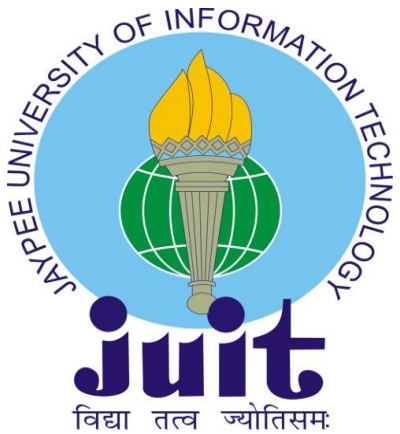
FRUIT QUALITY, PHYTOCHEMICAL AND DIVERSITY STUDIES OF APRICOT (*Prunus armeniaca* L.) ALONG AN ALTITUDINAL GRADIENT IN TRANS-HIMALAYAN LADAKH, INDIA

Thesis submitted in fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

By

AVILEKH



Department of Biotechnology & Bioinformatics

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY

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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.

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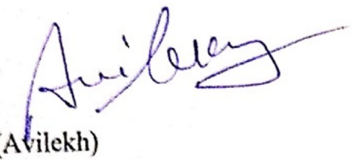
DECLARATION BY THE SCHOLAR

I hereby declare 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 at **Jaypee University of Information Technology, Waknaghat, India** is an authentic record of my work carried out under the supervision of **Dr. Anil Kant** and **Dr. Tsering Stobdan**. I have not submitted this work elsewhere for any other degree or diploma.



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(Avilekh)

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LIST OF ABBREVIATIONS

ALT	Altitude
AMOVA	Analysis of Molecular Variance
AMY	Amygdalin
ANOVA	Analysis of Variance
ARA	Arabitol
BA	Blush area
BC	Before Christ
CTAB	Cetyltrimethyl ammonium bromide
CV	Coefficient of variation
DM	Dry matter
DNA	Deoxyribonucleic acid
DW	Dry weight
FB	Date of full bloom
FrL	Fruit length
FrW	Fruit weight
FrWd	Fruit width
FW	Flesh weight
GLU	Glucose
GPS	Ground positioning system
GPS	Global positioning system
H	Nei’s genetic diversity
HPLC	High performance liquid chromatography
I	Shannon’s information index
IC	Ion chromatography
INO	Inositol
JD	Julian days
KD_g	Kernel geometric mean diameter
K*S*	Kernel surface area
KT	Kernel taste
KW	Kernel weight
KΦ	Kernel sphericity

MC	Moisture content
Na	Number of alleles
Ne	Effective number of alleles
NPL	Number of Polymorphic Loci
NPL	Number of polymorphic loci
ORFs	Open reading frames
PPL	Percentage of polymorphic loci
SC	Stone colour
SCT	Seed coat thickness
SCT	Seed coat thickness
SD	Standard deviation
SD_g	Seed geometric mean diameter
SL	Seed length
SM	Seed moisture content
SOR	Sorbitol
SPSS	Statistical Package for Social Sciences
SRAP	Sequence-related amplified polymorphism
S*S*	Seed surface area
ST	Seed thickness
SUC	Sucrose
SW	Seed weight
SW	Stone weight
SWd	Seed width
SΦ	Seed sphericity
TA	Total acid
TS	Total sugar
TSS	Total soluble solids
UPGMA	Unweighted Pair Group Method with Averages
USDA	United States Department of Agriculture

CHAPTER 1
INTRODUCTION

APRICOT

Apricot (*Prunus armeniaca* L., Rosaceae) is the 3rd most economically important stone fruit crops in the world [1], [2]. The annual production of apricots is about 4.26 million metric tons in 2017. Turkey, Iran, Uzbekistan, Algeria, and Italy are the top five apricot producing countries in the world. India ranked 30th among the top apricot producing countries, and the production volume was 15.07 thousand metric tons in 2016. The apricot tree grows up to 7-10 m height and has a spreading and dense canopy. Leaves are ovate, finely serrated and pointed at the tip. Flowers bloom in a cluster in early spring before the leaves, with 5-petaled whitepink coloured flowers. A long period of subzero temperatures forces the apricot tree to remain in a dormant condition. Dormant buds get sprouted on the onset of spring, and fruit is harvested in late summers [3].

Figure 1.1: Dormant apricot trees at Takmachik village in trans-Himalayan Ladakh (a); apricot in full bloom in Ladakh (b); apricot flower (c); and apricot fruit (d)

1.1 Origin and distribution

Apricot is believed to originate from Armenia and hence named as *armeniaca*. However, according to Vavilov [4] apricot has three main centers of origin: (1) Chinese centre of origin which includes mountainous region of Central and Western China, (2) Central Asiatic centre of origin that comprise Uzbekistan, Tajikistan, Afghanistan, North west India and Pakistan, Xinjiang province of China, and (3) Near-Eastern centre of origin which includes interior of Asia Minor, Iran, Transcaucasia and Turkmenistan.

There are three distinct perspectives on the spread of apricot from Central Asia to the rest of the world. The 1st view is that dried fruits and stones in Fergana Valley (border of Tajikistan, Uzbekistan, and Kyrgyzstan) were brought to Anatolia by soldiers of Great Alexander in BC 334 to Transcaucasia and Iran [5]. The 2nd view is that apricot was moved to Anatolia through Silk Road from Central Asia and China by the merchants, and later carried to Italy by Roman soldiers [6]. The 3rd view is that during 2 BC Romans took apricot with them to the west during their expeditions to Near East (Caucasus, Iran, and Syria) [5]. In 1524 or 1548 apricot was moved to England from Italy [7]. Apricot was taken to America continent in 1626 by the Spanish.

1.2 Apricot in trans-Himalayan Ladakh

Apricot is a hardy and important tree which grows in the dry and temperate climate of North-western Himalayas. Apricots are known for their quality in trans-Himalayan Ladakh. Because of High Mountain as a natural barrier and geographical isolation this region has not reported the introduction of apricot cultivars from outside the region. The popularity of Ladakhi apricot remains restricted to the region because of limited production [3]. The local Ladakhi apricot, colloquially known as *Chuli* has been classified into two distinct groups based on kernel taste. The *Khante* being the one with the bitter kernel, and the *Ngarmo* consists of the fruit with sweet kernel [8]. *Ngarmo* is further categorized into two groups on the bases of stone colour. Fruit with white coloured stone is locally called *Raktsey Karpo*, while fruit with the sweet kernel and brown coloured stone are known as *Nyarmo*. Fruits that are large in size are called *Chenmo* meaning large, while those with small size are called *Chun* meaning small. Apricots with white coloured stone are unique to this region and are always linked to the sweet kernel, high TSS and bright coloured fruits. Fruits of *Raktsey Karpo* are known for their sweetness and used for fresh consumption [3].

Figure 1.2: Fruit stone of apricots of trans-Himalayan Ladakh with white (a) and brown (b) colour

1.3 Traditional and economic importance

Apricot has multiple uses. No part of the fruit goes waste. Apricots is found most suitable for human consumption either in the form of fresh and dried, or as various confectionery products such as jam, juice, puree, jelly, marmalade, frozen dessert, paste, ice cream, wine, liquor, and vinegar [9], [10], [11], [12] . The sweet kernel is used for human consumption and also as a good substitute for almonds and nuts. The bitter kernel is used to extract oil for cosmetic products, soap composition and pharmaceutical industry [8], [13]. In some regions of Asia, apricot is grown primarily for the edible seed and oil, which are more consequential than the fruit [5].

In the Ladakh region of Jammu & Kashmir, apricot is directly associated with the traditions and cultures of the region. It is one of the major sources of livelihood. Dried apricot is the only horticultural product that is in high demand within and outside Ladakh region. Dried fruit is rehydrated by hot water overnight and is served with bread. The sweet kernel is dried and the powder is mixed with barley flour and is served in weddings as well as during field work. Apricot oil also has religious importance and is used to light lamps in monasteries. The oil is also used in the traditional *Amchi* system of medicine. Bitter kernel oil is used as hair oil and is believed to control hair fall and dandruff. It is also found to prevent joint pain and dryness of skin [8]. Lakdan et al. [14] estimated an overall gross return of Rs 1.6 lakh and net return of Rs. 1.32 lakh from 100 apricot trees in Ladakh.

1.4 Nutritive value

Apricot is a nutritious fruit. The nutritive value of fruit (fresh and dried) as per the USDA nutrient database and other studies [15], [16] is shown in Table 1.1 and that of the kernel is shown in Table 1.2.

1.5 Fruit quality and consumers preferences

Fruit quality is the major concern for acceptability of apricot cultivars by the buyers, particularly as concerned with the current situation the high competition requirement in the market with the existence of many modern cultivars, fruits, and other food [17]. Fruit quality embraces sensory attributes, nutritive values, mechanical properties, functional properties and chemical constituents [18], [19], [20]. Fruit choices made by consumers are predominantly determined by attributes such as colour, shape, size and external defects. Consumer's interest is mainly focused on juiciness, aroma, and flavor of apricot fruit, especially on the sugar content which is the most considerable quality [17], [21]. These quality attributes are strongly linked with the variety and ripening stages of the fruit [22]. Visual appeal is also one of the major quality attributes of fruit which is most demanded in the fresh market [23], and consumers are attracted to bright coloured apricots [24]. Therefore, new apricot genotypes and cultivars with high fruit quality traits are needed for consumers' satisfaction [17]. Lack of awareness regarding perceived apricot fruit quality is also another weak point. When compared with apples, where consumers are well known with varieties of fruit, a survey conducted in France demonstrates that about 81% of the questioners were not able to identify a single variety of apricots. They considered fresh apricots as a generic product [24]. Since fresh apricot fruit are available for fresh consumption in the market for a very short time, it is very difficult for consumers to make up a sound picture of quality fruit based on particular phenotypic traits as for apples [30].

Numerous pomological and sensory traits such as fruit size, colour, firmness, taste, aroma, and texture determine fruit quality [31], [32].The sensorial properties of apricots are influenced by organic acids, sugars, size, colour, texture and volatile compounds content [33]. Reports with analytical data for quality control of apricots are available [34], [35].

Table 1.1: Nutritional value of apricot (fresh and dried) as per USDA

Nutrients	Unit	Value/100gm		Nutrients	Unit	Value/100gm	
		Fresh apricot	Dried Apricot			Fresh apricot	Dried Apricot
Proximates				Beta carotene	µg	1094	2163
Water	g	86.35	30.89	Total choline	mg	2.8	13.9
Protein	g	1.4	3.39	Folate, total	µg	9.0	10.0
Energy	kcal	48	241	Niacin	mg	0.6	2.589
Total lipid (fat)	g	0.39	0.51	Pantothenic acid	mg	0.24	0.516
Ash	g	0.75	2.57	Riboflavin	mg	0.04	0.074
Total dietary, fiber	g	2.00	7.36	Thiamin	mg	0.03	0.015
Carbohydrate	g	11.12	62.64	Lipids (Fatty acids)			
Glucose	g	2.37	33.08	Total saturated	g	0.027	0.017
Fructose	g	0.94	12.47	Total monounsaturated	g	0.17	0.74
Sucrose	g	5.87	7.89	Total polyunsaturated	g	0.077	0.074
Total sugars	g	9.24	53.44	Amino Acids			
Minerals				Tryptophan	g	0.015	0.016
Calcium, Ca	mg	13.0	55.0	Threonine	g	0.047	0.073
Copper, Cu	mg	0.078	0.34	Isoleucine	g	0.041	0.063
Iron, Fe	mg	0.39	2.66	Leucine	g	0.077	0.105
Magnesium, Mg	mg	10.0	32.0	Lysine	g	0.097	0.083
Manganese, Mn	mg	0.077	0.24	Methionine	g	0.006	0.015
Phosphorus, P	mg	23.0	72.0	Cystine	g	0.003	0.019
Potassium, K	mg	259	1162	Phenylalanine	g	0.052	0.062
Sodium, Na	mg	1.0	10.0	Tyrosine	g	0.029	0.039
Selenium, Se	µg	0.1	2.2	Valine	g	0.047	0.078
Zinc, Zn	mg	0.2	0.39	Arginine	g	0.045	0.066
Vitamins				Histidine	g	0.027	0.047
Vitamin A, RAE	µg	96	180	Alanine	g	0.068	0.11
Vitamin A, IU	iu	1926	3604	Aspartic acid	g	0.314	0.937
Vitamin B-6	mg	0.054	0.143	Glutamic acid	g	0.157	0.188
Vitamin C	mg	10.0	1.0	Glycine	g	0.04	0.07
Vitamin E (alpha-tocopherol)	mg	0.89	4.33	Proline	g	0.101	0.821
Vitamin K (phylloquinone)	µg	3.3	3.1	Serine	g	0.083	0.087

Table 1.2: Nutritional value of apricot kernel

Nutrient	Unit	Value/100gm	Reference
Proximates			
Energy	Kcal	884	USDA [25]
Total lipid (fat)	g	100.0	
Protein	g	14.1-45.4	Alpaslan and Hayta [26]
Oil content	g	27.7-66.7	
Ash content	g	1.7-2.9	
Moisture	g	6.86	Bachheti et al. [27]
Fiber	g	1.94	
Carbohydrates	g	15.61	
Minerals			
Calcium (Ca)	mg	1.8-2.4	Normakhmatov et al. [28] and
Iron (Fe)	mg	2.14-2.82	Pala et al. [29]
Magnesium (Mg)	mg	113-290	
Potassium (K)	mg	473-570	
Sodium (Na)	mg	35.2-36.8	
Zink (Zn)	mg	3.15-2.33	
Nickel (Ni)	mg	0.14	Normakhmatov et al. [28]
Manganese (Mn)	mg	0.48	
Vitamin			
Thiamin	mg	0.12-0.38	Alpaslan and Hayta [26]
Riboflavin	mg	0.18-0.26	
Niacin	mg	2.03-6.07	
Vitamin C	mg	1.05-2.14	
Vitamin E (alpha-tocopherol)	mg	4.0	USDA [25]
Lipids (Fatty acids)			
Total saturated	g	6.30	USDA [25]
Total monosaturated	g	60.0	
Total polysaturated	g	29.3	

Azodaanlou et al. [36] proposed a model for quality assessment on the basis of TSS and total volatile compounds which provide limited information for good quality apricots. Quality parameters of apricots may not be autonomous to one another, and therefore interrelationships among these quality, parameters need to be studied.

Apart from fruit quality, consumers often criticize the organoleptic quality of apricot. Consumers are concern about the increase in poor taste of fresh apricots [37], especially the lack of sweetness in purchased apricots [38]. Sub-optimal fruit quality in terms of fruit aroma is frequent at the retail outlets [39]. Apricot fruit has limited post-harvest life because of high moisture content, so fresh apricots are available for sale for a very short period [40]. It is, therefore, hard for consumers to make up a strong image of high-quality apricot fruits. Therefore, there is a need to develop an easily identifiable phenotype that can be associated with quality apricots.

1.6 Sugar profiling and content

Sugar plays a fundamental role in plant metabolism, catabolism, signaling network, cell division, transportation of carbon and energy, regulators for gene expression and amalgamation of many complex molecules in plant [41]. Besides, it also plays an important role in induced stress-responsive genes, which alter the metabolic pathway modules of the plant during exposure to different environmental conditions [42]. Apricot fruit has high sugar content and has a perfect balance between organic acids and sugar content. This combination is combined with the rich aroma of fruit [43]. Taste of fruit is a desirable trait that contributes to the quality of fruit and is a predominant factor in breeding programs. Taste of fruit is determined by various forms of organic acids and soluble sugar content [44]. In apricots, the main contributor to fruit sweetness is fructose and sucrose. It also contributes to consumer satisfaction and sensory quality of fruit [45]. Sucrose is mostly reported as a major sugar present in apricots which is succeeded by glucose, fructose and sorbitol [37], [39], [45], [46]. In few studies higher glucose level than sucrose content [47]; and lower fructose [39] and glucose level [48] than sorbitol have also been documented. Akin et al. [48] reported sorbitol content as a major sugar in some varieties of apricot cultivars. The % age of these four major soluble sugars varied significantly in apricots grown in different parts of the world (glucose: 5.3-28.1%; sucrose: 17.6-81.8%; fructose: 1.9-16.6%; sorbitol: 0.3-32.5%). In apricot kernels,

the most common sugars are sucrose, glucose, and fructose. Femenia et al. [49] reported that bitter apricot kernels contain higher sugar as compare to sweet kernels. The difference in the individual levels of sugar could be the effect of both environmental conditions and the genotypic factor. It has been reported that individual sugar patterns is under the genetic control in apricots [50]. However, little is known on the effect of the environmental component on apricot fruit and kernel and on its sugar profiles.

1.7 Amygdalin

Amygdalin is a natural cyanogenic glucoside which gives a bitter taste to the kernel. Multiple cases of poisoning as a result of the consumption of bitter apricot kernel have been reported [51], [52]. It is reported that if the kernel is consumed directly without chewing then less amount of cyanide is released, than if the kernel is chewed completely [53]. Once chewed, the kernel released cyanide which reacts in small intestine under the alkaline environment, with the process of emulsification it gets quickly absorbed and circulates in the body. Cyanide causes anoxia which blocks the cytochrome oxidase to the tissues at the cellular level due to which anaerobic metabolism occurs and causes hypoxia and lactic acid is produced [51]. Amygdalin has been mistaken for laetrile. Both laetrile and amygdalin are promoted as vitamin B17, which is used for cancer treatment, but these are not really a vitamin [54]. The disputable utilization of amygdalin in the treatment of cancer by numerous alternative therapists is well known [55]. However, it is completely ineffective and toxic [56] and known to cause cyanide poisoning [57]. A systematic review concluded that there is no adequate data from any controlled clinical trials which support that amygdalin has any advantageous role for cancer treatment [58], [59]. Therefore, the presence of amygdalin content in the apricot kernel is considered as an undesirable character for human consumption. It is well documented that the amygdalin level in the bitter kernel is significantly greater than that of the sweet kernel [49], [60], [61]. Within the sweet kernel it ranged from less than 0.08 to 15.84 $mg.g^{-1}$ DW [60], [61]. Similarly, it ranged from 13.96 to 55 $mg.g^{-1}$ DW in bitter apricot kernel [49], [61]. The level of amygdalin differed highly in apricot kernel documented from many apricot growing locations throughout the world. The difference in the levels of amygdalin could be the result of both environmental and genotypic factors, but no literature is available about the effect of environmental conditions on amygdalin content.

1.8 Genetic Diversity

Genetic diversity (GD) is the core of biological diversity. The presence of plant genetic diversity is essential to ensure its long-term adaptation to the changing ecological environment [62]. Abundant plant genetic diversity can provide a wide range of genetic backgrounds for crop genetics and breeding research [63]. The assessment of the GD of crop germplasm is very important for the protection of endangered resources and sustainable use of the resources.

Central Asia is the primary and oldest source of genetically diverse apricot germplasm in central Asian accessions. In order to perform an assessment of the GD of the germplasm of cultivated plants, both in collections and in the field, the use of molecular markers has become an essential tool. The analysis of DNA with these molecular marker tools allows resolving the identification of genotypes which is impossible to obtain with morphological observation alone. There are several molecular techniques which are not affected by environmental changes, including inter-simple sequence repeats (ISSRs), sequence-related amplified polymorphism (SRAPs), simple sequences repeats (SSRs) and amplified fragment length polymorphism (AFLP). These can screen the whole genome to describe the diversity and genetic characterization of apricot germplasm worldwide. However, the genetic diversity of apricots of trans-Himalaya has not been studied in detail.

Thus, considering the above research gaps in apricots, the present study has been conducted with the following objectives:

1. Fruit quality, consumer perception and quality assessment for fresh apricots of Ladakh

2. Change in phenology and fruit quality of fresh apricot along an altitudinal gradient in trans-Himalaya

3. Altitudinal effect on sugar content and sugar profile in dried apricots

4. Effect of altitude and seed phenotypic characters on amygdalin and sugar content in apricot kernel

5. Morphological and SRAP markers based genetic diversity studies of apricots of trans-Himalaya

CHAPTER 2

FRUIT QUALITY, CONSUMER PERCEPTION AND QUALITY ASSESSMENT FOR FRESH APRICOT (*Prunus armeniaca* L.) OF LADAKH

ABSTRACT

Forty-seven apricot genotypes with white and brown fruit stone were studied by both instrumental and sensory methods. Attributes such as aroma, juiciness, sweetness, flesh colour, stone colour, fruit shape, and size were the main factors affecting overall acceptability of apricots. Fruit with white stone ranked first in terms of liking for sweetness, juiciness, aroma, stone colour, flesh colour, fruit shape, and size. It scored the highest hedonic score for overall appreciation (8.0±0.2). It also had the highest total soluble solids (TSS) (27.5±3.6°Brix), reducing sugars (17.5±1.9%) and total sugars (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 stone phenotype. Relationships between the instrumental data and sensory panel score were established. Overall appreciation showed positive significant relation with TSS (R^2 = 0.177), TSS/total acid (R^2 = 0.118), reducing sugar (R^2 = 0.140), total sugar (R^2 = 0.177) and fruit weight (R^2 = 0.230). A statistically significant negative relationship was observed between overall appreciation and fruit moisture content (R^2 = 0.168). The study demonstrated that white stone coat phenotype could be considered as a marker for high quality apricots in terms of aroma, sweetness, juiciness and overall appreciation.

