Himalaya, therefore, have lower moisture content as compared to previous reports[27,28], which may be because of drier climatic conditions in higher elevations. Fruit moisture content is an important factor at commercial maturity stage. Apricots with high moisture content are sensitive to transportation and handling. High moisture contents cause fruit to spoil earlier[27]. Blush area and seed dimensional properties showed inverse relationship with altitude, however, the values are not highly significant ($p\leq$0.05).

3.3 Altitude Effects on Fruit TSS Content

Altitude showed linear relationship with fruit TSS content (R^2=0.726, $p\leq$0.01) (Fig.1(d)). For every 100 m increase in elevation, the fruit TSS increased by 1.2ºBrix. Our result is in agreement with reports on mandarin[26], where high TSS

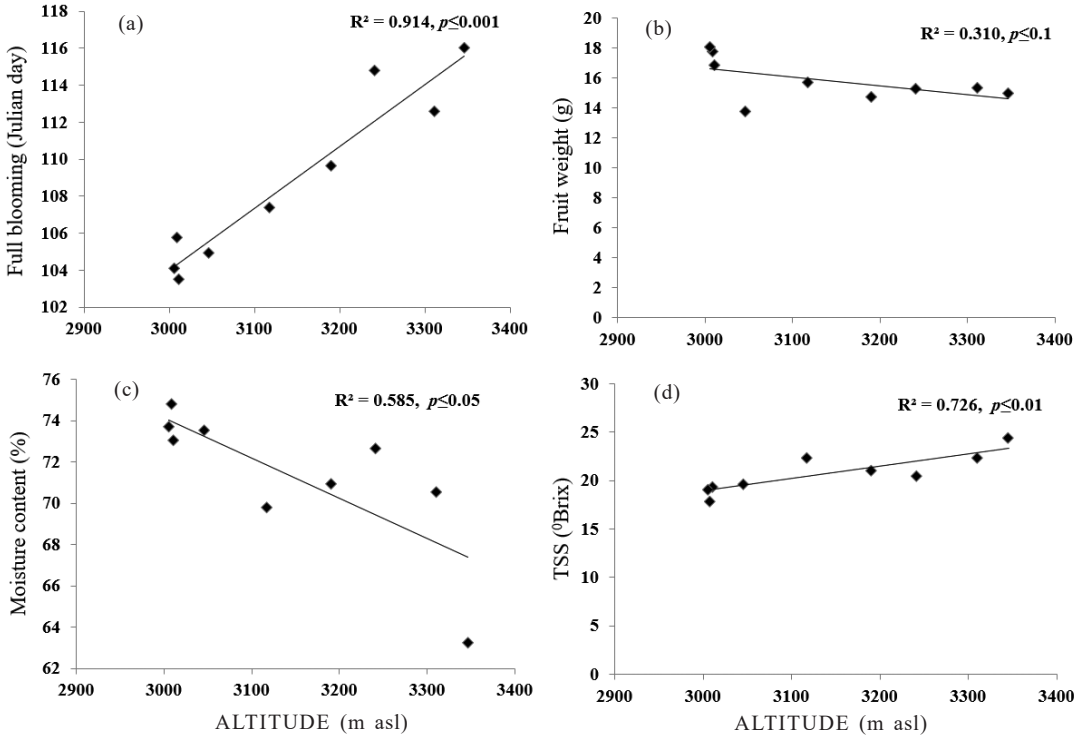

Figure 1. Altitudinal variation in apricot (a) flowering, (b) fruit weight, (c) fruit moisture contents, and (d) fruit TSS in trans-Himalayan region.

was found at higher altitude. However, inverse relation was observed when 'Wonderful' pomegranate cultivar was harvested at commercial harvest stage at 222, 662 and 898 m asl[29]. Similarly, low altitude (229 m asl) persimmon fruits have higher TSS content than those from a high elevation (770 m asl) region[30]. Altitude has no influence on fruit TSS content in sweet cherry[23] and blueberry fruits[31].

The evaluated genotypes showed marked variability in TSS content ranging from 10.7-37.6ºBrix with average value of 20.7±5.1 (Fig. 3). In comparison, the value ranged from 11-27ºBrix among 128 apricot cultivars and types in Turkey[20], 15.7-18.9ºBrix in 14 genotypes grown in Central Serbia[22], 10.6-16.3ºBrix in 43 selections and cultivars grown in Spain[19], 9.3-

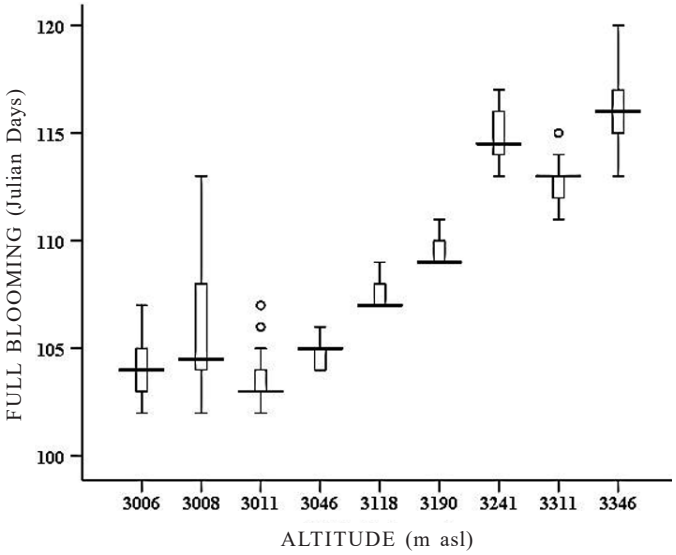

Figure 2. Box plot distribution of date of apricot full bloom along altitudinal gradient in trans-Himalayan region. The plot represents the minimum and maximum value (whiskers), the first and third quartile (box), the median (midline).

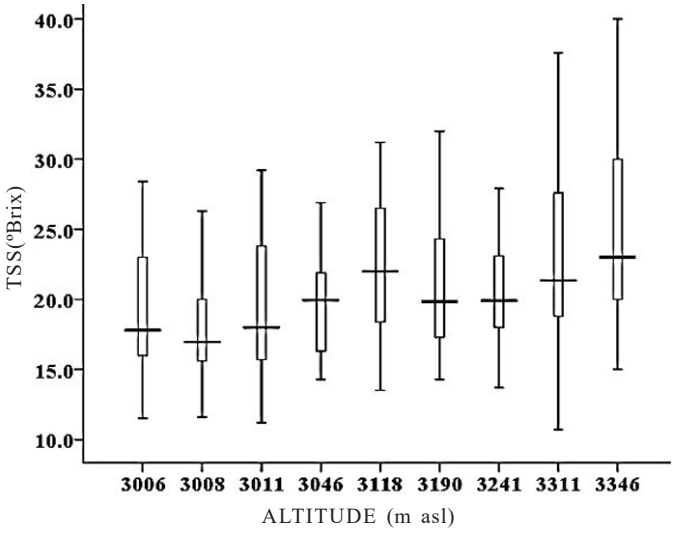

Figure 3. Box plot distribution of apricot fruit TSS along altitudinal gradient in trans-Himalayan region. The plot represents the minimum and maximum value (whiskers), the first and third quartile (box), the median (midline).

Table 2. Morphometric and pomological characteristics of apricot fruits of trans-Himalaya

Location	FB	FrW	SW	FW	TSS	MC	BA	FrL	FrWd	SL	SWd	ST	SCT
TAK	104.1±1.6^{b}	18.1±8.5^{d}	2.0±0.5^{d}	16.0±8.2^{c}	19.1±4.7^{ab}	73.7±5.8^{de}	163.4±294.8^{ab}	30.1±5.7^{cd}	32.1±5.0^{e}	21.1±2.3^{cd}	18.0±2.2^{e}	12.2±2.1^{b}	1.5±0.3^{a}
DOM	105.8±3.1^{d}	17.8±5.4^{cd}	1.9±0.4^{bcd}	15.9±5.1^{c}	17.8±3.7^{a}	74.8±4.2^{e}	225.7±218.7^{ab}	28.0±4.0^{abc}	30.2±4.0^{abc}	19.5±2.0^{ab}	16.5±1.9^{ab}	11.5±1.7^{ab}	1.5±0.3^{a}
KLS	103.5±1.4^{a}	16.8±3.6^{bcd}	1.9±0.4^{cd}	14.9±3.3^{bc}	19.4±5.0^{ab}	73.0±3.8^{cde}	265.6±236.2^{b}	30.5±3.8^{d}	30.9±3.1^{bc}	21.7±3.5^{d}	17.2±2.8^{abc}	11.9±2.6^{ab}	1.4±0.3^{a}
NUR	104.9±0.7^{c}	13.8±2.0^{a}	1.6±0.3^{a}	12.1±1.9^{a}	19.6±3.5^{abc}	73.6±3.2^{cde}	238.8±217.1^{ab}	27.3±1.9^{a}	29.2±1.7^{ab}	19.0±1.6^{a}	16.9±1.4^{abc}	11.6±1.6^{ab}	1.4±0.3^{a}
SPL	107.4±0.6^{e}	15.7±3.5^{abcd}	1.8±0.4^{abcd}	13.9±3.2^{abc}	22.3±5.2^{cd}	69.8±4.8^{b}	182.3±192.9^{ab}	27.9±2.7^{ab}	30.4±2.4^{bc}	19.8±1.4^{ab}	17.1±1.3^{abc}	11.6±1.6^{ab}	1.4±0.3^{a}
NMU	109.6±0.7^{f}	14.7±4.7^{ab}	1.7±0.3^{ab}	13.1±4.5^{ab}	21.1±4.4^{bc}	71.0±3.5^{bcd}	223.5±200.9^{ab}	28.1±2.8^{abc}	29.3±3.1^{ab}	19.6±1.4^{ab}	16.6±1.6^{ab}	11.1±1.5^{ab}	1.5±0.3^{a}
TSG	114.9±1.5^{h}	15.3±3.9^{abcd}	1.8±0.5^{abc}	13.5±3.5^{abc}	20.5±3.4^{abc}	72.7±3.5^{bcde}	230.8±237.1^{ab}	28.0±2.7^{abc}	29.3±2.2^{ab}	20.2±2.4^{abc}	16.8±1.6^{ab}	11.1±1.3^{ab}	1.5±0.4^{a}
TIA	112.7±1.0^{g}	15.3±5.1^{abcd}	1.7±0.5^{abc}	13.6±4.7^{abc}	22.3±6.0^{cd}	70.5±4.0^{bc}	154.6±220.8^{ab}	27.7±3.5^{a}	28.3±3.8^{a}	19.6±2.0^{ab}	16.3±1.9^{a}	11.1±1.7^{a}	1.3±0.2^{a}
LEH	116.0±1.5^{i}	14.9±3.5^{abc}	1.8±0.4^{abc}	13.2±3.3^{ab}	24.3±6.2^{d}	63.3±10.6^{a}	128.7±182.0^{a}	30.0±3.6^{bcd}	30.1±3.5^{ab}	20.6±2.2^{bcd}	17.5±2.4^{bc}	11.7±1.8^{ab}	1.4±0.2^{a}
Average	108.8±4.7	15.8±4.9	1.8±0.4	14.0±4.6	20.7±5.1	71.4±6.2	201.5±226.7	28.6±3.7	30.0±3.5	20.1±2.3	17.0±2.0	11.5±1.8	1.4±0.3

Values represented mean ± SD; for each column different lowercase letters indicate significantly differ ($P \leq 0.05$)
FB: date of full bloom (JD); FrW: fruit weight (g); SW: Seed weight (g); FW: flesh weight (g); TSS: total soluble solids (ºBrix); MC: Moisture content (%); BA: blush area (mm^2); FrL: fruit length (mm); FrWd: fruit width (mm); SL: seed length (mm); SWd: seed width (mm); ST: seed thickness (mm); SCT: seed coat thickness (mm).

18.7ºBrix in 55 cultivars at germplasm collection in Spain[32], 8.7-22.4ºBrix in 51 genotypes from germplasm collection in France[33], 12.3-15.8ºBrix in 18 genotypes belonging to the Italian and international germplasm[6], and 12.7-20.0ºBrix in six cultivars in Pakistan[27]. The present study showed that high altitudes are favourable for production of apricot fruit with high TSS content. Besides environmental factors, the genotypic factor of Central Asian apricots may also be responsible for higher sugar content. Higher fruit sugar content was observed when apricot germplasm from northern Pakistan were grown in California[1] suggesting that Central Asian apricot germplasm have inherent higher sugar content.

In trans-Himalayan Ladakh region apricot trees are cultivated in irrigated plantation. Irrigation water requirements are high due to high evaporative demand and scarcity of rainfall. Therefore, deficit irrigation is not uncommon in Ladakh, which may be a favourable factor for high TSS content of apricots. Studies have shown that deficit irrigation results in apricots with higher TSS[34]. Dry climatic conditions with low rainfall in high altitude regions appeared to be favourable factor for fruits with high sugar content. Previous research has reported that fruit TSS is associated with a dry climate in cactus pear[35]. Fruits from dry locations are often sweeter than those from humid or irrigated land[36]. Therefore, dry climatic conditions seem to be one of the important factors responsible for high TSS content of apricots of high altitude regions.

3.4 Correlation among Variables

Table 3 present correlations among variables. Seed stone colour showed significant correlation with kernel taste (r = 0.506), fruit weight (r = 0.530) and TSS (r = 0.451). Therefore, apricots with white stone are associated with sweet kernel, larger fruit and high TSS content. Sweet kernel taste is significantly correlated with fruit weight (r = 0.426) and TSS (r = 0.463). Date of flowering is significantly correlated with harvest date (r = 0.690). Fruit harvest date is positively correlated with TSS (r = 0.324). The result suggested that late maturing apricot genotypes have higher TSS content. Fruit weight showed correlations with TSS (r = 0.177). However, Caliskan[7], *et al*. did not find correlations between fruit weight and TSS.

4. CONCLUSIONS

The knowledge on altitudinal effect on apricot fruit quality is vital since it guide us in selecting orchard location towards improving fruit quality. The geographic elevation had a marked influence on flowering, fruit weight, moisture and TSS content. At higher elevation delayed flowering and fruit ripening occurs, and fruit remains smaller with low moisture content. Apricots from higher altitude regions are sweeter with high sugar content as compared to those grown at lower elevation. Dry climatic conditions with low rainfall appear to be favourable factor for fruits with high sugar content in high altitude regions.

Table 3. Pearson's correlation coefficients of fruit quality characteristics

	SC	KT	FB	HD	FrW	SW	FW	FW/SW	TSS	MC	BA	FrL	FrWd	SL	SWd	ST	SCT
SC	1	.506**	-.098*	.035	.531**	.375**	.530**	.327**	.451**	-.203**	.312**	.309**	.366**	-.044	.257**	.274**	-.208**
KT		1	-.015	.057	.421**	.228**	.426**	.356**	.463**	-.148**	.371**	.190**	.224**	.001	.046	.067	.136**
FB			1	.690**	-.154**	-.103*	-.154**	-.093*	.218**	-.335**	-.085	-.046	-.175**	-.014	-.063	-.121**	-.010
HD				1	-.120**	-.142**	-.115*	-.006	.324**	-.322**	-.032	-.059	-.210**	-.095*	-.123**	-.114*	-.121**
FrW					1	.694**	.998**	.609**	.177**	.098*	.284**	.673**	.726**	.332**	.426**	.274**	.075
SW						1	.644**	-.123**	.083	-.051	.016	.562**	.561**	.492**	.586**	.379**	.261**
FW							1	.659**	.180**	.109*	.300**	.663**	.719**	.307**	.398**	.256**	.055
FW/SW								1	.194**	.177**	.362**	.321**	.397**	-.076	-.040	-.032	-.161**
TSS									1	-.559**	.132**	.111*	.119**	-.070	.058	.102*	-.113*
MC										1	-.035	-.023	.053	-.031	-.082	-.100*	.059
BA											1	.239**	.268**	.061	.067	.181**	-.176**
FrL												1	.759**	.679**	.574**	.325**	.195**
FrWd													1	.454**	.648**	.416**	.169**
SL														1	.621**	.315**	.334**
SWd															1	.582**	.281**
ST																1	.091*
SCT																	1

* Significant at $p \leq 0.05$; ** Significant at $p \leq 0.01$;
SC: Stone colour; KT: kernel taste; FB: date of full bloom; HD: date of harvest; FrW: fruit weight; SW: Seed weight; FW: flesh weight; FW/SW: flesh and seed weight ratio; TSS: total soluble solids; MC: moisture content; BA: blush area; FrL: fruit length; FrWd: fruit width: SL: seed length; SWd: seed width; ST: seed thickness; SCT: seed coat thickness.

Conflict of Interest: None

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Gender-specific seasonal pattern and altitudinal variation in freeze tolerance responses of Seabuckthorn (*Hippophae rhamnoides* L.)

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Abstract.
BACKGROUND: In dioecious species the morphological and physiological adjustment to cold and freezing conditions may differ significantly between male and female individuals due to greater reproductive effort by females.
OBJECTIVES: To assess the importance of gender-specific responses of *H. rhamnoides* of cold acclimation and freeze tolerance in trans-Himalayan environments.
METHOD: We measured the proline contents in leaves and shoots in male and female *H. rhamnoides* from mid August to mid December in standing crop.
RESULTS: Proline content in leaves showed a significant increasing trend from August to October followed by a steady decline from November onwards in both the genders. Progression in season from August to December is related linearly to the increase in proline contents in both male ($R^2 = 0.967$) and female ($R^2 = 0.926$) shoots. Increase in altitude (3202–3812 m amsl) of plant origin is related linearly to increase in proline contents in both male ($R^2 = 0.676$) and female ($R^2 = 0.858$) shoots. The overall proline contents in both leaves and shoots were significantly higher in male (112 ± 77, 143 ± 66 μM g^{-1}, respectively) than in female (87 ± 46, 119 ± 82 μM g^{-1}, respectively).
CONCLUSION: The study suggested sexually dissimilar responses to cold and freezing conditions in *H. rhamnoides* and that male possess a better self protection mechanism than female. Leaves developed tolerance against cold stress more quickly than shoots.

Keywords: Abiotic stress, acclimation, chilling, climate change, himalaya, proline

1. Introduction

Woody plants in temperate regions are subjected to large seasonal variations of temperature. Plants adapt to such conditions by evolving annual growth cycle that alternates between active shoot growth and vegetative dormancy closely synchronized with the seasonal changes [1]. Most plant species have evolved a degree of cold tolerance, the extent of which is typically dependent on combination of the minimum temperature experienced and the length of exposure to cold stress [2]. Overwintering temperate plant species such as Seabuckthorn (*Hippophae rhamnoides* L.) are able to resist low temperature and freezing. Generally, in these species the level

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of freezing tolerance is season-dependent and can be modulated by a prior period of acclimation (pre-hardening) at low non-freezing temperatures, during which time a number of morphological, physiological, and molecular changes occur [3]. The full degree of tolerance (hardening) is achieved thereafter when plants are exposed to a period of sub-zero temperature. It is well documented that accumulation of low-molecular weight compounds is observed during exposure to low temperature. Changes in water-soluble carbohydrate [4] or in free amino acids, especially proline [5, 6], are associated with cold acclimation and acquisition of frost tolerance. It is suggested that proline might be involved in osmoregulation and in the protection of proteins against dehydration [7], membrane stabilization [8] or regulation of certain enzymatic systems [9] during low temperature stress.

The ability of plants to adapt to and survive freezing temperatures has many facets, which are often species specific, and is the result of the response to many environmental cues, rather than just low temperature [10]. However, most of the freeze tolerance responses in plants are studied in controlled conditions in growth chambers. There are significant differences between natural and artificial cold acclimations. Plants which have been cold acclimated in growth chambers may respond differently than those acclimated naturally [11, 12]. Varying diurnal temperatures that produce mixed signals in the field are in contrast to constant temperatures in a growth chamber. Any research designed to explore and understand cold hardiness should be verified in the context of the physiology, growth habit and life cycle of the plant grown under natural conditions [10].

In dioecious species the morphological and physiological adjustment to cold and freezing conditions may differ significantly between male and female individuals due to greater reproductive effort by females. The cost of reproduction involves prioritization of resources in fruit development rather than in vegetative growth or protection in females. A major investment in reproduction is generally associated with the disadvantage in terms of oxidative stress and cellular injuries, particularly under adverse conditions [13]. Not accounting for sexual variation could lead to incorrect assessment of a species response to frost. However, to the best of our knowledge no studies have been conducted that have addressed the relative importance of the gender of the plant for freeze tolerance in natural conditions particularly in the trans-Himalaya.

Hippophae rhamnoides L. is an ecologically and economically important dioecious plant. It is found in a large altitudinal range, from the sea shores in Europe to over 4694 m in trans-Himalayan Ladakh. Development of freeze tolerance is strongly influenced by gender and ecotype in this species [14]. Studies conducted in controlled conditions where plants were exposed to cold stress conditions (4°C for 0–24 h) suggested that males are more responsive to exposure to low temperature, and resulted in earlier cold acclimation and higher freeze tolerance than females [14, 15]. However, freeze tolerance of *H. rhamnoides* in natural conditions has not been investigated in concert with measurements of their progeny in common garden experiments. *H. rhamnoides* are easy to propagate by stem cuttings, and the availability of clonal material facilitates the testing of identical genotypes under different conditions. *H. rhamnoides*, therefore, presents an excellent opportunity to investigate the relative contributions of environmental and gender factors to the relationship between freeze tolerance and altitude. Therefore, the aim of this study was to assess the importance of gender-specific responses of *H. rhamnoides* on cold acclimation and freeze tolerance in trans-Himalayan environments. Proline contents were analyzed to use as a marker for freeze tolerance in standing crop in natural conditions. The research included two components: (a) field studies along an altitudinal gradient, and (b) common-garden approach, in which a large number of cuttings from several male and female shrubs collected along an altitudinal gradient were planted in an experimental plot. We expected to find gender differences and strong altitudinal variation for freeze tolerance.

2. Materials and Methods

2.1. Study site

We collected *H. rhamnoides* subsp. *turkestanica* from seven natural sites along an altitudinal gradient from 3202 to 3812 m amsl in trans-Himalayan Ladakh region. Common-garden experiment was carried out at an

experimental farm (34°08.2'N; 77°34.3'E, 3350 m amsl) on a horizontal site with direct sunshine at Defence Institute of High Altitude Research (DIHAR) in trans-Himalayan Ladakh, India. The mean maximum and minimum temperature during 2014 and 2015 recorded at DIHAR was 12.9 ± 8.8°C and -0.2 ± 9.0°C, respectively. The mean monthly temperature was highest in July (25.6°C), and lowest in January (–13.2°C). The mean maximum and minimum relative humidity was 31.0 ± 4.3% and 24.7 ± 3.7%, respectively. The average annual precipitation was 163 mm.

2.2. Leaf and shoot materials

In December 2014, we collected a single branch from 10 male and 10 female adult *H. rhamnoides* at each site in natural field condition. All branches were collected on the sunny side of the shrub. Fully grown leaf and shoot samples were collected from each branch for measurement of proline contents. Between 04 and 14 April 2015, dormant cuttings of pencil thickness were taken from each shrub and planted at an experimental farm at DIHAR. Fully expanded leaf and shoot samples were collected on 15th of every month from August to December 2015 from each of the rooted plants for measurement of proline contents in garden-grown plants.

2.3. Proline contents

The proline contents was determined using the method described earlier [16]. Proline was extracted from 0.5 g fresh weight plant material homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate was filtered through Whatman # 2 filter paper. 2 ml of filtrate was mixed with 2 ml acid-ninhdrin and 2 ml of glacial acetic acid in a test tube for 1 h at 100°C, and the reaction was terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene, mixed vigorously for 15–20 sec. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and absorbance at 520 nm was recorded in a micro-plate reader (SpectroMax M2 e, Molecular Devices, Sunnyvale, CA, United States) using toluene for the blank. The proline concentration was determined from a standard curve and calculated on fresh weight basis as follows:

[(μg proline/ml × ml toluene)/115.5 μg/μmole]/[(g sample)/5]=μmoles proline/g fresh weight material.

2.4. Statistical analysis

In all the experiments each data point was the mean of three replicates and comparisons with P-values ≤ 0.05 were considered significantly different. All statistical analysis were performed using SPSS (Statistical Program for Social Sciences, SPSS Corporation, Chicago, Illinois, USA) The values for the parameters were subjected to one-way analysis of variance and the mean differences were compared by *post hoc* analysis with 2-sided Tukey's HSD to check significant differences between different months. Student's *t* test was used to compare significant difference between male and female in each month.

3. Results

3.1. Gender-specific seasonal pattern in freeze tolerance

Seasonal pattern in acclimation and freeze tolerance is presented in Table 1. Proline contents in leaves and shoots varied significantly during the sampling period. The proline contents in leaves showed a significant increasing trend from August to October followed by a steady decline from November onwards (Table 1) in both gender. However, the shoot proline contents showed a steady increasing trend from August to December.

