## Altitude and Seed Phenotypic Effect on Amygdalin Content in Apricot (*Prunus armeniaca* L.) Kernel

## Avilekh Naryal<sup>1</sup>, Pushpender Bhardwaj<sup>1</sup>, Anil Kant<sup>2</sup>, OP Chaurasia<sup>1</sup>, Tsering Stobdan<sup>1\*</sup>

#### ABSTRACT

**Objective:** Genetic component influencing amygdalin content in apricot kernel is well documented. This study aims to evaluate influence of altitude and seed phenotypic characters on amygdalin content in apricot kernel. **Methods:** Fruits from 126 genotypes differing in kernel taste and seed coat colour were collected from seven locations from 3008-3346 m asl in trans-Himalaya. Amygdalin content in kernel was determined. **Results:** Amygdalin content in bitter kernel was significantly higher (44.6±9.0 mg.g<sup>-1</sup>) than that of sweet kernel (3.1±1.8 mg.g<sup>-1</sup>) with brown seed coat. The geographical elevation had no influence on kernel amygdalin content. Similarly, seed and kernel physical characters, except seed coat color, had no significant effect on kernel amygdalin content. High variability within genotypes was observed suggesting that genotype played significant role on amygdalin content in apricot kernel. Low amygdalin content (2.4±1.2 mg.g<sup>-1</sup>) in apricot kernel with white seed coat phenotype can be taken as a marker for low amygdalin content in future studies.

Key words: Elevation, Bitterness, Cyanide, Ladakh, Seed Coat, Sweetness

## **INTRODUCTION**

Amygdalin is a natural cyanogenic glucoside occurring widespread in apricot kernel. The compound gives a bitter taste to the kernel. It is well documented that amygdalin content in bitter kernel is significantly higher than that of sweet kernel.<sup>1-3</sup> Multiple cases of poisoning as a result of consumption of bitter apricot kernel have been reported.<sup>4-5</sup> If kernel is swallowed completely without chewing, less cyanide is released than if they are chewed.<sup>6</sup> After apricot kernels are eaten, cyanide is released in the alkaline environment of the small intestine and, with emulsification, is absorbed quickly and circulates in the body. Cyanide causes anoxia of tissues at cellular level by blocking the cytochrome oxidase. Anaerobic metabolism occurs owing to hypoxia and lactic acid is produced.

Amygdalin has been confused with laevomandelonitrile, also referred to as laetrile. Indeed both laetrile and amygdalin have been promoted as vitamin B17, a cure for cancer, but are not actually a vitamin.<sup>7</sup> The controversial use of amygdalin in the treatment of cancer by many alternative therapists is well known.<sup>8</sup> However, it is totally ineffective and potentially toxic<sup>9</sup> as a possible cause of cyanide poisoning.<sup>10</sup> Systematic review concluded that no sound data from any controlled clinical trial supports the claim that amygdalin has beneficial effects for patients with cancer.<sup>11-12</sup> Therefore, presence of amygdalin in apricot kernel is not a desirable character for human consumption. The level of amygdalin differed greatly in apricot kernel reported from different apricot growing regions around the world. Within the sweet kernel it ranged from <0.08 to 15.84 mg.g<sup>-1</sup> DW.<sup>2-3</sup> Similarly, it ranged from 13.96 to 55 mg.g<sup>-1</sup> DW in bitter apricot kernel.<sup>1-3</sup> Marked difference in amygdalin levels could be the result of both genotypic and environmental conditions. Previous studies demonstrated that amygdalin content in kernel depends on cultivars<sup>2-3</sup> and fruit developmental stages.<sup>13</sup> However, little is known about effect of environment and seed physical characters on kernel amygdalin level.

Altitudinal gradients are among the most powerful 'natural environments' for testing effect of environmental conditions on fruit quality characters. Altitude gradients are particularly interesting in that they are characterized by steep changes in numerous features of the physical environment, such as temperature, moisture, atmospheric pressure, hours of sunshine, ultraviolet radiation, wind, season length and geology.<sup>14</sup> Thus, to obtain a better understanding of factors affecting amygdalin content in apricot kernel, this study aimed to investigate the effect of

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growing locations with different altitudes on amygdalin content of apricot kernel. Besides, correlation between seed and kernel physical characters with kernel amygdalin content was also investigated.