2.1 Introduction

Apricot is broadly classified in trans-Himalayan Ladakh region into two main groups on the basis of kernel taste. Fruit with the bitter kernel is known as *Khante*, and those with the sweet kernel as *Ngarmo* [8]. The *Ngarmo* is further divided based on stone colour. Fruit with white stone is called *Raktsey Karpo*, and those with brown stone are known as *Nyarmo*. Large size fruit is called *Chenmo* meaning large, while those with small size are called *Chun* meaning small. Morphological diversity of apricots of the region is shown in Figure 2.1. Apricots with white stone are unique to Ladakh, and the phenotypic character is linked to brightly coloured fruit with high sugar and sweet kernel. Fruit of *Raktsey Karpo* is known for its sweetness and used for fresh consumption [3]. Accordingly, the purpose of this investigation was to determine whether white stone, which is an easily identifying phenotype, can be taken as a marker for high-quality apricots for fresh consumption. Knowledge about sensory properties and consumer appreciation of apricots with an easily identifying phenotype is vital since it provides a useful guide to create a good image of the fruit and earn consumer's loyalty based on the phenotypic trait.

Figure 2.1: Morphological diversity of apricot fruit of trans-Himalayan Ladakh

2.2 Materials and methods

2.2.1 Study site and fruit collection

We collected fruit samples in the year 2016 from an experimental orchard (77°34.3'E, 34°08.2'N) at 3340 m asl elevation at DIHAR in Leh Ladakh region. The weather data of the experimental site, from May to October, are shown in Table 2.1. The orchard contained twelve rows, eight trees per row of apricot genotypes from different parts of Ladakh region. Trees were grown at 4m × 4m spacing. All trees were 16-17 years and we followed standard organic cultural practices. Fruit of forty-seven genotypes was sampled at eating maturity stage. Fruit samples from different trees were grouped into six on the basis of kernel taste, stone colour, fruit size and drying quality (Table 2.2). Seven genotypes were grouped under Group-A with sweet kernel and white stone; 05 genotypes under Group-B with sweet kernel, brown stone, and high drying quality characteristics; 09 genotypes under Group-C with sweet kernel, brown stone, and large fruit size; 16 genotypes under Group-D with sweet kernel, brown stone, and small fruit size; 05 genotypes under Group-E with bitter kernel, brown stone, and large fruit size; and 05 genotypes under Group-F with bitter kernel, brown stone, and small fruit size.

Table 2.1: 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

2.2.2 Analysis of pomological and fruit quality traits

The standard fruit quality parameters and pomological traits (Table 2.2 & 2.3) were determined on the day of harvest. Stone and fruit weight was measured with an electronic

balance with 0.001 g accuracy. Dimensional properties were measured with a digimatic calliper with 0.01 mm accuracy. Fruit firmness was measured with a penetrometer equipped with an 8 mm cylindrical plunger. The perimeter of blush area was traced on a tracing paper and was used to determine fruit blush area using a graph paper. A refractometer (ATAGO, Tokyo) was used to determine total soluble solids (TSS) and the values were corrected at 20 ^{o}C. Total acid % (TA) was established by titration method using 0.1 N NaOH and the values were expressed as % malic acid [64]. Reducing sugars and sucrose were determined as outlined by Rangana [64]. Fruit moisture content was determined using an oven drying method and expressed as % of fresh weight.

2.2.3 Sensory evaluation and acceptance

Fruit samples were evaluated on the day of harvest by a 20-member semi-trained panel (eight women, twelve men, age 24-42 year) at room temperature with natural day light. Each panelist had previous experience with descriptive analysis. Panelists were presented with coded fruit samples from each genotype. Five or fewer samples were evaluated per session. The panelists rated the sensory attributes such as sweetness, juiciness, aroma, flesh colour, skin colour, stone colour, fruit shape, fruit size and overall acceptability. Each panelist rated different parameters and overall acceptability on a 9-1 scale (9 = extremely good to 1 = extremely bad).

2.2.4 Statistical analysis

The data were expressed as mean ± standard deviation (SD) using SPSS statistical tool. Analysis of variance (ANOVA) was performed to analyze significant differences. Sensory evaluation data were analyzed by descriptive statistics using the Spearman rank-order correlation. Spearman's correlation analysis was performed to determine a correlation between the different variables. Regression was performed using MS Excel 2007.

Table 2.2: Stone colour, kernel taste, fruit shape and colour of six groups of apricots of trans-Himalayan Ladakh

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

2.3 Results and discussion

2.3.1 Fruit quality characteristics and pomological traits

Pomological attributes of 47 apricot genotypes are shown in Table 2.3. Apricots with a white stone (Group-A) ranked second (21.7±4.9 g, 343.2±73.7 mm^2), after Group-E (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 found less than 40.0 g, which is the minimum weight of apricots required for fresh market in the United States [39]. A survey conducted in France suggested that elder people prefer large apricots while the majority of the consumers are looking for a medium-sized fruit [65]. *Raktsey Karpo* (Group-A) 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 apricots [66], [43], [67], [17], [68], [69], [70].

2.3.2 Sensory evaluation and appreciation

Consumer likings of apricots in terms of fruit shape, fruit size, fruit skin colour, flesh colour, stone colour, aroma, sweetness, juiciness and overall appreciation are shown in Table 2.4. *Raktsey Karpo* (Group-A) ranked first in terms of liking for sweetness, juiciness, aroma, flesh colour and stone colour. It also ranked first along with *Nyarmo Chenmo* (Group-C) for fruit shape. Group-A also ranked first for fruit size along with *Nyarmo Chenmo* (Group-C) and *Khante Chenmo* (Group-E). *Raktsey Karpo* scored the highest hedonic score for overall appreciation (8.0±0.2). Consumer dissatisfaction centered around lack of flavor, aroma and sweetness in apricots [71], [65], [38]. However, *Raktsey Karpo* has the characteristics of being aromatic and sweet. Therefore, the results suggested that white stone phenotype can be considered as a marker for high quality apricots in terms of aroma, sweetness, juiciness and overall appreciation.

2.3.3 Parameters for the distinction between quality classes

The instrumental data allowed obtaining distinction between the four quality classes (Table 2.5). TSS, sugar content, moisture content, and fruit weight were good parameters to differentiate between the four classes. However, the blush area and fruit firmness did not distinguish the six 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 ‘very good’ class. Distribution of apricots with high sugar content in ‘good’ and ‘very good’ class is as per previous report [36]. Fruit 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 ‘good’ and ‘very good’ classes. Piagnani et al. [72] also reported that cultivars with the best appreciation fell into the group with the highest TSS.

Consumers are fascinated with brightly coloured fruit [65]. In the present study, we found that fruit with dark orange skin was appreciated by consumers and fell under ‘good’ and ‘very good’ classes (Table 2.6). Seventy-five percent of the medium orange skin fruit were categorized as ‘good’. However, fruit with cream and light orange skin were disliked by the consumers. White stone phenotype was appreciated by the consumer and ranked as ‘good’, suggesting that consumers are attracted to the unique white stone phenotype.

Table 2.3: 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 (%)
A	*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}
B	*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}
C	*Nyarmo Chemno*	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}
D	*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}
E	*Khante Chenmo*	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}
F	*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 ($P \leq 0.05$)

Table 2.4: 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
A	*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}
B	*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}
C	*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}
D	*Nyarm 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}
E	*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}
F	*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 2.5: 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.(gm)	11.09±3.98[a]	20.26±4.86[b]	18.52±4.76[b]	26.98±8.87[c]
Stone Wt.(gm)	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 ($^{\circ}$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 column 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 2.6: 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
Stone 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.

2.3.4 Correlation between instrumental and sensory data

Relationships between data obtained by the instrumental method and sensory panel have been determined. A statistically significant positive relation between fruit weight and the consumer's overall appreciation was found (R^2 = 0.230). Similarly, overall appreciation showed positive significant relation with TSS (R^2 = 0.177), TSS/TA (R^2 = 0.118), reducing sugar (R^2 = 0.140), and total sugar (R^2 = 0.177). A statistically significant negative relationship was observed between overall appreciation and fruit moisture content (R^2 = 0.168) (Figure 2.2). The perceived fruit sweetness showed positive correlation with TSS (R^2 = 0.368), TSS/TA (R^2 = 0.231), reducing sugar (R^2 = 0.138) and total sugar (R^2 = 0.180). Sucrose and TA did not show a significant relation at $p \leq 0.05$. Perceived fruit juiciness showed a negative relation with fruit moisture content (R^2 = 0.207).

The results of coefficients of correlation (Table 2.7) suggested that 'aroma', 'sweetness', 'juiciness', 'stone colour', '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 2.8) showed that stone colour is significant correlation with fruit shape (0.593), fruit size (0.625), skin colour (0.486), flesh colour (0.6531), aroma (0.830); sweetness (0.823), juiciness (0.846), and taste (0.873). Similarly, we observed a significant correlation between other fruit quality characteristics and sensory variables.

Table 2.7: Coefficients of correlation between overall appreciation and sensory attributes of fresh apricots by the consumers

Attributes	**R Spearman**
Aroma	0.831**
Sweetness	0.925**
Juiciness	0.907**
Stone colour	0.896**
Shape	0.594**
Size	0.614**
Skin colour	0.493**
Flesh colour	0.662**
Taste	0.966**

** Significant at $p \leq 0.01$

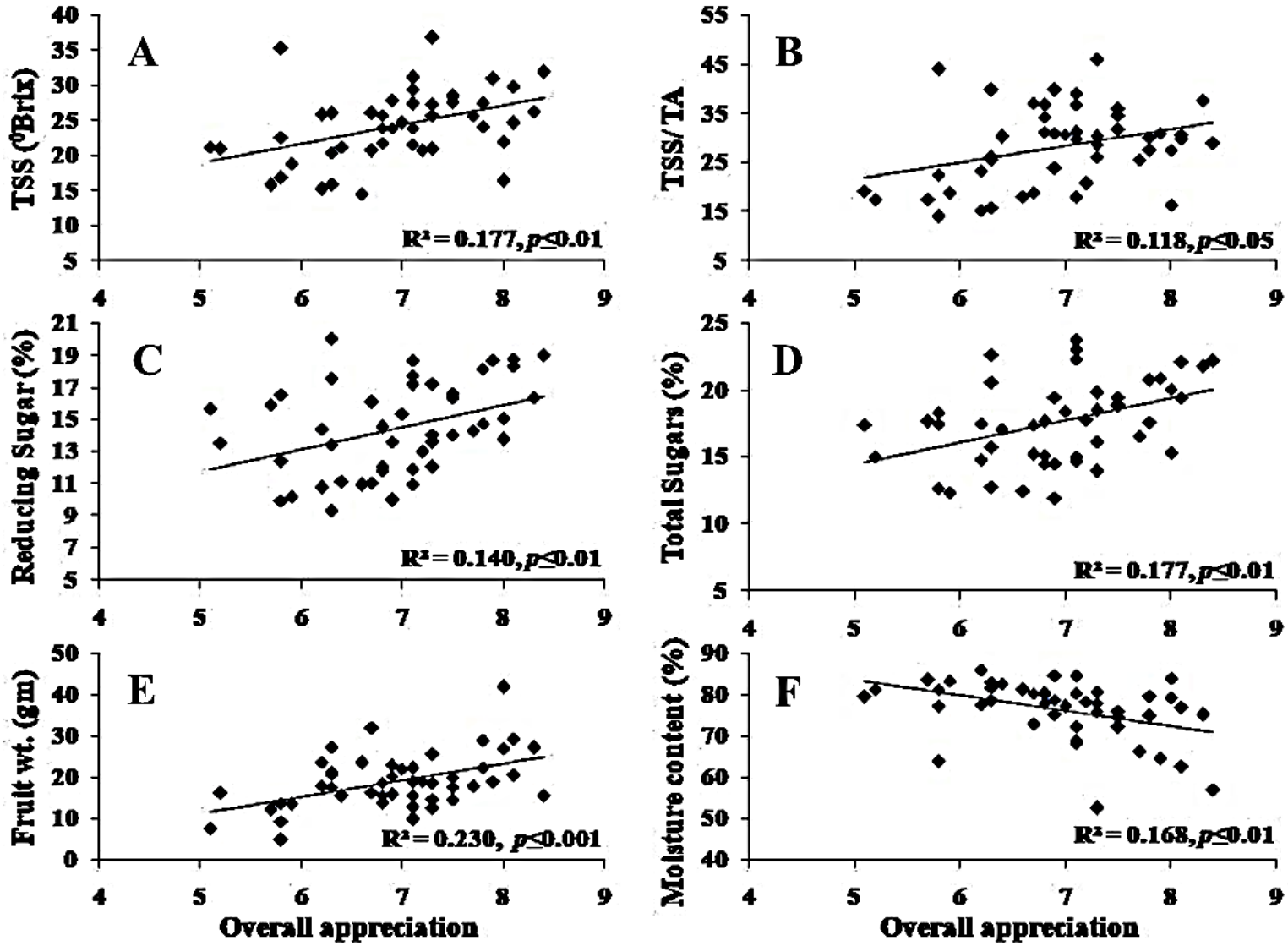


Figure 2.2: Relation between overall appreciation with TSS (A), TSS/TA (B), reducing sugar (C), total sugar (D), fruit weight (E) and fruit moisture content (F)

Table 2.8: 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	Stone 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**
Stone colour									1.00

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

2.4 Conclusion

The present study demonstrated that fruit with white stone phenotype can be considered as easily identifying a marker for quality apricots. 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 colour, stone colour, fruit shape, and fruit weight. Further research is suggested to identify molecular markers linked to white stone phenotype for early marker assisted selection in apricot breeding.

CHAPTER 3

CHANGE IN PHENOLOGY AND FRUIT QUALITY OF FRESH APRICOT ALONG AN ALTITUDINAL GRADIENT IN TRANS-HIMALAYA

Abstract

Consumer concern about the 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 the environmental effect on fruit quality. Therefore, this investigation aimed at studying the influence of elevation on phenological and fruit quality characters of apricot genotypes. Fruits from 162 trees were sampled from nine villages located at an elevation ranging from 3006 to 3346 meter asl in Ladakh. The altitude had a significant influence on the date of flowering, fruit weight, moisture, and TSS content. For every 100 meter rise in elevation, flowering and fruit ripening delayed by 3.3 and 7.1 days, respectively. An inverse relationship between fruit weight and elevation (R^2 = 0.310) was observed. The fruit weight decrease by 0.5 gm for every 100 meter increase in elevation. Fruit moisture content decreased significantly with increase in elevation (R^2 = 0.585). The decrease in moisture content was 1.9% for every 100 meter rise in elevation. Increase in elevation had a linear relationship with fruit TSS content (R^2 = 0.726). The fruit TSS increased by 1.2ºBrix for every 100 meter rise in elevation. Knowledge from the present study on the impact of altitude on fruit quality characters suggests guidance on selection of orchard site for improving apricot fruit quality.

3.1 Introduction

Trials conducted around the world have shown that the genetic factor determines quality characters of apricot fruits. As for the environmental factor, limited numbers of studies on a few cultivars are available in the literature on the variability in fruit quality traits. When Hasenbey and Şekerpare cultivars were grown at two elevations (850-1200; 1150-1600 meter asl), the fruit TSS increases and fruit size decreases at a higher elevation [73]. When three apricot cultivars were grown at 731, 855 and 1115 meter elevation, a linear relation was not observed between rising elevation and fruit quality traits such as TSS and fruit weight [74]. Contrasting observations have been recorded in other fruits on influence of elevation on the fruit quality. Because of the contrasting reports from limited research trials, investigations involving a bigger number of genotypes across different elevation are required to have a clearer understanding of the elevation effects on apricot fruit quality characters. While most of the studied were conducted below 1500 meter, there is little information from regions above 3000 meter. This knowledge is vital since it suggests guidance on selection of orchard site for improving apricot quality.

3.2 Materials and methods

3.2.1 Study sites

Apricots were collected from nine isolated villages spread across Ladakh region. The villages were located at an altitude ranging from 3006 to 3346 meter asl (Table 3.1)

3.2.2 Phenological, pomological and fruit quality traits

Date of flowering was determined when 50% of the floral buds attain full bloom stage. It was expressed in Julian days i.e. natural days from 1st January. Date of harvesting the fruit from each tree was established on the basis of fruit colour, firmness, and taste. Fruit samples (50 fruits per tree) were randomly handpicked at eating maturity stage. Standard pomological and fruit quality traits were determined on the date of harvest (Table 3.2). Stone and fruit weight was measured with a balance with 0.001 g accuracy. TSS was determined with the refractometer and values were corrected at 20ºC. Oven drying method was used to determine the moisture content of the fruit and expressed as percentage fresh weight (AOAC 1990).

Table 3.1: Geographical locations and sampling site 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

3.2.3 Statistical analysis

Data was recorded in triplicate. Each replicate consisted of three fruits. The results were expressed as the mean ± standard deviation using SPSS 16 statistical tool and MS Excel 2007. ANOVA and post hoc analysis with 2-sided Tukey's HSD at $p \leq 0.05$ level were performed. Pearson's correlation analysis was done to find a correlation between the different variables.

3.3 Results and discussion

3.3.1 Altitude effect on flowering phenology and fruit ripening date

Altitude significantly affects flowering phenology (Table 3.2). The Linear relationship between date of flowering and the rise in elevation was observed (R^2=0.914) (Figure 3.1a). For every 100 meter increase elevation, flowering was delayed by 3.3 days. Flowering dates showed significant variability and it ranged from 104.0 at 3006 meters to 116 Julian days at 3346 meter elevation (Figure 3.2). Similarly, a linear relationship between harvest date and increasing elevation was seen (R^2 = 0.820) (Figure 3.1b). For every 100 meter rise in elevation, fruit ripening delayed by 7.1 days.

Altitude had a marked influence on flowering phenology. Delay in date of flowering was observed with increasing altitude. Flowering delayed by 3.3 days for every 100 meter increase in elevation. Flowering dates showed significant variability and ranged from averaged 104.0 at 3006 m to 116 Julian days at 3346 m elevation. Delay in flowering in higher altitude regions may be associated with decreasing temperature. Significant differences

in flowering dates in apricot have been published from different regions. Apricot blooming dates in Spain and Italy varied from 25 to 80 Julian days [75], 79.9 to 88.7 Julian days in Serbia [76] and 111 to 114 Julian days in Ladakh [3]. Results of the current study suggested that differences in date of flowering are largely due to environmental factors associated with altitude. Late flowering is a vital character to protect the flower from chilling effect, and hence, a desirable character in high altitude regions. Similarly, a linear relationship between harvest date and rising in elevation was seen (R^2 = 0.820). For every 100 meter rise in elevation, fruit ripening delayed by 7.1 days. Apricots from different regions are known for marked differences in date of fruit ripening. Apricots are harvested in May to June in Greece and America [46] while selections and cultivars in Spain are harvested in mid-May to late June [17]. Fruits attain maturity in late June and early July in Anatolia, Turkey [67] while fruit from Lake Van Region in Turkey attains maturity between late July to early August [77]. In trans-Himalayan Ladakh, fruits attain maturity in August and early September [3]. Knowledge from the present study highlighted that difference in apricot fruit harvesting dates from different apricot growing regions is primarily due to environmental effects.

3.3.2 Altitude effects on pomological traits

Pomological attributes of 162 apricot genotypes collected from nine locations are presented in Table 3.2. Fruit weight ranged from 5.3-52.5 g. We have seen the opposite relationship between altitude and fruit weight (R^2=0.310). For every 100 meter rise in elevation the fruit weight decrease by 0.5 gm. Fruit moisture content decreased significantly with increase in elevation (R^2=0.585) (Figure 3.1c). The decrease in moisture content was 1.9% for every 100 meter increase in elevation. Blush area and seed dimensional properties showed an opposite relationship with altitude, but not significant.

Marked variability in fruit weight was observed among the 162 genotypes, ranging from 5.3-52.5 g. In comparison, fruit weight of 24.2 to 48.3 g has been reported among apricots of the Lake Van region [77]. A much higher fruit weight ranging from 49.1 to 81.5 g has been reported in apricot genotypes from Central Serbia [68].