Table 1
Seasonal pattern in proline contents as a measure of cold hardiness in *Hippophae rhamnoides* originated from different altitude in year 2015

Tissue	Date	Proline Contents ($\mu M\ g^{-1}FW$)	
		Male	Female
Leaves	15 Aug	0.3 ± 0.2^{a}	0.3 ± 0.2^{a}
	15 Sept	26.8 ± 17.1^{b}	32.7 ± 23.5^{ab}
	15 Oct	234.3 ± 76.0^{d}***	130.7 ± 38.9^{cd}
	15 Nov	71.3 ± 23.5^{c}	69.1 ± 20.9^{b}
	15 Dec	46.9 ± 13.7^{bc}	31.8 ± 7.1^{ab}
Shoot	15 Aug	0.6 ± 0.4^{a}	0.5 ± 0.4^{a}
	15 Sept	88.1 ± 72.0^{c}	91.0 ± 62.7^{bc}
	15 Oct	219.2 ± 135.9^{d}***	108.5 ± 77.7^{bc}
	15 Nov	237.5 ± 113.1^{d}*	164.0 ± 120.4^{c}
	15 Dec	361.7 ± 158.6^{d}*	298.2 ± 132.5^{d}

Values represented as mean ± SD. For each column, different lowercase letters indicate significantly different at $p \leq 0.05$, as measured by 2-sided Tukey's HSD. *value significantly higher than that of opposite sex at $p \leq 0.01$, ***value significantly higher than that of opposite sex at $p \leq 0.001$.

Table 2
Minimum temperature recorded at night at the experimental site in trans-Himalayan region (3350 m amsl) in year 2015

Period	Mean temperature (°C)		Number of days minimum temperature recorded at			
	Min	Max	Above 16°C	15 to 6°C	5 to 0°C	Below –1°C
16 Jul-15 Aug	12.5 ± 1.9	26.0 ± 2.2	2	29	0	0
16 Aug-15 Sept	9.1 ± 3.1	22.1 ± 2.5	0	27	4	0
16 Sept-15 Oct	3.0 ± 2.7	16.6 ± 2.3	0	5	25	0
16 Oct-15 Nov	–2.6 ± 3.4	10.5 ± 2.8	0	0	8	23
16 Nov-15 Dec	–8.3 ± 3.7	6.1 ± 3.7	0	0	0	30

Progression in season from August to December is related linearly to the increase in proline contents in both male ($R^2 = 0.967$) and female ($R^2 = 0.926$) shoot. Leaves of male plants had significantly higher proline contents ($234 \pm 76\ \mu M\ g^{-1}$) than female ($131 \pm 39\ \mu M\ g^{-1}$) in October ($P \leq 0.001$, Student's *t*-test). However, in shoot the proline contents remained significantly higher in male than female from October to December (Table 1). Throughout the sampling period the proline content in shoots remained significantly higher than that in leaves except in October. Acclimation began in September with significant increased in proline contents in both leaves and shoots. Four incidents of temperatures between 0°C to 5°C occurred during the period (Table 2).

3.2. Altitudinal variation in freeze tolerance

Altitude of plant origin did not have a significant impact on leaves proline contents in both male and female *H. rhamnoides* (Table 3). No increasing or decreasing trend was observed in leaves proline contents with increasing altitude (Table 3). However, increasing altitude is related linearly to increase in proline contents in both male ($R^2 = 0.676$) and female ($R^2 = 0.858$) shoot. Males collected from 3340 m altitude contained significantly higher proline in both leaves and shoot than those of females ($P \leq 0.01$, Student's *t*-test). The overall proline contents in both leaves and shoot was significantly higher in male (112 ± 77, $143 \pm 66\ \mu M\ g^{-1}$, respectively) than female

Table 3
Altitudinal variation in proline contents in leaves and shoots as a measure of cold hardiness in *Hippophae rhamnoides* in December 2014 under natural field condition

Altitude (m asl) of origin	Proline Contents (μM g^{-1} FW)			
	Leaf		Shoot	
	Male	Female	Male	Female
3203 ± 5.6	67.4 ± 23.6[a]	64.9 ± 23.6[a]	84.2 ± 43.4[a]	59.6 ± 56.6[a]
3239 ± 5.0	145.8 ± 103.7[ab]	133.4 ± 61.4[b]	113.6 ± 48.2[ab]	73.0 ± 43.6[a]
3260 ± 4.6	67.6 ± 34.5[a]	64.1 ± 23.8[a]	121.3 ± 47.4[ab]	96.6 ± 66.1[abc]
3340 ± 8.7	177.5 ± 108.0[b**]	65.7 ± 30.3[a]	175.1 ± 75.8[b***]	75.0 ± 19.3[ab]
3464 ± 23.9	119.3 ± 23.7[ab]	101.4 ± 34.0[ab]	180.5 ± 65.9[b]	163.8 ± 53.8[bcd]
3636 ± 49.6	69.2 ± 25.4[a]	66.0 ± 24.8 [a]	165.0 ± 64.4[b]	166.3 ± 98.8 [cd]
3812 ± 24.8	134.6 ± 86.1[ab]	113.1 ± 55.6[ab]	159.6 ± 59.0[ab]	195.7 ± 90.8[d]
Mean	111.6 ± 77.4**	86.9 ± 46.0	142.9 ± 65.7***	118.6 ± 81.5

Values represented as mean ± SD. For each column, different lowercase letters indicate significantly different at $p \leq 0.05$, as measured by 2-sided Tukey's HSD. **Value significantly higher than that of opposite sex at $p \leq 0.01$.

(87 ± 46, 119 ± 82 μM g^{-1}, respectively). Within each sampling site, proline content was significantly lower in leaves than shoot irrespective of the gender. However, the opposite was observed in samples collected from 3239 ± 5 m amsl.

4. Discussion

H. rhamnoides has a great ability to withstand different environmental stresses. In this study, male and female *H. rhamnoides* were examined for proline accumulation during the process of cold acclimation and developing freeze tolerance. The study was conducted during their transition from active growth to dormant stage in field experiment running from August to December in natural conditions. Leaves and shoots were monitored in an attempt to characterize proline contents during the cold acclimation and frost tolerance process. The plants were able to acclimate to cold, as shown by increased proline contents, when exposed to cold stress. Acclimation began in September with significant increased in proline contents in both leaves and shoots. Four incidents of temperatures between 0°C to 5°C occurred during the period (Table 2). Different woody perennials acclimate differentially within a given temperature range [17]. In *Weigela* a range of cultivars acclimate late, with substantial hardening taking place concurrent with the minimum air temperature dropping below 5°C on several occasions [18]. Likewise, in two populations of *Leptospermum scoparium* the apparent threshold temperature for the onset of frost hardening was about 6°C [19]. Exposure of *Rhododendron* to 5°C in controlled condition is reported to confer cold tolerance [20]. In this study cold hardening occurred during mid October when temperatures below 5°C were observed on 25 days between mid September and mid October as marked by significant increase in proline contents in both leaves and shoots. Sub-zero temperature does not seem to be a prerequisite for hardening in *H. rhamnoides*. From mid October onwards sub-zero temperature was a common phenomenon and proline contents in shoot remained significantly high.

In this study, it was observed that plants exposed to temperature 0 to 5°C during mid September to mid October had significantly higher proline contents in both the sexes; however, males had much more proline contents than females. Increased proline contents in cold-stressed male suggest that males have a better osmoregulation mechanism than females, because proline are the major osmoregulation substances in the expanded leaves of many plants [21]. Proline is vital in preventing protein denaturation, a source for carbon and nitrogen, and for

acting as a hydroxyl radical scavenger [22, 23]. The increased proline found in male is a sign of better protection against environmental stress in male cells compared to female cells. These results suggest that response to cold and freezing by gender is significant and that males possess a better self protection mechanism than females in *H. rhamnoides*. The greater cold and freezing tolerance of leaves of male plants than females may be advantageous in terms of cold hardiness of the whole plant. Lennartsson and Ögren [24] suggested that even deciduous trees may benefit from maintaining leaves as long as possible during autumn to allow continued photosynthesis, which may be important in building up reserves needed for cold acclimation. Our observations that male and female *H. rhamnoides* respond differently to a change in environmental conditions display the importance of an unambiguous role of gender for assessing the response of dioecious species to climate change or any other experimental manipulations. Li et al. [25] observed that stressful environments have more negative impact on females and become male-biased in nature as resources become limited.

Plant organs varied in their response to cold stress - typically the leaves are much more sensitive than the shoots. Leaves respond more quickly than shoots when exposed to cold stress. These data clearly suggest that leaves are more sensitive to the environmental cues triggering acclimation than the shoot tissues. The result is in consistent with findings of Li et al. [26] where buds and leaves of Silver birch (*Betula pendula*) are found to be more responsive to the environmental cues than the stem tissue.

Altitude of plant origin demonstrated a significant impact on shoot proline content in *H. rhamnoides.* Linear relationship between increased proline contents and increasing altitude was observed. The results suggested that plants from higher altitude possess more effective mechanisms that prevent the frost damage. This result is in consistent with findings of Greer and Robinson [19] who reported that *Leptospermum scoparium* from high altitude origin are less affected by frost than that from low altitude. Similar result is reported in *Salix pentandra* [27].

5. Conclusion

Our results showed sexually different responses of *H. rhamnoides* to environmental cues in natural conditions. Females suffer more from negative effects of cold and freezing than males. Leaves respond more quickly than shoots when exposed to cold stress. We suggest that when *H. rhamnoides* is planted in cold regions, females should be provided with added protective measures to improve their chances for survival.

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Conflict of interest

The authors have no conflict of interest to report.

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RESEARCH REPORT

Sensory attributes and consumer appreciation of fresh apricots with white seed coats

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Abstract
Forty-seven apricot genotypes with white and brown seed coats were analyzed by both sensory and instrumental methods. The overall appreciation of apricots was mainly affected by attributes such as aroma, sweetness, juiciness, flesh color, stone color, and fruit shape and size. In terms of these attributes, fruits with white seed coats ranked first, scoring the highest hedonic score for overall appreciation (8.0 ± 0.2). They also had the highest total soluble solid (TSS) (27.5 ± 3.6°Brix), reducing sugar (17.5 ± 1.9%), and total sugar (20.3 ± 1.9%) values, while the moisture content (68.9 ± 8.3%) was the lowest among the analyzed genotypes. Consumers were attracted to the unique white seed coat phenotype. Relationships between data obtained by the sensory panel and instrumental methods were established. Overall appreciation showed positive significant relation with TSS ($R^2 = 0.177$, $p \leq 0.01$), TSS/total acid ($R^2 = 0.118$, $p \leq 0.05$), reducing sugar ($R^2 = 0.140$, $p \leq 0.01$), total sugar ($R^2 = 0.177$, $p \leq 0.01$), and fruit weight ($R^2 = 0.230$, $p \leq 0.001$). Statistically significant negative relation was observed between overall appreciation and fruit moisture content ($R^2 = 0.168$, $p \leq 0.01$). The study demonstrated that white seed coat phenotype can be considered a marker for high-quality apricots in terms of aroma, sweetness, juiciness, and overall appreciation.

Keywords Aroma · Fruit quality · Hedonic · *Prunus armeniaca* · Sweetness

1 Introduction

Apricot (*Prunus armeniaca* L.) is one of the most popular temperate fruit tree species, and the fruit are consumed mainly as fresh snacks (Bhuhn et al. 1991). While consumers cherish the beauty and aromatic flavor of high-quality apricots, there are often complaints about the suboptimal fruit quality in the marketplace (Ledbetter et al. 2006). Consumer concern about poor taste is increasing in certain European markets (Bassi and Selli 1990). Consumers are more disappointed with apricots than with fruit in general regarding average quality, freshness, and ripeness (Moreau-Rio 2006). A survey conducted showed that consumer satisfaction with the quality of apricots at a supermarket was the lowest compared to other fruit (Bhuhn et al. 1991). A lack of sugar or sweetness in purchased apricot fruit is among the most common of the consumer complaints (Moreau-Rio and Roty 1998). Consumers are attracted by the aesthetic features of modern apricot cultivars with large fruit size, orange color skin, and intense blush with firm fruit but are disappointed by the poor eating quality (Piagnani et al. 2013). Lack of awareness is yet another weak point of perceived apricot quality. In contrast to apple, in which varieties are well known by consumers, a survey conducted in France showed that fresh apricots could be considered a generic produce with 81% of the interviewers unable to cite a single variety (Moreau-Rio 2006). Since apricots are only available for fresh consumption in the market for a short period, it is difficult to build up a strong image of the product and call for consumer's loyalty to a specific phenotype as can be done for apples (Gatti et al. 2009). However, if an easily identifiable phenotype can be linked to high-quality apricots, consumer loyalty and a strong image of the product can be developed (Angmo et al. 2017).

Apricots of trans-Himalayan Ladakh are known for their quality, and the region has not witnessed introduction of cultivars from other regions. The gene pool is maintained

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due to geographical isolation and a natural high mountain barrier. Historically, the premium quality dried apricots, locally known as *Phating*, with high sugar and dry matter content, were one of the main trading commodities. However, in modern days, the popularity of Ladakhi apricots remains restricted to the region due to limited production (Angmo et al. 2017). Apricot, locally known as *Chuli*, is classified in Ladakh into two broad categories based on kernel taste. Fruit with a bitter kernel are called *Khante*, meaning bitter, while those with a sweet kernel are called *Ngarmo*, meaning sweet (Targais et al. 2011). The *Ngarmo* is further divided into two subgroups based on seed coat color. Fruits with white seed coats are called *Raktsey Karpo*, while those with brown seed coats are called *Nyarmo*. Large fruits are called *Chenmo*, meaning large, while small ones are called *Chun*, meaning small. Morphological diversity of apricots of the region is shown in Fig. 1. Apricots with white seed coats are unique to Ladakh and are associated with a sweet kernel and brightly colored fruit with high TSS. *Raktsey Karpo* fruits are known for their sweetness and are consumed fresh (Angmo et al. 2017). Accordingly, the purpose of this study was to determine whether white seed coat color, which is an easily identifying phenotype, can be linked to high-quality apricots for fresh consumption. Knowledge about sensory properties and consumer appreciation of apricots with an easily identifiable phenotype are important because they help create a strong image of the product and foster consumer loyalty to a specific phenotype.

Fig. 1 Morphological diversity of trans-Himalayan Ladakh apricots

2 Materials and methods

2.1 Study site and sample collection

Fruit samples were collected in 2016 from an experimental orchard (34°08.2′N; 77°34.3′E, elevation 3340 m) at the Defence Institute of High Altitude Research in trans-Himalayan Ladakh, India. The weather data of the orchard location, from May to October, are shown in Table 1. The orchard contained 12 rows, with 8 trees per row of genotypes representing the major germplasm from the Ladakh region. All genotypes were grafted on *Chuli* wild apricot rootstock. Trees were trained to the modified central leader system and planted at a spacing of 4 × 4 m. All trees were the same age (16–17 years old), and standard cultural practices were performed, without the use of chemical fertilizer and pesticides. Fruits of 47 genotypes were sampled from trees at eating maturity stage. Date of fruit harvesting from each genotype was determined by a panel of four assessors who identified the best ripening stage for fresh consumption based on fruit firmness, color, and taste. Fruit samples from different genotypes were grouped into six based on seed coat color, kernel taste, fruit size, and drying quality (Table 2). Seven genotypes fall under Group-I with white seed coats and sweet kernels; 5 genotypes fall under Group-II with brown seed coats, sweet kernels, and high drying quality characteristics; 9 genotypes fall under Group-III with brown seed coats, sweet kernels, and large fruit size; 16 genotypes fall under Group-IV with brown seed coats, sweet kernels, and small fruit size; 5 genotypes fall under Group-V with brown seed coats, bitter kernels, and large fruit size; and 5 genotypes under Group-VI with brown seed coats, bitter kernels, and small fruit size.

2.2 Analysis of pomological and quality traits

The pomological traits and standard fruit quality parameters (Tables 2, 3) were determined on the day of harvest. Fruit and stone weight were measured with an electronic balance (ER-120A, Afcoset, India) to an accuracy of 0.001 g.

Table 1 Weather data of the orchard location in trans-Himalayan Ladakh, from May to October (2016)

Month	Air temperature (°C)		Relative Humidity (%)		Average precipitation (mm)
	Min	Max	Min	Max	
May	4.9 ± 3.6	19.3 ± 2.0	21.2 ± 0.9	30.1 ± 2.0	Nil
June	11.5 ± 3.1	24.2 ± 2.3	20.3 ± 0.9	26.5 ± 1.8	Nil
July	14.0 ± 2.6	26.1 ± 2.6	20.0 ± 0.2	24.1 ± 1.3	Nil
August	13.4 ± 2.7	24.8 ± 3.0	20.2 ± 0.6	24.3 ± 1.4	8.4
September	7.7 ± 2.3	22.1 ± 2.3	20.6 ± 0.6	27.5 ± 1.1	Nil
October	-0.3 ± 3.5	15.0 ± 3.4	23.5 ± 1.6	31.0 ± 1.6	Nil

Table 2 Seed coat colour, kernel taste, fruit shape and colour of six groups of apricots of trans-Himalayan Ladakh

Group		Seed coat colour (%)	Kernel taste (%)	Fruit shape lateral view (%)	Fruit shape ventral view (%)	Fruit shape of apex (%)	Fruit skin colour (%)	Fruit flesh colour (%)
I	*Raktsey Karpo*	White-100.0	Sweet-100.0	Ovate-57.1	Ovate-57.1	Rounded-85.7	Yellowish-42.9	Cream-28.6
				Oblique rhombic-42.9	Oblong-14.3	Truncate-14.3	Yellow green-28.6	Light orange-57.1
					Elliptic-28.6		Light orange-28.6	Medium orange-14.3
II	*Halman*	Brown-100.0	Sweet-100.0	Circular-60.0	Elliptic-20.0	Rounded-40.0	Yellow green-20.0	Cream-20.0
				Obovate-20.0	Circular-80.0	Truncate-20.0	Light orange-20.0	Medium orange-40.0
				Oblique rhombic-20.0		Retuse-40.0	Medium orange-60.0	Dark orange-40.0
III	*Nyarmo Chemno*	Brown-100.0	Sweet-100.0	Ovate-33.3	Ovate-22.2	Acute-11.1	Yellowish-11.1	Cream-22.2
				Oblong-11.1	Oblong-11.1	Rounded-66.7	Yellow green-33.3	Light orange-22.2
				Circular-11.1	Elliptic-44.4	Truncate-11.1	Light orange-33.3	Medium orange-55.5
				Oblique rhombic-44.4	Circular-11.1	Retuse-11.1	Medium orange-22.2	
IV	*Nyarmo Chun*	Brown-100.0	Sweet-100.0	Triangular-6.2	Ovate-12.5	Acute-6.3	Yellowish-37.5	Cream-18.8
				Ovate-18.8	Oblong-31.2	Rounded-50.0	Yellow green-12.5	Light orange-43.7
				Oblong-31.2	Elliptic-31.2	Truncate-25.0	Light orange-31.2	Medium orange-18.8
				Circular-18.8	Circular-25.0	Retuse-18.7	Medium orange-18.8	Dark orange-18.8
				Oblique rhombic-25.0				
V	*Khante Chenmo*	Brown-100.0	Bitter-100.0	Circular-20.0	Ovate-40.0	Rounded-40.0	Yellowish-20.0	Light orange-60.0
				Oblique rhombic-80.0	Elliptic-20.0	Truncate-40.0	Yellow green-20.0	Medium orange-40.0
					Circular-40.0	Retuse-20.0	Light orange-30.0	
							Medium organge-30.0	
VI	*Khante Chun*	Brown-100.0	Bitter-100.0	Circular-20.0	Ovate-20.0	Rounded-40.0	Yellowish-20.0	Light orange-40.0
				Oblique rhombic-80.0	Elliptic-20.0	Truncate-20.0	Light orange-40.0	Medium orange-60.0
					Circular-60.0	Retuse-40.0	Medium orange-40.0	

Dimensional properties were measured with a digimatic calliper (CD-6″CS, Mitutoya, Japan) to an accuracy of 0.01 mm. Fruit firmness was measured with a penetrometer equipped with an 8-mm cylindrical plunger. The perimeter of the blush area was drawn on a tracing paper and used to determine fruit blush area using graph paper. TSSs were measured with a refractometer (ATAGO, Tokyo), and values were corrected at 20 °C. Total acid (TA) percentage was determined by titration using 0.1 N NaOH and values expressed as percent mallic acid (Rangana 1986). Reducing sugars and sucrose were determined as outlined by Rangana (1986). Fruit moisture content was determined using an oven drying method until the weight was constant and then expressed as a percentage of fresh weight.

2.3 Sensory evaluation and acceptance

Fruit samples were evaluated on the day of harvest by a 20-member semitrained panel (8 women, 12 men, 24–42 years old) at room temperature with natural

Table 3 Pomological traits and standard fruit quality parameters of apricots of trans-Himalayan Ladakh

Group		Fruit wt. (gm)	Stone wt. (gm)	Blush area (mm^2)	Firmness (kg/cm^2)	Moisture content (%)	TSS (°Brix)	Titratable acidity (%)	TSS/TA	Reducing Sugar (%)	Sucrose (%)	Total Sugars (%)
I	*Raktsey Karpo*	21.7 ± 4.9^{bc}	2.4 ± 0.2^{b}	343.2 ± 73.7^{a}	2.4 ± 1.4^{a}	68.9 ± 8.3^{a}	27.5 ± 3.6^{b}	1.0 ± 0.1^{ab}	28.7 ± 1.9^{ab}	17.5 ± 1.9^{b}	2.8 ± 1.3^{ab}	20.3 ± 1.9^{b}
II	*Halman*	16.4 ± 5.6^{ab}	1.6 ± 0.3^{a}	293.4 ± 56.3^{a}	1.8 ± 0.6^{a}	77.7 ± 5.9^{b}	25.0 ± 3.9^{ab}	0.9 ± 0.1^{ab}	27.8 ± 3.0^{ab}	13.4 ± 4.0^{a}	3.2 ± 1.0^{ab}	16.5 ± 4.1^{a}
III	*Nyarmo Chemmo*	26.6 ± 6.9^{c}	2.2 ± 0.4^{b}	307.6 ± 132.9^{a}	2.1 ± 1.2^{a}	79.8 ± 3.2^{b}	21.1 ± 4.5^{a}	0.9 ± 0.2^{a}	27.0 ± 10.2^{ab}	15.3 ± 1.8^{ab}	3.4 ± 1.7^{ab}	18.7 ± 2.8^{ab}
IV	*Nyarmo Chun*	15.1 ± 3.7^{a}	1.6 ± 0.4^{a}	292.3 ± 90.3^{a}	2.5 ± 0.8^{a}	75.3 ± 8.2^{ab}	26.1 ± 4.9^{ab}	0.8 ± 0.1^{a}	32.1 ± 8.1^{c}	13.1 ± 3.0^{a}	3.8 ± 1.2^{b}	16.9 ± 3.3^{ab}
V	*Khante Chemmo*	23.0 ± 4.0^{c}	2.3 ± 0.6^{b}	387.4 ± 158.5^{a}	2.0 ± 1.0^{a}	79.6 ± 4.5^{b}	21.7 ± 6.4^{ab}	0.9 ± 0.1^{a}	25.6 ± 8.4^{ab}	12.5 ± 1.8^{a}	3.0 ± 1.4^{ab}	15.6 ± 2.6^{a}
VI	*Khante Chun*	13.5 ± 4.0^{a}	1.6 ± 0.4^{a}	299.5 ± 63.8^{a}	1.6 ± 0.5^{a}	80.4 ± 2.8^{b}	20.6 ± 5.0^{a}	1.1 ± 0.2^{b}	20.0 ± 6.8^{a}	15.6 ± 1.2^{ab}	1.8 ± 0.4^{a}	17.5 ± 1.4^{ab}
	Average	19.1 ± 6.7	1.9 ± 0.5	313.8 ± 101.0	2.2 ± 1.0	76.4 ± 7.2	24.2 ± 5.2	0.9 ± 0.2	28.2 ± 8.0	14.4 ± 2.9	3.2 ± 1.3	17.6 ± 3.1

Values represented as mean ± SD; for each column different lowercase letters indicate significantly difference ($p \leq 0.05$)

daylight. Each panelist had previous experience with descriptive analysis. Panelists were presented with coded fruit samples from each genotype. Five or less samples were evaluated per session. Panelists were given water as a neutralizing beverage between sample testing. The subjects were asked to rate the following sensory attributes: aroma, sweetness, juiciness, skin colour, flesh colour, seed coat colour, fruit shape, fruit size, and overall quality. Each panelist rated different parameters and overall appreciation on a scale of 1–9 (1 = extremely bad; 9 = extremely good).

2.4 Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD) using statistical analysis with SPSS (Statistical Program for Social Sciences, SPSS Corporation, Chicago, Illinois, USA). Analysis of variance (ANOVA) was performed to analyze significant differences. Sensory evaluation data were analysed by descriptive statistics using the Spearman rank-order correlation. Spearman's correlation analysis was performed to find correlation between the variables. Regression was performed using MS Excel 2007.

3 Results and discussion

3.1 Pomological traits and fruit quality characteristics

Table 3 presents pomological attributes of 47 apricot genotypes. Apricots with white seed coats (Group-I) ranked second (21.7 ± 4.9 g, 343.2 ± 73.7 mm^2), after Group-V (23.0 ± 4.0 g, 387.4 ± 158.5 mm^2), in terms of fruit weight and blush area. However, average fruit weight of all the six groups was determined to be < 40.0 g, which is the minimum weight necessary for fresh market apricots in the United States (Ledbetter et al. 2006). A survey conducted in France showed that a majority of consumers are looking for a medium-sized fruit, although elderly people prefer large apricots (Moreau-Rio 2006). *Raktsey Karpo* (Group-I) had the lowest moisture content (68.9 ± 8.3%), while TSS (27.5 ± 3.6°Brix), reducing sugar (17.5 ± 1.9%), and total sugar (20.3 ± 1.9%) values were the highest among the six groups. The moisture content was significantly lower, while TSS and sugar values were significantly higher than previous reports in apricot (Badenes et al. 1998; Gurrieri et al. 2001; Asma and Ozturk 2005; Ruiz and Egea 2008; Milošević et al. 2010; Ali et al. 2011; Leccese et al. 2012). High sugar content in Ladakh apricots has recently being attributed to high-altitude environmental conditions (Naryal et al. 2019).