## **MATERIALS AND METHODS**

#### Study sites

Apricot fruits were collected from seven major apricot growing villages spread across trans-Himalayan Ladakh region. The altitude of collection sites ranged from 3008 to 3346 m asl (Table 1). Altitude and location of study sites were established using GARMIN GPS 72, Olathe, Kansas, USA. The mean maximum and minimum temperature of the region recorded daily during cropping season (April-October) in 2015 at an experimental orchard (34°08.2'N; 77°34.3'E, elevation 3340 m) was 18.8±5.4°C and 5.8±5.2°C, respectively, while the mean maximum and minimum relative humidity was 28.3±2.7% and 22.1±2.0%, respectively. The light intensity measured with Datalogging light meter (HD450, Extech Instruments) at noon in open field was 131194±43574 lux.<sup>15</sup> The average annual precipitation of the region is less than 200 mm of which more than 70% is in the form of snowfall.<sup>16</sup>

#### Physical properties of seed and kernel

The Length (L), Width (W), Thickness (T) and Weight (Wt) were measured in 40 randomly selected apricot seed and kernel of each genotype. Stone and kernel weight were measured with an electronic balance (ER-120A, Afcoset, India) to an accuracy of 0.001 g. Stone moisture content was determined using oven drying method till constant weight and expressed as percentage of fresh weight. Dimensional properties were measured with a digimaticcalliper (CD-6"CS, Mitutoya, Japan) to an accuracy of 0.01 mm. Geometric mean diameter [Dg = (LWT)<sup>0.333</sup>/L × 100] and surface area [ $S = \pi D_g^2$ ] were calculated as described earlier.<sup>17-19</sup>

#### Chemicals and reagents

All chemicals and reagents used were of analytical grade. Amygdalin (97%) standard was purchased from Sigma Aldrich (St. Louis, MO, USA). Ethanol (99.9 %) was used for extraction. Water was prepared using deionized water from RiOs<sup>®</sup> type I simplicity 185 (Millipore Waters, USA) with a resistivity of 18.2 M $\Omega$  cm.

#### Preparation of samples

Fresh fruit samples from 126 apricot genotypes were collected on the basis of the stone colour and kernel taste and grouped into three<sup>20</sup> ie. Group-I with brown stone with a bitter kernel; Group-II with brown stone with a sweet kernel; and Group-III with white stone with a sweet kernel. Apricots under Group-III are unique to Ladakh region in trans-

Himalaya and have not been reported from anywhere else in the world.<sup>15</sup> Representative sample of 50 fruits per genotype were hand harvested at physiological maturity stage. Seed was isolated from the fruit by manually removing the flesh while kernel was removed by physically breaking the seed coat. Dried kernels were grounded to a fine powder using pestle and mortar before amygdalin extraction.

#### Amygdalin extraction

Amygdalin was extracted with ethanol as described by Bolarinwa *et al.*<sup>21</sup> Powdered sample was mixed with ethanol and incubated in water bath at 78.5°C for 100 min and then centrifuge at 5000 rpm for 10 min at 25°C. The ethanol extract was filtered with 0.22 $\mu$ m filter syringe filter and dispensed into HPLC vials.

## Amygdalin estimation by HPLC

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 for detection and quantification of amygdalin. Separation was achieved using ZORBAX Eclipse  $C_{18}$  column (250mm x 4.60mm, 5µm: Agilent Technologies). The gradient mobile phase consisted 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 before use. The flow rate was adjusted to 1.0ml. min<sup>-1</sup> and wavelength detection was set to 214 nm with 5µl of sample injection. The results were expressed as mg.g<sup>-1</sup>DW of the sample.

## Statistical analysis

The experimental results were expressed as mean  $\pm$  standard deviation (SD) using statistical analysis with SPSS 16 (Statistical Program for Social Sciences, SPSS Corporation, Chicago, Illinois, USA) and MS excel 2007. One-way analysis of variance (ANOVA) and post hoc analysis with 2-sided Tukey's HSD at *p*≤0.05 level were performed. Box plots were produced to show minimum, median and maximum values of each variable. Pearson's correlation analysis was performed to find correlation between the variables.