Table 3.2: Morphometric and pomological characteristics of apricot fruits of trans-Himalaya

Location	FB (JD)	FrW (g)	SW (g)	FW (g)	TSS (^{0}Brix)	MC (%)	BA $(mm)^2$	FrL (mm)	FrWd (mm)	SL (mm)	SWd (mm)	ST (mm)	SCT (mm)
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^{c}	21.1 ±2.3^{cd}	18.0 ±2.2^{c}	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^{b-d}	15.9 ±5.1^{c}	17.8 ±3.7^{a}	74.8 ±4.2^{e}	225.7 ±218.7^{ab}	28.0 ±4.0^{a-c}	30.2 ±4.0^{a-c}	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^{b-d}	1.9 ±0.4^{cd}	14.9 ±3.3^{bc}	19.4 ±5.0^{ab}	73.0 ±3.8^{c-e}	265.6 ±236.2^{b}	30.5 ±3.8^{d}	30.9 ±3.1^{bc}	21.7 ±3.5^{d}	17.2 ±2.8^{a-c}	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^{a-c}	73.6 ±3.2^{c-e}	238.8 ±217.1^{ab}	27.3 ±1.9^{a}	29.2 ±1.7^{ab}	19.0 ±1.6^{a}	16.9 ±1.4^{a-c}	11.6 ±1.6^{ab}	1.4 ±0.3^{a}
SPL	107.4 ±0.6^{e}	15.7 ±3.5^{a-d}	1.8 ±0.4^{a-d}	13.9 ±3.2^{a-c}	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^{a-c}	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^{b-d}	223.5 ±200.9^{ab}	28.1 ±2.8^{a-c}	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^{a-d}	1.8 ±0.5^{a-c}	13.5 ±3.5^{a-c}	20.5 ±3.4^{a-c}	72.7 ±3.5^{b-e}	230.8 ±237.1^{ab}	28.0 ±2.7^{a-c}	29.3 ±2.2^{ab}	20.2 ±2.4^{a-c}	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^{a-d}	1.7 ±0.5^{a-c}	13.6 ±4.7^{a-c}	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^{a-c}	1.8 ±0.4^{a-c}	13.2 ±3.3^{ab}	24.3 ±6.2^{d}	63.3 ±10.6^{a}	128.7 ±182.0^{a}	30.0 ±3.6^{b-d}	30.1 ±3.5^{ab}	20.6 ±2.2^{b-d}	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 ($P \leq 0.05$) JD: Julian days; FB: date of full bloom; FrW: fruit weight; SW: Seed weight; FW: flesh weight; 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

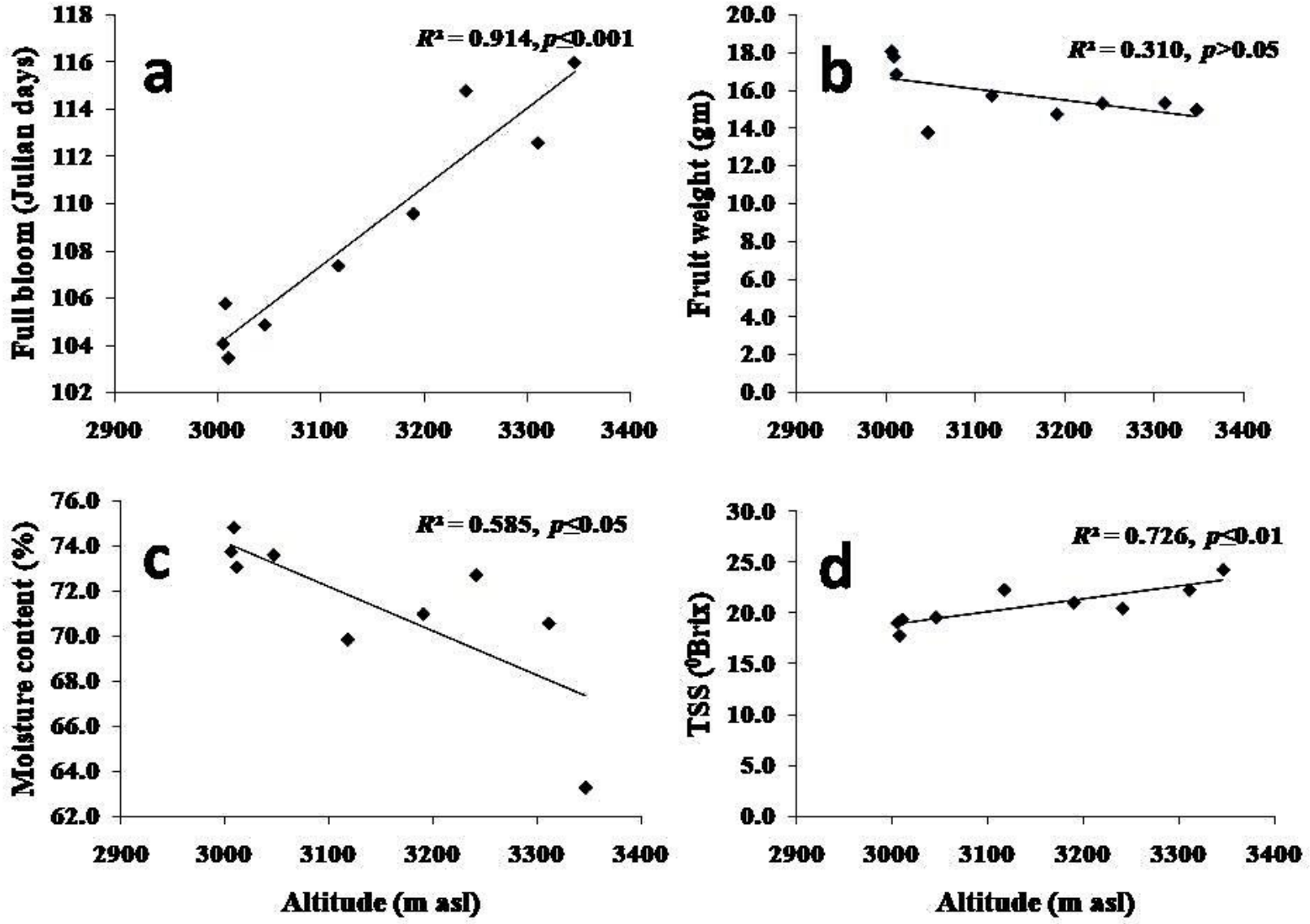


Figure 3.1 Altitudinal variation in apricot (a) flowering; (b) fruit weight; (c) fruit moisture content; (d) fruit TSS in trans-Himalayan region

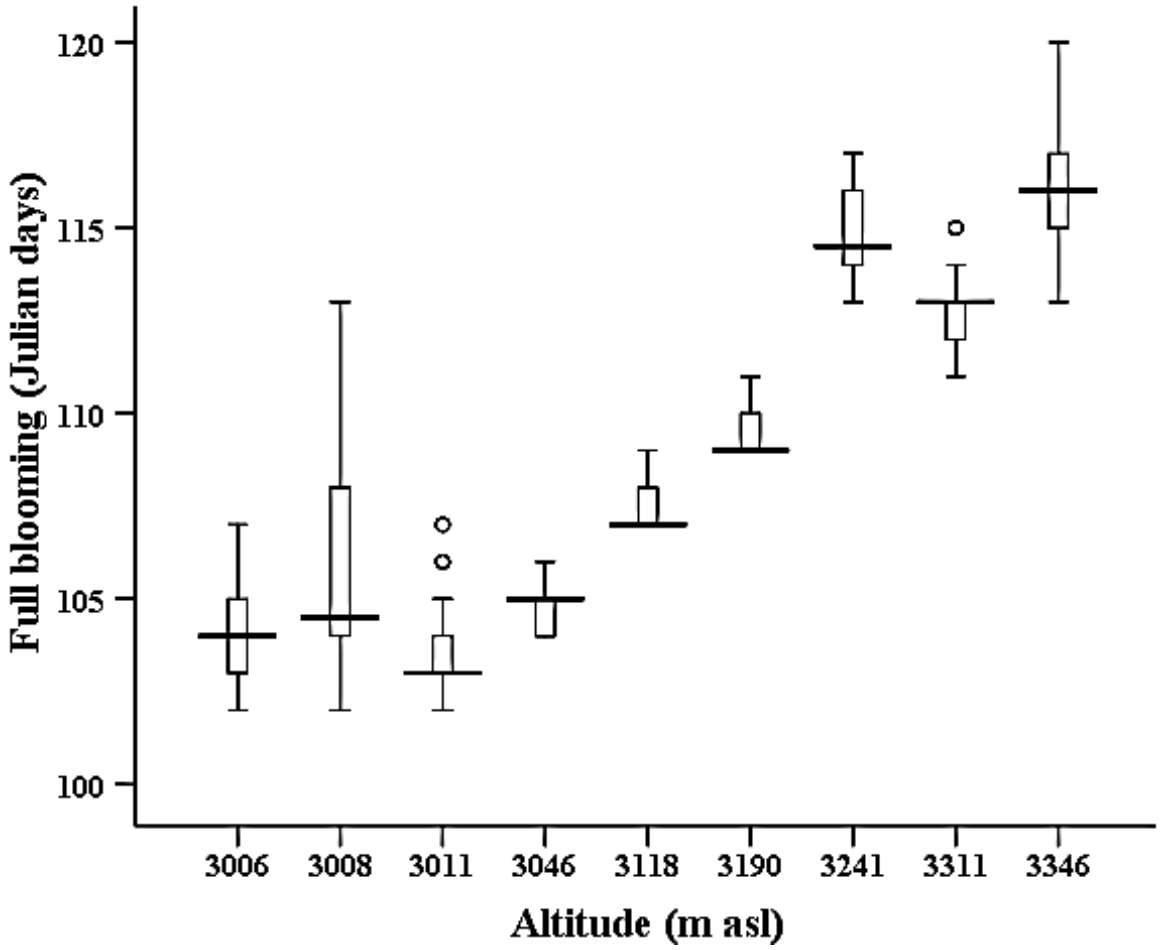


Figure 3.2. Box plot distribution of date of apricot full bloom along altitudinal gradient in trans-Himalayan region. The plot represents the minimum and maximum value (whiskers), the first and third quartile (box), the median (midline).

A much higher fruit weight ranging from 49.1 to 81.5 g has been reported in apricot genotypes from Central Serbia [68]. Twenty one cultivars from Canada, Ukraine, Czech Republic, and the USA ranged between 28.1 to 77.7 g [78]. Single fruit of twenty nine hybrids and cultivars of Greek and American origin weigh 36.5 to 105.3 g [46]. We have seen the opposite relationship between altitude and fruit weight (R^2 = 0.310). For every 100 meter rise in elevation the fruit weight decrease by 0.5 gm. When grown in California condition Central Asian apricot germplasm remained small (9.4 g) [39], and thus shows the importance of genotypic factor on fruit size. Wide variability in fruit weight (5.6 to105.3 g) among cultivated apricot is, therefore, the result of both environmental and genotypic factors.

Our data is partly in accordance with the observations made by Olmez et al. [74] 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 elevation from 731 to 855 meter asl and then showed a declining trend when grown at 1115 meter. Our data is in contrast with studied on other fruits such as fig [79] and chestnut [80], [81] where the increase in fruit size was observed with increasing elevation. It is suggested that larger fruit size could be due to antifreeze protein production at the higher elevation [80]. In sweet cherry and mandarin, no relation was found between fruit weight and altitude [82], [83]. Contrasting results in the previous studies could be due to the difference in altitude of studied areas. While most of the studied were conducted below 1500 meter, our study focuses on high altitude regions above 3000 meter asl.

Fruit moisture content showed a decreasing trend with the increase in elevation (R^2=0.585). Fruits of trans-Himalaya have, therefore, lower moisture content as compared to previous reports [69], [84], 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 content caused fruit to spoil earlier [69]. Blush area and seed dimensional properties showed an opposite relationship with elevation, but not highly significant ($p \leq 0.05$).

3.3.3 Altitude effects on fruit TSS content

Altitude showed a linear relationship with fruit TSS content (R^2=0.726) (Figure 3.1d). The evaluated genotypes showed marked variability in TSS ranging from 10.7-37.6ºBrix with an average of 20.7±5.1 (Figure 3.3). Fruit TSS content increased significantly with an increase in altitude (R^2=0.726). The fruit TSS increased by 1.2ºBrix for every 100 meter increase in elevation. Our result is similar to previous reports on mandarin [83], where high fruit TSS was observed at higher altitude. An opposite relation has been reported when pomegranate was harvested at commercial harvest stage at 222, 662 and 898 meters [85]. Similarly, higher TSS content in persimmon fruits grown at low altitude (229 meter) has been reported that those from a high elevation (770 meters) region [86]. No altitudinal effect on fruit TSS was observed in blueberry [87] and sweet cherry [82].

The evaluated genotypes showed marked variability in TSS content (10.7 to 37.6ºBrix). In comparison, it ranged from 5.7 to 18.9ºBrix in 14 genotypes in Central Serbia [68], 12.7 to 20.0ºBrix among six cultivars in Pakistan [69], 11 to 27ºBrix in 128 apricot types and cultivars in Turkey [67], 10.6 to 16.3ºBrix in 43 genotypes in Spain [17], 9.3 to 18.7ºBrix in 55 cultivars in Spain [66], 8.7 to 22.4ºBrix among 51 genotypes in France [43], 12.3 to

15.8ºBrix in 18 international germplasm collection [70]. The results showed environmental conditions at high altitude are conducive for the production of fruits with high TSS content. Besides the environmental conditions, the genotypic character of Central Asian apricots may also be contributing for higher sugar content. Higher sugar content was recorded when Pakistan origin apricots were grown in California [39]. The result suggested that Central Asian apricot genotypes have innate higher sugar content.

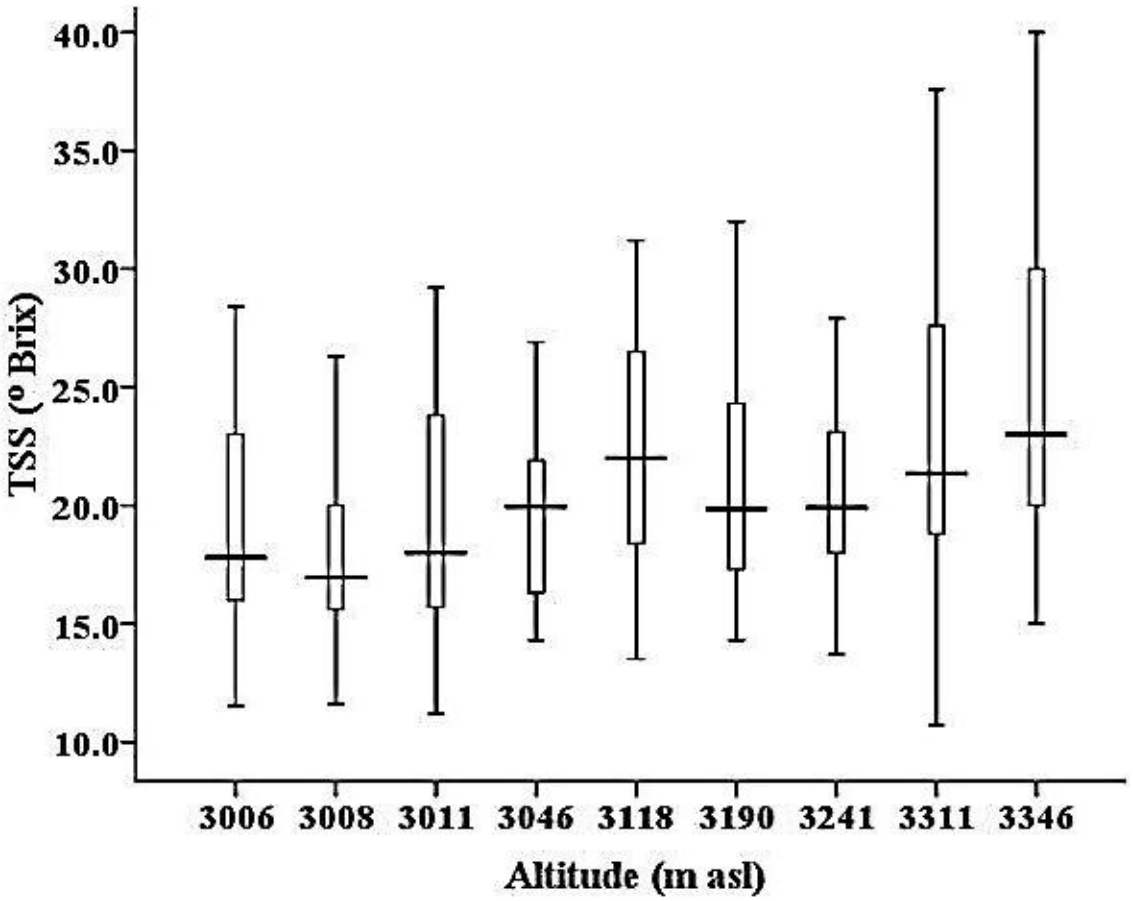


Figure 3.3: Box plot distribution of apricot fruit TSS along altitudinal gradient in trans-Himalayan region. The plot represents the minimum and maximum value (whiskers), the first and third quartile (box), the median (midline).

In the Ladakh region, apricot trees are grown only on irrigated land due to scanty rainfall and high evaporation. Therefore, deficit irrigation is not uncommon in Ladakh, which may be a favorable factor for high TSS content of apricots. Pérez-Pastor et al. [88] have shown apricots with higher TSS when grown under deficit irrigation condition. Dry climatic conditions with low rainfall in high altitude regions appeared to be a favorable factor for fruits with high sugar content. Previous research has reported that fruit TSS is associated with a dry

climate in cactus pear [89]. Fruits produced in dry climatic conditions are often sweeter as compared to those produced in irrigated or humid regions [90]. Therefore, dry climatic conditions seem to be one of the important factors responsible for high TSS content of apricot of high altitude regions.

3.3.4 Correlation

Correlations between the variables are presented in Table 3.3. Seed stone colour showed positive correlation with fruit weight (r = 0.530), kernel taste (r = 0.506) and TSS (r = 0.451). Therefore, apricots with white stone are linked with TSS (r = 0.463) and fruit weight (r = 0.426). Flowering date is significantly correlated with harvest date (r = 0.690). TSS is positively correlated with the apricot harvest date (r = 0.324). Late maturing apricots have higher fruit TSS content. Fruit weight showed correlations with TSS (r = 0.177). However, Caliskan et al. [91] did not find correlations between fruit weight and TSS.

Table 3.3: 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; Moisture content; BA: blush area; FrL; fruit length; FrWd; fruit width; SL: seed length; SWd: seed width; ST: seed thickness; SCT: seed coat thickness.

3.4 Conclusion

Knowledge about the effects of environmental conditions on fruit quality is important. The geographic elevation had a significant 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 the lower elevation. Dry climatic conditions with low rainfall appear to be a favorable factor for fruits with high sugar content in high altitude regions.

CHAPTER 4

ALTITUDINAL EFFECT ON SUGAR CONTENT AND SUGAR PROFILE IN DRIED APRICOTS

ABSTRACT

Apricot fruits from 108 genotypes were taken from six isolated villages located at an altitude ranging from 3006 to 3346 meter asl in Ladakh region. A linear relationship was observed between altitudinal elevation and total sugar content (R^2=0.877). Total fruit sugar increased by 64.8 mg.g^{-1} DW for every 100 meter rise in elevation. The most predominant individual sugar was sucrose (SUR) (57.8% of total sugar). The proportion of glucose (GLU), fructose (FRU), and sorbitol (SOR) was 19.4%, 14.3%, and 8.4% of total sugar, respectively. With altitudinal elevation, the relative proportion of SOR increased significantly in fruits with brown stone (R^2=0.849). A linear relationship was observed between the altitudinal elevation of the orchard and SUR (R^2 = 0.767). SUR content increased by 49.1 mg.g^{-1} DW for every 100 meter rise in elevation. Fruit SOR content showed a linear relationship with rising in elevation (R^2=0.899). GLU level increased with rising elevation, while FRU content showed the opposite relationship with elevation. Data from the investigation indicated that apricots produced at high elevation contain higher sugar, SUR and SOR content. This knowledge is vital since it provides guidance for selecting site for orchard establishment for improving apricot fruit sweetness.

4.1 Introduction

Sugar content in apricots showed significant variability ranging from 7.8 to 108.4 $mg.g^{-1}$ FW. It is well established through investigation that apricot fruit sugar content is determined by genetic factor [45], [91], [92]. As concern the environmental factor, very few investigations are reported on the variation in fruit sugar. No direct relation was observed between rising elevation and fruit TSS when three apricot cultivars were grown at three different elevations [74]. When two cultivars are grown at two elevations, the fruit TSS was found higher at the higher altitude [73]. Contrasting results have been reported in other fruit species on the effects of geographical elevation on fruit sugar content. More investigations involving a larger number of samples across different elevation are required to have a clearer understanding of the relationship between geographical elevation and apricots sugar content. Knowledge on the subject is vital since it provides a useful guidance on selection of orchard site for improving apricot fruit sweetness.

In general, surface air temperature diminishes with expanding altitude. This abatement in temperature with expanding altitude is broadly known as the temperature lapse rate [93]. In high altitude Qinghai-Tibet Plateau a lapse rate of -4.8°C per km has been reported [94]. Thus, to obtain a better understanding of the effect of environmental factors, this investigation was undertaken to study the effect of growing elevation on sugar content and sugar profile of dried apricots.

4.2 Materials and methods

4.2.1 Study sites

Fruits were collected from six different villages situated at an elevation of 3006 to 3346 meter asl in Ladakh region (Table 4.1).

4.2.2 Sample preparation

Fruits harvested from 108 apricot trees were grouped into three [8] i.e. Group-A with bitter kernel with brown stone; Group-B with sweet kernel with brown stone; and Group-C with sweet kernel with white stone. At maturity stage 100 fruits per tree were handpicked. Harvested fruits were dried in a solar dryer.

Table 4.1: Geographical locations and sampling site of apricots in trans-Himalaya

Sampling localities	Population ID	Latitude (N)	Longitude (E)	Altitude (m asl)	Sample size*		
					Group-A	Group-B	Group-C
Takmachik	TAK	34° 23.522"	76° 45.981"	3006	6	6	6
Domkhar	DOM	34° 23.522"	76° 45.984"	3008	6	6	6
Khalsi	KLS	34° 19.166"	76° 52.564"	3011	6	6	6
Nurla	NUR	34° 17.941'	76° 59.490"	3046	6	6	6
Nimmu	NMU	34° 11.357"	77° 20.437"	3190	6	6	6
Leh	LEH	34° 08.267"	77° 34.378"	3346	6	6	6

*Group-A: brown stone coat with bitter kernel; Group-B: brown stone coat with sweet kernel; Group-C: white stone coat with sweet kernel

4.2.3 Chemicals and reagents

All regents and chemicals used in the experiment were of analytical grade. Standards of FRU, GLU, SUR and SOR were procured from Sigma Aldrich. Aqueous solutions were prepared by using deionized water from RiOs™ type I simplicity 185 (Millipore Waters, USA) with the resistivity of 18.2 MΩ cm.