3.2 Sensory evaluation and appreciation

Consumer appreciation of apricots in terms of fruit shape, fruit size, fruit skin colour, flesh colour, seed coat colour, aroma, sweetness, juiciness, and overall quality are shown in Table 4. *Raktsey Karpo* (Group-I) ranked first in terms of sweetness, juiciness, aroma, flesh color, and stone color. It also ranked first, along with *Nyarmo Chenmo* (Group-III), for fruit shape. Group-I also ranked first for fruit size, along with *Nyarmo Chenmo* (Group-III) and *Khante Chenmo* (Group-V). *Raktsey Karpo* scored the highest hedonic score for overall appreciation (8.0 ± 0.2). Consumer dissatisfaction centered around lack of flavor, aroma, and sweetness (Bhuhn et al. 1991; Moreau-Rio 2006; Moreau-Rio and Roty 1998). However, *Raktsey Karpo* is aromatic and sweet. Therefore, the results suggested that the white seed coat phenotype can be considered a marker for high-quality apricots in terms of aroma, sweetness, juiciness, and overall value.

3.3 Parameters for distinction between quality classes

The instrumental data created a clear distinction between the four quality classes (Table 5). TSS, sugar content, moisture content, and fruit weight were good parameters to distinguish between the four quality classes. However, blush area and fruit firmness did not distinguish the four classes. Larger fruit (26.98 ± 8.87 g) with low moisture content (72.58 ± 10.39%), high reducing sugar (16.92 ± 2.16%), and high total sugar (20.17 ± 2.63%) were desirable characteristics, and fruit with such characteristics fell under the "very good" class. Distribution of apricots with high sugar content in "good" and "very good" classes is in agreement with a previous report (Azodanlou et al. 2003). Fruits with high TSS (25.17 ± 5.57, 26.79 ± 4.00°Brix) and medium acidity (0.89 ± 0.12, 0.9 ± 0.15%) fell under the "good" and "very good" classes. Piagnani et al. (2013) also reported that cultivars with the best appreciation fell into the group with the highest TSS.

Consumers are attracted to brightly colored fruit (Moreau-Rio 2006). In this study, we found that fruit with medium-orange skin were appreciated by consumers and fell under the "good" and "very good" classes (Table 6). Seventy-five percent of the fruits with light-orange skin were categorized as "good." However, fruits with cream and light-orange flesh were disliked by the consumers. Consumers appreciated the white seed coat phenotype and it was ranked as "good."

3.4 Correlation between sensory and instrumental data

Relationships between data obtained by the sensory panel and instrumental methods have been established.

Table 4 Hedonic score of fresh apricots of trans-Himalaya by sensory panel

Group		Shape	Size	Skin Colour	Flesh Colour	Aroma	Sweetness	Juiciness	Flavor	Stone colour	Overall appreciation
I	*Raktsey Karpo*	7.7 ± 0.5^{c}	7.7 ± 0.4^{b}	7.3 ± 0.6^{a}	7.4 ± 0.4^{b}	7.4 ± 0.3^{c}	8.0 ± 0.3^{c}	8.0 ± 0.3^{c}	8.0 ± 0.3^{c}	7.4 ± 0.2^{c}	8.0 ± 0.2^{c}
II	*Halman*	6.8 ± 0.5^{ab}	6.5 ± 0.7^{a}	7.3 ± 0.6^{a}	7.3 ± 0.6^{ab}	6.6 ± 0.1^{b}	7.2 ± 0.4^{b}	6.5 ± 0.3^{b}	7.0 ± 0.4^{b}	6.4 ± 0.3^{b}	6.9 ± 0.4^{b}
III	*Nyarmo Chemno*	7.5 ± 0.4^{c}	7.5 ± 0.4^{b}	7.2 ± 0.6^{a}	7.1 ± 0.6^{ab}	6.6 ± 0.4^{b}	7.0 ± 0.8^{b}	6.9 ± 0.7^{b}	6.9 ± 0.9^{b}	6.3 ± 0.6^{b}	7.1 ± 0.7^{b}
IV	*Nyarmo Chun*	6.5 ± 0.7^{a}	6.5 ± 0.7^{a}	6.8 ± 0.7^{a}	6.7 ± 0.5^{a}	6.4 ± 0.4^{ab}	6.7 ± 0.6^{b}	6.4 ± 0.5^{b}	6.6 ± 0.5^{b}	6.1 ± 0.4^{ab}	6.7 ± 0.5^{b}
V	*Khante Chenmo*	7.3 ± 0.5^{bc}	7.4 ± 0.4^{b}	7.2 ± 0.4^{a}	7.0 ± 0.4^{ab}	6.8 ± 0.5^{b}	6.9 ± 0.7^{b}	7.0 ± 0.6^{b}	6.9 ± 0.8^{b}	6.4 ± 0.2^{b}	7.1 ± 0.7^{b}
VI	*Khante Chun*	6.3 ± 0.9^{a}	6.1 ± 1.0^{a}	7.1 ± 0.5^{a}	6.8 ± 0.8^{ab}	6.0 ± 0.7^{a}	5.6 ± 1.3^{a}	5.7 ± 1.1^{a}	5.5 ± 1.1^{a}	5.8 ± 0.6^{a}	5.9 ± 1.0^{a}
	Average	6.9 ± 0.8	6.9 ± 0.8	7.1 ± 0.6	7.0 ± 0.6	6.6 ± 0.5	6.9 ± 0.9	6.7 ± 0.9	6.8 ± 0.9	6.4 ± 0.6	6.9 ± 0.8

Values represented as mean ± SD; for each column different lowercase letters indicate significantly differ ($p \leq 0.05$)

Table 5 Sample distribution and limit values of pomological and fruit quality characteristics used for quality classification of apricots based on overall consumer appreciation

Quantitative traits (unit)	Quality class			
	Bad	Medium	Good	Very Good
Fruit wt. (g)	11.09 ± 3.98^{a}	20.26 ± 4.86^{b}	18.52 ± 4.76^{b}	26.98 ± 8.87^{c}
Stone wt. (g)	1.49 ± 0.46^{a}	1.98 ± 0.48^{b}	1.84 ± 0.49^{ab}	2.47 ± 0.19^{c}
Blush area (mm^2)	243.90 ± 74.50^{a}	308.60 ± 121.07^{a}	341.42 ± 82.39^{a}	326.38 ± 101.62^{a}
Firmness (kg/cm^2)	1.97 ± 0.74^{a}	2.01 ± 0.95^{a}	2.52 ± 0.98^{a}	1.72 ± 1.04^{a}
Moisture content (%)	78.61 ± 6.79^{ab}	80.04 ± 3.33^{b}	73.68 ± 7.48^{ab}	72.58 ± 10.39^{a}
TSS (°Brix)	21.63 ± 6.46^{a}	22.05 ± 4.61^{a}	26.79 ± 4.00^{c}	25.17 ± 5.57^{ab}
Titratable acidity (%)	1.03 ± 0.15^{b}	0.83 ± 0.19^{a}	0.89 ± 0.12^{ab}	0.90 ± 0.15^{ab}
TSS/TA	21.97 ± 10.05^{a}	27.94 ± 8.32^{ab}	30.75 ± 6.46^{b}	28.52 ± 6.89^{ab}
Reducing sugar (%)	13.49 ± 2.73^{a}	12.93 ± 3.02^{a}	15.28 ± 2.44^{ab}	16.92 ± 2.16^{b}
Sucrose (%)	2.39 ± 1.26^{a}	3.28 ± 1.34^{a}	3.43 ± 1.16^{a}	3.25 ± 1.85^{a}
Total sugar (%)	15.87 ± 2.48^{a}	16.22 ± 2.96^{ab}	18.71 ± 2.80^{bc}	20.17 ± 2.63^{c}

Values represented mean ± SD; for each row different lowercase letters indicate significantly differ ($p \leq 0.05$)

Hedonic score: bad: ≤ 5.9; medium: 6–6.9; good: 7–7.9; very good: ≥ 8

Table 6 Sample distribution and limit values of fruit shape and colour characteristics used for quality classification of apricots based on consumer appreciation

Visual traits	Traits	Shape/colour	Frequency	Quality class (%)			
				Bad	Medium	Good	Very Good
Fruit shape	Lateral view	Triangular	1	100.0	0.0	0.0	0.0
		Obovate	1	0.0	100.0	0.0	0.0
		Oblong	6	50.0	33.3	16.7	0.0
		Circular	9	11.2	44.4	44.4	0.0
		Ovate	10	0.0	40.0	50.0	10.0
		Oblique rhombic	20	0.0	35.0	50.0	15.0
	Ventral view	Oblong	7	14.3	42.8	28.6	14.3
		Elliptic	14	14.3	28.6	50.0	7.1
		Circular	14	7.1	50.0	35.7	7.1
		Ovate	12	0.0	33.3	58.3	8.4
	Fruit shape of apex	Acute	2	0.0	0.0	100.0	0.0
		Retuse	9	22.2	33.3	44.4	0.0
		Truncate	10	0.0	50.0	20.0	30.0
		Rounded	26	7.7	38.5	50.0	3.8
Skin colour	Fruit skin colour	Yellow green	9	11.1	55.5	22.2	11.1
		Medium orange	10	0.0	0.0	80.0	20
		Yellowish	12	8.3	58.3	25.0	8.3
		Light orange	16	0.0	25.0	75.0	0.0
Flesh colour	Fruit flesh colour	Dark orange	5	0.0	0.0	100.0	0.0
		Cream	8	12.5	62.5	25.0	0.0
		Medium orange	16	6.3	18.7	75.0	0.0
		Light orange	18	5.6	55.6	27.7	11.1
Seed coat colour	Fruit stone colour	White	7	0.0	0.0	100.0	0.0
		Brown	40	35.0	60.0	5.0	0.0

Hedonic score: bad: ≤ 5.9; medium: 6–6.9; good: 7–7.9; very good: ≥ 8

A statistically significant positive relation between fruit weight and the consumer's overall appreciation was found ($R^2 = 0.230$, $p \leq 0.001$). Similarly, overall appreciation showed positive significant relation with TSS ($R^2 = 0.177$, $p \leq 0.01$), TSS/TA ($R^2 = 0.118$, $p \leq 0.05$), reducing sugar ($R^2 = 0.140$, $p \leq 0.01$), and total sugar ($R^2 = 0.177$, $p \leq 0.01$). A statistically significant negative relation was observed between overall appreciation and fruit moisture content

($R^2 = 0.168$, $p \leq 0.01$) (Fig. 2). The perceived fruit sweetness showed positive correlation with TSS ($R^2 = 0.368$, $p \leq 0.001$), TSS/TA ($R^2 = 0.231$, $p \leq 0.001$), reducing sugar ($R^2 = 0.138$, $p \leq 0.05$), and total sugar ($R^2 = 0.180$, $p \leq 0.01$). Sucrose and TA did not show significant relation at $p \leq 0.05$. Perceived fruit juiciness showed negative relation with fruit moisture content ($R^2 = 0.207$, $p \leq 0.01$).

Coefficients of correlation results (Table 7) suggested that aroma, sweetness, juiciness, seed coat color, fruit shape, fruit size, skin colour, flesh colour, and fruit taste were significant attributes ($p \leq 0.01$) to describe the quality of apricots. The results of Spearman's correlation (Table 8) showed that seed coat colour correlates significantly with fruit shape (0.593, $p \leq 0.01$), fruit size (0.625, $p \leq 0.01$), skin colour (0.486, $p \leq 0.01$), flesh colour (0.653, $p \leq 0.01$), aroma (0.830, $p \leq 0.01$), sweetness (0.823, $p \leq 0.01$), juiciness (0.846, $p \leq 0.01$), and taste (0.873, $p \leq 0.01$). Similarly, significant correlation was found between other fruit quality characteristics and sensory variables.

In conclusion, this study demonstrated that apricots with a white seed coat phenotype can be considered easily identifiable markers for quality. The phenotype can be explored as a key parameter in apricot breeding for selection criteria for consumer satisfaction. The important quality attributes for apricots were aroma, sweetness, juiciness, flesh color, stone color, fruit shape, and fruit weight. Further research is suggested to identify molecular markers linked to the white seed coat phenotype for early marker-assisted selection in apricot breeding.

Table 7 Coefficients of correlation between overall appreciation and sensory attributes of fresh apricots by the consumers

Attributes	R spearman
Aroma	.831**
Sweetness	.925**
Juiciness	.907**
Seed coat colour	.896**
Shape	.594**
Size	.614**
Skin colour	.493**
Flesh colour	.662**
Taste	.966**

**Significant at $p \leq 0.01$

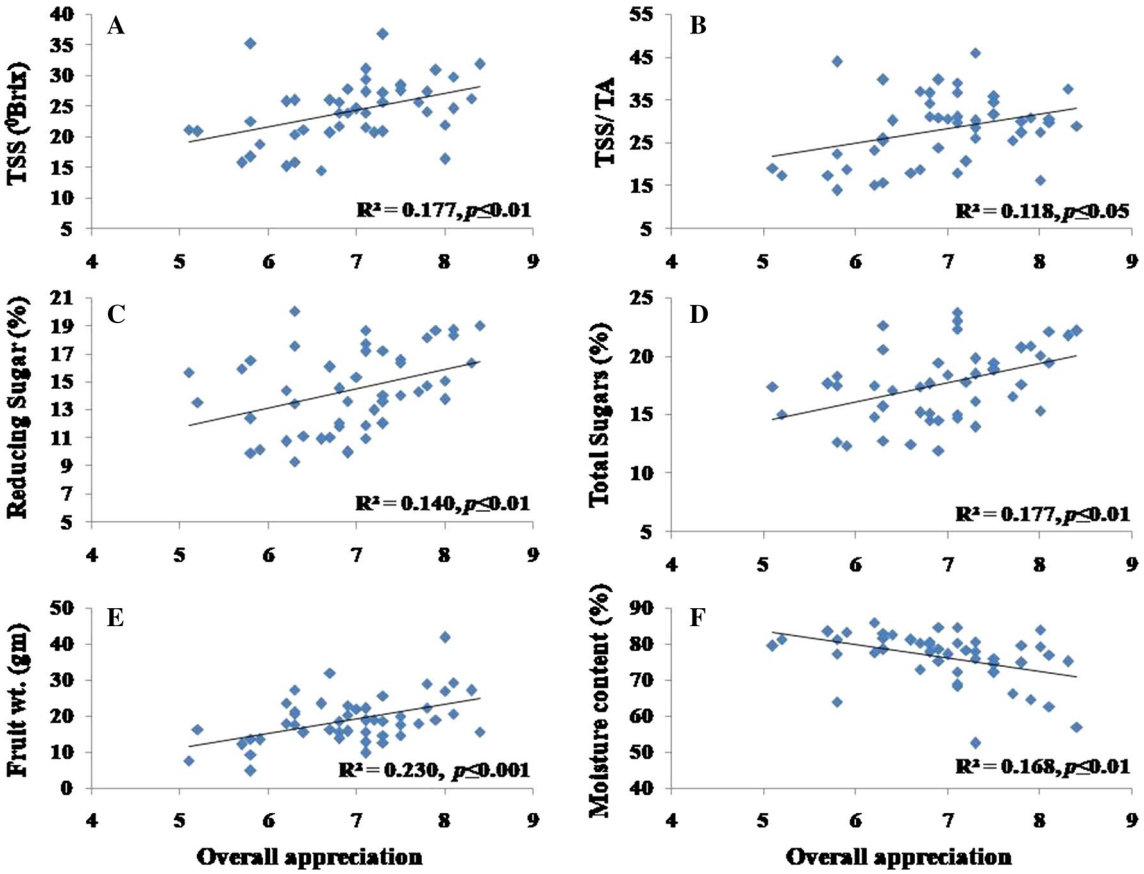

Fig. 2 Relation between overall appreciation with TSS (**a**), TSS/TA (**b**), reducing sugar (**c**), total sugar (**d**), fruit size (**e**), and fruit moisture content (**f**)

Table 8 Spearman's coefficients among sensory variables and fruit quality characteristics for fresh apricots of trans-Himalayan Ladakh

Attributes	Shape	Size	Skin Colour	Flesh Colour	Aroma	Sweetness	Juiciness	Taste	Seed coat colour
Shape	1.00	0.963**	0.595**	0.601**	0.702**	0.430**	0.592**	0.494**	0.593**
Size		1.00	0.538**	0.558**	0.732**	0.450**	0.629**	0.527**	0.625**
Skin Colour			1.00	0.879**	0.457**	0.332*	0.358*	0.389**	0.486**
Flesh Colour				1.00	0.599**	0.546**	0.539**	0.597**	0.653**
Aroma					1.00	0.798**	0.866**	0.815**	0.830**
Sweetness						1.00	0.903**	0.964**	0.823**
Juiciness							1.00	0.924**	0.846**
Taste								1.00	0.873**
Seed coat colour									1.00

*Significant at $p \leq 0.05$; **significant at $p \leq 0.01$

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Gender differences in phenotypic plasticity and adaptive response of Seabuckthorn (*Hippophae rhamnoides* L.) along an altitudinal gradient in trans-Himalaya

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Abstract.
BACKGROUND: In dioecious plants, morphological adjustment to climate change may differ between male and female individuals due to greater reproductive effort in females. Not accounting for sexual variation could lead to incorrect assessment of a species response to climate change.
OBJECTIVES: The aim of this study was to assess how important gender-specific responses are to *Hippophae rhamnoides* in changing trans-Himalayan environments.
METHOD: Leaf morphological characters of male and female *Hippophae rhamnoides* individuals along an altitudinal gradient (2797-4117 m) and plants raised in 'common-garden' experiment was measured.
RESULTS: Leaves become smaller in length and area, but became thicker with decreasing specific leaf area (SLA) with increasing altitude in both the gender. Leaf size, area, thickness, chlorophyll and petiole length were found to be higher in males than in females, while female had a higher SLA. When cuttings from the plants were grown in a common-garden experiment, the altitudinal effect disappeared for all morphological variables suggesting that most leaf morphological variation in *H. rhamnoides* is environmentally determined. In the event of climate change, our study showed that phenotypic plasticity would be a crucial determinant of plant response in mountainous region. Effect of altitudinal gradient on leaf morphology was more conspicuous in males suggesting that males are more responsive to change in environmental conditions.
CONCLUSION: The results suggested that males will adapt better to the changing climate and may lead to a male-biased population in the event of climate change. Stressful environments cause added detrimental impact on female than on male.

Keywords: Abiotic stress, adaptation, climate change, plasticity, Ladakh

1. Introduction

Climate change is altering the availability of resources and the conditions that are crucial to plant performance [1]. Over the last few decades, attention is being increasingly focused on evolutionary responses to rapid climate change [2]. One major concern in this context relates to the ability of long-lived species to cope with rapid change in climate [3–5]. Plant species can adjust to changing climate through environmentally induced shift

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in phenotype (phenotypic plasticity), adapt through natural selection (genetic response) or migrate to follow conditions to which they are adapted; these options are not mutually exclusive [1]. However, studies on climate change-induced evolution under simulated and natural climatic conditions have rarely integrated plastic and genetic evolutionary responses [6]. In dioecious plants, morphological adjustment to climate change may differ between male and female individuals due to greater reproductive effort in females. The cost of reproduction involves prioritization of resources for fruit development rather than for vegetative growth or protection in females. A major investment in reproduction is generally associated with the disadvantage in terms of oxidative stress and cellular injuries, particularly under adverse conditions [7]. Not accounting for sexual variation could lead to incorrect assessment of a species response to climate change [8]. However, this aspect has not been studied in detail, particularly in the fragile trans-Himalayan region.

Both abrupt and gradual climate changes will impose selection on plant population [1]. Abrupt climate changes will result in rapid harsh selection for more stress-tolerant genotypes, whereas gradual climate changes are expected to impose soft selection mediated by intraspecific interactions [6]. Altitudinal gradients in stressful mountain ecosystem provide an ideal experimental opportunity for studying the functional traits of plants in response to abrupt climate change. In mountainous regions, sharp changes in abiotic factors occur over short distances, leading to major changes in the selection pressures acting on plant life history traits [2].

Altitude has a major effect on leaf morphology and physiology within a species. Leaves generally decrease in length, width and area but become thicker with increasing altitude [9–11]. However, the problem in interpreting the well documented relationship between altitude and leaf morphology is the confounding of environmental and genetic factors [10]. There are evidences that plant originating from different altitudes remain different when grown at same altitude [10, 12, 13]. Cordell et al. [14] reported that leaf morphology is largely genetically determined but leaf anatomy and physiology are environmentally determined in tree species *Metrosideros polymorpha.* Hovenden and Vander Schoor [10] found that the morphological response to the environment generally overrides the genetic influence in *Nothofagus cunninghamii*. A similar study showed that the extensive altitudinal distribution of *Pennisetum setaceum* is the result of ecological tolerance rather than adaptation of specific ecotype [15]. At extreme altitude (above 2800 m) the relationship between leaf morphology and altitude differed from the conventional linear relationship along altitudinal gradients [16], but this aspect has not been studied in details. Thus it appears that the degree of environmental plasticity and adaptation is species and environment dependent.

Hippophae rhamnoides L. is an ecologically and economically important dioecious plant. It is found in a large altitudinal range, from the sea shores in Europe to over 4694 m in trans-Himalayan Ladakh. Leaf morphological characters including leaf size, thickness and specific leaf area (SLA) are strongly influenced by altitude and gender in this species [16]. However, leaf morphological traits of *H. rhamnoides* measured in natural conditions have not been investigated in concert with measurements of their progeny in common garden experiments. *H. rhamnoides* are easy to propagate by stem cuttings, and the availability of clonal material facilitates the testing of identical genotypes under different conditions. *H. rhamnoides*, therefore, presents an excellent opportunity to investigate the relative contributions of environmental, genetic and gender factors to the relationship between leaf morphology and altitude. The aim of this study was, therefore, to assess how important gender-specific responses are to *H. rhamnoides* in rapidly changing trans-Himalayan environments. This was done using a 'common-garden' experiment, in which large number of cuttings from several male and female shrubs at each of four altitudinal range was grown in a single experimental plot.

2. Materials and methods

2.1. Study site

The study was conducted in trans-Himalayan Ladakh region. The altitude of origin of field-grown plants ranged from 2797–4117 m amsl (Table 1). Common-garden experiment was carried out at an experimental farm (34°08.2'N; 77°34.3'E, elevation 3350 m) on a flat site with direct sunshine at Defence Institute of High

Table 1
Geographical location and sampling sites of *Hippophae rhamnoides* in trans-Himalaya Ladakh

Altitude ranges (m amsl)	Population	Altitude (m amsl)	No. of samples	
			Male	Female
2800–3000	Turtuk	2797	2	2
	Hundar	2890	1	1
	Diskit	2910	1	1
	Nurla	2990	1	1
3001–3300	Phey	3179	1	1
	Panamik	3180	1	1
	Shey	3232	2	2
	Achinathang	3255	1	1
3500–3800	Shayok I	3576	1	1
	Chemday	3680	1	1
	Shayok II	3740	1	1
	Durbuk	3850	1	1
3801–4200	Horzey	3885	2	2
	Sakti	3927	1	1
	Khardong	4117	1	1

Altitude Research (DIHAR) in trans-Himalayan Ladakh, India. The mean maximum and minimum temperature during 2014–2015 recorded at DIHAR was 12.9 ± 8.8°C and -0.2 ± 9.0°C, respectively. The monthly maximum temperature was highest in July (25.6°C), and the minimum temperature was recorded lowest in January (–13.2°C). The mean maximum and minimum relative humidity was $31.0 \pm 4.3\%$ and $24.7 \pm 3.7\%$, respectively. The average annual precipitation was 163 mm.

2.2. Leaf analysis

Between 13 and 25 August 2014, a single branch was collected from each of 8–10 adult *H. rhamnoides* shrubs from four altitudinal range. All branches were collected on the sunny side of the shrub. Samples of young fully expended leaves (10 leaves per plant) were collected from each branch to investigate the field-grown leaf characteristics. Between 04 and 14 April 2015, dormant cuttings of pencil thickness were taken from each shrub and planted at an experimental farm at DIHAR. Samples of fully expended leaves (10 leaves per plant) were collected in September 2015 from each of the rooted plants to record the garden-grown leaf characteristics. Both field and garden-grown leaves were evaluated for leaf gross morphology. Leaf length, width, thickness were measured using digital calipers (CD-6“CS, Mitutoya, Japan). Leaf length was measured from the base of petiole to leaf tip, while leaf width was recorded at the maximum width of the blade. Leaf thickness was measured from the central part of the lamina, half-way between midrib and margin. Petiole length was taken by cutting the petiole portion of the leaf from base of the leaf blade, while chlorophyll was measured with Chlorophyll Meter SPAD-502 (Konica Minolta Sensing Inc., Japan). Leaf area was measured with a portable leaf area meter (CI 202) (CID Inc, Camas, WA, USA). Leaves were shade-dried to constant mass at 60°C. SLA was calculated by dividing one-sided fresh leaf area by the dry mass.