## **RESULTS AND DISCUSSION**

#### Amygdalin content in bitter and sweet kernel

Significant difference in amygdalin content was observed between bitter and sweet kernel. Amygdalin content in bitter kernel was significantly higher (44.6 $\pm$ 9.0 mg.g<sup>-1</sup>) than that of sweet kernel (Group-II: 3.1 $\pm$ 1.8; Group-III: 2.4 $\pm$ 1.2 mg.g<sup>-1</sup>), which is in agreement with previous reports.<sup>1-3</sup> Marked variability was observed within the bitter kernel (Group-I) and ranged from averaged 35.1 $\pm$ 7.1 at 3311 m to 55.9 $\pm$ 8.1 mg.g<sup>-1</sup> DW at 3190 m

#### Table 1: Geographical locations and sampling site of apricots in trans-Himalaya.

Sampling	Altitude (m)	Population ID	Latitude	Longitude	Sample Size*				
localities	(asl)		(N)	(E)	Group-I	Group-II	Group-III		
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-I: brown stone coat with bitter kernel; Group-II: brown stone coat with sweet kernel; Group-I: white stone coat with sweet kernel

elevation (Table 2). Among individual genotypes the value ranged from 25.5-63.7 mg.g<sup>-1</sup> DW. In comparison, the value ranged from 13.96-44.41 mg.g<sup>-1</sup>among four bitter kernel apricot cultivars<sup>3</sup> and 44.1-63.5 mg.g<sup>-1</sup> in three bitter kernel apricot cultivars from Turkey.<sup>2</sup> Fememia *et al.*<sup>1</sup> reported 51mg.g<sup>-1</sup> DW in a bitter apricot cultivar from Spain.

Amygdalin content within sweet kernel (Group-II and Group-III) also showed marked variability and the averaged value ranged from  $1.4\pm0.1$ at 3008 m to  $4.3\pm1.2$  mg.g<sup>-1</sup> DW at 3190 m. Among individual genotypes the value ranged from 1.3-7.9 mg.g<sup>-1</sup> DW (Table 2). In comparison, the value ranged from <0.08-0.4 mg.g<sup>-1</sup> among nine sweet kernel apricot cultivars<sup>3</sup> and 6.04-15.84 mg.g<sup>-1</sup> in seven sweet kernel apricot cultivars from Turkey.<sup>2</sup> Fememia *et al.*<sup>1</sup> did not detected amygdalin in a sweet apricot cultivar from Spain.

#### Altitude effects on kernel amygdalin content

The geographical elevation did not showed marked influence on the kernel amygdalin content (Table 2). No increasing or decreasing trend in amygdalin content was observed with increasing altitude (Figure 1). The result was supported by Pearson's correlations and regression analysis (data not shown). The results of the present study inferred that environmental factors do not significantly influence amygdalin content in apricot kernel. However, high variability within genotypes suggested that genotype played a significant role on amygdalin content in apricot kernel.

#### Effect of seed coat color on amygdalin content

Genotypes with white seed coat are unique to Ladakh region and are associated with sweet kernel.<sup>15</sup> We observed low amygdalin content (1.3-5.5 mg.g<sup>-1</sup> DW) in genotypes with white seed coat phenotype (Group-III) (Table 2). In comparison, amygdalin content of genotypes having brown seed coat with sweet kernel (Group-II) ranged from 1.3-7.9 mg.g<sup>-1</sup> DW, while that of bitter kernel (Group-II) was 25.5-63.7 mg.g<sup>-1</sup> DW. The averaged value of amygdalin content (2.4±1.2 mg.g<sup>-1</sup> DW) in genotypes with white seed coat phenotype (Group-III) was significantly lower than that of bitter kernel (44.6±9.0 mg.g<sup>-1</sup> DW). However, the value was not significantly lower than that of genotypes having brown seed coat with sweet kernel (3.1±1.8 mg.g<sup>-1</sup> DW). Low amygdalin content in kernel of genotypes with white seed coat phenotype confirmed our earlier finding that the white phenotypic marker is



**Figure 1:** Box plot distribution of kernel amygdalin contents (mg.g<sup>-1</sup> 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).

associated with sweet kernel.<sup>15</sup> Therefore, white seed coat phenotype can be taken as a marker for low amygdalin content in future studies.