4.2.4 Sugar profiling

Randomly selected 10-20 dried fruits per tree were grounded using pestle and mortar. Powdered samples were blended at high speed in deionized water (Millipore Waters, USA) using homogeniser (IKA T 10 basic ULTRA-TURRAX, Germany). Homogenised samples were sonicated using the ultrasonic bath (Ultrasonic cleaner YJ5120-1, India) at 40 ^{0}C for 30 min and then centrifuge at 5000 rpm for 10 minute, and filter through Whatman filter paper no 1. Further dilutions were carried out for analysis. The final diluted sample was passed through a 25 mm diameter and 0.22 μm pore filter. A sample of 20 μL was directly injected onto a 4.1 mm × 250 mm RCX-307µm (Hamilton) column. The mobile phase, 0.1M NaOH, was used as eluent with a flow rate of 1.0 ml minute^{-1} (930Compact IC flex Metrom). Separated sugars were detected using an amperometric detector (Metrohm 945 Profession detector version 1).

4.2.5 Statistical analysis

The experiment was performed in triplicates and results were expressed as mean ± standard deviation (SD) using statistical analysis with SPSS 16 and MS Excel 2007. One way

ANOVA and post hoc analysis with 2-sided Tukey's HSD at $p \leq 0.05$ significant level were performed. Box plots were produced to show minimum, median and maximum values. Pearson's correlation analysis was performed to find a correlation between the variables. Three-way ANOVA was used to test the relationship of fruit sugar with stone colour, kernel taste, and altitudinal gradient.

4.1 Results and discussion

4.3.1. Altitude effect on total sugar content

Increase in elevation had a significant impact on the sugar content of dried apricots (Table 4.2). A linear relationship was observed between sugar content and rise elevation (R^2=0.877) (Fig. 4.1a). For every 100 meter rise in elevation, total sugar increased by 64.8 mg.g^{-1} DW. The influence of elevation on sugar content was supported by the data obtained from Pearson's correlations (Table 4.3) and ANOVA (Table 4.4). The result is in concurrence with the report on mandarin [83], where high TSS was recorded at higher altitude. However, an opposite relation was seen when pomegranate was grown at 898, 662 and 222 meter elevation [85]. Persimmon fruits from low elevation (229 meter) have been reported to have higher TSS content than those grows at a higher elevation (770 meter) [86]. Elevation has no effect on fruit TSS content in cherry [82] and blueberry [87]. The results from the current investigation surmise that high elevated regions are suitable for growing of fruit with high sugar. In addition to the environmental conditions, the genotypic effect of Central Asian apricots may also be responsible for higher sugar content. High sugar content was observed in apricots when Pakistan origin apricots were grown in California [39], which showed that Central Asian apricot genotypes have innate higher sugar content. Other environmental conditions which may results in higher sugar content in apricots of high altitude regions could be dry climatic conditions and deficit irrigation. Apricots when grown in deficit irrigation are known to results in fruits with higher TSS [88]. Previous studies found that fruits from dry regions were often sweeter as compared to those from irrigated or humid region [90].

Significant variability in apricot sugar content was observed, and average value ranged from 354.4±40.6 at 3006 meter to 565.3±46.7 mg.g^{-1} DW at 3346 meter altitude (Fig 4.2). In comparison, sugar content of apricots grown in China ranged from 74.2 to 146 mg.g^{-1} [45]. It ranged from 22.3 to 101.5 mg.g^{-1} FW in Spain [95], 9.7 mg.g^{-1} in Korea [47], 7.8 mg.g^{-1} FW in France [96], 0.69 to 0.94 mg.g^{-1} DW in Turkey [48], 73.9 to 108.4 mg.g^{-1} FW in Italy [37]. Comparison of the data showed that the apricots of Ladakh region have higher sugar content.

4.3.2. Altitude effect on fruit sugar profile

Sugar profile of 108 apricot genotypes collected from six different locations are listed in Table 4.2. SUC was the major sugar (57.8%). It was followed by GLU (19.4%), FRU (14.3%) and SOR (8.4% of total sugar). The order of the sugars in the analysed fruit is in agreement with earlier reports [37], [39], [46], [45]. In contrast, higher GLU than SUC [47], and higher SOR than FRU [39] and GLU [48] have also been recorded. SOR is the main fruit sugar reported in selected cultivars of apricots [48]. With increasing elevation, no significant decreasing or increasing pattern was observed in relative proportion (%) of SUC, GLU and FRU. However, the relative proportion of the SOR content increased with increasing elevation in apricots with brown stone (Fig. 4.4). The result showed that trends of sugar content in apricots are affected by environmental factors.

We observed that SUC was the main sugar (Table 4.2). Among the three Groups significant difference in SUR content was recorded (Fig 3). Overall, apricots with the sweet kernel (Group-B & C) contained significantly higher SUC (289.2±137.5, 261.9±78.9 $mg.g^{-1}$ DW, respectively) than that of apricots with the bitter kernel (Group-A) (211±80.3 $mg.g^{-1}$ DW). Effect of kernel taste on SUC content was supported by results obtained from ANOVA (Table 4.4). SUR content constitutes 53.2, 63.2 and 56.9% of the total sugar in Group-A, -B and -C, respectively. In comparison, in apricot fruit SUC is known to range from 17.6% [47] to 81.8% [37] of the total sugar. The altitudinal gradient had a significant effect on the fruit SUC content (Table 4.2). A linear relationship between SUC and rise in elevation was recorded (R^2=0.767) (Fig. 1b). For every 100 meter rise in elevation, SUC increased by 49.1 $mg.g^{-1}$ DW. The effect of elevation on SUC was supported by Pearson's correlations (Table 4.3) and ANOVA (Table 4.4).

Marked variation in SOR level was observed in the genotypes (2.1-212.4 $mg.g^{-1}$ DW). A linear relationship was observed between SOR and rising elevation (R^2=0.899) (Fig. 4.1c). SOR increased by 11.9 $mg.g^{-1}$ DW for every 100 meter rise in elevation. Overall, Group-C contained higher SOR (53.6±26.5 mg/g DW). It was followed by fruits under Group-B (34.6±27.0 $mg.g^{-1}$ DW) and Group-A (23.8±36.1 $mg.g^{-1}$ DW). The trends remain the same in all six locations. The effect of stone colour, elevation and kernel taste on SOR content was supported by results obtained from Pearson's correlations (Table 4.3) and ANOVA (Table 4.4). In comparison, SOR content varied from 0.48 to 4.67 $mg.g^{-1}$ FW in apricots grown in

Table 4.2: Level of individual sugar, sugar ratio and dry matter of dried apricots of 108 genotypes of trans-Himalayan Ladakh grouped based on stone colour and kernel taste for dried apricot from different localities

Localities	Group*	SUR (mg/g)	GLU (mg/g)	FRU (mg/g)	SOR (mg/g)	Total sugar (mg/g)	Total: SOR	GLU: FRU	FRU: SOR	Dry matter (%)
TAK	A	163.4±34.5^{bc}	82.3±24.5^{c-g}	61.5±21.2^{b-d}	7.4±4.0^{a}	314.5±32.1^{a}	42.5	1.3	8.3	28.1±5.4^{a-c}
	B	294.0±61.3^{e-g}	37.3±40.6^{a}	27.6±27.8^{a}	18.4±5.0^{ab}	377.3±31.2^{b-e}	20.5	1.4	1.5	24.9±6.4^{a}
	C	198.8±7.7^{b-d}	80.9±13.1^{b-g}	71.5±16.4^{cd}	20.3±11.3^{a-c}	371.5±24.4^{b-d}	18.3	1.1	3.5	25.8±5.3^{ab}
DOM	A	182.6±57.7^{bc}	76.5±27.1^{b-g}	62.4±22.3^{b-d}	9.3±3.5^{a}	330.8±15.2^{a}	35.6	1.2	6.7	24.1±3.3^{a}
	B	303.0±7.2^{f-h}	46.7±14.9^{ab}	28.1±5.8^{a}	19.7±6.6^{a-c}	397.5±17.6^{c-f}	20.2	1.7	1.4	25.7±6.0^{ab}
	C	201.3±37.5^{b-d}	82.6±25.0^{c-g}	72.7±18.4^{cd}	62.9±11.2^{e}	419.5±5.1^{f}	6.7	1.1	1.2	25.8±2.4^{ab}
KLS	A	160.6±28.3^{b}	98.3±22.6^{e-h}	75.3±16.1^{cd}	12.2±4.5^{a}	346.3±14.9^{ab}	28.4	1.3	6.2	24.9±3.9^{a}
	B	227.9±62.6^{c-e}	68.7±24.8^{a-e}	45.1±17.4^{a-c}	25.1±9.1^{a-c}	366.9±34.7^{bc}	14.6	1.5	1.8	26.3±2.3^{ab}
	C	227.0±26.1^{b-e}	72.4±10.2^{b-f}	67.5±9.7^{cd}	49.4±11.4^{c-e}	416.2±24.6^{f}	8.4	1.1	1.4	29.6±3.4^{a-c}
NUR	A	184.6±74.0^{bc}	106.4±42.9^{f-h}	76.2±34.6^{cd}	24.8±6.8^{a-c}	391.9±29.5^{c-f}	13.1	1.9	1.1	26.7±4.6^{ab}
	B	67.9±62.0^{a}	152.9±34.0^{i}	155.3±57.5^{e}	30.8±8.4^{a-d}	406.8±41.5^{ef}	8.6	1.5	1.1	26.3±2.4^{ab}
	C	254.1±19.1^{d-f}	54.3±3.9^{a-c}	46.6±3.8^{a-c}	49.0±3.4^{c-e}	404.0±21.6^{d-f}	8.2	1.2	1.0	26.3±2.0^{ab}
NMU	A	319.3±6.4^{f-h}	106.8±19.3^{f-h}	57.2±21.3^{a-d}	32.9±28.8^{a-d}	516.1±21.4^{g}	15.7	1.9	1.7	29.5±2.8^{a-c}
	B	405.5±93.7^{ij}	94.5±33.8^{d-h}	50.1±30.9^{a-d}	45.4±14.4^{b-e}	595.4±37.3^{h}	13.1	1.9	1.1	28.4±4.2^{a-c}
	C	323.6±60.2^{gh}	108.4±35.6^{gh}	71.8±25.4^{cd}	66.2±20.6^{e}	570.0±27.1^{h}	8.6	1.5	1.1	29.2±3.4^{a-c}
LEH	A	255.8±92.3^{d-f}	125.7±48.4^{hi}	81.4±49.9^{d}	56.4±73.7^{de}	519.3±22.9^{g}	9.2	1.5	1.4	31.4±3.2^{bc}
	B	437.1±70.7^{j}	62.3±39.2^{a-d}	31.2±25.9^{ab}	68.2±47.1^{e}	598.8±43.8^{h}	8.8	2.0	0.5	33.5±11.5^{c}
	C	366.5±89.2^{hi}	79.7±30.7^{b-g}	58.1±23.4^{a-d}	73.6±41.3^{e}	577.8±27.5^{h}	7.9	1.4	0.8	45.3±9.4^{d}
Average	A	211.0±80.3$_{a}$	99.3±35.9$_{b}$	69.0±30.5$_{a}$	23.8±36.1$_{a}$	403.2±87.8$_{a}$	16.9	1.4	2.9	27.5±4.6$_{a}$
	B	289.2±137.5$_{b}$	77.0±49.9$_{a}$	56.2±54.9$_{a}$	34.6±27.0$_{b}$	457.1±106.1$_{b}$	13.2	1.4	1.6	27.5±6.8$_{a}$
	C	261.9±78.9$_{b}$	79.7±27.5$_{a}$	64.7±19.9$_{ab}$	53.6±26.5$_{c}$	459.8±85.6$_{b}$	8.6	1.2	1.2	30.3±8.5$_{b}$
Overall Average		254.1±107.3	85.4±40.0	63.3±38.3	37.3±32.5	440.0±96.9	11.8	1.3	1.7	28.4±6.9

Values represented mean ± SD; for each column different lowercase letters indicate significantly different at $p < 0.05$; *Group-A: brown stone with bitter kernel; Group-B: brown stone with sweet kernel; Group-C: white stone with sweet kernel.

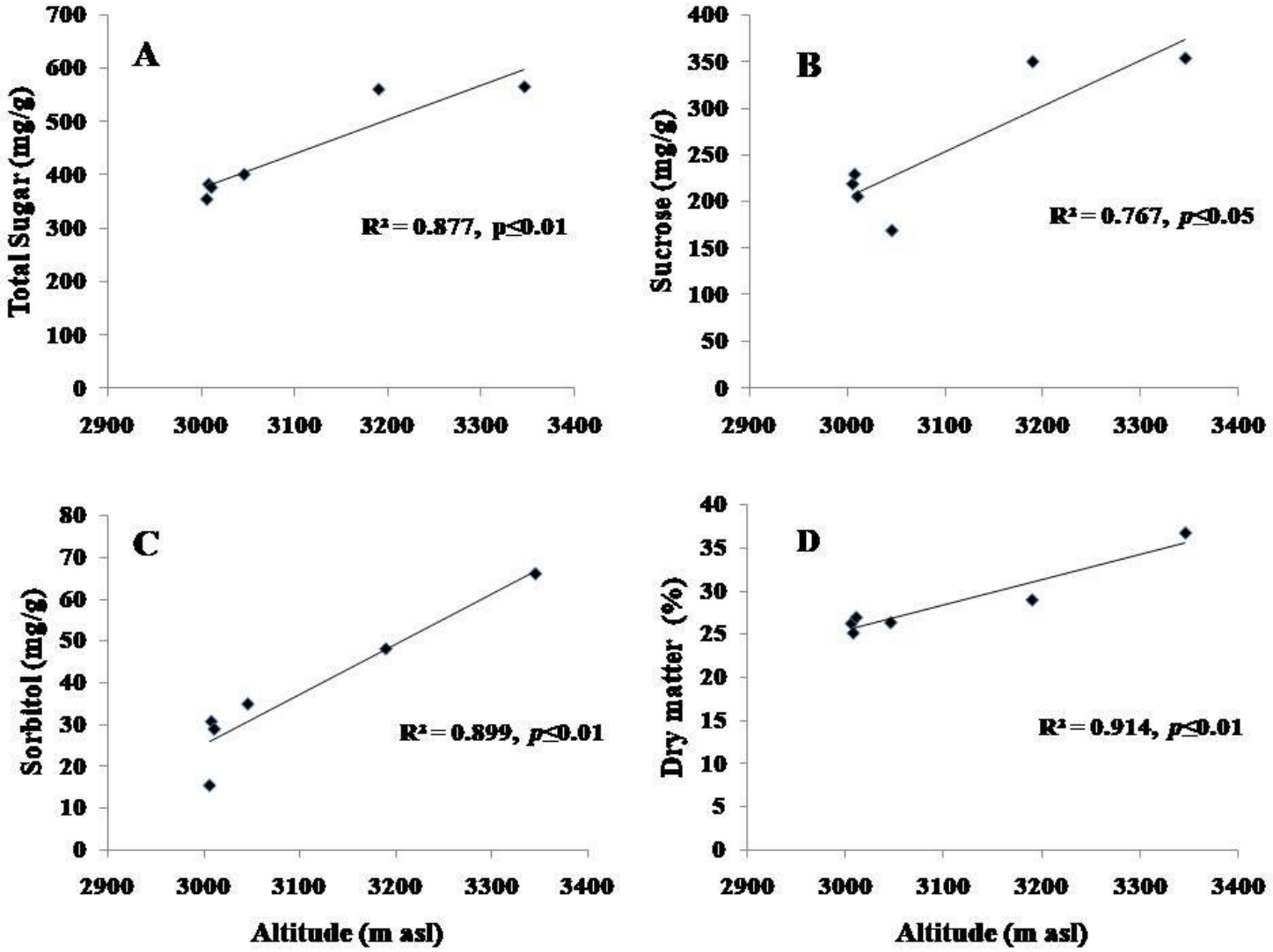


Figure 4.1: Relation between altitude and dried apricot fruit (A) total sugar; (B) SUR; (C) SOR; (D) dry matter contents in trans-Himalayan region

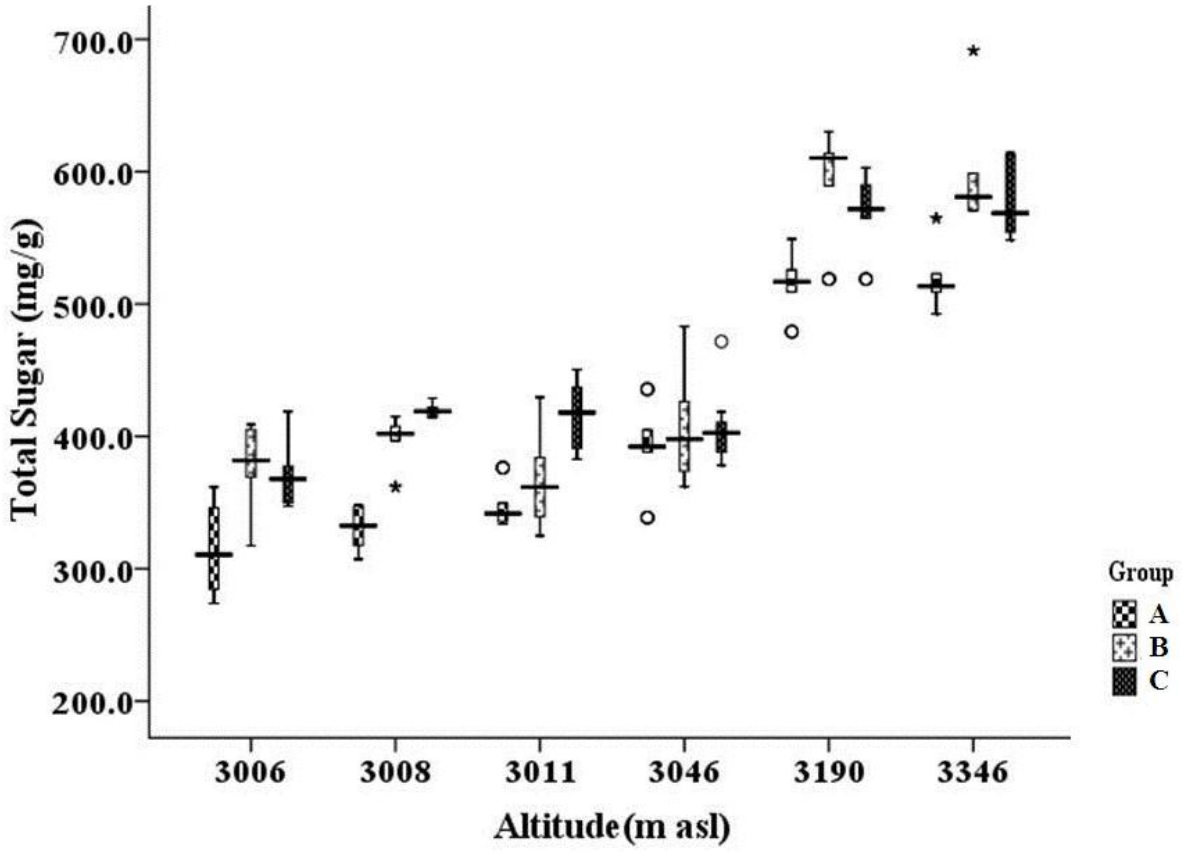


Figure 4.2: Box plot distribution of total fruit sugar content (mg/g DW) along altitudinal gradient in trans-Himalayan region. The plot represents the minimum and maximum value (whiskers), the first and third quartile (box), the median (midline), mild outlier (hollow circle) and extreme outlier (star).

Italy [37] and 0.169 to 0.268 $mg.g^{-1}$ DW in apricots of Malatya region in Turkey [48]. SOR is one of the minor sugar in apricots. SOR content varied from 0.2% to 0.5% FW in 11 Italian cultivars [97]. It constitute 2.7% of total sugar of apricots in Korea [47] and 0.7 to 6% of apricot fruit sugars in Italy [37]. In Chinese apricot SOR constitutes 5.4% of total sugar [45]. However, SOR is reported to be major sugar in Çataloğlu (30.4% of total sugar) and Hacihaliloğlu (32.5%) cultivar of Turkey [48]. In comparison, SOR content in the present investigation represents 5.9, 7.6 and 11.7% of the total sugar under Group-A, -B and –C, respectively. Apricots of northern Pakistan when grown in California contained over two-times higher SOR content (29.4±14.1 $mg.g^{-1}$) than its level of FRU (12.0±1.7 $mg.g^{-1}$) [39], which emphasize the importance of genotypic effect on apricots SOR content. Therefore, high SOR level in apricots of Ladakh region is the outcome of both environmental and genotypic factors. High SOR content than published reports is an important quality parameter of apricots of the trans-Himalayan region. There is a growing interest in apricots that are rich in SOR

content [39]. SOR can be used as an alternative natural sweetener for SUC and it is a good substitute of GLU for diabetics [98]. Plant breeder takes a keen interest in fruits rich in SOR. SOR content has a positive effect on browning of apricots during the drying process since it does not take part in Maillard reactions, which is linked with drying process. SOR also acts as humectants which keep the dry products softer and more pliable. During storage, SOR also acts as a natural preservative to keep the dry quality of fruit [39].