2.3. Statistical analysis

Assumptions of normality were checked for all variables with Kolmogorov-Smirnov test and variables that significantly deviated from normality were log transformed. Tukey's HSD test was performed at $p \leq 0.05$ level for mean comparison. One-way analysis of variance (ANOVA) and regression was conducted with altitude as the fixed factor and leaf morphological parameters as dependent variable. A two-way ANOVA was used to test the relationship of gender, altitude and their interaction with leaf morphological characters. Coefficient of variation (CV) for each trait as a complementary index to interpret the plasticity was computed using the formula: CV = standard deviation × 100/mean. Statistical analysis was carried out in MS excel 2007 and SPSS software package v.17.0 for Windows (SPSS Inc. released in 2008).

3. Results

3.1. Gender differences in phenotypic variation along altitudinal gradient

Both altitude and gender had a significant impact on selected leaf morphology of field-grown *H. rhamnoides* (Table 2). Leaves become smaller in length and area, but became thicker with decreasing SLA with increasing altitude in both the gender. Effect of altitudinal gradient on leaf thickness was more evident in male. The linear regression showed almost linear ($R^2 = 0.82$) relation between leaf thickness with altitude in male, but there was no linear relationship in female ($R^2 = 0.02$) (Table 3). Petiole length in males increased significantly with increasing altitude ($R^2 = 0.44$), but similar pattern was not observed in females ($R^2 = 0.04$). No significant relationship with altitude was observed for leaf width in both the gender. The chlorophyll contents varied between 'high' and 'low' altitudes, with leaves from higher altitude (3500–4200 m) having more than those from lower altitude (2800–3300 m) in both the gender.

Within each altitude, there was a significant influence of gender on selected morphological characters measured. Leaf length, width, thickness, area and chlorophyll contents were higher in males than in females (Table 2). The effect of altitude and gender on leaf morphology was supported by results of two-way ANOVA (Table 4). Predominant effects of altitude on leaf length ($F_1 = 3.8$, $p \leq 0.05$), thickness ($F_1 = 3.8$, $p \leq 0.05$), petiole length ($F_1 = 3.3$, $p \leq 0.05$), chlorophyll contents ($F_1 = 3.0$, $p \leq 0.05$), and SLA ($F_1 = 12.0$, $p \leq 0.001$) was observed. Similarly, significant effect of gender was observed on leaf width ($F_1 = 7.0$, $p \leq 0.05$), thickness ($F_1 = 4.4$, $p \leq 0.05$), area ($F_1 = 7.5$, $p \leq 0.05$) and SLA ($F_1 = 7.3$, $p \leq 0.01$). Males showed more variability than females in all the leaf morphological traits except for leaf thickness (Table 5).

3.2. Genetic differentiation in the common-garden experiment

Altitude of plant origin did not have significant impact on leaf morphology of garden-grown *H. rhamnoides* (Table 2). No increasing or decreasing trend was observed in leaf length, width, thickness and petiole length with increasing altitude of origin in both the gender. However, a relationship between leaf chlorophyll contents and altitude was observed ($R^2 = 0.37$) in males, with lower chlorophyll contents in plants from higher altitude origin. Similar relationship was observed between altitude and SLA of garden-grown male plants ($R^2 = 0.26$), with decreasing SLA with increasing altitude of origin (Table 3). However, within each altitude of origin, there was a significant influence of gender on all morphological characters measured. Leaf length, petiole length, leaf area and chlorophyll contents were higher in males than in females (Table 2). SLA was significantly higher in females of all altitude of origin except those from 2800–3000 m asl. However, two-way ANOVA results did not support significant effect of gender on leaf morphology in garden-grown plants (Table 4). A reduced variability in leaf morphology was observed in garden-grown plant as compared to field-grown plant in both the gender (Table 5). However, the exceptions were chlorophyll contents and SLA which remained unchanged in one of the gender. SLA showed opposite trend in field-grown females.

Table 2

One way ANOVA in filed grown and common garden grown plants and their difference in different sexes

Gender	Growing condition	Altitude (m amsl)	LL	LW	LT	LA	PL	CC	SLA
Male	Field	2800–3000	35.60 ± 7.85^{d}	3.75 ± 0.32^{a}	0.26 ± 0.01^{ab}	1.41 ± 0.30^{d}	1.66 ± 0.12^{a}	58.35 ± 5.46^{bcdef}	0.21 ± 0.05^{ab}
		3001–3300	30.65 ± 6.62^{abcd}	4.37 ± 0.73^{a}	0.28 ± 0.03^{abcd}	1.28 ± 0.40^{ab}	2.10 ± 0.69^{ab}	62.15 ± 11.78^{cdef}	0.13 ± 0.04^{ab}
		3500–3800	31.73 ± 3.49^{abcd}	4.31 ± 1.23^{a}	0.36 ± 0.03^{bc}	1.27 ± 0.40^{abcd}	2.23 ± 0.1^{ab}	72.59 ± 6.76^{f}	0.14 ± 0.06^{ab}
		3801–4200	28.99 ± 5.09^{abcd}	3.68 ± 0.57^{a}	0.37 ± 0.03^{c}	1.14 ± 0.26^{abcd}	2.64 ± 0.28^{b}	71.74 ± 4.86^{ef}	0.11 ± 0.03^{a}
	Garden	2800–3000	32.52 ± 5.95^{bcd}	3.58 ± 0.86^{a}	0.28 ± 0.05^{abc}	1.12 ± 0.37^{abcd}	1.65 ± 0.38^{a}	63.47 ± 6.21^{cdef}	0.15 ± 0.03^{ab}
		3001–3300	26.08 ± 4.72^{abcd}	3.51 ± 0.75^{a}	0.29 ± 0.07^{abc}	0.86 ± 0.35^{abcd}	1.85 ± 0.52^{ab}	74.79 ± 4.61^{f}	0.11 ± 0.05^{a}
		3500–3800	33.20 ± 5.28^{cd}	3.04 ± 0.37^{a}	0.25 ± 0.09^{a}	0.94 ± 0.16^{abcd}	2.17 ± 0.12^{ab}	69.57 ± 7.42^{def}	0.09 ± 0.03^{a}
		3801–4200	29.07 ± 2.80^{abcd}	3.61 ± 0.25^{a}	0.31 ± 0.04^{abc}	0.96 ± 0.16^{abcd}	2.17 ± 0.24^{ab}	69.36 ± 6.89^{def}	0.09 ± 0.02^{a}
Female	Field	2800–3000	24.79 ± 1.73^{abcd}	3.05 ± 0.31^{a}	0.28 ± 0.04^{abc}	0.75 ± 0.12^{abc}	1.58 ± 0.36^{a}	40.48 ± 1.05^{a}	0.27 ± 0.11^{b}
		3001–3300	23.21 ± 2.23^{abc}	3.58 ± 0.61^{a}	0.26 ± 0.03^{ab}	0.73 ± 0.16^{abc}	1.45 ± 0.16^{a}	50.45 ± 4.30^{abc}	0.26 ± 0.11^{ab}
		3500–3800	21.78 ± 4.16^{ab}	3.34 ± 0.62^{a}	0.29 ± 0.04^{abc}	0.71 ± 0.29^{abcd}	1.41 ± 0.17^{a}	55.16 ± 9.85^{abcde}	0.17 ± 0.02^{ab}
		3801–4200	20.97 ± 4.98^{a}	3.12 ± 0.60^{a}	0.32 ± 0.06^{abc}	0.62 ± 0.21^{ab}	1.46 ± 0.14^{a}	52.70 ± 1.94^{abcd}	0.17 ± 0.05^{ab}
	Garden	2800–3000	21.71 ± 4.64^{ab}	3.43 ± 0.37^{a}	0.28 ± 0.02^{abc}	0.74 ± 0.14^{abc}	1.54 ± 0.15^{a}	41.14 ± 5.99^{ab}	0.23 ± 0.04^{ab}
		3001–3300	21.53 ± 2.23^{ab}	3.28 ± 0.39^{a}	0.28 ± 0.03^{abc}	0.66 ± 0.09^{abc}	1.56 ± 0.35^{a}	59.38 ± 12.19^{cdef}	0.19 ± 0.05^{ab}
		3500–3800	21.47 ± 2.36^{ab}	2.99 ± 0.24^{a}	0.25 ± 0.05^{a}	0.55 ± 0.09^{a}	1.52 ± 0.12^{a}	52.95 ± 2.88^{abcd}	0.21 ± 0.07^{ab}
		3801–4200	21.95 ± 3.65^{abc}	3.33 ± 0.61^{a}	0.29 ± 0.02^{abc}	0.64 ± 0.17^{abc}	1.39 ± 0.10^{a}	54.60 ± 3.61^{abcde}	0.17 ± 0.04^{ab}

LL = leaf length (mm), LW = leaf width (mm), LT = Leaf thickness (mm), PL = Petiole length (mm), LA = Leaf Area (cm^2), CC = Chlorophyll contents (SPAD VALUE), SLA = Specific leaf area (cm^2/mg). Values represented as mean ± SD; for each column, different lowercase letters indicate significantly different at $P \leq 0.05$, as measured by 2-sided Tukey's HSD.

Table 3
Regression analysis with altitude as independent variable and field- and garden-grown leaf of *Hippophae rhamnoides* as dependent variable

Parameters	Male				Female				Mixed (male + female)			
	Field-grown		Garden-grown		Field-grown		Garden-grown		Field-grown		Garden-grown	
	R^2	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2	P
Leaf Length (mm)	0.08	0.243	0.55	0.351	0.01	0.840	0.00	0.892	0.05	0.374	0.02	0.607
Leaf width (mm)	0.02	0.592	0.03	0.484	0.02	0.583	0.02	0.739	0.05	0.377	0.01	0.708
Leaf thickness (mm)	**0.82**	**0.000**	**0.20**	**0.050**	0.02	0.542	0.01	0.728	**0.51**	**0.001**	**0.23**	**0.045**
Petiole Length (mm)	**0.44**	**0.003**	0.03	0.522	0.04	0.230	0.09	0.237	**0.41**	**0.004**	0.11	0.178
Leaf Area (cm^2)	0.06	0.330	0.01	0.712	0.03	0.518	0.05	0.379	0.07	0.303	0.04	0.452
Chlorophyll contents (SPAD)	0.09	0.234	**0.37**	**0.008**	0.00	0.868	0.13	0.140	0.04	0.412	**0.28**	**0.025**
Specific leaf area (cm^2/mg)	**0.34**	**0.010**	**0.26**	**0.033**	**0.31**	**0.016**	0.08	0.247	**0.48**	**0.002**	**0.37**	**0.008**

Table 4
Two-way ANOVA for leaf morphological characters of *Hippophae rhamnoides* with gender and altitude as main effects

Growing condition	Source	*Df*	*F*						
			LL	LW	LT	PL	LA	CC	SLA
Field-grown	Sex	1	0.775	7.037*	4.413*	1.855	7.456*	1.462	7.274**
	Altitude	3	3.817*	0.354	3.864*	3.298*	2.349	2.971*	12.003***
	SXA	3	0.865	1.670	3.316*	0.347	0.494	0.788	0.292
Garden-grown	Sex	1	0.555	0.315	1.216	0.274	1.094	1.036	0.803
	Altitude	3	0.419	0.949	1.530	0.474	0.714	3.538*	1.788
	SXA	3	0.515	1.421	1.582	0.264	0.737	1.190	1.210

LL = leaf length (mm), LW = leaf width (mm), LT = Leaf thickness (mm), PL = Petiole length (mm), LA = Leaf Area (cm^2), CC = Chlorophyll contents (SPAD VALUE), SLA = Specific leaf area ($cm^{2/}$/mg). Significance: *** = $P < 0.001$; ** = $P < 0.01$; and * = $P < 0.05$.

Table 5
Interpopulation variability (coefficient of variation) of leaf morphological traits in field- and garden-grown *Hippophae rhamnoides* in trans-Himalaya

Parameters	Coefficient of variation			
	Male		Female	
	Field-grown	Garden-grown	Field-grown	Garden-grown
Leaf Length (mm)	0.19	0.14	0.17	0.13
Leaf width (mm)	0.21	0.15	0.19	0.13
Leaf thickness (mm)	0.17	0.15	0.21	0.11
Petiole Length (mm)	0.26	0.13	0.21	0.15
Leaf Area (cm^2)	0.32	0.25	0.29	0.19
Chlorophyll contents (SPAD)	0.12	0.12	0.09	0.18
Specific leaf area (cm^2/mg)	0.37	0.33	0.36	0.36

The leaves of the low altitude origin (2800–3300 m) garden-grown plants were smaller in length than those collected from the field in both the gender. However, the opposite trend was observed in plants of higher altitude

origin (3500–4200 m). Leaf area of garden-grown male plants was smaller than field-grown plants irrespective of their altitude of origin but the values were not significant at high altitudes. However, the trend was not observed in leaves of female plants. Chlorophyll contents of garden-grown leaves remained higher in males of low altitude origin (2800–3300 m) than field-grown plant. However, a lowering trend was observed in leaves of garden-grown plants of higher altitude origin (3500–4200 m). In contrast, no such increasing or decreasing trend was observed in females. The SLA of garden-grown leaves of male plants was lower than those collected from the field. However, in females the trend was observed only in plants of low altitude origin (2800–3300 m).

4. Discussion

Some of the leaf morphological characters in *H. rhamnoides* are significantly affected by altitude. Leaf size decreased in both the gender with increasing altitude. This trend is consistent with the findings of previous studies [2, 10, 11]. Reduction in size is an important strategy employed by plants at high altitude to withstand decrease in temperature and reduced nutrient availability. At high altitude, plants increase supercooling capacity by decreasing cell size and intercellular spaces [17]. Plants decrease the size of their parts to reduce water loss through transpiration, which is a crucial factor in the rain shadowed trans-Himalayan region [11]. Colder soils reduce the water uptake of the root system and induce water stress [18], which might result in reduced size at high altitude. In common-garden experiment, no significant trend was observed in leaf size, suggesting that phenotypic variability along the gradient is due to environmental effect. This trend is consistent with findings of previous studies in other species [2, 10]. In contrast, genetic variation between population from contrasting environments has been reported for leaf size in *Populus deltoides* [19] and *Alchemilla alpina* [20], suggesting that diversifying selection with altitude may be responsible for leaf size differentiation. Within each altitude, leaf size was smaller in female than male. Reduced leaf size in females may be due to greater demand for nutrient and carbon for seed and fruit production. Reduced shoot length in females is reported in *H. rhamnoides* [16]. Garden-grown leaves of low altitude origin (2800–3300 m) were smaller in length than those collected from the field in both the gender. However, the opposite trend was observed in plants from higher altitude origin (3500–4200 m). Our result is in agreement with those of Hovenden and Vander Schoor [10] who reported similar trends in *N. cunninghamii*. Increase in leaf size of plants of higher altitude origin (3500–4200 m) in garden experiment may be due to more conducive environmental conditions for plant growth at lower altitude. The results elucidate the phenotypic plasticity in response to change in environmental conditions.

Leaf thickness increased with elevation, which is consistent with trends observed in other species along altitudinal gradients [21, 22]. Leaves become thicker with elevation due to increase in intensity of solar radiation and decline in nutrient availability [22]. Increased leaf thickness is an adaptation mechanism against stressful environmental conditions. Leaf thickness is important in terms of carbon assimilation as, so long as light is not limiting, thicker leaves tend to have a higher photosynthetic rate per unit leaf area [12]. Plants from higher altitudes have higher carbon assimilation rates per unit area [13], and there is a genetic basis for this difference [12], which supports the proposition that thicker leaves would be selected for with increasing altitude. Leaf longevity increased with leaf thickness [23, 24] and thus results in an increased residence time of nutrient within the leaves [22], which is beneficial in nutrient-poor environments such as the trans-Himalayan region. Effect of altitudinal gradient on leaf thickness was more prominent in males. Therefore, males are more responsive to change in environmental conditions resulting in greater adaptation. In case of climate change the females are more likely to be adversely affected than males.

We found that there is a decrease in SLA with increasing altitude in both the gender in field-grown plants. The result is consistent with most of the earlier studies [2, 10, 25], although a study by Schoettle and Rochelle [26] highlighted an increase in SLA with increasing altitude in *Pinux flexilis*. It has been pointed out that leaves with low SLA generally contain more photosynthetic machinery per unit area [27], increasing water use efficiency and photosynthetic capacity at high altitude [28]. The development of a low SLA is often considered

a strategy to increase the longevity of a leaf, in order to optimize the use of scarce nutrients [29, 30]. Our results showed that most leaf morphological variation in *H. rhamnoides* is environmentally determined, but SLA and leaf thickness are also dependent on genotype. However, environmental influence was stronger than genetic influence (Table 3). This mix of genetic and environmental influences on morphology of *H. rhamnoides* leaves is also seen in various other species that occur along environmental gradients, including *Metrosideros polymorpha* [14] and *N. cunninghamii* [10].

Petiole length in male increased significantly with increasing altitude ($R^2 = 0.44$), but similar pattern was not observed in female ($R^2 = 0.04$) (Table 3). Elongation of petiole in response to shading is known to increase resource capture under low light condition [31]. However, low light condition alone was an unlikely factor for increased petiole length with increasing altitude in trans-Himalaya. The intensity of solar radiation increases with elevation due to a decline in the optical thickness of the atmosphere [22]. Therefore, the increase in petiole length may be an adaptive response to capture more light to compensate the smaller leaf with increasing altitude. In common-garden experiment, no trend was found for petiole length in both the gender (Table 3), suggesting that this trait is essentially an environmental determinism. The chlorophyll contents differed between 'high' and 'low' altitudes, with leaves from higher altitude (3500–4200 m) having more than those from lower altitude (2800–3300 m) in both the gender. Increased chlorophyll contents at higher altitude may be an adaptive mechanism to offset the decline in concentration of CO_2 with elevation. Within each altitude, chlorophyll contents was significantly higher in male than female (Table 2), which may be evolutionary advantageous for male for higher photosynthetic activity. Our result is in contrast with that of tropical origin *Piper betle*, where female contained nearly two fold more chlorophyll than male counterparts [32].

Few studies on tree species [33, 34] have shown that the field-grown phenotypic variability may result partly from the local genetic adaptations of populations over the altitudinal gradient. In our study, it was observed that variation was always greater in field-grown populations than those in the common-garden experiment, indicating the importance of environmental factors. One of the concerns about the potential confounding of genetic and environmental controls of leaf morphology is that the genetic control may mask the climate signals [35]. This would be evident particularly in the long-lived species. This is unlikely to be the case for *H. rhamnoides* for leaf size characteristics would be useful indicators of environmental conditions.

5. Conclusion

It may be concluded from the results of this study that *H. rhamnoides* is a prime candidate to investigate gender response to climate change. The morphological variation in leaves of *H. rhamnoides* is primarily environmentally determined. Our study showed that in the event of climate change the phenotypic plasticity would be a crucial determinant of plant response in mountainous region. Stressful environments will have an added detrimental impact on female than on male. The results elucidated that male will adapt better to the changing climate and may lead to a male-biased population in the event of climate change.

Acknowledgments

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Conflict of interest

The authors have no conflict of interest to report.

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MEMORANDUM OF UNDERSTANDING

Between

Magbro Healthcare Pvt. Ltd

and

Jaypee University of Information Technology - Solan Himachal Pradesh

JUIT Waknaghat offers a challenging academic environment to its students. It aims to instill the habit of life-long learning and therefore, provides a learner-centric rather than a teacher-centric educational process. The Department of Biotechnology and Bioinformatics – Jaypee University of Information Technology Waknaghat imparts education to equip students with modern skills compatible to the needs of industry, academia, government and non-government organizations. The Department is actively involved in research by having the distinction of running externally funded R & D projects worth Rs. 25 crores from various Govt. of India agencies such as the Department of Biotechnology, Department of Science & Technology, DRDO, Ministry of Environment and Forest, ICMR, and industries. Recently Department has been funded by DBT under Skill Vigyan Program in Biotechnology and aspiring similar type of funding in future as well. The major objective of this program is to provide skills to the young graduates to upgrade their existing skills, so that the students are able to setting up self-employment ventures and for salaried jobs in the relevant industries.

For the accomplishment of the mentioned objective, Jaypee University of Information Technology-Solan Himachal Pradesh offers and agrees to enter into mutual consent with the "**Magbro Healthcare Pvt Ltd**" in the form included in the tie-up document to provide a platform to the young talent in the form of skills development for sustainable livelihood. One of the identified areas is Health care industries.

It shall be pleasure for us to pay a token of honorarium and transportation expenses for your visit and kind participation in the mentioned activities as per your availability in this long term venture.

We await with very delight to have your positive response in this activity.

Prof. Vinod Kumar
Vice Chancellor
Jaypee University of Information Technology-
Solan, HP - 173234

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY * Vice Chancellor

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CERTIFICATE- I

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TO WHOM IT MAY CONCERN

This is to certify that Ms. Pallavi D/o Mr.Devender Singh Parihar Roll No-197818 student of M.Sc Biotechnology, Jaypee University of Information Technology Waknaghat Distt, Soaln (H.P) **has successfully completed her Industrial Training under the supervision of Miss. Rishpa Sharma, Manager Q.C./ Q.A. & Mr. Sanjay Gupta, Manager Production from 08.02.2021 to 08.05.2021 in this organization.**

We wish her all the best in her future endeavors.

For Magbro Healthcare Pvt. Ltd.

Authorized Signatory

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MEMORANDUM OF UNDERSTANDING
between
Alves Health Care Pvt. Ltd.
&
Jaypee University of Information Technology - Solan Himachal Pradesh

Jaypee University of Information Technology (JUIT)-Solan offers a challenging academic environment to its students. It aims to instill the habit of life-long learning and therefore, provides a learner-centric rather than a teacher-centric educational process. The major objective of the department is to provide skills to the young graduate to upgrade their existing skills, so that the students are able to setting up self-employment ventures and for salaried jobs in the relevant industries.

Purpose: The purpose of this agreement is to promote collaborative work in the area of education and technology between the Department of Biotechnology & Bioinformatics, Jaypee University of Information Technology (JUIT)-Solan INDIA and Alves Health Care Pvt. Ltd/ (Alves group company).

- Both the organization agreed to cooperate within the framework of below mentioned principles:
  - Create opportunity to work on joint research projects in the fields of mutual interests.
  - Facilitating undergraduate and postgraduate students of JUIT for internship & training at Alves group company- as per company norms.
  - Opportunities for eligible employees of Alves group for pursuing M.Tech/PhD degree in JUIT; The eligibility criteria for selection will be as per norms of JUIT.
  - Training of Alves group Limited personnel through continuing education program conducted by JUIT in areas of interest to Alves group.
  - Time to time any other appropriate mode of interaction agreed upon between JUIT and Alves group company.
  - JUIT and Alves group independently have the right to amend this collaboration.

It shall be pleasure for us to pay a token of honorarium and transportation expenses for your visit and kind participation in the mentioned activities as per your availability in this long term venture.

We would be very delight to have your positive response in this activity.

05/02/2021

- Prof. Vinod Kumar
  Vice Chancellor
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05/02/2021

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Onion genotypes Red Cereole, followed by Katarina Red 3 and Katarina Red 7 are superior with respect to post harvest quality parameters

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Abstract

Onion bulbs of long-day genotypes, viz. Red Cereole, Katarina Red 3, Katarina Red 7, Supreme, Cyrus, Lock Roy, Legend, Wall Brown, Brown Spanish, and a local cultivar were stored for 50, 100, and 150 days in a controlled atmosphere at 2± 1 °C and 75±1% relative humidity. The experiment was laid in a randomised block design with three replicates. Dry matter, TSS, hardiness of bulb, total sugar, non-reducing sugar, and reducing sugar loss in weight (%), rotating (%) sprouting (%), sprout length (cm), incident black mold (%), and marketable bulbs (%) were recorded throughout the storage period. In all genotypes, dry matter, TSS, total sugar, and non-reducing sugars, rotting (%) sprouting (%), sprout length (cm), and incidence of black mold increased gradually during storage. In contrast, hardiness/firmness of bulb, ascorbic acid, reducing sugar, physiological losses in weight, marketable bulbs decreased gradually during the storage period. Similar patterns of increase and decrease in all the observed traits were observed for all the genotypes. Furthermore, at the genotypic level, significant variation was observed in storage potential. The genotypes Red Cereole, Katarina Red 3, and Katarina Red 7 were superior to many of the post-harvest traits. They gave the highest marketable bulb at the end of storage. Therefore, it is concluded that onion genotypes Red Cereole, Katarina Red 3, and Katarina Red 7 have good storage potential that could be stored overwinter at high altitudes. Therefore, it is recommended to cultivate these onion genotypes for long-term storage in temperate regions.

Keywords: Cold desert region, onion genotypes, physico-chemical, post-harvest traits, total soluble solids.
Abbreviations: DHA_Dehydroascorbic Acid , DM_Dry Matter, TSS_Total Soluble Solids.

Introduction

Onion (Allium cepa var. Cepa L. family Alliaceae) is one of the most valuable vegetable crops grown worldwide. India is the second largest producer after China and ranks third in exports; however, it suffers from great fluctuations in supply and prices due to the effect of climate and weather on production and post-harvest losses. The shelf life of onion is more relevant in India because it is produced in the production hot spot states of Maharashtra, Gujarat, and Karnataka and transported to long-distance markets. Post-harvest losses may be as high as 66% due to poor postharvest management, absence of adequate cold storage or preservation facilities, and poor transport infrastructure, resulting in poor quality and shortage of onion in relation to the requirement (Adnan et al., 2014; Margaret et al., 1993).