GLU content increase with rising elevation. It showed an opposite relationship with increasing elevation. The ratios of specific sugar content are useful tools for determining the genuineness of juice samples. If GLU:FRU ratio is over than 3.3 it is an indication that apricot juice have been adulterated [97]. In this study the GLU:FRU ratio varied between 1.1 to 2.0. The GLU:FRU ratio increase with increasing elevation. The FRU:SOR and total sugar:SOR ratios showed opposite relationship with elevation but not significant.

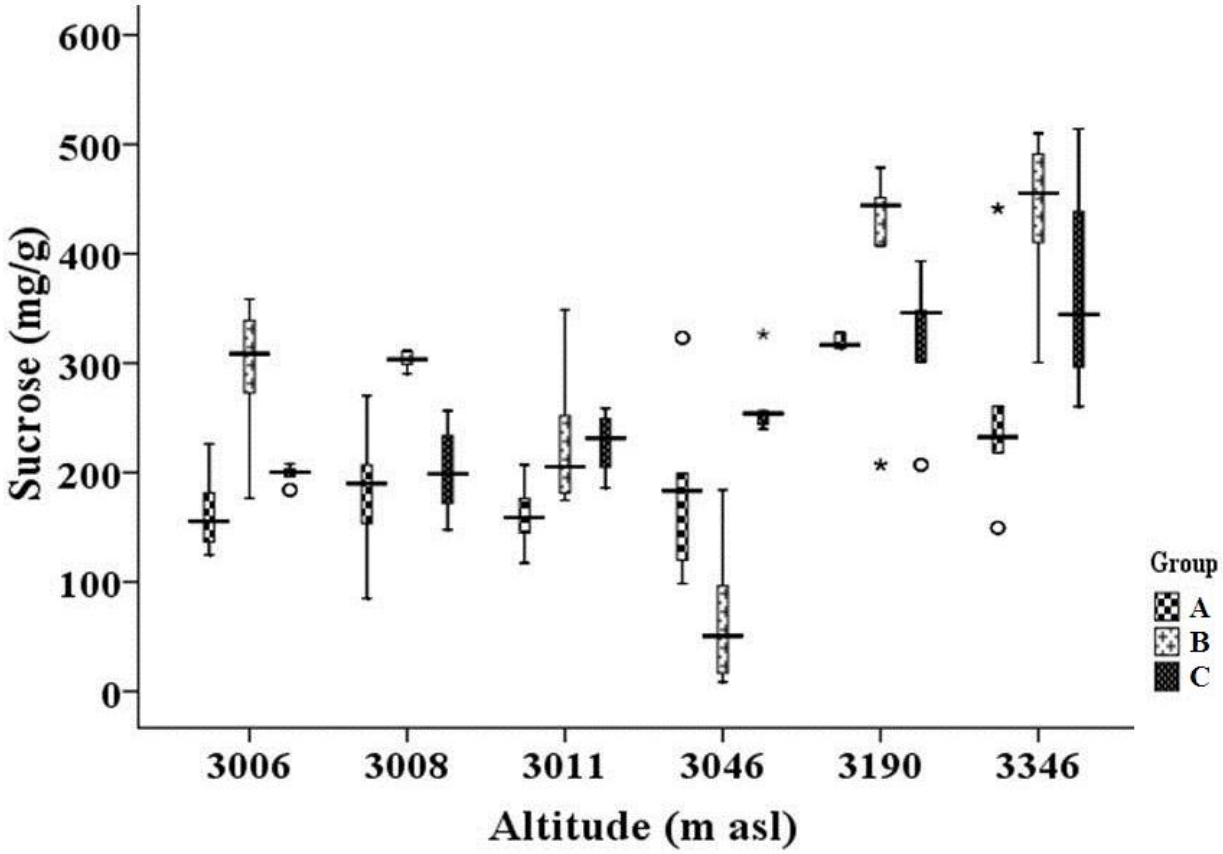


Figure 4.3: Box plot distribution of fruit SUR contents (mg/g DW) along altitudinal gradient in trans-Himalayan region. The plot represents the minimum and maximum value (whiskers), the first and third quartile (box), the median (midline), mild outlier (hollow circle) and extreme outlier (star).

4.3.3. Altitude effect on fruit dry matter

Fruit dry matter content in the present investigation varied from 15.4 to 64% with an average value of 28.4±6.9%. Dry matter is important parameters which affects commercial value of apricot fruits. Broadly, apricots cultivars with low dry matter are preferred for fresh consumption and the ones with high dry matter content are preferred for drying processes [48]. Hacihaliloğlu cultivar, which is the most important cultivar for dried apricots production in Turkey, contains 24.8±0.4% dry matter [48]. The results suggested that apricots of Ladakh region have higher dry matter content. A strong linear relationship was observed between geographical elevation and fruit dry matter content (R^2=0.914) (Fig. 1d). Dry matter increased by 2.9% for every 100 meter rise in elevation. Apricots under Group-B showed a stronger linear relationship with elevation (R^2=0.951) than those under Group-A (R^2=0.749) and Group-C (R^2=0.808). Environmental factors could be the reason for high dry matter content in apricots of Ladakh region.

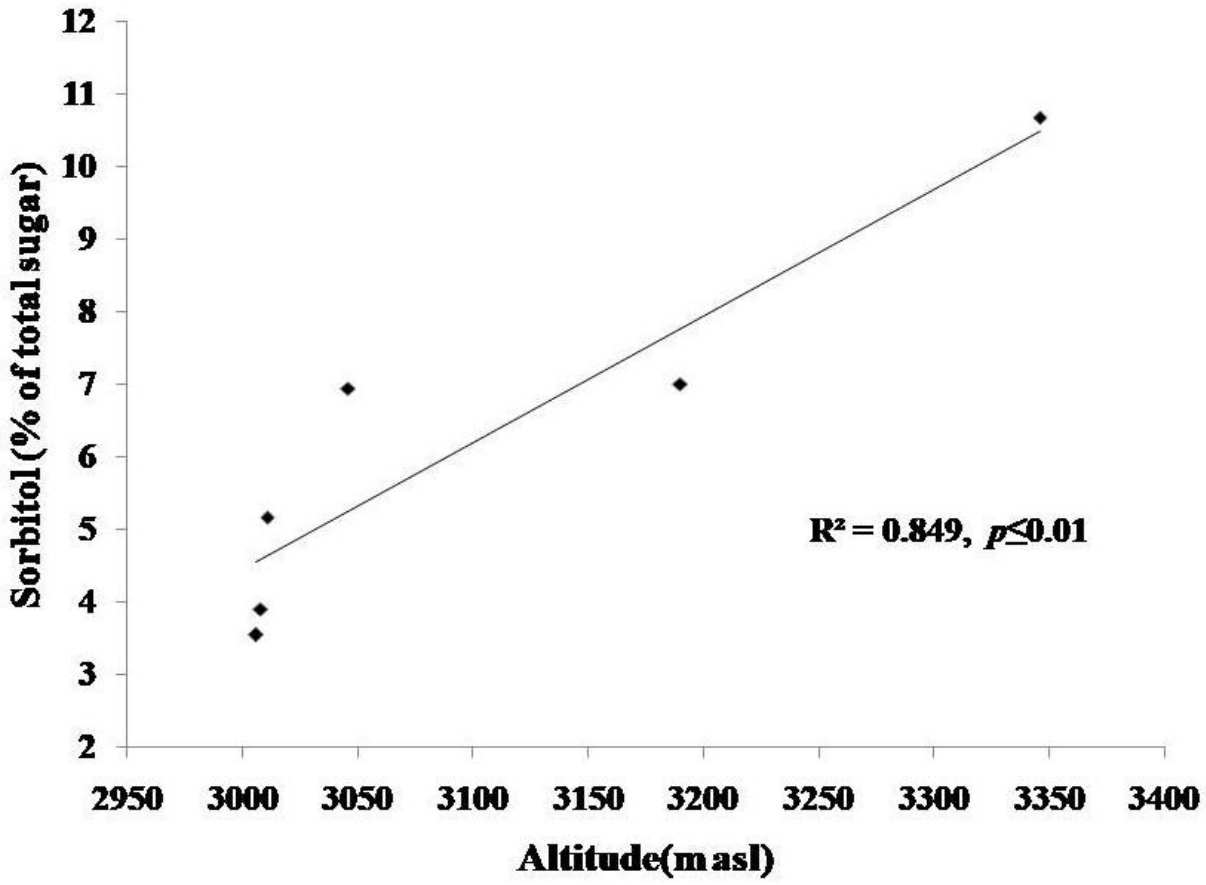


Figure 4.4: Relation between altitude and relative proportion (%) of SOR in dried apricot fruit of trans-Himalayan region.

4.3.4. Correlation

Correlations among the variables are shown in Table 4.3. Elevation had marked positive correlation with total sugar (r = 0.851), SOR (r = 0.469), SUC (r = 0.583), GLU:FRU ratio (r = 0.499), and dry matter (r = 0.539). Significant opposite correlation was seen between altitude with FRU:SOR ratio (r = -0.421) and total sugar:SOR ratio (r = -0.448). White stone colour was positively correlated with total sugar:SOR ratio (r = 0.451, GLU:FRU ratio (r = 0.582), and kernel taste (r = 0.500). Sweet kernel taste was positively correlated with FRU:SOR ratio (r = 0.605), and total sugar:SOR ratio (r = 0.573). Dry matter showed positive correlation with total sugar (r = 0.494), and SOR content (r = 0.489). Significant positive correlation was observed between SOR content and total sugar (r = 0.582). Significant negative correlations between SUC and SOR (r = -0.986) has been reported by Bae et al. [47]. However, our results showed a positive correlation between the two sugars (r = 0.225). Positive correlations has been reported between GLU and SUC contents (r = 0.973) [47]. In contrast, we observed marked negative relationship between the two sugar contents (r = -0.555). Significant positive correlation between GLU and FRU content (r = 0.858) is in accordance with earlier report [39].

4.3.5. ANOVA

ANOVA results are presented in Table 4.4. The results revealed that the elevation of growing region significantly affects SUC, GLU, FRU, SOR, total sugar and dry matter contents. Stone colour had a significant effect on SOR, SUC and dry matter content. Kernel taste had a significant effect on GLU, FRU, SUC and total sugar. The interaction between elevation, kernel taste, and stone colour showed a significant effect on GLU, FRU, SUC, total sugar and fruit dry matter.

Table 4.3: Pearson's correlation coefficients of altitude, seed characteristics and fruits sugars of apricots of trans-Himalayan Ladakh

Variables	ALT	SC	KT	DM	SOR	GLU	FRU	SUC	TS	TS:SOR	GLU:FRU	FRU:SOR
ALT	1	0.000	0.000	0.539**	0.469**	0.174**	-0.058	0.583**	0.851**	-0.448**	0.499**	-0.421**
SC		1	0.500**	-0.193**	-0.353**	0.100	-0.026	-0.052	-0.145**	0.451**	0.582**	0.260**
KT			1	-0.100	-0.294**	0.247**	0.105	-0.284**	-0.269**	0.573**	0.108	0.605**
DM				1	0.489**	0.076	-0.032	0.281**	0.494**	-0.253**	0.130*	-0.218**
SOR					1	0.089	-0.099	0.225**	0.582**	-0.525**	0.084	-0.447**
GLU						1	0.858**	-0.555**	0.168**	-0.128*	0.119*	-0.012
FRU							1	-0.690**	-0.047	-0.111*	-0.136*	0.034
SUC								1	0.681**	-0.292**	0.460**	-0.410**
TS									1	-0.597**	0.533**	-0.596**
TS:SOR										1	-0.198**	0.933**
GLU:FRU											1	-0.397**
FRU:SOR												1

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

ALT: altitude, SC: Stone colour, KT: kernel taste, DM: fruit dry matter, SOR: SOR, GLU: GLU, SUC: SUR, TS: total sugar

Table 4.4: Three-way ANOVA for altitude, stone colour, and kernel taste and their interactions on fruit sugars and dry matter

Source	d.f	*F*	Source	d.f	*F*
		Total sugar			**FRU**
ALT	5	642.636***	ALT	5	15.977***
SC	1	0.505	SC	1	5.237*
KT	1	201.565***	KT	1	11.896***
ALT *SC*KT	10	8.753***	ALT *SC*KT	10	24.248***
		SUR			**SOR**
Altitude	5	100.616***	ALT	5	26.120***
SC	1	12.370***	SC	1	30.330***
KT	1	101.053***	KT	1	9.808**
ALT *SC*KT	10	22.455***	ALT *SC*KT	10	2.088*
		GLU			**Dry matter**
ALT	5	16.619***	ALT	5	36.420***
SC	1	0.433	SC	1	15.714***
KT	1	30.479***	KT	1	0.010
ALT *SC*KT	10	16.193***	ALT *SC*KT	10	6.647***

df - Degrees of freedom; *F* – F ratio; *Significant at $p \leq 0.05$; **Significant at $p \leq 0.01$; ***Significant at $p \leq 0.001$.

ALT: altitude, SC: Stone colour, KT: kernel taste

4.4. Conclusion

The altitude of fruit growing region had a significant effect on apricots sugar content. Linear relationship between rise in elevation with SOR, SUR and total sugar content was seen. The major sugar was SUC followed by GLU, FRU, and SOR. The trend of each sugar in apricots was not affected by environmental factors. However, the relative proportion of SOR significantly increased with rise in elevation in apricots with the brown stone. A strong relationship was observed between elevation and dry matter content. Therefore, apricots growing at high altitude contain higher SUC, SOR, total sugar, and dry matter content.

CHAPTER 5

EFFECT OF ALTITUDE AND SEED PHENOTYPIC CHARACTERS ON AMYGDALIN AND SUGAR CONTENT IN APRICOT KERNEL

ABSTRACT

This investigation aimed to analyse the effect of geographical elevation and seed phenotypic characters on amygdalin and sugar content in the apricot kernel. Apricots from 126 genotypes differing in stone colour and kernel taste were collected from seven different villages from 3008-3346 meter asl spread across trans-Himalaya Ladakh region. Amygdalin and sugar content in the kernel were determined in the apricot kernel. Amygdalin level in the bitter kernel was significantly higher (44.6±9.0 $mg.g^{-1}$) than sweet kernel (3.1±1.8 $mg.g^{-1}$) with brown stone. The altitudinal gradient had no influence on kernel amygdalin level, whereas altitude had a significant impact on the kernel sugar content. A linear relationship was observed between total sugar and rising elevation (R^2=0.103). Total sugar increased by 2.9 $mg.g^{-1}$ DW for every 100 meter rise in elevation, Sucrose (SUC) was the major sugar (47.9%). It was followed by glucose (GLU) (24.6%), fructose (FRU) (23.6%), inositol (INO) (3.5%) and arabitol (ARA) (3.5% of total sugar). A linear relationship was observed between SUC and increasing elevation (R^2=0.186). For every 100 meter increase in geographical elevation, SUC content increased by 11.0 $mg.g^{-1}$ DW. Kernel INO content showed an inverse relationship with increase in elevation (R^2=0.149). Similarly, seed and kernel phenotypic characters, except stone colour, had no significant effect on amygdalin and sugar content. Therefore, data from the present investigation indicated that apricots growing at high geographical elevation contain higher sugar, SUC and low INO content in the kernel. Low amygdalin content (2.4±1.2 $mg.g^{-1}$) in apricot kernel with white stone phenotype confirmed our previous finding that white stone apricot is always linked to the sweet kernel.

5.1 Introduction

Amygdalin is a widespread natural cyanogenic glucoside in the bitter apricot kernel, which gives a bitter taste to the kernel. It is well documented that amygdalin content is significantly higher in the bitter kernel than that of the sweet kernel [49], [60], [61]. The level of amygdalin varied significantly in apricot kernel. It ranged from less than 0.08 to 15.84 $mg.g^{-1}$ DW in a sweet apricot kernel [60], [61]. Similarly, within bitter apricot kernel, it ranged from 13.96 to 55 $mg.g^{-1}$ DW [49], [60], [61]. The difference in amygdalin level could be the result of both environmental and genotypic conditions. Previous studies suggested that in apricot kernel level of amygdalin depend on cultivar [60], [61] and fruit developmental stages [99]. However, there are very few reports on the effect of environment and seed physical characters on amygdalin content in the apricot kernel.

Apricot kernel contains sugar. However, there are very few reports with limited numbers of cultivars on sugar content in the apricot kernel. Femenia et al. [49] reported that sweet apricot kernel contains less soluble sugars (7 $g.l00g^{-1}$) than bitter kernels (14 $g.l00g^{-1}$). SUC, FRU, and GLU are the most important sugars in apricot kernels [29]. High sugar content in kernels of promising hybrids suggested that it is possible to breed apricot kernel with high sugar content through conventional breeding. Besides, the kernel sugar pattern can be used as a method to study diverse germplasm and fruit at different maturity stages. Sugars such as FRU, SUC, ARA, maltose, sorbitol, ribose, trehalose, galactitol and iso-maltotriose are reported as most important sugars for such discrimination [100].

5.2 Materials and methods

5.2.1 Study sites and samples preparation

Fresh apricot fruit from 126 genotypes were collected from seven villages situated at the altitude that ranged from 3008 to 3346 meter asl in trans-Himalayan Ladakh region (Table 5.1). The fruits were grouped into three (Group-A: brown stone and bitter kernel; Group-B: brown stone and sweet kernel; and Group-C: white stone and sweet kernel). Representative fruit samples (50 fruits per tree) were handpicked at maturity stage. Seed was isolated manually by removing the flesh, while the kernel was taken out by physically breaking of stone. Shade dried kernel was grounded to a fine powder.

Table 5.1: Geographical locations and sampling site of apricots in trans-Himalaya

Sampling localities	Altitude (m) (asl)	Population ID	Latitude (N)	Longitude (E)	Sample Size*		
					Group-A	Group-B	Group-C
Domkhar	3008	DOM	34° 23.522"	76° 45.984"	6	6	6
Khalsi	3011	KLS	34° 19.166"	76° 52.564"	6	6	6
Nurla	3046	NUR	34° 17.941'	76° 59.490"	6	6	6
Saspol	3116	SPL	34° 14.251"	77° 10.194"	6	6	6
Nimmu	3190	NMU	34° 11.357"	77° 20.437"	6	6	6
Tia Khaling	3311	TIA	34° 19.979"	76° 58.685"	6	6	6
Leh	3346	LEH	34° 08.267"	77° 34.378"	6	6	6

*Group-A: brown stone with bitter kernel; Group-B: brown stone with sweet kernel; Group-C: white stone with sweet kernel

5.2.2 Physical properties of kernel and seed

For each apricot genotype weight (Wt), width (W), length (L) and thickness (T) were determined in 40 randomly selected fruits. Kernel and stone weight was measured with a balance with 0.001g accuracy. Stone moisture content was determined by using an oven drying method. Dimensional properties of stone and kernel were measured with a digimatic calliper with 0.01 mm accuracy. Sphericity, geometric mean diameter and surface area were calculated as described earlier [101, 102, 103].

5.2.3 Chemicals and reagents

Analytical grade reagents and chemicals were used in the experiment. Amygdalin, GLU, FRU, SUC, INO and ARA standards were procured from Sigma Aldrich. Ethanol (99.9 %) was used for amygdalin extraction and deionized water for sugar extraction. Aqueous solutions were prepared by using deionized water from RiOs™ type I simplicity 185 (Millipore Waters, USA) with resistivity of 18.2 MΩ cm.

5.2.4 Amygdalin and sugar extraction

Amygdalin extraction was carried out with ethanol as described previously [104]. Powdered kernel was mixed with ethanol and incubated in preheated water bath at 78.5°C for 100 minutes and then centrifuge at 5000 rpm for 10 minutes at 25°C. The ethanol extract was

filtered through 0.22µm filter syringe filter and dispensed into HPLC vials for further analysis.

Sugar extraction was carried by hot water extraction. Powdered kernel was blended at high speed in deionized water for 1 min using homogeniser (IKA T 10 basic ULTRA-TURRAX, Germany). Homogenised samples were sonicated using the ultrasonic bath (Ultrasonic cleaner YJ5120-1, India) at 40 ^{0}C for 30 minute and then centrifuge at 5000 rpm for 10 minute. It was then filtered through Whatman Filter Paper no 1.

5.2.5 Amygdalin estimation by HPLC

For amygdalin quantification, an Agilent 1260 Infinity series HPLC (Agilent Technologies, Santa Clara, CA) equipped with Quaternary Pump VL (G1311C) and degasser, 1260 ALS auto-sampler (G1329B) and 1260 DAD VL detector (G1315D) was used. Separation was performed by using ZORBAX Eclipse C_{18} column (250mm x 4.60mm, 5µm: Agilent Technologies). Gradient mobile phase made up of water (A) and methanol (B; 0-7 min: 30%; 10 min: 100%; 15min: 100%; 17 min: 30%; 25 min: 30%). The mobile phase was sonicated for 20 min at 22°C to remove gas bubbles and the flow rate was adjusted to 1.0 ml.min^{-1}. The wavelength was set to 214 nm, and 5µl of sample was injected in the HPLC. The results were expressed as mg.g^{-1} DW of kernel.