Rotting, sprouting, physiological weight loss, and post-harvest diseases are reasons for the huge post-harvest loss reduction in marketable quality. A higher rate of respiration at room temperature generates heat, resulting in sprouting, causing loss of moisture and weight from bulbs (Petropoulos et al., 2017; Tanaka 1991; Gubb and MacTavish, 2002). Several studies have been carried out regarding onion storage at various temperatures. It was found that refrigeration temperature lowers respiration rate and inhibits sprouting and decay, which helps retain the quality and increase the shelf life of onion. Hot and humid storage conditions are suitable for the growth of black mold (Aspergillus niger) (Arowora and Adetunji, 2014; Tanaka, 1991; Yoo et al., 1989; Yang et al., 2004) bacterial soft rot (Pseudomonas gladioli) (Vintila et al., 2014; Wright, 1993) and other storage diseases in onion bulbs (Tripathi and Lawande, 2019). The quality of onion bulbs was better retained at low temperatures (0 °C).

Studies indicate that postharvest losses can be minimised by postharvest management and preharvest management, such as improved varieties and proper cultural practices. Some cultivars cannot maintain their quality for a long duration at ambient temperatures; however, some cultivars can be stored for more than six months under ambient conditions without deteriorating the quality. The selection of such long-storing cultivars could help maintain and enhance the physicochemical characteristics of onion bulbs.

Many physiological changes occur during post-harvest storage, resulting in a decline in the quality of the produce. There are two types of patterns for changing the sugar content in onion bulbs during storage. First, the sugar content changes with storage period following a regular trend, which may be monotonous increase, decrease, or a stable pattern. Second, there may be sharp fluctuations in

the concentration, with the amplitude and period of fluctuations showing no specific behaviour (Sharma and Lee, 2016). Sugar content in onion during storage depends on the type of cultivar, storage temperature, and postharvest treatments and techniques showing either a constant or fluctuating behaviour. Hence, there are conflicting reports in the literature and findings (Chope et al., 2007; Hansen, 1999). The ascorbic acid content in onion differs from cultivar to cultivar. Ascorbate oxidase is a copper-containing enzyme that oxidises ascorbic acid to dehydroascorbic acid (DHA) in the presence of molecular oxygen (Saari et al., 1995). Ascorbate oxidase is associated with rapidly growing regions in the plant and bound to cell walls and soluble protein in the cytosol. Under stress, such as pathogen or chemical exposure, ascorbate oxidase levels increase (Bielen et al., 2013; Loewus et al., 1987). Thus, ascorbic acid decreased throughout the storage period.

Although several studies on the shelf life of onion have been performed, there is no research on the storage behaviour of long-day onion cultivated in Leh-Ladakh. This motivated the researchers to conduct experiments on the shelf life of long-day onion genotypes. This study aims to understand the physicochemical changes occurring during storage of onion bulbs under controlled climatic conditions of Leh-Ladakh. The main objective is to elucidate the storage behaviour of different long-day onion genotypes.

Results and Discussion

Impact of Postharvest Storage on Important Quality parameters

Dry Matter Content

The observations of data indicate that the dry matter content range in different genotypes of onion was found to be from 8.03% to 14.83%, 8.21% to 15.07%, 8.33% to 15.12%, and 8.46% to 15.89% at 0, 50, 100, and 150 days of onion bulb storage (Table 1). The highest dry matter content was found in the Red Creole genotype, ranging from 14.83% to 15.89% during storage, followed by Katarina Red 3 (13.31% to 14.42%). The highest dry matter content (15.89%) was found at 150 days of storage of onion bulbs, whereas the lowest (8.03%) was found in Wall Brown at 0 days of storage. There was a significant difference in dry matter content during storage. The increase in dry matter content during storage could be attributed to the decrease in moisture content of the bulbs and increase in chemical constituents, resulting in more dry matter. Similar findings on the characterisation of stored onions and shallots by high dry matter content have been reported (Kahsay et al., 2013). Among the cultivars of bulb onions, dry matter content consisting mostly of fibre and sugars is an important quality factor determining bulb use; such high-dry-matter onions are required for dehydration.

Hardiness of Onion Bulbs

The hardiness of onion bulbs of different genotypes varied from 5.77 Kg/cm2 to 11.61 Kg/cm2, 5.11 Kg/cm2 to 11.53 Kg/cm2, 4.23 Kg/cm2 to 11.41 Kg/cm2, and 3.48 Kg/cm2 to 9.94 Kg/cm2 during 0, 50, 100, and 150 days of storage, respectively (Table 2). The maximum hardiness of the bulb was recorded as 11.61 kg/cm2, 11.53 kg/cm2, 11.41 and 9.94 kg/cm2 during 0, 50, 100, and 150 days of storage, respectively in the genotype Red Creole followed by Katarina Red 3 (11.09 kg/cm2, 10.75 kg/cm2, 10.01 kg/cm2, and 9.13 kg/cm2) at 0, 50, 100, and 150 days of storage, respectively. The hardness of bulbs decreased significantly during storage. Highest hardiness of the bulb was observed in fresh onion (11.61 Kg/cm2 in Red Creole) and lowest hardiness of the bulb was observed at 150 days of storage (3.48 Kg/cm2 in genotype Local cultivar). According to the findings of (Darbyshire and Henry, 1979), onions with high dry matter content are likely to be much firmer and can be stored for longer periods before shoot growth, and disease incidence reduces the marketable bulbs. Similar findings were reported by (Rutherford and Whittle, 1982) who classified cultivars by dry matter content from the highest to the lowest content that matches the ranking in terms of storage life from longest to shortest. Moreover, (Suzuki and Cutliffe, 1989) stated that higher dry matter content resulted in firmer bulbs.

Weight loss and Rotting

The physiological weight loss in different genotypes of onion bulbs ranged from 2.99% to 26.47% at 50 days of storage, 3.94% to 29.50% at 100 days of storage, and 8.65% to 61.28% at 150 days of storage (Table 3). The physiological loss in fresh onion bulbs was considered to be zero. The physiological loss in weight of the bulb was found to be maximum in the local cultivar (26.47%, 29.50%, and 61.28%) at 50, 100, and 150 days of storage. The lowest physiological loss in weight (2.99%, 3.94%, and 8.65%) at 50, 100, and 150 days of storage was found in the Red Creole genotype. The physiological weight loss in all genotypes increased significantly during the storage period. It was found to be lowest in Red Creole (8.65%) and highest in Local cultivar (61.28%) at 150 days of storage. Physiological loss in weight of onion bulbs during storage occurs due to moisture loss by respiration and hence depends on temperature. Therefore, weight loss decreases significantly with storage at low temperatures (Ko et al., 2002). Weight loss in onion bulbs was different in various genotypes; thus, results were observed to agree with the findings reported earlier (Kahsay et al., 2013) wherein it is reported that 'Bombay Red' and 'Melkam' varieties showed a significantly higher percentage of bulb weight loss.

The rotting percentage of the onion bulb of different genotypes varied from 0.66 % to 6.92%, 1.21% to 13.45%, and 2.58% to 30.44% at 50, 100, and 150 days of storage, respectively (Table 3). The minimum rotting percent of the bulb was recorded in Red Creole at 0%, 0.84%, and 2% at 50, 100, and 150 days of storage, respectively, followed by Katarina Red 3 at 0.83%, 1.21%, and 3.31%, and Katarina red 7 0.84%, 1.19%, and 3.63% at 50, 100, and 150 days of storage, respectively. Furthermore, the maximum rotting percentage of the bulb was in the local cultivar (6.92%, 13.45%, and 30.44% at 50, 100, and 150 days of storage). The percentage of rotting increased significantly during the storage of onion bulbs of all genotypes. Microbial spoilage is a major constraint in improving the storability of onion bulbs. They multiply and infect the bulb surface when congenital conditions prevail. Onion bulbs are affected by various postharvest diseases, such as black mold, neck rot, white rot, and soft rot. Among these, the only major postharvest disease responsible for the rotting of bulbs during storage was identified as black mold rot caused by Aspergillus niger. It is interesting to note that rotting and black mold were very low after 50 days of storage. The rotting and black mold percentages of bulbs significantly increased during 100 and 150 days of storage. The higher

rotting and black mold percentage may be due to the buildup of respiratory heat and humidity within the onion pile, creating favourable conditions for the proliferation of spoilage pathogens. Storage life can also be associated with dry matter (DM) content. This result is in accordance with a previous report where DM was also reported to be negatively correlated with the level of rotten bulbs (Rafika et al., 2006).

Sprouting

The data showed that the sprouting percentage in different genotypes of onion was found to be in the range of 0.67% to 7.01%, 1.00% to 16.53%, and 2.96% to 42.11% at 50, 100, and 150 days of storage (Table 4). In the genotype Red Creole, the sprouting percent was found to be minimum, which was recorded as 0.67%, 0.83 %, and 2.96% at 50,100, and 150 days of storage followed by 0.67, 1.00, 3.57% and 0.83, 1.10, 5.11% were found in the genotypes Katerina Red 3, Katerina Red 7, respectively. According to (Ghulam et al., 2013), the increasing sprouting percentage for different storage durations might be due to the increasing rate of respiration and metabolic processes.

The sprouting length in onion bulbs varied from 0.33 cm to 2.72 cm, 0.33 cm to 5.82 cm, and 1.21 to 9.00 cm during 50, 100, and 150 days of storage, respectively (Table 4). The sprouting length was found to be minimum (0.33, 0.33, and 1.21 cm) in Red Creole, followed by Katarina Red 3 (0.30, 0.47, and 1.28 cm), whereas the maximum sprouting length (2.72, 5.82, and 9.00 cm) was found at 50,100, and 150 days of storage, respectively, in the local cultivar. Sprouting was found to be augmented among all samples throughout the storage period in all genotypes. (Vintila et al., 2014) reported that sprouting was common to all genotypes of onions stored for different periods. (Kukanoor, 2005) reported that sprouting triggered the shrivelling of bulbs, resulting in the loss of marketable quality.

Disease incidence and Marketable Bulbs

The percentage incidence of black mold in onion bulbs ranged from 0.67% to 3.43% at 50 days of storage, 0.82% to 12.58% at 100 days of storage, and 1.80% to 15.48% at 150 days of storage (Table 5). The lowest percentage incidence of black mold 0.67%, 0.82%, and 1.80% at 50, 100, and 150 days of storage, respectively were found in genotype Red Cereole followed by Katarina Red 3 0.68, 0.82 and 1.81% and Katarina red 7 0.83, 1.34 and 1.84%, respectively. The incidence of black mold percentage was found to be a maximum of 3.43%, 12.58%, and 15.48% at 50, 100, and 150 days of storage, respectively) in the local cultivar. In fresh onion bulbs, the percentage incidence of black mold was found to be the lowest in all the genotypes, which significantly increased at 150 days of storage.

The marketable bulb percentages of different genotypes of onion were found to be in the range of 64.95% to 97.01%, 61.32% to 96.06%, and 24.43% to 88.77% during 50, 100, and 150 days of storage, respectively (Table 5). The maximum marketable bulb percentage (97.01%, 96.06%, and 88.77%) at 50, 100, and 150 days of storage, respectively, were found in the red cereole genotype. Furthermore, the minimum marketable bulb percentage was found in the local cultivar, which was recorded as 64.95%, 61.32%, and 24.43% at 50, 100, and 150 days of storage, respectively. This might be due to the genetic potential of these genotypes which produces high amounts of TSS and dry matter content that minimises weight loss, sprouting percentage, and incidence of black mold during storage.

Impact of Postharvest storage on key metabolites composition

Total Soluble Solids

The TSS content in onion bulbs ranged from 6.74% to 13.58% at 0 days of storage, 6.78% to 14.21% at 50 days of , 6.93% to 14.46%, and 7.71% to 14.79% after 150 days of storage (Table 6). The TSS was found to have maximum values of 13.58%, 14.21%, 14.46%, and 14.79% at 0, 50, 100, and 150 days of storage in the Red Creole genotype, followed by Katarina Red 3 at 11%, 11.88, 12.04, and 13%, respectively. Among all genotypes, the highest TS was found at 150 days of storage. A significant difference was observed in all genotypes during storage. TSS content was significantly influenced by different storage durations. Among the various storage durations tested, the maximum TSS was recorded at 150 days of storage, similar to the others. The higher percentage of TSS may be due to a greater loss of moisture and an increase in the dry matter content of the bulb, leading to an increase in the TSS content. These results are in close agreement with previous reports (Saimbhi and Randhawa, 1982; Patil and Kale, 1989). The lowest TSS was observed in bulbs after 0 days of storage. This may be due to the lack of metabolic reactions in freshly harvested bulbs and the high moisture content in the bulb.

Ascorbic Acid

The Ascorbic acid content in onion bulbs varied from 8.29 mg/100 g to 18.72 mg/100 g, 8.15 mg/100 g to 18.05 mg/100 g, 7.04 mg/100 g to 15.50 mg/100 g, and 5.78 mg/100 g to 12.55 mg/100 g during 0, 50, 100 and 150 days of storage, respectively (Table 7). The Ascorbic acid content was found maximum (18.72 mg/100 g, 18.05 mg/100 g, 15.50 mg/100 g, and 12.55 mg/100 g during 0, 50, 100 and 150 days of storage respectively) in the local cultivar followed by Cyrus (16.37 mg/100 g, 16.24 mg/100 g, 14.84 mg/100 g, 12.54 mg/100 g) and brown Spanish (16.08 mg/100 g, 15.86 mg/100 g, 14.27 mg/100 g, 12.52 mg/100 g) during 0, 50, 100, and 150 days of storage, respectively. The ascorbic acid content of the onion bulbs decreased significantly during storage. It was found to be the highest in fresh onions. There was a gradual decrease in the ascorbic acid content with increasing storage duration. This may be due to the oxidative destruction of ascorbic acid in the presence of molecular oxygen by the ascorbic acid oxidase enzyme.

Sugar Content

The total sugar content in different genotypes of onion was found to be in the range of 4.98% to 8.06%, 5.11% to 8.15%, 5.29% to 8.28%, and 5.98% to 8.90% at 0, 50, 100, and 150 days of onion bulb storage (Table 8). The highest total sugar content was found in the Red Creole genotype (8.90%), followed by Katarina Red 7 (8.72%) and Katarina Red 3 (8.46%) after 150 days of storage, whereas the lowest (4.98%, 5.11%, 5.29%, and 5.98%) in the Nasik Red genotype at 0,50,100, and 150 days of storage, respectively. The total sugar content increased significantly during the storage of onion bulbs in all genotypes. The reducing sugar content in different genotypes of onion was found to be in the range of 3.32% to 4.92%, 3.16% to 4.84%, 2.97% to 4.56%, and 2.56% to 3.98% at 0, 50, 100, and 150 days of storage of onion bulbs, respectively (Table S1). The maximum reducing sugar

Table 1. Effect of different storage durations on dry matter content.

		Dry matter (%)			
	Genotypes	0 DOS (control)	50 DOS	100 DOS	150 DOS
1.	Red Cereole	14.83±0.30^{n}	15.07±0.16^{no}	15.12±0.16^{no}	15.89±0.51^{p}
2.	Katarina Red 3	13.31±0.55^{klm}	13.53±0.51^{lmn}	13.67±0.52^{lm}	14.42±0.43^{mn}
3.	Katarina Red 7	11.22±0.40^{fgh}	11.35±0.44^{fgh}	11.85±0.31^{fghij}	12.67±0.17^{jkl}
4.	Supreme	8.85±0.31^{abcde}	9.18±0.37^{bcde}	9.33±0.41^{cde}	9.70±0.28^{e}
5.	Cyrus	8.84±0.21^{abcde}	9.07±0.32^{abcde}	9.42±0.30^{de}	9.73±0.20^{e}
6.	Lock Roy	8.18±0.28^{ab}	8.39±0.29^{abcd}	8.44±0.27^{abcd}	8.74±0.22^{abcde}
7.	Legend	8.15±0.26^{ab}	8.28±0.23^{abc}	8.44±0.12^{abcd}	8.99±0.11^{abcde}
8.	Wall Brown	8.03±0.25^{a}	8.21±0.24^{ab}	8.33±0.28^{a}	8.46±0.35^{abcd}
9.	Brown Spanish	12.02±0.20^{ghij}	12.02±0.39^{ghij}	12.18±0.35^{hij}	12.46±0.23^{ijk}
10.	Local Cultivar	8.22±0.27^{ab}	8.32±0.25^{abc}	8.46±0.24^{abcd}	8.67±0.17^{abcd}
11.	Nasik Red	10.92±0.36^{f}	11.05±0.41^{fg}	11.56±0.48^{fghi}	11.95±0.22^{ghij}
	Range	8.03-14.83	8.28-15.07	8.33-15.12	8.46-15.89

Values are represented as mean ± SE; for each column, different lowercase letters indicate significant differences at p<0.05, as measured by 2-sided Tukey's HSD among genotypes. Values bearing a common superscript (abcd) within the column did not vary significantly.

Table 2. Effect of different storage durations on Hardiness of bulb.

S.no.	Hardness of the bulb (kg/cm^2)				
	Genotypes	0 DOS (control)	50 DOS	100 DOS	150 DOS
1.	Red Cereole	11.61±0.50^{o}	11.53±0.49^{o}	11.41±0.62^{o}	9.94±0.14^{nm}
2.	Katarina Red 3	11.09±0.44^{no}	10.75±0.55^{no}	10.01±0.47^{no}	9.13±0.29^{lm}
3.	Katarina Red 7	10.69±0.29^{no}	10.57±0.49^{no}	10.47±0.29^{no}	9.12±0.33^{lm}
4.	Supreme	7.80±0.70^{ghij}	7.34±0.78^{hijk}	4.76±0.16^{cde}	4.19±0.32^{bcd}
5.	Cyrus	6.57±0.28^{ghi}	6.42±0.72^{ghi}	4.31±0.23^{bcd}	3.80±0.34^{abc}
6.	Lock Roy	8.90±0.34^{jk}	8.47±0.37^{kl}	5.42±0.12^{bcd}	5.20±0.23^{def}
7.	Legend	6.87±0.14^{fgh}	6.77±0.15^{ghij}	4.94±0.18^{cde}	4.90±0.10^{cde}
8.	Wall Brown	7.63±0.29^{ghi}	7.53±0.48^{ijk}	4.89±0.09^{bcd}	4.79±0.20^{cde}
9.	Brown Spanish	6.15±0.19^{fgh}	5.23±0.40^{def}	4.62±0.11^{bcde}	3.92±0.33^{a}
10.	Local Cultivar	5.77±0.23^{cde}	5.11±0.28^{def}	4.23±0.06^{bcd}	3.48±0.19^{ab}
11.	Nasik Red	6.75±0.31^{ghij}	7.55±0.58^{ijk}	5.81±0.26^{efg}	4.30±0.29^{bcd}
	Range	5.77-11.61	5.11-11.53	4.23-11.41	3.48-9.94

Values are represented as mean ± SE; for each column, different lowercase letters indicate significant differences at p<0.05, as measured by 2-sided Tukey's HSD among genotypes. Values bearing a common superscript (abcd) within the column did not vary significantly.

Table 3. Effect of different storage durations on weight loss and Rotting.

S.no.		Physiological loss in weight (%)			Rotting (%)		
	Genotypes	50DOS	100DOS	150DOS	50DOS	100DOS	150DOS
1.	Red Cereole	2.99±0.08^{a}	3.94±0.21^{a}	8.65±0.42^{bcd}	0.67±0.17^{a}	0.84±0.34^{a}	2.58±0.22^{abc}
2.	Katarina Red 3	3.08±0.22^{a}	4.07±0.25^{a}	9.41±0.56^{cdef}	0.83±0.17^{a}	1.21±0.15^{ab}	3.31±0.37^{abcd}
3.	Katarina Red 7	3.75±0.16^{a}	4.90±0.16^{ab}	10.98±0.62^{cdef}	0.84±0.17^{a}	1.19±0.37^{ab}	3.63±0.37^{bcd}
4.	Supreme	6.84±0.77^{abc}	12.45±1.03^{def}	31.56±3.38^{i}	4.87±0.33^{cde}	10.48±0.77^{fg}	22.89±2.75^{l}
5.	Cyrus	10.27±1.02^{cdef}	13.08±1.17^{ef}	35.77±2.97^{j}	5.43±0.42^{de}	11.84±0.53^{fgh}	21.85±1.20^{kl}
6.	Lock Roy	11.05±0.36^{cdef}	11.26±0.86^{def}	35.41±1.81^{j}	4.50±0.19^{cde}	9.41±0.35^{f}	19.90±1.24^{jk}
7.	Legend	3.82±0.32^{a}	8.62±0.86^{bcd}	13.55±0.63^{f}	0.87±0.19^{a}	9.99±0.29^{f}	16.61±1.05^{i}
8.	Wall Brown	10.32±0.20^{cdef}	12.80±0.99^{def}	31.10±1.81^{i}	4.83±0.11^{cde}	13.40±1.14^{h}	25.60±1.61^{m}
9.	Brown Spanish	10.54±0.06^{cdef}	12.58±0.45^{def}	36.88±1.55^{j}	6.31±0.27^{e}	12.60±0.50^{gh}	14.29±1.19^{n}
10.	Local Cultivar	26.47±1.22^{h}	29.50±1.56^{hi}	61.28±2.86^{k}	6.92±0.28^{e}	13.45±0.50^{f}	30.44±0.80^{h}
11.	Nasik Red	8.99±0.85^{cde}	12.07±0.91^{def}	22.04±1.66^{g}	5.05±0.47^{cde}	10.03±0.66^{f}	18.85±1.30^{ij}
	Range	2.99-26.47	3.94-29.50	8.65-61.28	0.67-6.92	0.84-13.45	2.58-30.44

Values are represented as mean ± SE; for each column, different lowercase letters indicate significant differences at p<0.05, as measured by 2-sided Tukey's HSD among genotypes. Values bearing a common superscript (abcd) within the column did not vary significantly.

Table 4. Effect of different storage durations on sprouting.

S.no.	Genotypes	Sprouting (%)			Sprouting length (cm)		
		50DOS	100DOS	150DOS	50DOS	100DOS	150DOS
1.	Red Cereole	0.67±0.17^{a}	0.83±0.29^{ab}	2.96±0.34^{abcd}	0.33±0.09^{a}	0.33±0.06^{a}	1.21±0.06^{bc}
2.	Katarina Red 3	0.67±0.17^{a}	1.00±0.33^{a}	3.59±0.43^{cd}	0.30±0.06^{a}	0.47±0.09^{ab}	1.28±0.11^{bc}
3.	Katarina Red 7	0.83±0.17^{a}	1.10±0.29^{ab}	5.11±0.26^{de}	0.27±0.09^{a}	0.34±0.09^{a}	1.75±0.24^{c}
4.	Supreme	2.20±0.23^{abc}	10.70±0.69^{h}	15.73±1.14^{i}	1.20±0.10^{bc}	4.13±0.37^{ef}	5.99±0.56^{ijk}
5.	Cyrus	2.87±0.24^{abcd}	9.86±0.46^{ij}	18.32±1.21^{j}	1.29±0.15^{bc}	4.59±0.35^{fg}	6.50±0.39^{jk}
6.	Lock Roy	3.07±0.21^{abcd}	8.03±0.13^{hi}	16.14±1.12^{ij}	1.61±0.20^{c}	4.98±0.20^{gh}	7.44±0.50^{l}
7.	Legend	1.34±0.17^{abc}	1.96±0.15^{abc}	6.33±0.28^{ef}	0.34±0.09^{a}	2.51±0.04^{d}	3.69±0.16^{e}
8.	Wall Brown	3.82±0.23^{cd}	9.38±0.48^{ij}	14.63±0.76^{i}	1.66±0.25^{c}	5.37±0.47^{ghi}	7.44±0.36^{l}
9.	Brown Spanish	3.62±0.23^{cd}	8.34±0.39^{hi}	32.19±1.58^{k}	1.37±0.12^{c}	5.65±0.20^{hi}	8.41±0.32^{m}
10.	Local Cultivar	7.01±0.30^{ef}	16.53±0.88^{ij}	42.11±2.70^{l}	2.72±0.17^{d}	5.82±0.22^{ij}	9.00±0.35^{m}
11.	Nasik Red	3.38±0.10^{bcd}	7.56±0.53^{hi}	14.20±0.69^{i}	1.11±0.07^{abc}	4.70±0.33^{fg}	6.73±0.48^{kl}
	Range	0.67-7.01	0.83-16.53	2.96-42.11	0.33-2.72	0.33-5.82	1.21-9.00

Values are represented as mean ± SE; for each column, different lowercase letters indicate significant differences at p<0.05, as measured by 2-sided Tukey's HSD among genotypes. Values bearing a common superscript (abcd) within the column did not vary significantly.

Table 5. Effect of different storage durations on Incidence of black mold and Marketable bulb.