5.2.6 Sugar profiling

Sugar profiling was done using ion chromatography (930Compact IC flex Metrom). Diluted samples were passed through a 25 mm diameter and 0.22 μm pore filter. A sample of 20 μL was directly injected onto a 4.1 mm × 250 mm RCX-307µm (Hamilton) column. The mobile phase, 0.1M NaOH, was used as eluent with a flow rate of 1.0 ml.min^{-1}. Separated sugars were detected using an amperometric detector (Metrohm 945 Profession detector varion 1). Results were expressed as mg.g^{-1} DW of kernel.

5.2.7 Statistical analysis

Experimental results were expressed as mean ± standard deviation using statistical analysis with SPSS 16 and MS Excel 2007. One-way ANOVA and post hoc analysis with 2-sided Tukey's HSD at $p \leq 0.05$ level were performed. Box plots were produced to show the minimum, median and maximum values of each variable. Pearson's correlation analysis was performed to find a correlation between the variables.

5.3 Results and discussion

5.3.1 Amygdalin and sugar content kernel

Significant difference in amygdalin was observed between bitter and sweet apricot kernel. Amygdalin in the bitter kernel was significantly higher (44.6±9.0 $mg.g^{-1}$) than that of the sweet apricot kernel (Group-B: 3.1±1.8; Group-C: 2.4±1.2 $mg.g^{-1}$), which is in accordance with previous studies [49], [60], [61]. Marked variability was observed within the bitter apricot kernel (Group-A) which ranged from 35.1±7.1 at 3311 m to 55.9±8.1 $mg.g^{-1}$ DW at 3190 m altitude (Table 5.2). Among individual genotypes, amygdalin ranged from 25.5 - 63.7 $mg.g^{-1}$ DW. In comparison, the value ranged from 13.96-44.41 $mg.g^{-1}$ in four bitter kernel apricot cultivars [61] and 44.1-63.5 $mg.g^{-1}$ among three bitter kernel apricot cultivars in Turkey [60]. Femenia et al., [49] reported 51 $mg.g^{-1}$ DW in a bitter kernel apricot cultivar in Spain.

Amygdalin within the sweet apricot kernel (Group-B and Group-C) also showed marked variability and the averaged value ranged from 1.4±0.1 at 3008 meters to 4.3±1.2 $mg.g^{-1}$ DW at 3190 meter altitude. Among individual genotypes, amygdalin content ranged from 1.3-7.9 $mg.g^{-1}$ DW (Table 5.2). In comparison, the amygdalin level ranged from <0.08-0.4 $mg.g^{-1}$ among nine sweet kernel apricot cultivars [61] and 6.04-15.84 $mg.g^{-1}$ in seven sweet kernel apricot cultivars from Turkey [60]. Femenia et al. [49] did not detect amygdalin in a sweet kernel apricot cultivar in Spain.

SUC was the major individual sugar (47.9%) followed by GLU (24.6%), FRU (23.6%), INO (3.5%) and ARA (3.5% of total sugar) (Table 5.2). Kernel sugar content showed significant variability and average value ranged from 100.1±56.9 at 3008 meters to 155.9±53.7 $mg.g^{-1}$ DW at 3346 meter asl. We observed that bitter kernel (Group-A) apricot genotypes have a significantly higher proportion of GLU (52.1±21.3), SUC (58.2±27.5) and total sugar (141.3±28.5) than that of Group-B and -C.

Table 5.2: Level of individual sugar of dried apricots kernels of 126 genotypes of trans-Himalayan Ladakh grouped based on fruit stone colour and kernel taste for dried apricot from different localities

Localities	Group*	Amygdalin (mg/g)	INO (mg/g)	ARA (mg/g)	GLU (mg/g)	FRU (mg/g)	SUC (mg/g)	Total Sugar(mg/g)
DOM	A	46.7±9.5^{d}	2.5±1.5^{ab}	6.2±2.9^{d}	64.2±7.0^{e}	21.1±6.5^{b-g}	77.6±26.2^{gh}	171.6±23.8^{i}
	B	1.4±0.1^{a}	4.8±0.8^{cd}	3.7±0.9^{a-c}	1.8±1.5^{a}	12.1±9.0^{a-c}	28.4±20.4^{a-c}	50.8±23.5^{ab}
	C	1.9±0.3^{a}	3.7±0.8^{a-c}	4.8±0.9^{c}	11.7±6.5^{ab}	27.1±3.8^{d-h}	30.9±13.7^{a-d}	78.1±16.0^{b-d}
KLS	A	36.5±4.0^{b}	6.0±3.6^{d}	3.7±0.7^{a-c}	31.4±19.9^{cd}	34.7±25.0^{hi}	52.3±11.1^{d-g}	128.0±20.1^{fg}
	B	1.9±0.9^{a}	4.5±1.4^{a-c}	3.1±0.2^{ab}	4.6±3.2^{a}	9.4±8.5^{ab}	27.4±6.0^{a-c}	49.0±11.5^{ab}
	C	2.8±1.4^{a}	6.0±2.0^{d}	3.4±0.5^{a-c}	3.9±3.3^{a}	33.2±3.3^{g-i}	55.1±20.5^{d-h}	101.6±27.0^{d-f}
NUR	A	42.3±5.5^{c}	3.1±1.2^{a-c}	3.6±1.0^{a-c}	39.6±21.1^{d}	18.9±2.6^{a-f}	56.3±10.4^{d-h}	121.4±23.5^{e-g}
	B	3.9±1.8^{a}	4.4±0.6^{b-d}	3.0±1.4^{ab}	13.5±25.6^{ab}	8.0±8.8^{a}	18.3±30.0^{ab}	47.1±67.5^{ab}
	C	4.3±1.2^{a}	5.7±1.2^{d}	3.5±1.0^{a-c}	4.3±2.7^{a}	27.6±6.8^{e-i}	41.2±30.7^{c-f}	82.3±40.2^{b-d}
SPL	A	48.5±4.1^{d}	2.1±0.3^{a}	3.1±0.7^{ab}	77.8±6.0^{f}	34.2±6.5^{hi}	17.6±9.8^{ab}	134.8±18.8^{f-h}
	B	5.1±2.0^{a}	2.8±0.8^{ab}	2.2±0.2^{a}	4.7±6.1^{a}	29.1±11.7^{f-i}	8.9±8.1^{a}	47.6±18.9^{ab}
	C	1.6±0.2^{a}	3.5±0.7^{a-c}	3.4±0.3^{a-c}	11.1±7.2^{ab}	30.7±10.2^{f-i}	14.4±12.0^{ab}	63.0±27.9^{a-c}
NMU	A	55.9±8.1^{e}	1.7±0.3^{a}	2.9±0.2^{ab}	60.5±12.2^{e}	20.3±5.8^{a-f}	57.2±23.7^{d-h}	142.5±30.0^{g-i}
	B	3.6±1.7^{a}	2.4±1.4^{a}	2.3±0.2^{a}	5.2±4.8^{a}	10.9±6.0^{ab}	9.3±9.7^{a}	30.1±16.0^{a}
	C	2.1±0.4^{a}	2.0±1.0^{a}	3.0±0.6^{ab}	23.3±3.5^{bc}	24.0±10.7^{c-h}	36.4±13.9^{b-e}	88.8±16.1^{c-e}
TIA	A	35.1±7.1^{b}	1.9±0.6^{a}	2.7±0.3^{ab}	35.8±18.5^{cd}	14.7±5.3^{a-d}	68.9±26.2^{f-h}	123.9±22.8^{e-g}
	B	3.6±0.4^{a}	2.3±0.7^{a}	2.4±0.4^{a}	7.3±7.9^{a}	19.7±8.4^{a-f}	67.7±22.7^{f-h}	99.4±31.2^{c-f}
	C	2.2±1.1^{a}	3.6±0.9^{a-c}	3.1±0.5^{ab}	5.8±4.9^{a}	29.7±9.1^{f-i}	61.1±23.8^{e-h}	103.3±21.3^{d-f}
LEH	A	46.9±3.8^{d}	3.2±1.7^{a-c}	4.3±1.7^{bc}	55.7±14.7^{e}	26.3±10.1^{d-h}	77.3±29.7^{gh}	166.7±19.6^{hi}
	B	1.9±1.1^{a}	3.0±2.6^{a-c}	3.3±1.1^{a-c}	7.6±6.5^{a}	15.4±7.1^{a-e}	68.2±33.1^{f-h}	97.5±36.5^{c-f}
	C	1.8±0.5^{a}	3.0±1.8^{a-c}	4.7±2.7^{c}	37.5±8.5^{d}	41.0±9.0^{i}	117.4±21.5^{h}	203.7±33.2^{j}
Average	A	44.6±9.0$_{c}$	2.9±2.1$_{a}$	3.8±1.7$_{b}$	52.1±21.3$_{c}$	24.3±12.6$_{b}$	58.2±27.5$_{b}$	141.3±28.5$_{c}$
	B	3.1±1.8$_{a}$	3.4±1.6$_{ab}$	2.9±0.9$_{a}$	6.4±10.7$_{a}$	14.9±10.6$_{a}$	32.6±31.0$_{a}$	60.2±40.4$_{a}$
	C	2.4±1.2$_{a}$	3.9±1.8$_{b}$	3.7±1.3$_{b}$	14.0±12.7$_{b}$	30.5±9.0$_{c}$	50.9±36.3$_{b}$	103.0±50.3$_{b}$
Overall Average		16.7±20.61	3.4±1.9	3.4±1.4	24.2±25.4	23.2±12.5	47.2±33.3	101.5±52.3

Note: Values represented mean ± SD; for each column different lowercase letters indicate significantly different at $p < 0.05$

*Group-A: brown stone with bitter kernel; Group-B: brown stone with sweet kernel; Group-C: white stone with sweet kernel

Table 5.3: Seed and kernel physical characteristics of 126 apricot genotypes of trans-Himalayan Ladakh grouped based on seed coat colour and kernel taste

Localities	Group*	SW	SM	KW	SD_g	KD_g	SΦ	KΦ	*SS*	*KS*	SCT
DOM	A	1.6±0.3^{cd}	25.8±5.1^{b-d}	0.4±0.1^{ab}	16.6±1.3^{a-c}	9.4±0.8^{ab}	77.2±5.6^{a-e}	65.4±2.6^{a}	871.5±132.0^{a-c}	281.6±44.9^{ab}	1.7±0.1^{f}
	B	1.2±0.3^{a-c}	28.8±2.5^{b-e}	0.4±0.1^{ab}	15.0±1.9^{a}	8.8±1.0^{a}	74.8±5.9^{ab}	63.0±9.2^{a}	719.8±178.8^{a}	248.3±53.7^{a}	1.4±0.4^{a-e}
	C	1.4±0.2^{a-d}	32.7±3.4^{de}	0.4±0.1^{ab}	16.1±0.6^{a-c}	9.7±0.5^{a-c}	83.0±2.3^{c}	66.3±1.3^{a}	817.4±66.3^{a-c}	297.5±31.7^{a-c}	1.4±0.1^{a-e}
KLS	A	1.4±0.4^{a-d}	24.3±2.6^{bc}	0.5±0.1^{a-c}	17.1±4.0^{bc}	9.7±1.0^{a-c}	76.9±7.1^{a-e}	67.0±7.3^{a}	964.3±508.4^{c}	301.7±60.6^{a-c}	1.4±0.2^{a-e}
	B	1.4±0.2^{a-d}	27.2±2.0^{b-e}	0.5±0.1^{a-c}	16.2±0.9^{a-c}	9.8±0.7^{a-c}	72.6±6.3^{a}	61.8±6.0^{a}	827.0±90.5^{a-c}	302.2±41.4^{a-c}	1.5±0.3^{b-f}
	C	1.5±0.1^{b-d}	31.8±1.7^{c-e}	0.5±0.1^{bc}	16.5±0.7^{a-c}	10.0±0.8^{bc}	80.1±2.3^{b-e}	64.1±2.8^{a}	856.5±72.3^{a-c}	317.4±47.4^{bc}	1.3±0.2^{abc}
NUR	A	1.3±0.3^{a-d}	25.0±1.2^{b-d}	0.5±0.1^{a-c}	15.9±1.5^{a-c}	9.8±0.5^{a-c}	79.1±5.5^{b-e}	68.8±7.9^{a}	803.9±158.6^{a-c}	301.4±28.8^{a-c}	1.6±0.4^{c-f}
	B	1.3±0.2^{a-d}	27.4±1.7^{b-e}	0.5±0.1^{a-c}	15.7±0.8^{a-c}	9.8±0.7^{a-c}	78.4±8.5^{a-e}	69.2±7.3^{a}	772.3±83.8^{a-c}	300.0±42.7^{a-c}	1.3±0.2^{a-d}
	C	1.1±0.1^{a}	31.9±1.2^{c-e}	0.4±0.0^{ab}	15.2±0.3^{ab}	9.7±0.4^{a-c}	81.5±3.2^{c-e}	69.2±5.5^{a}	727.5±28.6^{ab}	295.2±26.8^{a-c}	1.2±0.1^{a}
SPL	A	1.4±0.3^{a-d}	24.5±2.6^{bc}	0.4±0.1^{ab}	15.8±1.3^{a-c}	9.6±1.0^{ab}	76.1±2.8^{a-d}	65.9±4.6^{a}	786.9±137.5^{a-c}	294.0±61.4^{a-c}	1.4±0.3^{a-f}
	B	1.3±0.2^{a-d}	25.9±2.0^{b-d}	0.4±0.1^{ab}	15.7±0.7^{a-c}	9.6±0.6^{ab}	79.7±3.7^{b-e}	66.0±2.5^{a}	775.9±68.7^{a-c}	288.7±36.4^{ab}	1.6±0.2^{def}
	C	1.5±0.1^{b-d}	30.5±1.6^{b-e}	0.5±0.1^{a-c}	15.9±0.4^{a-c}	9.7±0.6^{a-c}	81.4±1.4^{c-e}	67.4±5.0^{a}	798.3±36.2^{a-c}	297.7±35.8^{a-c}	1.4±0.1^{a-e}
NMU	A	1.2±0.3^{a-c}	25.8±3.3^{b-d}	0.4±0.1^{ab}	15.9±1.0^{a-c}	9.2±0.8^{ab}	77.3±4.0^{a-e}	65.4±3.1^{a}	801.5±93.7^{a-c}	269.0±45.1^{ab}	1.7±0.4^{ef}
	B	1.3±0.4^{a-d}	25.0±1.2^{b-d}	0.4±0.0^{ab}	15.0±1.4^{a}	9.8±0.6^{a-c}	75.6±5.3^{a-c}	67.5±6.7^{a}	714.2±142.9^{a}	300.3±35.8^{a-c}	1.5±0.2^{b-f}
	C	1.3±0.2^{a-d}	29.5±1.9^{b-e}	0.5±0.1^{a-c}	15.8±0.9^{a-c}	10.0±0.8^{bc}	81.4±4.2^{c-e}	67.1±3.0^{a}	790.1±91.5^{a-c}	314.9±46.9^{bc}	1.3±0.1^{ab}
TIA	A	1.4±0.3^{a-d}	23.5±2.8^{b}	0.4±0.1^{a}	14.8±1.3^{a}	9.0±1.2^{ab}	76.1±7.0^{a-d}	67.5±9.4^{a}	691.3±128.4^{a}	260.7±69.2^{ab}	1.4±0.1^{a-f}
	B	1.2±0.4^{a-c}	24.8±1.6^{b-d}	0.4±0.1^{a-c}	14.9±1.6^{a}	9.7±1.0^{a-c}	74.5±1.9^{ab}	65.9±3.5^{a}	703.1±155.0^{a}	296.4±60.0^{a-c}	1.3±0.1^{a-c}
	C	1.6±0.2^{d}	28.0±4.0^{b-e}	0.5±0.1^{a-c}	16.2±0.5^{a-c}	9.7±0.5^{a-c}	80.5±1.2^{b-e}	63.0±2.8^{a}	831.2±54.2^{a-c}	298.4±32.4^{a-c}	1.3±0.1^{a-c}
LEH	A	1.2±0.3^{b}	35.0±20.7^{e}	0.4±0.1^{a-c}	16.2±1.3^{a-c}	9.5±0.5^{ab}	76.6±3.1^{a-e}	65.7±3.9^{a}	832.9±141.7^{a-c}	284.4±28.2^{ab}	1.5±0.3^{b-f}
	B	1.3±0.2^{a-d}	13.3±7.1^{a}	0.4±0.1^{ab}	15.2±0.8^{ab}	9.7±0.8^{a-c}	76.3±4.8^{a-d}	67.3±6.1^{a}	726.0±76.5^{ab}	294.9±52.7^{a-c}	1.4±0.1^{a-e}
	C	1.6±0.3^{cd}	26.1±9.6^{b-d}	0.6±0.1^{c}	17.2±1.4^{c}	10.7±0.4^{c}	82.3±3.3^{de}	68.6±7.2^{a}	941.0±157.1^{bc}	356.4±25.1^{c}	1.5±0.2^{a-f}
Average	A	1.3±0.3$_{ab}$	26.3±8.5$_{a}$	0.4±0.1$_{a}$	16.0±1.9$_{b}$	9.5±0.8$_{a}$	77.0±4.9$_{a}$	66.5±5.7$_{a}$	821.7±225.1$_{b}$	284.7±49.0$_{a}$	1.5±0.3$_{b}$
	B	1.3±0.3$_{a}$	24.5±5.7$_{a}$	0.4±0.1$_{ab}$	15.3±1.2$_{a}$	9.5±0.8$_{a}$	76.0±5.7$_{a}$	65.9±6.3$_{a}$	745.2±119.1$_{a}$	288.9±46.7$_{a}$	1.4±0.3$_{b}$
	C	1.4±0.2$_{b}$	30.1±4.6$_{b}$	0.5±0.1$_{b}$	16.1±0.9$_{b}$	9.9±0.6$_{b}$	81.5±2.7$_{b}$	66.5±4.6$_{a}$	823.1±98.1$_{b}$	311.1±39.2$_{b}$	1.3±0.1$_{a}$
Overall Average		1.3±0.3	27.0±6.8	0.4±0.1	15.8±1.4	9.7±0.8	78.1±5.1	66.3±5.5	797.7±160.2	295.3±46.3	1.4±0.2

Note: Values represented mean ± SD; for each column different lowercase letters indicate significantly different at $p < 0.05$

*Group-A: brown stone coat with bitter kernel; Group-B: brown stone coat with sweet kernel; Group-C: white stone coat with sweet kernel SW: stone weight (g); SM: seed moisture content (%); KW: kernel weight (g); SD_g: seed geometric mean diameter (mm); KD_g: kernel geometric mean diameter (mm); SΦ: seed sphericity (%); KΦ: seed sphericity (%); *SS*: seed surface area (mm); *KS*: kernel surface area (mm); SCT: seed coat thickness (mm).

5.3.2 Altitude effects on kernel amygdalin and sugar content

The geographical elevation did not show any marked influence on amygdalin content of apricot kernel (Table 5.2). No increasing or decreasing pattern in amygdalin level was observed with increasing elevation. This finding was further supported by the Pearson's correlations. The results of the present investigation conclude that environmental factors do not significantly influence amygdalin level in the apricot kernel. However, high variability within genotypes was observed, which suggested that genotype played a significant role on amygdalin level in the apricot kernel.

The increasing elevation showed a significant impact on the kernel sugar content (Table 5.2). A linear relationship was observed between increasing elevation and sugar content (R^2=0.103) (Figure 5.1C). For every 100 meter rise in elevation, kernel total sugar increased by 12.9 mg.g^{-1} DW. The influence of geographical elevation on sugar content was further supported by Pearson's correlations (Table 5.4). SUC content also showed a linear relationship with increasing elevation (R^2=0.186) (Figure 5.1B). SUC content increased by 11.0 mg.g^{-1} DW for every 100 meter rise in elevation. For INO content inverse relationship was observed with increasing elevation (R^2=0.149) (Figure 5.1A). INO content decreased by 0.5 mg.g^{-1} DW for every 100 meter increase in elevation. Data from the present investigation indicated that apricots growing at high altitude contain higher sugar, SUC but low INO in the kernels.

5.3.3 Effect of stone colour on amygdalin content

Apricots having white stone are unique to Ladakh region and are linked to sweet kernel [3]. Low amygdalin content was observed (1.3-5.5 mg.g^{-1} DW) in genotypes with white stone phenotype (Group-C) (Table 5.2). Whereas amygdalin content of genotypes having brown stone and sweet apricot kernel (Group-B) ranged from 1.3-7.9 mg.g^{-1} DW, while that of bitter kernel (Group-A) was 25.5-63.7 mg.g^{-1} DW. Genotypes with white stone phenotype (Group-C) contain significantly lower amygdalin content (2.4±1.2 mg.g^{-1} DW) than that of the bitter kernel (44.6±9.0 mg.g^{-1} DW). However, the value was not significantly different than that of genotypes having brown stone with the sweet kernel (3.1±1.8 mg.g^{-1} DW). Low amygdalin level in kernel of apricot genotypes with white stone phenotype confirmed our earlier finding that white stone phenotypic marker is always associated with the sweet kernel [3]. Therefore, it is suggested from the present study that white stone phenotype can be taken as a marker for low amygdalin content in future studies.

5.3.4 Correlation between seed physical properties, amygdalin and sugar content

The physical characteristics of apricot seed and kernel determined for 126 genotypes are presented in Table 5.3. Wide variation was observed in physical characteristics among the three groups. Significant differences in physical properties of apricot seed and kernel showed differences between the genotypes. Table 5.4 presents correlations among variables. Geographical elevation showed a positive correlation with SUC (r=0.432), total sugar (r= -0.322) and significant negative correlation with INO. Sweet kernel were negative correlation with amygdalin (r=-0.965). Amygdalin showed a significant positive correlation with GLU (r=0.789) and total sugar (r=0.531). Seed and kernel physical characteristics have no significant effect on amygdalin and sugar content in the apricot kernel. Similarly, the altitude did not show significant correlation with amygdalin content.

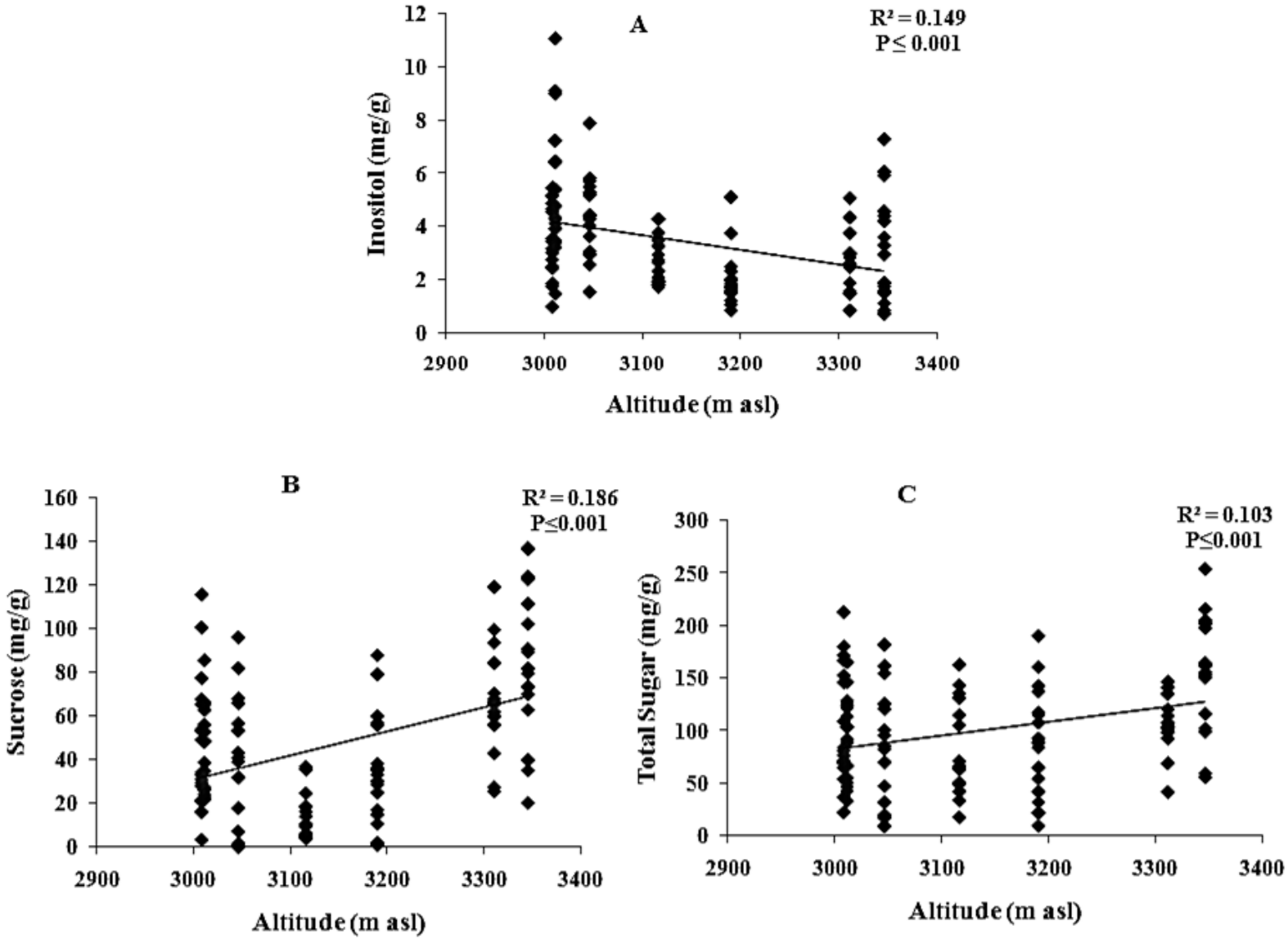


Figure 5.1: Relation between altitude with INO (A), SUC (B), total sugar (C)

Table 5.4: Pearson's correlation coefficients of altitude, seed physical characters, amygdalin and sugar content in apricot kernels of trans-Himalayan Ladakh

	ALT	AMY	INO	ARA	GLU	FRU	SUC	TS	SW	SM	KW	SD_g	KD_g	SΦ	KΦ	S *S*	K*S*	SCT
ALT	1	.002	-.386**	-.146	.103	.062	.432**	.322**	.020	-.199*	.025	-.088	.056	-.026	.048	-.085	.058	-.040
AMY		1	-.220*	.159	.791**	.058	.219*	.533**	-.025	-.079	-.122	.088	-.177*	-.167	.006	.097	-.166	.327**
INO			1	.063	-.375**	.216*	-.133	-.177*	.014	.182*	.067	.029	.035	-.016	-.033	.031	.024	-.168
ARA				1	.270**	.313**	.424**	.505**	.207*	-.055	.084	.151	.096	.127	.064	.143	.094	-.028
GLU					1	.198*	.276**	.702**	.032	-.049	-.032	.118	-.023	.010	.066	.105	-.015	.289**
FRU						1	.239**	.504**	.150	.109	.157	.104	.207*	.217*	.062	.083	.213*	-.172
SUC							1	.835**	.130	-.102	.083	.111	.055	.044	.027	.117	.060	.036
TS								1	.140	-.058	.079	.158	.077	.088	.064	.150	.085	.115
SW									1	-.157	.486**	.632**	.498**	-.125	-.310**	.586**	.496**	.311**
SM										1	.073	.124	.071	.182*	-.040	.094	.064	.042
KW											1	.496**	.800**	.088	.136	.450**	.805**	-.013
SD_g												1	.483**	.081	-.254**	.990**	.476**	.354**
KD_g													1	.183*	.313**	.425**	.998**	-.029
SΦ														1	.624**	.071	.180*	-.244**
KΦ															1	-.253**	.314**	-.334**
S*S*																1	.419**	.332**
K*S*																	1	-.033
SCT																		1

Note: * Significant at $p \leq 0.05$; ** Significant at $p \leq 0.01$ levels; ALT: altitude; AMY: amygdalin; INO: INO; ARA: ARA; GLU: GLU, FRU: FRU; SUC: SUC, TS: total sugar SW: stone weight (DW); SM: seed moisture content; KW: kernel weight (DW); SD_g: seed geometric mean diameter; KD_g: kernel geometric mean diameter; SΦ: seed sphericity; KΦ: kernel sphericity; S*S*: seed surface area; K*S*: kernel surface area; SCT: seed coat thickness.

5.4 Conclusion

Altitudinal gradient had no significant influence on kernel amygdalin content. However, geographical elevation had a significant influence on kernel sugar content. A linear relationship was seen between increasing elevation with SUC and total sugar content. A strong inverse relationship was observed between increasing elevation and INO. SUC was the major individual sugar, which was followed by GLU, FRU, INO, and ARA. The pattern of each sugar in the kernel was not affected by environmental factors. Therefore, apricots of high altitude contain higher SUC and total sugar, and low INO content in the kernel. Similarly, seed and kernel phenotypic characters have no significant effect on amygdalin content in the apricot kernel. High variability within genotypes suggested that genotype played a significant role on amygdalin content in apricot kernel. Low amygdalin content in genotypes with white stone phenotype confirmed our earlier findings that white stone phenotypic marker is associated with the sweet kernel. Therefore, the white stone phenotype can be taken as a marker for low amygdalin content in future studies.

CHAPTER 6

MORPHOLOGICAL AND SRAP MARKERS BASED GENETIC DIVERSITY STUDIES OF APRICOTS OF TRANS-HIMALAYA

ABSTRACT

Forty seven apricot genotypes were used to assess genetic diversity (GD) based on morphological and SRAP (Sequence-related amplified polymorphism). Six qualitative and 16 quantitative characters were studied among the genotypes. Twenty combinations of SRAP markers were used and 115 polymorphic bands out of 134 bands were observed. The overall GD estimated as percentage polymorphic loci (85.06%), Nei's genetic diversity (0.27±0.19) and Shannon's information index (0.40±0.25) were high. Analysis of molecular variance (AMOVA) revealed higher GD (92%) within the groups of apricot. The unweighted pair group method (UPGMA) demonstrated that the apricot genotypes had a similarity range from 0.96 to 0.48 with a mean value of 0.72 similarity coefficient. Furthermore, UPGMA clustering and Bayesian-based STRUCTURE analysis revealed an intermixing in the clustering of apricot genotypes. There is no clear grouping between apricots according to their kernel taste and stone colour which revealed that these genotypes have a similar genetic background. Knowledge gained from the present study has practical utility in the management of germplasm conservation and in breeding programs.

6.1 Introduction

Knowledge of genetic diversity (GD) is key for efficient preservation, management, and utilization of plant genetic resources [105]. Knowledge about genetic relationship and diversity among breeding materials could be useful in crop improvement strategies [106]. Ample GD in plants can provide a wide background for genetic research and crop breeding programmes [107]. Preservation of crop genetic resources is based on the continuous introduction of new genetic material from traditional and wild varieties for breeding of highly productive and resistant verities [108].

Cultivar characterization and discrimination are required for breeding and commercialization of apricot cultivars [109]. Knowledge of GD and relationship among the germplasm resource will be useful for protecting and utilizing local apricot varieties [110]. Several investigations have been carried out to determine diversity in apricots with pomological, phenological and morphological characters [66], [111]. These traditional approaches to diversity study are slow and subject to environmental influences. Apricot can be adapted to particular microclimates and shows significantly different morphological changes when proceeding to one microclimate to others [112]. Therefore, for reliable identification and discrimination of genotype and cultivars, independent markers from the environmental factor are required. Accordingly, new methods based on the molecular studies must be included in breeding programs and to study the genetic relationships among cultivars. Various types of molecular markers such as AFLP, ISSR, RAPD, SRAP, RFLP and SSR have been employed for analysis of plant GD and characterization. Among them, SRAP (Sequence-related amplified polymorphism) marker has been commonly used method for diversity study and population genetics analysis [113].

The SRAPs is an efficient and simple marker system and has several advantages over other markers system [113], [114]. SRAP targets coding sequences in the genome and results in a moderate number of co dominant markers [115]. The information given by SRAP markers are more conformable to morphological variability and evolutionary history of the morphotypes than of AFLP marker [116]. SRAP markers are more effective, quicker and less expensive over SSR marker [115].

The aim of the current work was to characterize the morphological and GD of apricot genotypes and to investigate the genetic and morphometric relationship among the genotypes to estimate the extent of GD in apricot genotypes between and within classified groups

according to kernel taste and stone colour. Furthermore, a model based clustering method was used to determine the optimal number of genetic and morphometric clustering in the genotypes. To best of our knowledge use of SRAP marker along with morphological characters for assessment of structure and GD in apricots has not been reported.

6.2 Materials and methods

6.2.1 Plant material and DNA extraction

Leaf sample of 47 genotypes was collected from an experimental orchard in Ladakh and kept in -80^{0}C freezer until DNA extraction. Apricot genotypes were grouped into three. Fourteen genotypes fall under Group-A: brown stone and bitter kernel; 23 genotypes under Group-B: brown stone and sweet kernel and 10 genotypes under Group-C: white stone and sweet kernel. Genomic DNA was isolated using a CTAB method [117]. The DNA sample was diluted to the final concentration of 30 ng.µl^{-1} before PCR amplification. Further, the genotypic data was also used for a coalition with earlier determined morphological data of these 47 individuals as mentioned in Chapter 2.

6.2.2 SRAP analysis and PCR amplification

SRAP markers developed previously were adopted in this study [114]. Twenty primer combinations using seven forward (Me 1-7) and nine reverse (Em1-9) (Table 6.1) were tested and selected based on proper amplification and reproducibility for diversity studies. The PCR amplification was performed in PCR tubes with total volume of 20 µl SRAP PCR reaction consisted 0.9µM of primers, 0.2mM of dNTPs, 2.5 mM of $MgCl_2$, 1.5 units of Taq DNA polymerase, genomic DNA at 30 ng and nuclease free water up to 20 µl reaction volume. Amplification was carried out in a 96 well thermocycler (BioRad T100^{TM}) programmed with the initial step at 95^{0}C for 3 min followed by 5 cycles of three steps: 1 min of denaturing at 94^{0}C, annealing at 35^{0}C for 1 min and extension at 72^{0}C for 1 min. In the subsequent 35 cycles, the annealing temperature was increased to 50^{0}C and extension step consist of one cycle for 5 min at 72^{0}C. Amplified products were electrophoresed on 1.5 % agarose gel and molecular size of amplicons was determined using a 50 bp-10 kb DNA ladders. After electrophoresis, the gels were documented in a gel documentation system (BioRad, Gel Doc^{TM} XR+).

Table 6.1: SRAP primers used for diversity studies of apricots of trans-Himalayan Ladakh

Forward primer	Reverse primer
Me1: TGAGTCCAAACCGGATA	Em1: GACTGCGTACGAATTAAT
Me2: TGAGTCCAAACCGGAGC	Em2: GACTGCGTACGAATTTGC
Me3: TGAGTCCAAACCGGAAT	Em3: GACTGCGTACGAATTGAC
Me4: TGAGTCCAAACCGGACC	Em4: GACTGCGTACGAATTTGA
Me5: TGAGTCCAAACCGGAAG	Em5: GACTGCGTACGAATTAAC
Me6: TGAGTCCAAACCGGTAA	Em6: GACTGCGTACGAATTGCA
Me7: TGAGTCCAAACCGGTCC	Em7: GACTGCGTACGAATTGAG
	Em8: GACTGCGTACGAATTGCC
	Em9: GACTGCGTACGAATTTCA

6.2.3 Morphological and genetic data analysis

Six qualitative and 16 quantitative characters were used in the multivariate statistical analysis of morphological data (Table 6.2). A multivariate approach was used to classify the plant population based on quantitative morphological characters using Gower general similarity coefficient [118] in the PAST software (Paleontological statistics, Version 3.22).

The band with same mobility was considered as an identical band whereas polymorphism was scored by the presence (1) or absence (0) of the band. POPGENE version 1.32 [119] was used to calculate the different GD parameters: number of alleles (Na), effective number of alleles (Ne), Nei's genetic diversity (H), Shannon's information index (I), number of polymorphic loci (NPL), percentage polymorphic loci (PPL). In order to describe genetic variability within and among the groups, the non-parametric analysis of molecular variance (AMOVA) was performed using squared Euclidean distances among all genotypes to partition the variation into two hierarchical levels; individual and groups [120] using GenAlEx v. 6.3 software [121]. Interpopulation genetic distance and genetic identity were calculated by Nei's method using GenAlEx software. STRUCTURE version 2.3 [122], [123] was used to predict number of clusters (K) and the probability of individual assigned to each cluster. The parameters sets assumed were admixture allele model with correlated allele frequencies and with no prior group's information. The number of clusters set from K=1 to 10 with five simulations for each K and for each simulation we have fixed burn-in period of 100000 steps followed by 250000 Monte Carlo Markov chain replicates. Results obtained

Table 6.2: Descriptive statistics related to morphological variables among the 47 genotypes (minimum, maximum and mean values measured for each variable, SD: standard deviation, CV: coefficient of variation

Variables	Mean ±SD	Min	Max	CV (%)
Fruit shape Lateral	6±3	1	8	43.4
Fruit shape Ventricle	4±1	2	5	32.5
Fruit shape of apex	3±1	1	4	32.7
Fruit skin colour	4±1	3	6	25.0
Flesh colour	4±1	3	6	20.9
Stone shape	2±1	1	5	45.2
Fresh fruit Wt. (gm)	20.2±8.5	5.0	41.9	41.8
Fresh stone Wt. (gm)	2.0±0.6	0.7	4.2	31.0
Moisture fruit %	71.4±7.6	48.7	84.7	10.7
TSS	23.7±5.5	14.5	36.8	23.3
Fruit Length (mm)	32.5±4.9	21.2	43.3	15.0
Fruit Width (mm)	32.6±5.0	21.5	44.0	15.3
Fruit Thickness (mm)	30.0±4.8	19.6	40.4	16.1
Stone Length (mm)	21.7±2.5	14.7	26.4	11.6
Stone Width (mm)	17.9±2.3	13.1	25.0	12.8
Stone Thickness (mm)	11.4±1.6	8.1	15.9	13.8
Blush area (mm)	223.6±247.7	0.0	831.5	110.8
Kernel Wt.(gm)	0.5±0.2	0.1	1.0	35.3
Kernel Length (mm)	15.1±1.7	10.7	18.3	11.3
Kernel Width (mm)	10.8±1.3	7.6	14.4	11.6
Kernel Thickness (mm)	6.2±1.3	3.1	9.9	20.6
Seed coat thickness (mm)	1.5±0.2	1.0	2.4	16.0

from STRUCTURE were interpreted by online available tool STRUCTURE HARVESTER [124] which implements Evanno's method [125] for calculation of a correct number of clusters (K).

6.3 Result and discussion

6.3.1 SRAP amplification

The selected 20 SRAP primer combinations pairs results in reproducible amplification and polymorphic bands were used to analysis polymorphism in 47 genotypes. A total of 134 bands were scored, out of which 115 (85.8%) bands were polymorphic (Table 6.3). An average of 6.7 bands per primer set was obtained, and an average 5.75 polymorphic band was obtained per primer set. The number of bands observed was higher than previous reports on apricot by Uzun et al. [112] (5.4 bands per primer set and 73% polymorphism rate), Pinar et al [126] (4.9 bands per primer set and 64.3% polymorphism rate). However, Ai et al. [114] obtain 19.1 bands per primer set and 88.11% polymorphism rate, which was greater than that of our study.

6.3.2 Genetic diversity

At the genotypic level, the GD were higher (NPL=114, PPL%=85.07, Na=1.85±0.36, Ne=1.46±0.37, H=0.27±0.19, I=0.40±0.25) (Table 6.4). The GD parameters at the level of grouped genotypes were highest in Group-B (NPL=103, PPL%=76.87, Na=1.77±0.42, Ne=1.46±0.38, H=0.26±0.20, I=0.39±0.28). Group-C showed the lowest GD (NPL=82, PPL%=61.19, Na=1.61±0.49, Ne=1.37±0.39, H=0.21±0.21, I=0.32±0.29). AMOVA also showed that the major part of total variance is partitioned resides within the population (92%) and only 8% of the variance was partitioned among populations (Table 6.5 and Figure 6.1). Thus, AMOVA analysis of SRAP marker suggests a better panmictic and not genetic divergent. The Nei's genetic distance and genetic identity of apricot groups are presented in Table 6.6. The highest value of genetic identity (0.9631) and the lowest genetic distance (0.0376) were obtained between Group-A and Group-B, followed by Group-B and Group-C (genetic identity = 0.9344, genetic distance = 0.0679) and the lowest value of genetic identity (0.9343) and highest genetic distance (0.0680) were observed between Group-A and Group-C.

Table 6.3: Polymorphism revealed by twenty SRAP primer combinations

Primer combination	Total band	Polymorphic band	Percentage of polymorphic band	Primer combination	Total band	Polymorphic band	Percentage of polymorphic band
Me1/Em1	6	5	83.3	Me4/Em6	7	5	71.4
Me1/Em2	9	8	88.9	Me5/Em2	8	7	87.5
Me1/Em4	9	9	100.0	Me5/Em6	6	3	50.0
Me1/Em5	4	3	75.0	Me5/Em8	8	8	100.0
Me2/Em2	7	7	100.0	Me5/Em9	8	7	87.5
Me2/Em6	8	7	87.5	Me6/Em2	4	3	75.0
Me2/Em7	5	3	60.0	Me6/Em5	8	7	87.5
Me3/Em2	6	5	83.3	Me6/Em6	6	5	83.3
Me3/Em4	5	5	100.0	Me7/Em3	8	7	87.5
Me4/Em3	6	5	83.3	Total	134	115	85.8
Me4/Em4	6	6	83.3	Average	6.7	5.75	85.8

Table 6.4: Summary of genetic variation statistics for all loci of SRAP among the apricot genotypes of trans-Himalayan Ladakh

Group*	Sample size	Na (Mean ± SD)	Ne (Mean ± SD)	H (Mean ± SD)	I (Mean ± SD)	NPL	PPL
Group-A	14	1.63±0.48	1.40±0.39	0.23±0.21	0.34±0.29	85	63.43
Group-B	23	1.77±0.42	1.46±0.38	0.26±0.20	0.39±0.28	103	76.87
Group-C	10	1.61±0.49	1.37±0.39	0.21±0.21	0.32±0.29	82	61.19
Overall genetic variability	47	1.85±0.36	1.46±0.37	0.27±0.19	0.40±0.25	114	85.07

Na = number of alleles; Ne = effective number of alleles; H = Nei's genetic diversity; I = Shannon's information index; NPL= number of polymorphic loci, PPL= Percentage of polymorphic loci. *Group-A: brown stone and bitter kernel; Group-B: brown stone and sweet kernel; Group-C: white stone and sweet kernel

Table 6.5: Total genetic variance analysis (AMOVA) of Apricot brought from SRAP results

Source	Df	SS	MS	Est. Var.	%
Among Pops	2	70.352	35.176	1.323	8%
Within Pops	44	690.627	15.696	15.696	92%
Total	46	760.979		17.019	100%

Figure 6.1: Total genetic variance analysis (AMOVA) of apricot based on SRAP results

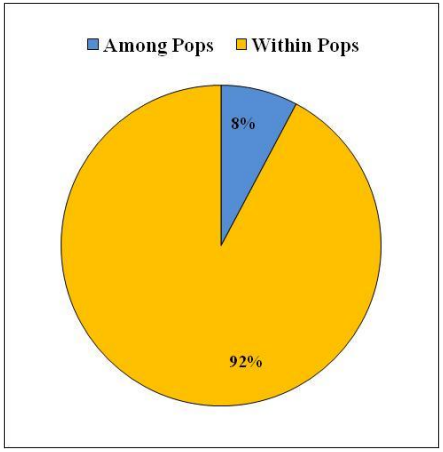


Table 6.6: Inter-population genetic distances and genetic identity calculated by Nei's method in apricot groups

Group*	A	B	C
A	***	0.9631	0.9343
B	0.0376	***	0.9344
C	0.0680	0.0679	***

Nei's genetic identity is above diagonal and genetic distances is below diagonal.