S.no.	Genotypes	Incidence of black mold			Marketable bulb (%)		
		50DOS	100DOS	150DOS	50DOS	100DOS	150DOS
1.	Red Cereole	0.67±0.17^{a}	0.82±0.32^{ab}	1.80±0.11^{ab}	97.01±0.08^{m}	96.06±0.21^{m}	88.77±0.35^{l}
2.	Katarina Red 3	0.68±0.17^{a}	0.82±0.32^{ab}	1.81±0.11^{ab}	96.92±0.22^{m}	95.93±0.25^{m}	87.28±0.36^{kl}
3.	Katarina Red 7	0.83±0.17^{a}	1.34±0.17^{ab}	1.80±0.23^{ab}	96.25±0.16^{m}	95.10±0.16^{m}	85.38±0.68^{ijkl}
4.	Supreme	1.07±0.07^{ab}	3.74±0.17^{abc}	8.42±1.25^{f}	83.68±0.81^{hijk}	82.68±0.09^{hijk}	45.55±5.45^{c}
5.	Cyrus	1.20±0.12^{ab}	4.45±0.12^{ab}	12.43±1.04^{g}	84.29±1.34^{hijkl}	76.74±0.26^{fg}	42.38±4.17^{c}
6.	Lock Roy	1.90±0.21^{ab}	5.73±0.34^{cdef}	13.07±1.40^{g}	84.46±0.55^{hijkl}	80.66±0.62^{ghi}	44.69±0.61^{c}
7.	Legend	1.40±0.31^{ab}	6.03±0.20^{cdef}	12.16±0.87^{g}	96.18±0.32^{m}	81.38±0.72^{ghij}	69.84±1.43^{e}
8.	Wall Brown	2.07±0.18^{ab}	8.11±0.44^{ef}	13.55±1.63^{g}	84.19±0.61^{hijkl}	75.08±1.86^{f}	43.30±0.52^{c}
9.	Brown Spanish	2.00±0.12^{ab}	7.53±0.68^{def}	12.35±1.45^{g}	80.14±0.73^{gh}	74.82±0.95^{f}	32.68±0.42^{b}
10.	Local Cultivar	3.43±0.46^{abc}	12.58±1.57^{g}	15.48±1.31^{g}	64.95±0.77^{d}	61.32±1.30^{d}	24.43±3.66^{a}
11.	Nasik Red	1.07±0.07^{ab}	4.56±0.60^{bcde}	8.36±1.03^{f}	85.96±1.04^{jkl}	79.93±0.75^{gh}	64.34±0.01^{d}
	Range	0.67-3.43	0.82-12.58	1.80-15.48	64.95-97.01	61.32-96.06	24.43-88.77

Values are represented as mean ± SE; for each column, different lowercase letters indicate significant differences at p<0.05, as measured by 2-sided Tukey's HSD among genotypes. A value bearing a common superscript (abcd) within the column does not vary significantly.

Table 6. Effect of different storage durations on total soluble solids (TSS).

S.no.	Genotypes	Total soluble solids (TSS, %)			
		0 DOS (control)	50 DOS	100 DOS	150 DOS
1.	Red Cereole	13.58±0.48^{lm}	14.21±0.56^{lmn}	14.46±0.69^{mn}	14.79±0.43^{n}
2.	Katarina Red 3	11.30±0.18^{ijk}	11.88±0.52^{jk}	12.04±0.48^{k}	13.37±0.44^{l}
3.	Katarina Red 7	10.56±0.35^{hi}	10.59±0.33^{hi}	10.85±0.41^{hij}	12.15±0.08^{k}
4.	Supreme	8.50±0.38^{def}	8.62±0.30^{ef}	8.69±0.32^{ef}	9.09±0.36^{fg}
5.	Cyrus	6.77±0.14^{ab}	6.92±0.11^{a}	6.97±0.09^{a}	9.10±0.34^{fg}
6.	Lock Roy	7.39±0.36^{abcd}	7.45±0.43^{abcd}	7.49±0.39^{abcd}	8.27±0.18^{cdef}
7.	Legend	7.18±0.63^{abc}	7.50±0.32^{abcd}	7.34±0.71^{abc}	8.29±0.23^{cdef}
8.	Wall Brown	6.78±0.03^{a}	6.93±0.17^{ab}	6.93±0.20^{ab}	7.71±0.33^{abcde}
9.	Brown Spanish	11.23±0.06^{ijk}	11.28±0.04^{ijk}	11.52±0.06^{ijk}	11.50±0.17^{ijk}
10.	Local Cultivar	7.28±0.26^{abc}	7.32±0.28^{abc}	7.44±0.32^{abcd}	7.92±0.28^{bcde}
11.	Nasik Red	9.92±0.19^{gh}	10.02±0.10^{gh}	10.11±0.22^{gh}	10.72±0.34^{hi}
	Range	6.77-13.58	6.92-14.21	6.93-14.46	7.71-14.79

Values are represented as mean ± SE; for each column, different lowercase letters indicate significant differences at p<0.05, as measured by 2-sided Tukey's HSD among genotypes. Values bearing a common superscript (abcd) within the column did not vary significantly.

Table 7. Effect of different storage durations on ascorbic acid.

S.no.	Ascorbic acid (mg/100 g bulb)				
	Genotypes	0 DOS (control)	50 DOS	100 DOS	150 DOS
1.	Red Cereole	13.07±0.22^{efgh}	13.09±0.17^{efgh}	11.77±0.59^{def}	7.58±0.68^{bc}
2.	Katarina Red 3	13.12±0.19^{efgh}	13.08±0.22^{efgh}	11.98±0.97^{defg}	8.52±0.74^{bc}
3.	Katarina Red 7	14.11±0.06^{hij}	13.75±0.16^{hi}	12.98±0.64^{efgh}	8.77±0.28^{c}
4.	Supreme	15.69±0.45^{jkl}	15.65±0.48^{jkl}	14.11±1.25^{hij}	10.30±0.95^{d}
5.	Cyrus	16.37±0.37^{kl}	16.24±0.33^{kl}	14.84±0.40^{ijk}	12.54±0.55^{def}
6.	Lock Roy	15.57±0.53^{jk}	15.54±0.55^{jk}	13.61±0.83^{ghi}	11.39±0.87^{de}
7.	Legend	8.29±0.21^{bc}	8.15±0.13^{bc}	7.04±0.14^{ab}	5.78±0.29^{a}
8.	Wall Brown	15.83±0.60^{kl}	15.81±0.61^{kl}	13.35±0.48^{fghi}	10.58±0.49^{d}
9.	Brown Spanish	16.08±0.62^{m}	15.86±0.73^{m}	14.27±0.60^{l}	12.52±0.55^{efgh}
10.	Local Cultivar	18.72±0.33^{n}	18.05±0.77^{n}	15.50±0.56^{m}	12.55±0.69^{de}
11.	Nasik Red	12.78±0.35^{efgh}	12.71±0.40^{efgh}	10.55±0.56^{d}	7.22±0.38^{abc}
	Range	8.29-18.72	8.14-18.05	7.04-15.50	5.78-12.55

Values are represented as mean ± SE; for each column, different lowercase letters indicate significant differences at p<0.05, as measured by 2-sided Tukey's HSD among genotypes. Values bearing a common superscript (abcd) within the column did not vary significantly.

Table 8. Effect of different storage durations on Total sugar.

S.no.	Total sugar (%)				
	Genotypes	0 DOS (control)	50 DOS	100 DOS	150 DOS
1.	Red Cereole	8.06±0.16^{qrst}	8.15±0.14^{qrstu}	8.28±0.21^{qrstuv}	8.90±0.07^{v}
2.	Katarina Red 3	7.36±0.14^{klmnop}	7.49±0.15^{lmnopq}	7.58±0.13^{mnopq}	8.46±0.13^{tuv}
3.	Katarina Red 7	7.62±0.32^{nopqr}	7.78±0.33^{pqrs}	8.35±0.14^{stuv}	8.72±0.08^{uv}
4.	Supreme	7.47±0.18^{lmnopq}	7.61±0.16^{nopqr}	7.76±0.16^{pqrs}	8.10±0.13^{qrstu}
5.	Cyrus	6.56±0.35^{fghi}	6.69±0.35^{ghij}	7.02±0.25^{ijklmno}	7.64±0.09^{nopqr}
6.	Lock Roy	7.52±0.42^{lmnopq}	7.65±0.41^{nopqr}	7.80±0.44^{pqrs}	8.58±0.21^{tuv}
7.	Legend	5.97±0.17^{cdef}	6.19±0.18^{defg}	6.91±0.10^{ijklm}	7.33±0.13^{jklmnop}
8.	Wall Brown	7.31±0.16^{jklmnop}	7.49±0.17^{lmnopq}	7.69±0.24^{opqrs}	8.11±0.18^{qrstu}
9.	Brown Spanish	6.71±0.39^{qhijk}	6.88±0.35^{hijkl}	6.98±0.43^{ijklmn}	7.56±0.28^{mnopq}
10.	Local Cultivar	5.38±0.07^{abc}	5.56±0.07^{abcd}	5.85±0.18^{bcde}	6.26±0.20^{efgh}
11.	Nasik Red	4.98±0.08^{a}	5.11±0.06^{a}	5.29±0.03^{ab}	5.98±0.11^{cdef}
	Range	4.98-8.06	5.11-8.15	5.29-8.28	5.98-8.90

Values are represented as mean ± SE; for each column, different lowercase letters indicate significant differences at p<0.05, as measured by 2-sided Tukey's HSD among genotypes. Values bearing a common superscript (abcd) within the column did not vary significantly.

content (4.92%, 4.84%, 4.56%, and 3.98%) after 50, 100, and 150 days of storage, respectively, was found in the Red Creole genotype, followed by Katarina Red 7(4.77%, 4.67%, 4.38%, and 3.95% at 0,50,100, and 150 days of storage, respectively). The highest reducing sugar content (4.92% in Red Creole, 4.77% in Katarina Red 7) was found in fresh onion bulbs, whereas the lowest (3.98% in Red Creole, 3.95% in Katarina Red 7) was found at 150 days of storage. The reducing sugar content decreased significantly during 150 days of storage.

The non-reducing sugar content in onion bulbs varied from 1.66% to 3.63%, 1.95% to 3.93%, 2.32% to 4.32%, and 3.37% to 5.62% at 0, 50, 100, and 150 days of storage, respectively (Table S2). It was found to be maximum (3.63%, 3.93%, 4.32%, and 5.62%) at 0, 50, 100, and 150 DOS, respectively, in the genotype Lock Roy, followed by Brown Spanish 3.33%, 3.58%, 3.97%, and 4.98% at 0, 50, 100, and 150 DOS, respectively, and Red Creole 3.13%, 3.41%, 3.87%, and 4.92%) at 0, 50, 100 and 150 DOS respectively). A significant variation was observed in the non-reducing sugar content during storage. The highest non-reducing sugar content was recorded after 150 d of storage of onion bulbs. The increase in total sugar content could be due to the enzymatic hydrolysis of fructans to fructose and glucose during the storage period (Shivakumar and Chandrashekar, 2014). All genotypes showed a decreasing trend in reducing sugars during all storage durations. The differences between the varieties and storage durations were found to be significant. The decreasing trend may be due to the conversion of reducing sugars to starch during storage at low temperatures (Kukanoor, 2005; Bogevska et al., 2016). It wasobserved that the cultivar, postharvest treatments, and temperature can affect sugar content during storage, showing either a constant or an unstable pattern (Chope et al., 2007). The sugar content may be correlated with other physiological factors, such as dormancy break and sprouting (Sharma and Lee, 2016). There are two types of sugar content behaviour. According to the first one, the concentration of sugar changes with storage time following a regular pattern, such as a monotonous increase, decrease, or stable behaviour. Another type of behaviour consists of strong fluctuations in the sugar content, with the amplitude and period of fluctuations showing no regular pattern (Sharma et al., 2015).

Materials and methods

Conduction of study

The present study was conducted during two consecutive cropping seasons at the Vegetable Research Unit of the Defense Institute of High Altitude Research, Defense Research and Development Organization, which lies at

latitude 34°8'16.119' 'N, longitude 77°34'19.2216 '' E at an elevation of 3500 m msl in Leh-Ladakh, India. The climate of the area is typically dry temperate, with extreme fluctuations in temperature, and precipitation is negligible.

Plant material

The experimental material consisted of nine long-day genotypes and one local cultivar of onion: Red Creole, Katarina Red 3, Katarina Red 7, Supreme, Cyrus, Lock Roy, Legend, Wall Brown, Brown Spanish, Local Cultivar, and Nasik Red. The seeds were sown in trenches with 5 cm spacing between lines during the first week of April, and all standard agronomic practices recommended for onion cultivation were carried out. After 60 days, when the seedlings reached the optimum set/baby bulb size, they were harvested and cured in the shade for one month. They were stored at 2±1 0C and 75±2 % relative humidity in an onion store. Sets were planted to produce mature onion bulbs using standard agronomic practices. The crop was harvested at maturity when 70 % of the plants showed drying and falling of their tops. The plants were pulled along with leaves and kept for three days in the field for curing. The dry aerial parts were removed with sharp clean knives leaving a 2.5 cm top above the bulb. These bulbs were kept under 50 % shade for curing for 20 days. The cured onion bulbs were sorted out; any diseased or damaged bulbs were discarded before storage, and 5 kg of healthy bulbs from each treatment were packed in thin gunny bags and stored for storage. Bulbs were stored under controlled atmosphere (CA) conditions (2± 1 °C and 75±1% relative humidity).

Experimental design

The experiment was conducted in a randomised block design with three replicates. The observations were recorded at 50, 100, and 150 days of storage for the traits, such as physiological loss in weight (%), rotation (%), sprouting (%), sprout length (cm), incidence of black mold (%), and marketable bulbs (%).

Dry matter (%)

Bulbs were randomly selected from each treatment and cut into small pieces using a stainless steel knife. A known weight of the sample was dried in a hot air oven at 60 °C until a constant weight was obtained. The percentage of dry matter was calculated using the following formula: Dry matter percent = (Dry weight of sample)/(Fresh weight of sample)×100

Total Soluble Solids (%)

The total soluble solids (TSS) of onions were determined using a hand refractometer (Attago, Japan). The values were expressed as the percentage of total soluble solids of the bulbs.

Hardness of the bulb (kg/cm2)

The hardness of the onion bulbs was measured using a hand penetrometer (Fruit Pressure Tester, Make: Effegi, Model: PT 327), and the pressure required to penetrate the bulb was recorded in kg/cm2.

Ascorbic Acid (mg/100 g)

Vitamin C (ascorbic acid) in the onion bulb was calculated using the titrimetric method (AOAC, 2005). A fresh onion bulb (100 g) was crushed the whole material into a pestle and mortar by adding 100 ml 2 per cent oxalic acid solution. The whole content was finally transferred into a pre-weighed beaker, and the weight of the crushed slurry was recorded on a digital balance. Twenty grams of crushed slurry was transferred into a 100 ml capacity conical flask and the volume was made up to 100 ml by adding one per cent oxalic acid solution. The content of the conical flask was filtered using filter paper, and the filtrate was collected into another flask. The filtrate (5 ml) of each flask was taken and the whole content of the conical flask was titrated against the dye solution (2, 6-dichloro phenol endophenol) until the end point (pink colour) was achieved, and the titer value was noted. A 5 ml standard ascorbic acid solution was placed in another conical flask and the whole content was titrated against the dye solution until the end point was obtained. The results were expressed as milligrammes of ascorbic acid per 100 g of fresh sample.

Total Sugars (%)

Total sugar was measured using the phenol-sulfuric acid method (Dubois et al., 1951). Water (0.1 ml of sample, water was added to a volume of 2 ml. To this solution 0.05 ml phenol reagent and 5 ml sulfuric acid were added rapidly one after another and allowed to remain at room temperature for 30 min. The absorbance was recorded at 490 nm against a reagent blank. A standard curve using an aqueous stock solution containing 10-120 µg of D-glucose was plotted to estimate total sugar in the samples.

Estimation of Reducing Sugars

Reducing sugar (%) was determined using the dinitrosalicylic acid method. One gram of sample was taken and crushed properly in a mortar and pestle. It was transferred to a test tube, and the volume was made up to 1 ml with distilled water. Then, 3 ml DNS reagent was added and incubated in a boiling water bath for 20 min and cooled for 5 min at room temperature. The sample was then diluted to make up 20 ml (necessary to obtain a percentage between 20% and 80%). A glucose standard curve was produced in the range of 0.25-6.0 mg of glucose per ml using the same procedure. The absorbance was recorded at 540 nm against the blank reagent when the colour stability developed until 72 h.

Non-reducing sugar (%)

The percentage of non-reducing sugar was obtained by subtracting the values of reducing sugar from that of total sugar and multiplying it with 0.95, as described below.

Nonreducing sugar (%)=(Total sugar-reducing sugar)×0.95

Weight Loss (%)

The weight of the bulbs was measured using an electronic balance. The cumulative weight loss of the bulbs was calculated and expressed as percent weight loss.

Weight loss (%) = ((W0-W1-W2-W3))/W0×100

where W0 is the initial weight of the bulbs, W1 is the weight loss at 50 days of storage, W2 is the weight loss at 100 days of storage, and W3 is the weight loss at 150 days of storage.

Rotting (%)

The weight of the rotted bulbs at the end of 50, 100, and 150 days of storage (DOS) was recorded under each storage condition, and the rotting percentage was calculated using the formula.

Rotting (%) = (Weight of rotted bulbs)/(Initial weight of the bulbs)×100

Sprouting (%)

To determine the sprouting percentage on stipulated days of storage, the bulbs showing a sprout were separated from the lot and weighed on an electronic balance. The sprouting percentage, which indicates the weight of the bulbs sprouted at 50, 100, and 150 days of storage (DOS) was calculated.

Sprouting percentage = (Weight of sprouted bulbs)/(Initial weight of the bulbs)×100

Sprout length (cm)

Five sprouted bulbs were randomly selected, and the length of the sprouts in each bulb was measured. The mean length of the sprouts was expressed in centimetres.

Incidence of black mold (%)

The incidence of black mold was expressed as the percentage of bulbs affected per 100 bulbs.

Marketable bulbs (%)

At the end of the storage period (150 DOS), the rotted and sprouted bulbs were separated, and the weight of healthy bulbs was recorded. The recovery of marketable bulbs was calculated using the following formula:

Marketable bulbs (%) = (Weight of the healthy bulbs obtained)/(Initial weight of the bulbs stored)×100

Statistical Analysis

All statistical analyses were performed using the statistical package "SPSS" for Windows Version 21. The least significant difference between mean values was calculated using Duncan's multiple range test (DMRT) at the 5% significance level.

Conclusion

Different physiological and biochemical changes occur during the storage period in all genotypes, causing significant post-harvest storage deterioration and reduce the marketable bulb quality. It can be concluded that the quality of the stored material depends on the genetic potential of the studied genotypes and storage duration. The highest dry matter and total soluble solids were observed in the Red Cereole genotype, which increased with the number of days of storage, whereas ascorbic acid was the highest in the Local cultivar. Total sugar and reducing sugar were highest in red cereole, which decreased with the increase in days of storage. The least weight loss, rotting, the incidence of black mold, sprouting was observed in red cereole, and the highest marketable bulb yield was observed in red cereole. Therefore, among all the studied genotypes, Red Cereole, Katarina Red 3, and Katarina Red 7 were characterised as having a longer storage life with the highest proportion of marketable bulbs.

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SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the PhD thesis titled **"Characterization and Evaluation of Onion (Allium cepa L.) Germplasm at Leh, trans-Himalaya, India"** submitted by **Jagdish Singh Arya** at **Jaypee University of Information Technology, Waknaghat, Solan, India** is a bonafide record of his original work carried out under our supervision. This work has not been submitted elsewhere for any other degree or diploma.

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Sexual differences and seasonal variations in total phenolics and antioxidant properties in *Hippophae rhamnoides* leaves

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Abstract.
BACKGROUND: Seabuckthorn (SBT) leaves are used for extraction of health promoting compounds and product development.
OBJECTIVE: The aim of the work was to find out gender differences and seasonal variation in total polyphenol content (TPC) and total antioxidant capacity (TAC).
METHOD: Leaves of six natural population of SBT, which comprised 200 plants (100 males, 100 females) from the trans-Himalaya were harvested, and their methanolic and acetone extracts were studied for TPC and TAC.
RESULTS: Males exhibit significantly higher TPC (100.8 ± 23.9 mg GAE/g DW) than females (95.0 ± 23.8 mg GAE/g DW). Similarly, ferric reducing activity was significantly higher in males (6.5 ± 1.1 Fe^{2+} mmol/g DW) than females (6.1 ± 1.2 Fe^{2+} mmol/g DW). Significant increase in TPC was observed in male leaves from July to October followed by a significant decrease in November. However, a trend of increase in TPC upto August and a steady decline thereafter was observed in leaves of female SBT. Similarly a trend of an increasing TAC was observed in both the sexes but female leaves were observed to be on an increasing TAC from July to October.
CONCLUSION: Male SBT leaves exhibit higher TPC and TAC than females; October is the best time for harvesting SBT leaves and; SBT leaves contain significantly higher hydrophilic than lipophilic phenolics and antioxidants.

Keywords: Dioecious, Himalaya, Polyphenols, reproductive effort, Seabuckthorn

1. Introduction

Seabuckthorn (*Hippophae rhamnoides* L.) is an ecologically and economically important plant [1]. The species is native to Europe and Asia, but nowadays it is widely grown all over the world. Seabuckthorn (SBT) is dioecious and wind pollinated plant. Traditionally, every part of the plant is being used for a variety of purpose. There are over a hundred popular SBT-based formulations in various pharmacopoeias of *Sowa Rigpa* (Tibetan medicine) [2]. Although the nutritional and medicinal properties of SBT berries are usually the focus of attention, SBT leaf has been receiving much attention in recent years for its medicinal and therapeutic applications. SBT leaves possess

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antimicrobial [3–5], anti-viral [6], anti-radiation [7], hepatoprotective [8], cytoprotective [3], adaptogenic [9] and immunoprotective [10] activities. Many of these activities are attributed to high antioxidant capacity including the phenolics. Dried SBT leaves are used for tea and processed for nutraceuticals products.

Antioxidant properties of SBT leaves have been extensively studied [4, 8, 11, 12]. However, SBT is a dioecious plant and hence sexual differences in presence of health promoting compounds in its vegetative parts is expected due to greater reproductive effort in females. Limited studies have been carried on sex-related differences in antioxidant activity and phenolic content in SBT leaves. Górnaś et al. [13] studied lipophilic antioxidants in mixed SBT samples of two females and 10 males. It was found that lipophilic antioxidant is higher in male as compared to female leaves. In contrast, Šnē et al. [14] reported higher total phenolics and antioxidant in female SBT leaves. The antioxidant compounds viz. α-tocopherol, β-tocopherol, γ-tocopherol, plastochromanol-8 and β-carotene were observed to be higher in female than in male SBT leaves [15]. In view of the contrasting results from limited studies, it is felt that more studies involving larger number of samples are needed to better understand the sexual differences in TPC and TAC. Besides the gender differences, the season of harvesting is also known to have a significant effect on the content of antioxidant compounds in leaves [16–19]. SBT leaves can be harvested from June to November with a varying ease of harvesting. But only a small number of studies have been carried on to see the influence of season of harvesting on antioxidant properties of SBT leaves. Morgenstern et al. [20] studied change in antioxidant capacity and phenols during SBT leaf development from April to July. Górnaś et al. [13] studied antioxidants in mixed SBT samples of two female and 10 male harvested in June and October. To the best of our knowledge, studies involving a large number of samples over an extended period of harvesting have not been conducted thus far. In view of emerging importance of SBT leaves for medicinal and therapeutic applications, the present study was undertaken on a larger number of samples with the objective to investigate the role of sexual differences and seasonal variation in phenolic content and antioxidant capacities in SBT leaves.

2. Materials and methods

2.1. Sample collection

Six natural population of *Hippophae rhamnoides* subsp. *turkestanica* consisting of 100 male and 100 female plants were sampled across the major distribution sites from the Indian trans-Himalaya in October 2014 to study the gender-related differences in TPC and TAC in leaves. Leaves (5 g/plant) were harvested and freeze dried in a Laboratory freeze dryer (ALPHA 2–4 LD plus, Fisher Bioblock Scientific, France) and stored until analysis. The altitude of collection sites ranged from 3203 to 3885 m asl (Table 1). Altitude and location of study sites was established using GARMIN GPS 72, Olathe, Kansas, USA. Ten individual plants (five male and five female each) growing at experimental farm at Defence Institute of High Altitude Research (DIHAR) were selected for studying the seasonal variations in TPC and TAC. Leaves (2 g/plant) were harvested every month in the first week of July to November, freeze dried and stored until analysis.

2.2. Chemicals

Solvents and Folin-Ciocalteu reagent were obtained from Merck, Germany. 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 2,4,6-tripyridyl-*s*-triazine (TPTZ), gallic acid and ferrous sulfate hexahydrate, were from Sigma-Aldrich, USA. All the other chemicals used were of analytical grade.

2.3. Extraction

Two cycles of extraction, hydrophilic and lipophilic, were performed using the method previously described [21]. Hydrophilic extraction was performed with methanol while lipophilic extraction was done with acetone.

Table 1
Locations of six natural populations of *H. rhamnoides* L. from Indian trans-Himalaya

Sampling Locations	Population ID	Latitude (N)	Longitude (E)	Altitude (m) ASL	Sample size (numbers)	
					Male	Female
Spituk	SPT	34°07′7″	77°30′4″	3203 ± 5.6	20	20
Chuchot	CHU	34°05′4″	77°35′9″	3239 ± 5.0	17	17
Shey	SHY	34°04′1″	77°37′7″	3260 ± 4.6	17	17
Phyang	PHY	34°11′5″	77°30′1″	3636 ± 49.6	16	16
Horzey	HOR	34°12′1″	77°35′3″	3812 ± 24.8	15	15
Sakti	SKT	34°02′1″	77°48′6″	3885 ± 37.3	15	15

Powdered leaf samples (20 to 40 mg) was extracted ($n = 3$) for 15 min with 1.5 ml methanol in a 2 ml micro centrifuge tube and vortexed at room temperature. The sample was centrifuged at 5600 g for 10 min and the supernatant was recovered. The residue was mixed with 1.5 ml of acetone and the process was repeated as described above. TPC and FRAP were measured directly in the methanolic and acetone extracts and the values were combined mathematically. DPPH was measured in the combined methanolic and acetone extract.