6.3.3 Morphometric and genetic relationship

The dendrogram generated from the UPGMA cluster analysis based on Gower similarity index from morphological traits classified the 47 genotypes included in this study into two main groups (Figure 6.2). The first cluster includes only one genotype with brown stone with the sweet kernel. The second cluster includes most of the genotypes from Group-A, B, C. The second cluster was further grouped into three subcluster. First and third subcluster shows intermixing of genotypes from Group-A and B, whereas Group-C is clustered into separate sub group.

To determine the genetic similarity among the genotypes, a dendrogram for the genotypes were obtained (Figure 6.3) with Jaccard similarity coefficient and UPGMA cluster analysis using PAST (Paleontological Statistics, Version3.22). The similarity index values range from 0.49 to 0.96. All these results reflect high genetic variability in apricots grown in Ladakh region. Apricot genotypes were grouped into nonspecific groups and did not follow our earlier assumption of three groups based on kernel taste and stone colour.

Figure 6.2: UPGMA cluster analysis based on Gower similarty index from morphometric parameters

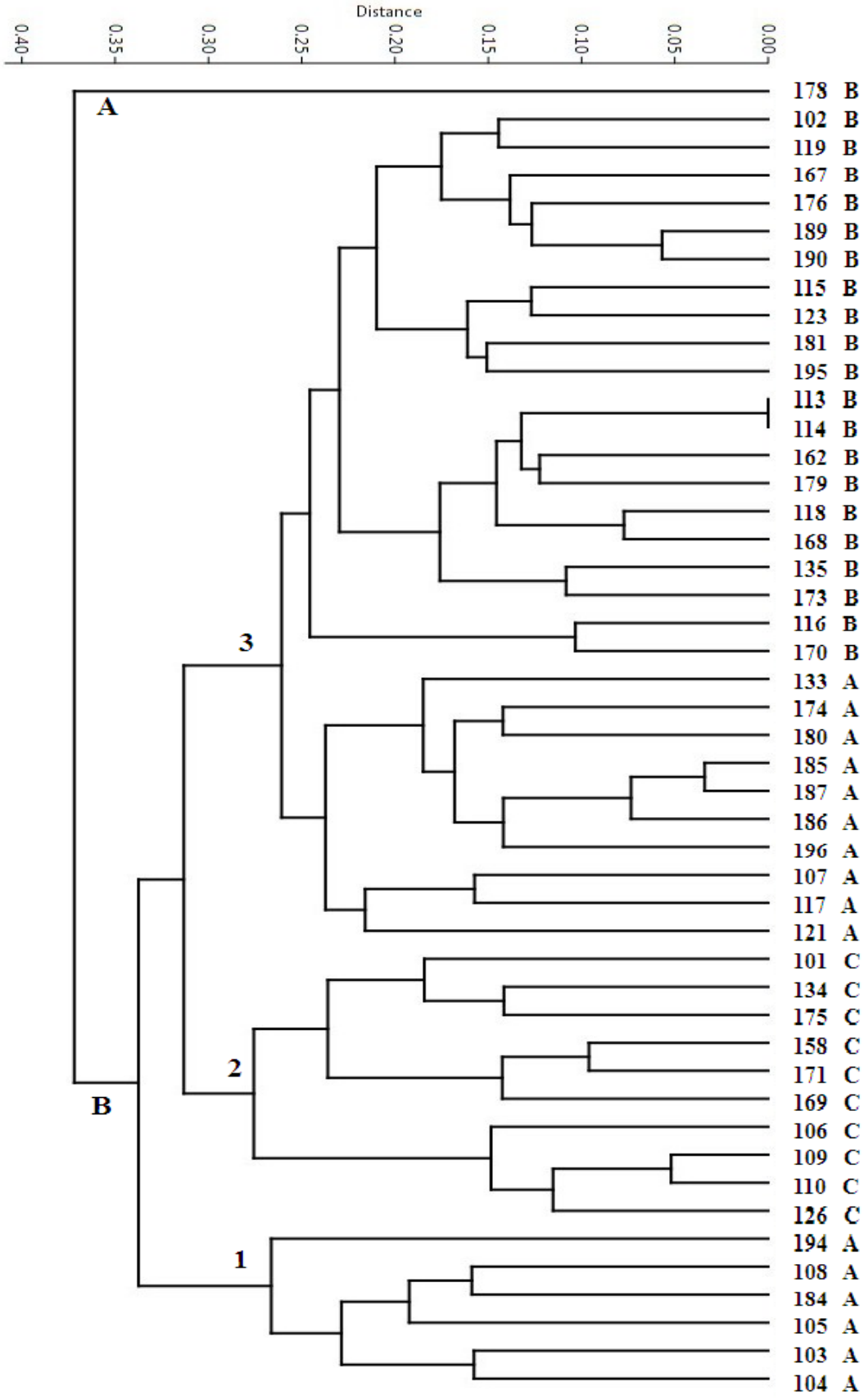
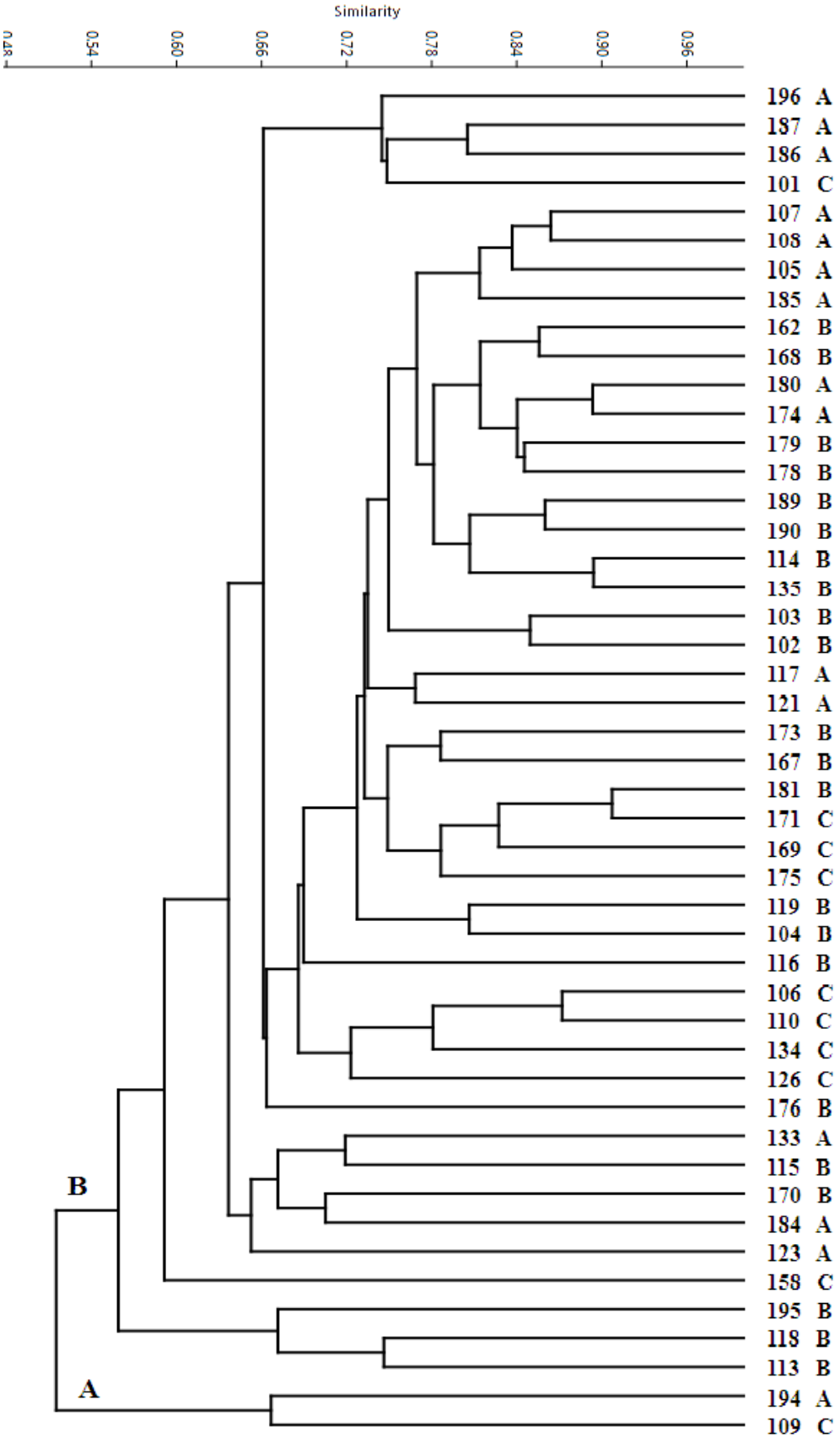


Figure 6.3 : SRAP based UPGMA cluster analysis by Jaccard similarity coefficient



6.3.4 Genetic structure

The genetic structure investigated on 47 genotypes, by applying Bayesian model based clustering algorithm approach in STRUCTURE software. Delta K values were plotted against the K numbers. The modal value of distribution of K number identified two clusters; DK, when graphed against K, showed a maximum peak at K = 2, dropping down to near zero at K = 3 (Figure 6.4). Structure analysis using SRAP markers revealed independent distribution of genotypes with respect to their groups. The study also indicates that apricot genotypes from trans-Himalayan region are highly genetically diverse. Structure analysis did not support our grouping of apricots into three based on kernel taste and stone colour (Group-A, -B, -C).

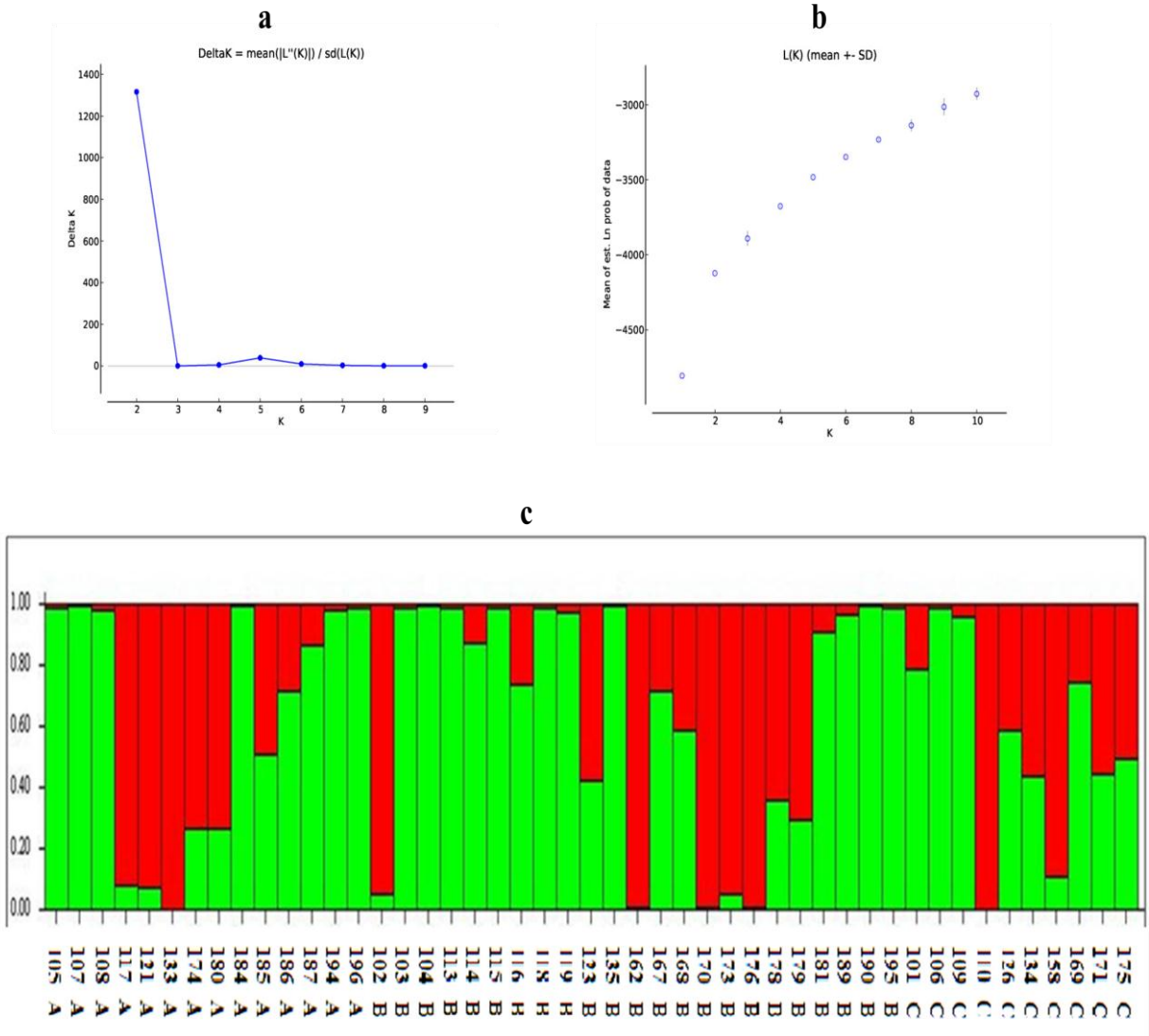


Figure 6.4: STRUCTURE analysis (a): the relationship between K and delta K; (b): the relationship between K and Ln P (D); (c): Membership probability of assigning individuals of the all populations to different clusters when K=10

6.4 Conclusion

SRAP markers efficiently distinguish apricot genotypes with a high level of polymorphism. UPGMA cluster analysis reflects that apricots of Ladakh region are morphologically divergent. High GD within the group and less among the three groups was observed. The study, therefore, revealed that germplasm conservation should not be done purely based on kernel taste and stone colour.

SUMMARY

Fruit quality, consumer perception and quality assessment for fresh apricots of Ladakh

Forty-seven apricot genotypes with white and brown fruit stone were studied by both instrumental and sensory methods. Attributes such as aroma, juiciness, sweetness, flesh colour, stone colour, fruit shape, and size were the main factors affecting overall acceptability of apricots. Fruit with white stone ranked first in terms of liking for sweetness, juiciness, aroma, stone colour, flesh colour, fruit shape, and size. It scored the highest hedonic score for overall appreciation (8.0±0.2). It also had the highest total soluble solids (TSS) (27.5±3.6°Brix), reducing sugars (17.5±1.9%) and total sugars (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 stone phenotype. Relationships between the instrumental data and sensory panel score were established. Overall appreciation showed positive significant relation with TSS (R^2 = 0.177), TSS/total acid (R^2 = 0.118), reducing sugar (R^2 = 0.140), total sugar (R^2 = 0.177) and fruit weight (R^2 = 0.230). A statistically significant negative relationship was observed between overall appreciation and fruit moisture content (R^2 = 0.168). The study demonstrated that white stone coat phenotype could be considered as a marker for high quality apricots in terms of aroma, sweetness, juiciness and overall appreciation.

Change in phenology and fruit quality of fresh apricot along an altitudinal gradient in trans-Himalaya

Consumer concern about the 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 the environmental effect on fruit quality. Therefore, this investigation aimed at studying the influence of elevation on phenological and fruit quality characters of apricot genotypes. Fruits from 162 trees were sampled from nine villages located at an elevation ranging from 3006 to 3346 meter asl in Ladakh. The altitude had a significant influence on the date of flowering, fruit weight, moisture, and TSS content. For every 100 meter rise in elevation, flowering and fruit ripening delayed by 3.3 and 7.1 days, respectively. An inverse relationship between fruit weight and elevation (R^2 = 0.310) was observed. The fruit weight decrease by 0.5 gm. for every 100 meter increase in elevation. Fruit moisture content decreased significantly with increase in elevation (R^2 = 0.585). The decrease in moisture content was 1.9% for every 100

meter rise in elevation. Increase in elevation had a linear relationship with fruit TSS content (R^2 = 0.726). The fruit TSS increased by 1.2ºBrix for every 100 meter rise in elevation. Knowledge from the present study on the impact of altitude on fruit quality characters suggests guidance on selection of orchard site for improving apricot fruit quality.

Altitudinal effect on sugar content and sugar profile in dried apricots

Apricot fruits from 108 genotypes were taken from six isolated villages located at an altitude ranging from 3006 to 3346 meter asl in Ladakh region. A linear relationship was observed between altitudinal elevation and total sugar content (R^2=0.877). Total fruit sugar increased by 64.8 mg.g^{-1} DW for every 100 meter rise in elevation. The most predominant individual sugar was sucrose (SUR) (57.8% of total sugar). The proportion of glucose (GLU), fructose (FRU), and sorbitol (SOR) was 19.4%, 14.3%, and 8.4% of total sugar, respectively. With altitudinal elevation, the relative proportion of SOR increased significantly in fruits with brown stone (R^2=0.849). A linear relationship was observed between the altitudinal elevation of the orchard and SUR (R^2 = 0.767). SUR content increased by 49.1 mg.g^{-1} DW for every 100 meter rise in elevation. Fruit SOR content showed a linear relationship with rising in elevation (R^2=0.899). GLU level increased with rising elevation, while FRU content showed the opposite relationship with elevation. Therefore, data indicated that apricots of high elevation contain higher sugar, SUR and SOR content. This knowledge is vital since it provides guidance for selecting site for orchard establishment for improving apricot fruit sweetness.

Effect of altitude and seed phenotypic characters on amygdalin and sugar content in apricot kernel

Apricots from 126 genotypes differing in stone colour and kernel taste were collected from seven different villages from 3008-3346 m asl spread across trans-Himalaya Ladakh region. Amygdalin and sugar content in the kernel were determined in the apricot kernel. Amygdalin level in the bitter kernel was significantly higher (44.6±9.0 mg.g^{-1}) than sweet kernel (3.1±1.8 mg.g^{-1}) with brown stone. The altitudinal gradient had no influence on kernel amygdalin level, whereas altitude had a significant impact on the kernel sugar content. A linear relationship was observed between total sugar content and rising elevation (R^2=0.103). Total sugar increased by 2.9 mg.g^{-1} DW for every 100 meter rise in elevation, Sucrose (SUC)

was the major sugar (47.9%). It was followed by glucose (GLU) (24.6%), fructose (FRU) (23.6%), inositol (INO) (3.5%) and arabitol (ARA) (3.5% of total sugar). A linear relationship was observed between SUC and increasing elevation (R^2=0.186). For every 100 meter increase in geographical elevation, SUC content increased by 11.0 $mg.g^{-1}$ DW. Kernel INO content showed an inverse relationship with increase in elevation (R^2=0.149). Similarly, seed and kernel phenotypic characters, except stone colour, had no significant effect on amygdalin and sugar content. Therefore, apricots growing at high geographical elevation contain higher sugar, SUC and low INO content in the kernel. Low amygdalin content (2.4±1.2 $mg.g^{-1}$) in apricot kernel with white stone phenotype confirmed our previous finding that white stone apricot is always linked to the sweet kernel.

Morphological and SRAP markers based genetic diversity studies of apricots of trans-Himalaya

Forty seven apricot genotypes were used to assess GD based on morphological and SRAP (Sequence-related amplified polymorphism). Six qualitative and 16 quantitative characters were studied among the genotypes. Twenty combinations of SRAP markers were used and 115 polymorphic bands out of 134 with an average of 5.75 polymorphic bands per combination were observed. The overall GD estimated as percentage polymorphic loci (85.06%), Nei's genetic diversity (0.27±0.19) and Shannon's information index (0.40±0.25) were high. Analysis of molecular variance (AMOVA) revealed higher GD (92%) within the groups of apricot. The unweighted pair group method (UPGMA) demonstrated that the apricot genotypes had a similarity range from 0.96 to 0.48 with a mean value of 0.72 similarity coefficient. Furthermore, UPGMA clustering and Bayesian-based STRUCTURE analysis revealed an intermixing in the clustering of apricot genotypes. There is no clear grouping between apricots according to their kernel taste and stone colour which revealed that these genotypes have a similar genetic background. Knowledge gained from the present study has practical utility in the management of germplasm conservation and in breeding programs.

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PUBLICATION IN PEER-REVIEWED JOURNAL

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1. Sahil Kapoor., Ashwani K Bhardwaj., Ashish R Warghat., **Avilekh Naryal**., Om P Chaurasia., *"Ethnobotanical, phytochemical and pharmacological properties of genus Rhodiola (L.): a high- altitude plant with potential medicinal applications*." Apple Academic Press, USA. (In-Press)

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