2.4. Determination of total phenolic content

The Folin-Ciocalteu reagent assay was used to determine the TPC [22]. An aliquot of the samples (30 µl) was introduced into 96 well micro-plate followed by 150 µl Folin-Ciocalteu reagent, which was previously diluted with distilled water (1 : 10) and 120 µl sodium carbonate (75 g/l). The micro-plates were vortexed, covered with parafilm and allowed to stand for 30 min. Absorbance at 765 nm was recorded in a micro-plate reader (SpectroMax M2 e, Molecular Devices, Sunnyvale, CA, United States). TPC was expressed in gallic acid equivalents (GAE mg/g DW). The calibration equation for gallic acid was $y = 0.014 \times -0.003$ ($R^2 = 0.995$) where y is the absorbance at 765 nm and x is the concentration of gallic acid in mg/l.

2.5. Determination of antioxidant capacity

Ferric reducing antioxidant potential (FRAP) assay was conducted using the method previously described [23] with minor modifications [21]. A total of 7.5 µl of extract and 22.5 µl of distilled water were added to 225 µl of freshly prepared FRAP reagent (10 parts of 300 mmol/l sodium acetate buffer at pH 3.6, one part of 10 mmol/l 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ) solution and one part of 20 mmol/l $FeCl_3 0.6H_2O$) and the reaction mixture was incubated for 30 min. The increase in absorbance was measured at 593 nm. The FRAP value was expressed as $FeSO_4 0.7H_2O$ mmol/g DW. The calibration equation for $FeSO_4 0.7H_2O$ was $y = 0.323 \times -0.103$ ($R^2 = 0.983$) where y is the absorbance at 593 nm and x is the concentration of $FeSO_4 0.7H_2O$ in mM. Free radical scavenging method by DPPH developed by Brand-Williams et al. [24] was followed with minor modification [21]. A 0.1 mmol/l solution of DPPH in methanol was prepared and 300 µl of the solution was treated with 15 µl of the methanolic and acetone extracted sample. Control was treated with 15 µl of solvent instead of the extract. The mixture was left to stand at room temperature for 30 min before the decrease in absorbance at 517 nm was recorded. Antioxidant value was expressed as IC_{50}, the amount of sample extracted into 1 ml solution necessary to decrease by 50% the initial DPPH concentration. IC_{50} was derived from the % disappearance vs. concentration plot (concentration means mg of SBT leaf on DW basis extracted into 1 ml solution).

2.6. Statistical analysis

All the experiments were performed in triplicate. The experimental results were expressed as mean ± standard deviation (SD) using statistical analysis with SPSS (Statistical Program for Social Sciences, SPSS Corporation, Chicago, Illinois, USA). One way analysis of variance (ANOVA) and *post hoc* analysis with 2-sided Tukey's HSD at $p \leq 0.05$ level were performed. Student's *t* test and Pearson's correlation analysis were performed to compare and find the correlations among parameters. Regression was performed using MS Excel. Box plots were produced using XLSTAT software.

3. Results and discussion

3.1. Effect of plant sex on total polyphenol content and antioxidant activity

High variability in TPC within and among genotypes from different populations was observed. The TPC ranged from 47.2 to 173.1 in male and 29.9 to 165.8 mg GAE/g DW in female between genotypes. Therefore, a variation of 1–3.7 fold in male and 1–5.5 fold in female in TPC was observed. Effect of plant sex on TPC is presented in Table 2. Significantly high variability was observed between the populations. Males showed significantly higher TPC than females ($P < 0.001$, Student's *t*-test) in three out of the six populations. Females showed higher values in two populations and no significant gender differences was observed in the remaining single population. However, the overall mean TPC value of the six population was significantly higher in males (100.8 ± 23.9 mg GAE/g DW) than females (95.0 ± 23.8 mg GAE/g DW) at $p \leq 0.01$. In contrast, Šnē et al. [14] reported higher total phenolics in female SBT leaves which could be because of small sample size as observed in PHY and HOR populations in the present study. The overall difference in TPC between male and female SBT leaves in the present study was 5.8%, which is significantly lower than 45% higher TPC reported in male *Ginkgo biloba* leaves that females [25].

FRAC and DPPH assay are widely used method to test the antioxidant capacity in berries [26, 27]. The ferric reducing activity ranged from 3.9 to 9.5 in male and 2.6 to 9.1 Fe^{2+} mmol/g DW in female. The difference in FRAP value between the genotypes showing the highest and lowest value was 1–2.4 fold in male and 1–3.5 fold in female. Gender effect on FRAP is presented in Table 3. Significantly high variability was observed between the populations. Males showed significantly higher ferric reducing activity than females ($P < 0.001$, Student's *t*-test) in two populations and no significant gender differences was observed in the remaining four populations. However, the overall mean FRAP value was significantly higher in males (6.5 ± 1.1 Fe^{2+} mmol/g DW) than females (6.1 ± 1.2 Fe^{2+} mmol/g DW). The result is in agreement with studies by Górnaś et al. [13] who reported higher antioxidant activities in mixed 10 SBT males than two females. In contrast, Šnē et al. [14] reported higher ferric reducing activity in female than male SBT leaves. The antioxidant compounds viz. α-tocopherol, β-tocopherol, γ-tocopherol, plastochromanol-8 and β-carotene were observed higher in female than in male SBT leaves [15].

Free radical scavenging activity of leaves extract expressed as IC_{50} is presented in Table 3. A single population showed significantly higher scavenging activities in males than females ($P < 0.001$, Student's *t*-test) and no significant gender differences was observed in the remaining five populations. The overall mean scavenging value was higher in males but the difference was not statistically significant. Higher TPC and TAC with acetone suggests that SBT leaves contains significantly higher hydrophilic than lipophilic antioxidants.

Higher TPC and TAC in male leaves in the present study is in agreement with the fact that in dioecious species the cost of reproduction involves prioritization of resources in fruit development rather than in vegetative growth or protection in females. A major investment in reproduction is generally associated with the disadvantage in terms of oxidative stress and cellular injuries, particularly under adverse conditions [28].

Table 2
Sexual difference in total phenolic content (mg GAE/g DW) of *H. rhamnoides* (100 males, 100 females) leaves

Population ID	Male leaves					Female leaves				
	Hydrophilic	Lipophilic	Combined[1]			Hydrophilic	Lipophilic	Combined[1]		
		Mean ± SD		Min	Max		Mean ± SD		Min	Max
SPT	101.60 ± 20.47^{b}	2.27 ± 0.26^{bc}	103.86 ± 20.58^{b}	61.53	142.45	107.76 ± 19.95^{c}	2.27 ± 0.40^{bc}	110.03 ± 20.05^{c}	76.72	165.83
CHU	$123.88 \pm 21.83^{c***}$	2.19 ± 0.22^{ab}	$126.07 \pm 21.84^{c***}$	93.03	173.06	91.67 ± 25.43^{b}	$2.95 \pm 0.40^{e***}$	94.62 ± 25.34^{b}	51.02	152.44
SHY	$86.87 \pm 18.28^{a***}$	2.48 ± 0.40^{c}	$89.66 \pm 18.90^{a***}$	47.19	131.67	74.98 ± 10.95^{a}	2.53 ± 0.49^{d}	77.51 ± 11.15^{a}	51.99	97.03
PHY	93.43 ± 15.18^{ab}	2.03 ± 0.28^{a}	95.46 ± 15.37^{ab}	63.69	124.28	$109.59 \pm 16.45^{c***}$	2.11 ± 0.30^{ab}	$111.70 \pm 16.70^{c***}$	81.86	144.57
HOR	84.71 ± 12.82^{a}	2.49 ± 0.39^{c}	87.21 ± 12.88^{a}	67.04	120.58	$94.36 \pm 13.65^{b***}$	2.38 ± 0.35^{cd}	$96.89 \pm 14.10^{b***}$	73.41	125.42
SKT	$99.91 \pm 29.63^{b***}$	$3.12 \pm 0.66^{d***}$	$103.02 \pm 29.70^{b***}$	58.07	170.36	76.76 ± 26.49^{a}	2.01 ± 0.38^{a}	78.77 ± 26.76^{a}	29.88	132.01
Average	$98.24 \pm 23.82^{**}$	2.41 ± 0.60	$100.83 \pm 23.92^{**}$	47.19	173.06	92.64 ± 23.66	2.37 ± 0.49	95.02 ± 23.82	29.88	165.83

Values represented as mean ± SD. For each column, different lowercase letters indicate significantly different at $p < 0.05$, as measured by 2-sided Tukey's HSD between populations. [1]Combined: Values of hydrophilic and lipophilic extract combined mathematically. **Value significantly higher than that of opposite sex at $p \leq 0.01$; ***Value significantly higher than that of opposite sex at $p \leq 0.001$.

Table 3
Sexual difference in total antioxidant capacity of *H. rhamnoides* (100 males, 100 females) leaves

Population ID	Male leaves				Female leaves			
	[1]FRAP ($FeSO_4.7H_2O$ mmol/g DW)			[2]IC_{50} (mg/ml)	[1]FRAP ($FeSO_4.7H_2O$ mmol/g DW)			[2]IC_{50} (mg/ml)
	Hydrophilic	Lipophilic	[3]Combined	[4]Combined	Hydrophilic	Lipophilic	[3]Combined	[4]Combined
SPT	7.07 ± 1.17^{c}	0.13 ± 0.01^{a}	7.21 ± 1.18^{c}	0.34 ± 0.10^{a}	7.21 ± 1.04^{c}	0.14 ± 0.02^{ab}	7.35 ± 1.05^{c}	0.32 ± 0.18^{a}
CHU	$7.01 \pm 0.61^{c***}$	0.14 ± 0.02^{ab}	$7.15 \pm 0.60^{c***}$	$0.33 \pm 0.03^{a***}$	6.05 ± 1.20^{b}	$0.18 \pm 0.03^{d***}$	6.23 ± 1.21^{b}	0.37 ± 0.03^{a}
SHY	5.75 ± 0.71^{ab}	0.15 ± 0.02^{bc}	5.90 ± 0.73^{b}	0.40 ± 0.27^{ab}	5.63 ± 0.62^{ab}	$0.17 \pm 0.02^{c***}$	5.80 ± 0.62^{b}	0.42 ± 0.29^{a}
PHY	$6.86 \pm 0.63^{c***}$	$0.16 \pm 0.02^{c**}$	$7.02 \pm 0.64^{c***}$	0.38 ± 0.05^{ab}	5.54 ± 0.70^{ab}	0.15 ± 0.02^{b}	5.69 ± 0.72^{ab}	0.37 ± 0.03^{a}
HOR	6.09 ± 0.91^{b}	$0.15 \pm 0.02^{c***}$	6.24 ± 0.91^{b}	0.46 ± 0.17^{bc}	6.08 ± 0.82^{b}	0.13 ± 0.02^{a}	6.21 ± 0.83^{b}	0.44 ± 0.13^{a}
SKT	5.28 ± 1.26^{a}	$0.15 \pm 0.03^{c***}$	5.08 ± 1.80^{a}	0.54 ± 0.20^{c}	5.07 ± 1.32^{a}	0.13 ± 0.02^{a}	5.19 ± 1.34^{a}	0.65 ± 0.35^{b}
Average	$6.37 \pm 1.14^{***}$	0.15 ± 0.2	$6.51 \pm 1.14^{***}$	0.41 ± 0.18	5.96 ± 1.19	0.15 ± 0.03	6.11 ± 1.20	0.43 ± 0.23

Values represented as mean ± SD. For each column, different lowercase letters indicate significantly different at $p < 0.05$, as measured by 2-sided Tukey's HSD between populations. [1]FRAP: Ferric reducing antioxidant potential. [2]IC_{50}: Inhibitory concentration, the amount of sample extracted into 1 ml solution necessary to decrease by 50% the initial DPPH concentration. [3]Combined: Values of hydrophilic and lipophilic extract combined mathematically. [4]Combined: Values of combined hydrophilic and lipophilic extract measured. **Value significantly higher than that of opposite sex at $p \leq 0.01$, ***Value significantly higher than that of opposite sex at $p \leq 0.001$.

3.2. Effect of harvest season on total polyphenol content and antioxidant activity

Effect of the season of harvest on TPC is presented in Table 4. TPC varied significantly during the sampling period. Significant increase in TPC was observed in male leaves from July (66.75 ± 7.16 mg GAE/g DW) to October (93.25 ± 7.14 mg GAE/g DW) followed by a significant decrease in November (7390.4 ± 1096.5 mg GAE/100 g DW). However, increase in TPC was observed upto August in female leaves and then showed a steady declining trend. Decline in TPC from August onward in female as compare to October in male leaves is may be due to higher reproductive efforts by female during the study period (July-November), females developing fruits while males not reproducing. Male contained significantly higher TPC than female leaves from August to November harvesting months ($P < 0.001$, Student's *t*-test). Similar trend was observed in TAC in both the sexes except that female also showed increasing TAC from July to October (Table 4). Progression in harvest season from July to October is related linearly to the increase in TPC ($R^2 = 0.937$) and FRAP ($R^2 = 0.976$) in male leaves (Fig. 1). However, in females the trend of increase was not observed in TPC. In comparison, Morgenstern et al. [20] studied the change in antioxidant capacity and phenols during SBT leaves development from April to July. Antioxidant capacity increased in first week of May and then decreased in third week of the month. A steady increase was observed from June onwards. The phenols decreased initially and then increased steadily during the study period. However, changes in antioxidant capacity and phenols were not studied beyond July. Górnaś et al. [13] studied antioxidants in mixed SBT samples of two female and 10 male harvested in June and October. Higher antioxidant was observed in samples collected in autumn than in summer in both male and female leaves. Results obtained in the present study over an extended harvesting period suggest that October is the best time for harvesting SBT leaves. Ercisli et al. [17] also observed similar trend in antioxidant activity of tea leaves harvested at three commercial harvest seasons (May 15, July 15, September 15). Highest antioxidant activity was observed at 2nd harvest. Increase in TPC and TAC from July to October may be linked to accumulation of health promoting compounds during leaf developmental stages. Decline in TPC and TAC in November may be due to the beginning of leaf senescence in the plant.

3.3. Correlation analysis

Table 5 displays the correlation between TPC and antioxidant activity. TPC of male, female and combined samples was significantly correlated with FRAP (0.423, 0.717, 0.581, respectively, $p \leq 0.01$) and DPPH (–0.208,

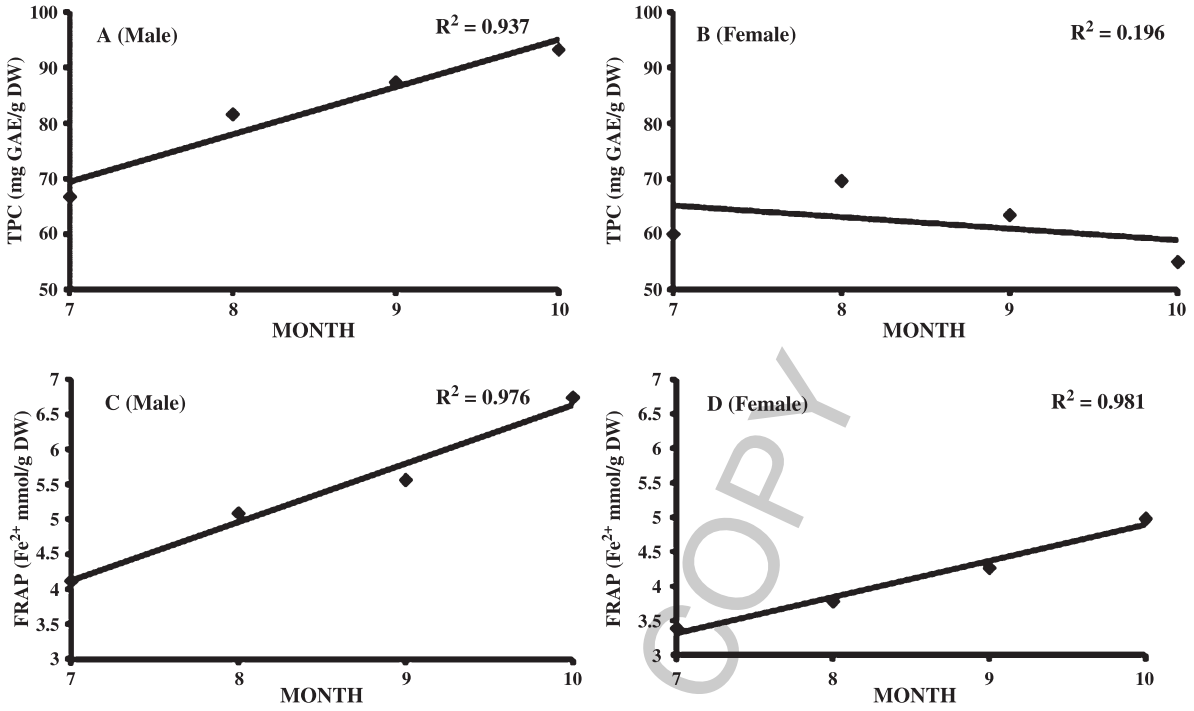

Fig. 1. Relation between total phenolic content (A-B) and antioxidant capacity (C-D) in male and female Seabuckthorn leaves with harvest season (July-October).

Table 4

Seasonal variation in total phenolic content and total antioxidant capacity of *H. rhamnoides* (5 males, 5 females) leaves

Month	Male			Female		
	[1]TPC	[2]FRAP	[3]IC_{50}	[1]TPC	[2]FRAP	[3]IC_{50}
July	66.75 ± 7.15^{a}	$4.12 \pm 0.20^{a***}$	0.52 ± 0.20^{c}	59.97 ± 16.63^{abc}	3.39 ± 0.50^{a}	0.69 ± 0.57^{a}
August	$81.59 \pm 10.71^{bc**}$	$5.09 \pm 0.49^{b***}$	$0.26 \pm 0.09^{b***}$	69.57 ± 13.04^{c}	3.78 ± 0.37^{ab}	0.52 ± 0.16^{a}
September	$87.38 \pm 6.13^{cd***}$	$5.62 \pm 0.51^{bc***}$	$0.19 \pm 0.08^{ab***}$	63.45 ± 8.02^{bc}	4.27 ± 0.32^{c}	0.73 ± 0.16^{a}
October	$93.25 \pm 7.14^{d***}$	$6.74 \pm 0.57^{d***}$	$0.12 \pm 0.04^{a***}$	55.02 ± 8.04^{ab}	4.98 ± 0.33^{d}	0.85 ± 0.63^{a}
November	$73.90 \pm 10.97^{ab***}$	$5.68 \pm 0.85^{c***}$	$0.31 \pm 0.18^{b***}$	51.47 ± 6.60^{a}	4.09 ± 0.47^{bc}	0.94 ± 0.43^{a}
Average	$80.57 \pm 12.68^{***}$	$5.45 \pm 1.02^{***}$	$0.28 \pm 0.19^{***}$	59.89 ± 12.56	4.10 ± 0.66	0.75 ± 0.45

Values represented as mean ± SD. For each column, different lowercase letters indicate significantly different at $p < 0.05$, as measured by 2-sided Tukey's HSD between months. [1]TPC: Total phenolic content (mg GAE/g DW). [2]FRAP: Ferric reducing antioxidant potential ($FeSO_4.7H_2O$ mmol/g DW). [3]IC_{50}: Inhibitory concentration, the amount of sample extracted into 1 ml solution necessary to decrease by 50% the initial DPPH concentration. **Value significantly higher than that of opposite sex at $p \leq 0.01$; ***Value significantly higher than that of opposite sex at $p \leq 0.001$.

–0.551, –0.399, respectively, $p \leq 0.01$). Similar result was observed in SBT berry from the trans-Himalaya [29]. Similarly, DPPH scavenging activity (IC_{50}) of male, female and combined samples was significantly correlated with FRAP (–0.48, –0.577, –0.533, respectively $p \leq 0.01$).

Table 5
Pearson correlation to estimate the interrelationship between TPC, IC_{50}, and FRAP

	Male			Female			Combined		
	[1]TPC	[2]FRAP	[3]IC_{50}	[1]TPC	[2]FRAP	[3]IC_{50}	[1]TPC	[2]FRAP	[3]IC_{50}
[1]TPC	1	0.423**	–0.208**	1	0.717**	–0.551**	1	0.581**	–0.399**
[2]FRAP		1	–0.481**		1	–0.577**		1	–0.533**
[3]IC_{50}			1			1			1

**Correlation is significant at $p \leq 0.01$. [1]TPC: Total phenolic content (mg GAE/g DW). [2]FRAP: Ferric reducing antioxidant potential ($FeSO_4 0.7H_2O$ mmol/g DW). [3]IC_{50}: Inhibitory concentration, the amount of sample extracted into 1 ml solution necessary to decrease by 50% the initial DPPH concentration.

4. Conclusion

Sexual differences and seasonal variation in TPC and TAC in SBT leaves was demonstrated. Males exhibit significantly higher TPC and ferric reducing activity than females. Significant seasonal variation in TPC and TAC was observed in both the sexes. Significant increase in TPC was observed in male leaves from July to October followed by a significant decrease in November. However, increase in TPC in female leaves was observed upto August and then a steady declining trend afterwards due to greater reproductive effort in females. October is the best time for harvesting SBT leaves for higher health promoting compounds content. Leaves contain significantly higher hydrophilic than lipophilic phenolics and antioxidants. Results obtained in this study can be considered for harvesting of SBT leaves for extraction of health promoting compounds and product development.

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Conflict of interest

The authors have no conflict of interest to report.

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Report

Semester even 2021

Title: ***Identification of potential pathogen in Ladakh for early disease detection using mobile app in economically important crop"***

INTRODUCTION:

Ladakh, a newly formed union territory in India covers approximately 60,000 sq miles (100,000 sq km) and is surrounded by Ladakh range in the east, Karakoram in the north east, Zanskar range in the east and the Himalayan range in the North West. However, due to the prolonged and extreme winter condition, the agriculture season extends for a short period of time and the agriculture produce is not able to meet the requirement of local population as well as the tiny settlements over there (Bhatt.*et.al* 2015)

Plant contribute to almost 80% of the human diet .They are essential for food security and bio security concern especially in Ladakh as it is situated in a border area .The local inhabitants as well as the heavy settlement of army in the region are dependent upon the native plant diversity as such the region remains inaccessible for the winter months due to the harsh environmental condition .

Potato is one of the valuable food crop grown in Ladakh .The area under potato production is 253 hactares producing 8970 metric tones.(Stobdan *et.al*.2018) However , there are there are number of production constraints leading to its low yield and quality such as occurrence of many fungal diseases viz. Early blight *(Alternaria solani),* Late blight *(Phytopthora infestans),* Powdery scab *(Spongospora subterranean), Wart (Synchytrium endobioticu),* Leaf black *(Cercospora concors),* Fusarium wilt *(Fusarium solani f. sp radicicola),* Black scurf *(Rhizoctonia solani f.sp. radicicola) and* charcoal rot *(Macrophomina phaseolin* .Among early blight is the major economic constraint for potato production worldwide. Early blight is caused by *Alternaria solani* .It affects mainly potato foliage and leads to leaf necrosis and premature defoliation .Symptoms include characteristics "target like" lesions of concentric rings that appear dark and sunken and become papery. Lesions enlarge, coalesce and cause cell

death. The disease also causes major crop loss accounting upto 75% of the total production. (Dey and Chakraborty, 2012).

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Objective:

1. Identification of early blight of potato through morphological study.
2. Yield loss analysis associated with early blight in Ladakh.

METHODOLOGY:

I. Identification of the early blight of potato in Ladakh

a) Survey and trial:

Field experiments were conducted in the growing season in different potato growing villages of Ladakh(Saspol : 34.1435^{o} N , 77.1011^{o} E ;Nang : 34.2748^{o} N , 77.44599^{o} E ;Leh: 34.13938^{o} N, 77.57298^{o} E) which showed highest disease incidence and severity according to previous studies .The size of the plots were 2* 2 m^2 with three replications each for positive control , three replication for negative control and three replication for treated control .

b) Collection of samples:

During the month June to September , when diseased leave samples started exhibiting early blight symptoms initially as small irregular spots to circular dark spots on leaves which gradually progresses to concentric circles were collected and kept at 4^{o}C till further processing .

c) Isolation of pathogens :

For isolation of alternaria,symptomatic leaves were cut into small pieces, sterilized with Sodium hypo chloride(1%) , rinsed with sterile water and cultured on PDA plates. The plates were incubated in darkness at 28^{o}C for 3- 5 days.

d) Morphological identification :

The fungal pathogen were identified based on colony and morphological characters on the PDA plates .

h) Yield loss analysis :

Assesment of yield loss by early blight was done in the harvested tubers obtained from experimental plots **.** Yield loss due to early blight was calculated according to walker 1990.

$$W = \frac{(m-y)}{m} * 100$$

Where,W=Yield loss (%),m =yield in sprayed plot ,y= yield in unsprayed plot

RESULTS:

a) Survey and trial:

Experimental plots in surveyed region of ladakh was selected for trial. Sowing of tubers was done on 2 April ,2020 in Saspol , 8 April , 2020 in Nang and 12 April 2020 in Leh in the year 2010.

b) Collection of samples:

Random sampling were done from experimental plots .Leave samples similar to early blight having early diseased symptoms as irregular smalls spots , having numerous concentric circles and leaves having coalescence of concentric circles merged were collected and processed further.(Fig 3)

Fig 1: Infected potato leaf showing early blight symptoms a)leaves having numerous concentric circles between veins b) coalescence of concentric circles merged together on leaf surface

c) Isolation of fungal culture:

60 pure fungal cultures were obtained from isolated cultures .

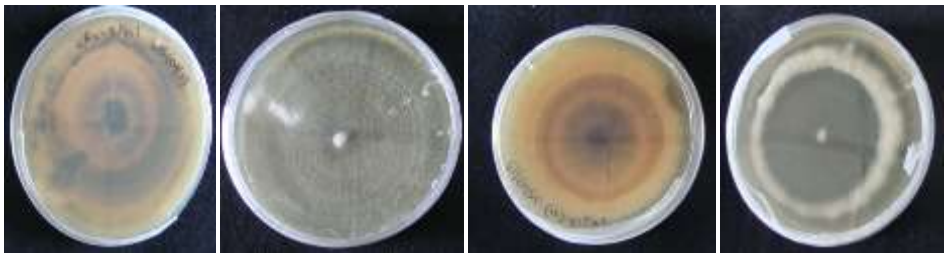


Fig 2 :Pure fungal cultures isolated on PDA plates

d) Morphological identification:

Morphological characteristics based on 60 colony culture on PDA were studied. The isolates exhibited a variable diversity in terms of colony color and morphology. The colour of isolated cultures varies from dark green, light green, grey and brown. The morphological characteristics of some of the cultures are given below :

Fungal Cultures on PDA	Reverse side	Morphological characteristics	Fungal Cultures on PDA	Reverse side	Morphological characteristics
		Brown with concentric circles **Reverse**:Dark green with concentric circles , white Margin			Light grey at center ,raised colony,white margin **Reverse-** Brown at center with white margin
		Light to dark brown concentric circles ,cottony texture **Reverse**:Dark green color ,cottony texture ,white margin			Grey with white margin, cottony texture **Reverse**: Dark brown in center with light yellow margin

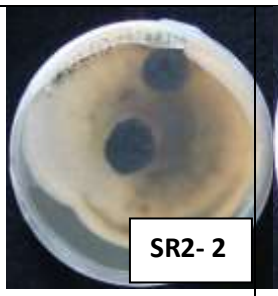
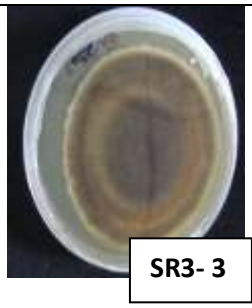
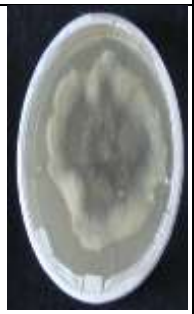
		Dark green with white margin , cottony mycelia **Reverse** - black color with white margin			Green with white mycelia margin **Reverse-** Black at center with light brown
		Dark green with cottony mycelia in center , yellow margin **Reverse** – Black to grey color , light yellow margin			Green with cottony texture ,white mycelia growth **Reverse-** light to dark brown ,concentric circles , white margin
		Dark green color , cottony texture , whilte margin **Reverse-** light to dark brown with concentric circles , white margin			Greyish with filamentous growth , growth rate:fast **Reverse;** dark brown in color

Table 1:Morphological characteristics of some of the cultures

f) Yield loss analysis:

Assesment of yield loss were done at harvesting stage .In Nang, average yield from harvested tuber obtained from positive plots accounted upto 36% whereas the average yield of tubers harvested from the treated plots were 39% .The result indicated that there is 3% yield loss in positive plots as compared to the treated plots with herbal bioformulation In Saspol , average yield from harvested tuber of positive plots accounted upto 42% whereas the average yield of tubers harvested from the three replicates of treated plots were 30% .The result showed a higher yield was obtained in positive as compared to treated. In Leh ,average yield from harvested tuber of positive plots accounted upto 26% whereas the average yield of tubers harvested from the three replicates of treated plots were 39% .It indicated that there is a significant margin of 13% yield loss in positive plots as compared to the treated plots.

	Yield(Kg/m2) (Saspol)	**Yield(Kg/m2) (Nang)**	**Yield(Kg/m2) (Leh)**
Positive (unsprayed)	11.6	13.08	7.07
Treated (Srayed)	8.3	14.02	11.20

Fig 6: Table showing yield (kg/m2) in obtained Saspol village , Nang and Leh villages of Ladakh

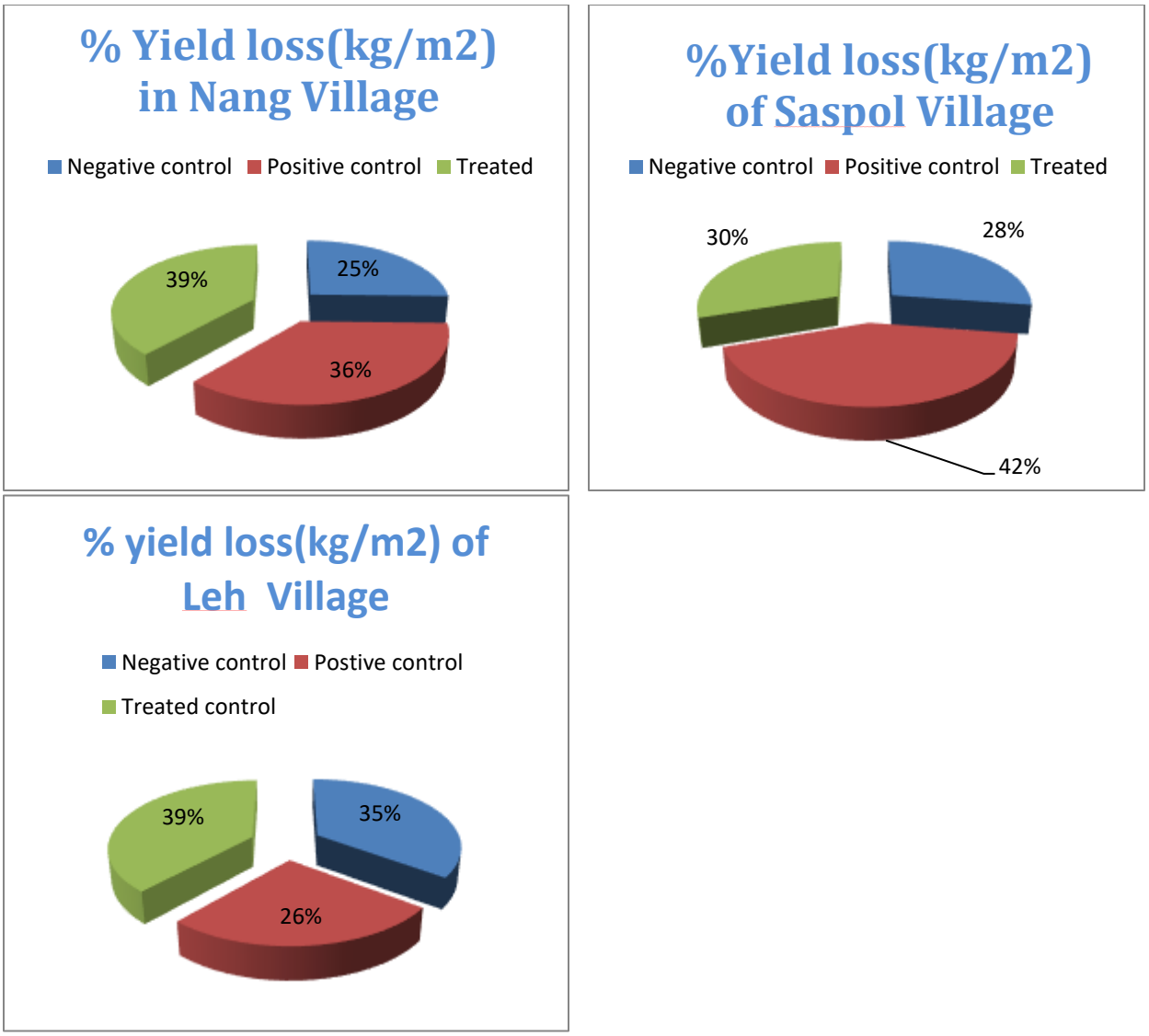


Fig 6: Yield loss(kg/m2) in Saspol village , Nang and Leh villages of Ladakh

g) Creation of mobile based app:

The study will provide a collection of large number of data that will be carried out for the development of mobile based app for early disease detection to save crop loss .

Conclusion:

Incidences of early blight was in Potato growing villages in Ladakh were found .Quantitative yield loss was assessed and it showed significant yield loss .Higher yield reduction was observed in Leh than Nang and Saspol . It also indicates that the early blight infection was more severe and causes more tuber loss in unsprayed plots as compared to the plots treated with

bioformulation as revealed by the fact that yield obtained from treated (Sprayed) plots were higher than the unsprayed plots having more severe infection of early blight.

References:

1) R.K Bhatt,M.S Raghuvanshi ,Rajwant K . Kalia ;Achieving Sustainable Livelihood in Cold Arid Region of India through Multienterprise option ;Annals of Arid zone ;2015:54(3&4):1-12
2) A.H.M Ali Reza; Bio-diversity of Jammu and Kashmir; www.kashmirnetwork.com/wildlife/biodiversity.html
3) Dey, S. and chakraborty, A. (2012); Varietal reaction against early blight of potato in plains of West Bengal. *J. Crop Weed*., 8 (1): 181-183.
4) Tsering Stobdan, Stanzin Angmo, Dorjey Angchok, Eli Paljor, Thinles Dawa, Tashi Tsetan, and O.P. Chaurasia;Vegetable Production Scenario in Trans –Himalayan Leh Ladakh Region ;Defence life Science Journal;2018:3(1):85-90
5) S.A Ganie,M.Y Ghani,Qazi Nissar, Nayeeema Jabeen,Qaisar Anjum,F.A Ahanger and Aadil Ayaz;Status and symptomatology of early blight (Alternaria solani of potato (Solanum tuberosumL.) in Kashmir Valley;Academic Journals;2013:8(41)5104-5115
5) Walker PT. 1990. Insect pest-loss relationship: Characteristics and importance. In: *Crop Loss Assessment in Ric*e.International Rice Research Institute, Philippines. Pp. 71-184.
6) Cetas RC, Jones ED, 1962. New soil fungicides for potato scab and *Rhizoctonia* control. *Plant Disease Reporter* **46**, 601–5

G. Any other e.g. Patent filed, please specify:

12.5 Work Plan for the next Semester:
Molecular study and pathogenicity testing of the isolated cultures

12.6 Percentage of Ph.D work completed by Scholar in assessment of Scholar & Supervisor(s)

S. Mandal

Jata Shankar

(Signature of Scholar) (Signature of Supervisor(s))

13. Comments and Recommendations: *(Supervisor(s) and DPMC Members are requested to write here a few sentences regarding the actual Ph.D work done during the current semester and the specific outcomes reported by the Scholar above. Further, they are requested to record their observations/comments in regard to the conduct, regularity in meeting and discussions with the candidates during the semester. They should give their comments on the performance in the seminar, overall assessment of progress of the Scholar so far on a ten point scale and should make specific recommendation regarding the continuation of registration in the next semester.)*

14. Topic of Seminar:
Identification of early blight in Ladakh for development of mobile based app for early disease detection to save crop loss.

Date of Seminar:

(i) Supervisor(s)Progress sastisfactory8/10

Jata Shankar

(Jitendra Singh)

(Supervisor-1) (Supervisor-2) (External Supervisor)

(ii) DPMC Members

(DPMC Member-1) (DPMC Member-2) (DPMC Member-3)

9. ***Proposed broad areas and topics of interest in order of priority***

(i) Identification of potential phytopathogen in important crops in Ladakh and its impact on Crop Yield .

ii) Management of identified disease .

S. Yandol 30-1-2023

Signature of Ph.D. Scholar
with date

Authentication by Registrar

Signature of Registrar
with date

Enrollment No. Allotted _____ .

NOTE: - One copy of this form duly completed is to be submitted to Registrar and the other to the concerned HoD immediately after enrollment.

10. Supervisor(s) allotted:

	Name	***Designation***	***Department / Address***
(i) Supervisor – 1:	***Dr Jata Shanker***	***Associate Professor***	***Biotechnology and Bioinformatics, JUIT, Solan***
(ii) Supervisor – 2:	Dr.Narendra Singh	Sc "F"	Vegetable Science Division DIHAR-DRDO,C/0 56APO Leh Ladakh

Ph.D FORM - 1 (Enrollment) **Effective: 01 April 2019**

Ph.D ENROLLMENT
JUIT, Waknaghat

(To be filled up by the Candidate at the time of enrollment)

Semester: Odd√ / Even (strike out semester n/a), Session: 2021-

Personal Data **Dated: 30/4/2021**

(The Personal Data should be correct and the Candidate may save a soft copy for ready reference so that the same personal data can be used in all subsequent forms.)

1. ***Name (Full):*** Skalzang Youdol
2. ***Date of Birth:*** 11.09.1989
3. ***Department: Biotechnology and Bioinformatics***
4. ***Enrollment Date:*** 5. ***Enrollment No:*** 206555
6. ***Category (Tick): (i) Full Time (ii) Part Time (iii) JUIT Faculty (iv) Sponsored (Specify) √*** (Defence Institute Of High Altitude Research , DRDO ,Leh Ladakh)
7. ***Academic Qualifications (Beginning with Class 10th)***

	Name of Board / Institute /University	*Year of Passing*	*Grades / % of Marks / Credits obtained out of ____*	*Main Subjects Studied*
(i) Class 10th	C.B.S.E	2006	81%	English, Science,Social Studies Maths, Hindi
(ii) Class 12th (10+2)	C.B.S.E	2008	65%	Physics,Chem,Maths Biology, Func.English
(iii) Bachelor's Degree (B.Sc /B.Com /BA / B.Tech etc.)	*Guru Gobind Singh Indraprastha University*	2013	64.86%	Mol. Biotech, Agri. Biotech , Immunology
(iv) Master's Degree (M.Sc /M.Tech /MBA / *M. Phil etc.)*	*Guru Gobind* Singh Indraprastha *University*	2015	75.53%	Genomics,Proteomics IPR,Bioethics
(v) Any other specify				

8. ***Score in any national examination :Nil*** ***Name & Year:*** ***Score/Rank:***

11. Proposed list of two DPMC members including one from other Department (as per the Ph.D Ordinance)

DPMC Members

	Name	Designation	Department
(i)	Dr. Anil Kant	Associate Prof.	BT & BI 30/01/2021
(ii)	Dr. Saurabh Bansal	Assist. Prof.	BT & BI 30/01/2021
(iii)	Dr. Shanti Jain	Assoc. Prof	ECE (Other Dept.) 30/01/2021

Other Ex-officio DPMC Members:

(iv) Supervisor-1

(v) Supervisor-2

(vi) External Supervisor

(vii) HoD

(viii) Dean (A&R)

Shanker 30/01/2020
Signature of Supervisor-1 (Tatashankar)

Signature of Supervisor-2 (Narendra Singh)

External Supervisor

30/01/2021
Signature of HoD (Dr. Sudhir Kumar)

Signature of Dean (A&R)

Approved/ Approved as Modified/ Not Approved

Signature of Vice Chancellor.

12. Course work assigned

(a) Mandatory Courses to be registered in the subsequent semester

	Course Name	Course Code	Credits	To register in (Subsequent Semester - Specify Odd/Even)
(i)	Research Methodologies including Quantitative Methods and Computer Applications	10P1NGE201	3	
(ii)	Literature Survey	17P1WGE101	2	
(iii)	Ethics, Intellectual Property Issues and Plagiarism	17P1WGE102	1	

(b) Other Mandatory and more Courses (may be assigned later).

Course Name	Course Code	Credits	To register in (Later Semesters -- Specify Odd/Even)
(iv) Functional Genomics	KMMBT213	03	
(v)			
(vi)			
(vii)			

(c) No objection by Supervisor(s) on leaving the University due to any reason on Ph.D Scholars continuation in event of the following

(i) *Ph.D work in the specialized area of research can continue irrespective of IPR.*

(ii) *Research work on the same topic.*

(iii) *In the event of non availability of new Supervisor to guide in the area of research for which the Scholar is registered, HoD of the Department in consultation with Dean (A&R) and DPMC members assigning a new topic to the Scholar.*

Khanka 30/01/2021
Signature of Supervisor-1
(Jata Shankar)

Signature of Supervisor-2

Signature of Ext. Supervisor

30/01/2021
Signature of DPMC Mem-1
Dr. Anil Kant

DBansal
(DR. SAURABH BANSAL)
Signature of DPMC Mem-2

30/01/2021
Signature of DPMC Mem-3
Dr. Shaukat Jamal

30/01/2021
Signature of HoD
(Dr. Sudhir Kumar)

Signature of Dean (A&R)

Registrar

9. ***Proposed broad areas and topics of interest in order of priority***

(i) Identification of potential phytopathogen in important crops in Ladakh and its impact on Crop Yield .

ii) Management of identified disease .

S. Yadav 30-1-2023

Signature of Ph.D. Scholar
with date

Authentication by Registrar

Signature of Registrar
with date

Enrollment No. Allotted _____ .

NOTE: - One copy of this form duly completed is to be submitted to Registrar and the other to the concerned HoD immediately after enrollment.

10. Supervisor(s) allotted:

	Name	***Designation***	***Department / Address***
(i) Supervisor – 1:	***Dr Jata Shankar***	***Associate Professor***	***Biotechnology and Bioinformatics, JUIT, Solan***
(ii) Supervisor – 2:	Dr.Narendra Singh	Sc "F"	Vegetable Science Division DIHAR-DRDO,C/0 56APO Leh Ladakh

(iii) External Supervisor:

Suggested Topic: Identification of potential phytopathogen in important crops of Ladakh for early disease detection to save crop loss.

Signature of HoD with date

Signature of Dean (A&R) with date

To

The Vice Chancellor
Jaypee University of Information Technology
Waknaghat Solan

Subject: Request to approve inclusion of Dr Sundresha Siddapa as Co-guide in the DPMC of Ms Suhani Bhagta (196552) PhD

Sir

(A) It is requested to approve the inclusion of name of Dr Sundresha Siddapa as Co-supervisor of my PhD student Ms Suhani Bhagta (196552). Ms Suhani has been assigned research topic "RNAi meditated spray-induced silencing of multiple genes of *Venturia inaequalis* for apple scab management". Dr Sundresha is working of RNAi since long and we will be able to utilize the lab facilities and expertise of Dr Sundresha by doing so, at CPRI, Shimla. We already have an MoU with CPRI Shimla and outcome of this research work would be shared by both the institutes.

Dr Anil Kahl 12/02/2020

(A) Dr. Sundresha may be approved as Co-PI of Ms. Suhani Bhagta (196552) (Ph.D. student)

12/02/2020.

(B)

Dean Academic

12/02/2020

R | AR

Agbd 13/02/2020

(B) may be approved subject to systemic support towards our students & scholars being benefitted for academic gain by use of CPRI infra-structure

VC

13/02/2020

पंजीकृत पोस्ट
REGISTERED POST

Tele : 01982 252096
Fax : 01982 252096
Army : 2475
E-mail : dihardrdo@gmail.com

Bharat Sarkar
Raksha Mantralaya
Anusandhan Tatha Vikas Sangthan
Defence Institute of High Altitude Research
PIN – 901205
C/o 56 APO

DIHAR/01/CARS/2015

30 Jan, 2015

Director
Jaypee University of Information Technology
Waknaghat, PO: Waknaghat
Solan-173215
Himachal Pradesh

Sub: Sanction of CARS

Dear Sir,

1. Refer your project proposal for financial assistance under Contract for Acquisition of Research Services (CARS) submitted by Dr. Anil Kant.

2. The competent authority has sanctioned the CARS entitled 'Transcriptome analysis of seabuckthorn male and female buds (DIHAR/01/CARS/2015). The cost of the CARS is Rs 9.996 Lakh and PDC is 26 Jan 2017 (sanction letter attached).

3. If you need any clarification, kindly contact us.

Yours sincerely,

(Dr Tsering Stobdan)
OIC Tech Coord
For Director

Copy to:

Dr. Anil Kant
Biotechnology Department
Jaypee University of Information Technology
Waknaghat, PO: Waknaghat
Solan-173215
Himachal Pradesh

Accounts Officer (R&D)

Tele/Fax : 01982-252096

Government of India
Ministry of Defence
Defence Research & Development Organisation
Defence Institute of High Altitude Research (DIHAR)
PIN 901205, C/o 56 APO

118/6/JUIT-I/15 /DIHAR

27 Jan 2015

Director
Jaypee University of Information Technology
Waknaghat, Solan 173215
Himachal Pradesh

SANCTION OF CARS ENTITLED "TRANSCRIPTOME ANALYSIS OF SEABUCKTHORN MALE AND FEMALE FLOWER BUDS"

1. Sanction is hereby accorded for undertaking the following CARS within the estimated cost indicated below:-

Title of the CARS & No	Sanction cost (Rs in Lakh)	PDC
"TRANSCRIPTOME ANALYSIS OF SEABUCKTHORN MALE AND FEMALE FLOWER BUDS "(DIHAR /01/ CARS /2015)	9.996 Lakh	24 Months

2. Normal purchase and accounting procedure will be followed.

3. Based on this sanction budget provision of funds for actual expenditure will be made through periodical Budget Estimates under the relevant heads of accounts and expenditure will be debited to Major Head 2080 and Minor Head 110 of Defence Services Research and Development.

4. Dr. Tsering Stobdan, Scientist of DIHAR will be coordinator of the project.

5. The Principal Investigator (PI) will submit financial closure report of the CARS after PDC.

6. The PI will submit the Audited Detailed Statement of Expenditure, Utilization Certificate and Closure Report.

7. IPR issues viz Patent / Publication will be shared jointly with prior approval.

Auth: No. DRDO/ DBFA/FA/83226/M/01/2031/D(R&D)dated 30 July 2010.

Encls : As above

(Dr. RB Srivastava)
OS & Director

Copy to :

Director General (Life Sciences)
DRDO Bhawan, Rajaji Marg - for info and necessary action, please.
New Delhi -110011

Director (PM)
O/o Director General (Life Sciences)
DRDO Bhawan, Rajaji Marg - for info and necessary action, please.
New Delhi -110011

Accounts Officer (R&D)
Himparisar, Sector 37-A - for info and necessary action, please.
Chandigarh

Dr. Anil Kant
Jaypee University of Information Technology - for info and necessary action, please.

МИНОБРНАУКИ РОССИИ

Федеральное государственное автономное образовательное
учреждение высшего образования «Южный федеральный университет»
(ЮЖНЫЙ ФЕДЕРАЛЬНЫЙ УНИВЕРСИТЕТ)

ПРИКАЗ

«17» января 2022 г. № 50

г. Ростов-на-Дону

О результатах конкурса по программе постдоков Института компьютерных технологий и информационной безопасности Южного федерального университета

С целью привлечения на конкурсной основе высококвалифицированных специалистов из числа талантливых молодых ученых для выполнения научных проектов, формирования конкурентной среды, повышения эффективности научно-исследовательской деятельности и укрепления кадрового состава за счет выпускников программы постдоков, на основании приказа «О проведении конкурса по программе постдоков Института компьютерных технологий и информационной безопасности Южного федерального университета» от 14.09.2021 №1710 и протокола комиссии по конкурсному отбору от 01.12.2021 п р и к а з ы в а ю :

1. Утвердить темы научно-исследовательских проектов, выполняемых в ИКТИБ в рамках программы постдоков, руководителей и исполнителей проектов (Приложение №1).
2. Установить срок выполнения проектов с 10.01.2022 по 31.12.2024.
3. Утвердить источник финансирования и смету проектов на первый год выполнения проектов (Приложение №2).
4. Департаменту кадров и правового сопровождения трудовых отношений университета заключить срочные трудовые договоры о приёме на работу с победителями конкурсного отбора по программе постдоков на период с 10.01.2022 по 31.12.2022 г.
5. Контроль за исполнением настоящего приказа возложить на проректора по стратегическому и инновационному развитию Муханова Е.Л.

Ректор И.К. Шевченко

ПРИЛОЖЕНИЕ № 1
к приказу Южного федерального университета

от «17» января 2022 г. № 50

Научно-исследовательские проекты, выполняемые в ИКТИБ в рамках программы постдоков

№ п/п	Тема проекта	Научный руководитель проекта	Исполнитель проекта
1	Распределенные вычисления для киберфизических систем	Вяткин Валерий Владимирович – д.т.н., профессор Института компьютерных технологий и информационной безопасности	Muthanna, Mohammed Saleh Ali, гражданин Йемена (имеет вид на жительство в РФ)
2	Теория и приложения графовых нейронных сетей, мягкие алгоритмы машинного обучения	Боженюк Александр Витальевич – д.т.н., профессор, кафедры Информационно-аналитических систем безопасности имени профессора Л.С. Берштейна	Sharma, Amit, гражданин Индии

ПРИЛОЖЕНИЕ № 2
к приказу Южного федерального университета

от «17» января 2022 г. № 50

Смета и источник финансирования проекта на период 10.01.2022 – 31.12.2022

№ п/п	Наименование расходов	Сумма в рублях	Источник финансирования
1.	Фонд оплаты труда	1 723 405,32	Фонд оплаты труда ИКТИБ
1.1.	Заработная плата, в том числе:	1 323 660,00	
	Заработная плата руководителя проекта, 26 805 руб. в месяц	321 660,00	
	Заработная плата Постдока, 83 500 руб. в месяц	1 002 000,00	
1.2.	Начисления на выплаты по оплате труда	399 745, 32	
2.	Транспортные расходы (оплата проезда Постдока из места проживания в г. Таганрог)	100 000,00	Средства государственного задания Программы развития ИКТИБ
3.	Командировочные расходы, оплата публикаций, расходные материалы	676 594,68	
	ИТОГО:	2 500 000,00	