# Correlation between seed physical properties and amygdalin content

The physical properties of seed and kernel determined for 126 genotypes are shown in Table 3. Wide variation in physical properties was observed among the three groups. Significant differences in physical properties of seed and kernel signify differences between the genotypes. Table 4 present correlations among variables. Seed and kernel physical characteristics

Altitude (m)(asl)	Amygdalin (mg.g <sup>-1</sup> )									
	C	Gr	oup-ll		Gre	oup-III		Average		
	Mean ±SD	Min	Мах	Mean ±SD	Min	Max	Mean ±SD	Min	Max	
3008	46.7±9.5 <sup>b</sup>	29.2	56.2	1.4±0.1ª	1.3	1.5	1.9±0.3 <sup>ab</sup>	1.5	2.1	16.7
3011	$36.5 {\pm} 4.0^{a}$	28.4	39.0	$1.9{\pm}0.9^{a}$	1.3	3.6	$2.8 \pm 1.4^{b}$	1.3	4.8	13.7
3046	$42.3 \pm 5.5^{ab}$	34.7	47.5	$3.9{\pm}1.8^{\text{b}}$	2.7	6.2	4.3±1.2°	2.5	5.5	16.8
3116	$48.5\pm4.1^{bc}$	42.1	54.7	5.1±2.0 <sup>b</sup>	1.8	7.9	1.6±0.2ª	1.4	1.8	18.4
3190	55.9±8.1°	40.8	63.7	$3.6 \pm 1.7^{b}$	2.1	5.8	$2.1\pm0.4^{ab}$	1.7	2.7	20.5
3311	35.1±7.1ª	25.5	44.5	$3.6 \pm 0.4^{b}$	2.9	4.0	$2.2 \pm 1.1^{ab}$	1.5	4.3	13.6
3346	$46.9 \pm 3.8^{b}$	42.2	51.7	$1.9{\pm}1.1^{a}$	1.3	4.1	$1.8 \pm 0.5^{ab}$	1.0	2.6	16.9
Average	44.6±9.0			3.1±1.8			2.4±1.2			16.7

Table 2: Level of amygdalin content in apricot kernel of 126 genotypes of trans-Himalayan Ladakh grouped based on seed stone colour and kernel taste from different localities.

Values represented mean  $\pm$  SD; for each column different uppercase letters indicate significantly different at p < 0.05

Group-II: brown stone coat with bitter kernel; Group-II: brown stone coat with sweet kernel; Group-III: white stone coat with sweet kernel

Table 3: Seed	l and kernel $\mathfrak{k}$	ohysical characte	eristics of 126 apri	cot genotypes of	f trans-Himalayaı	n Ladakh groupe	d based on seed	coat colour and	l kernel taste.		
Localities	Group*	SW	SM	КW	SDg	KDg	SΦ	КФ	SS	ĸs	SCT
DOM	Ι	$1.6\pm0.3^{cd}$	$25.8\pm5.1^{bcd}$	$0.4\pm0.1^{ab}$	16.6±1.3 <sup>abc</sup>	$9.4{\pm}0.8^{\mathrm{ab}}$	$77.2\pm 5.6^{a-e}$	$65.4\pm 2.6^{a}$	871.5±132.0 <sup>abc</sup>	$281.6\pm 44.9^{ab}$	$1.7\pm0.1^{f}$
	II	$1.2\pm0.3^{\rm abc}$	$28.8\pm2.5^{b-e}$	$0.4\pm0.1^{ab}$	$15.0\pm 1.9^{a}$	$8.8{\pm}1.0^{a}$	$74.8\pm5.9^{ab}$	63.0±9.2ª	$719.8\pm178.8^{a}$	$248.3\pm53.7^{a}$	$1.4 \pm 0.4^{a-e}$
	III	$1.4{\pm}0.2^{\rm a-d}$	$32.7\pm3.4^{de}$	$0.4\pm0.1^{ab}$	$16.1\pm0.6^{\rm abc}$	$9.7\pm0.5^{\rm abc}$	83.0±2.3°	$66.3\pm1.3^{a}$	817.4±66.3 <sup>abc</sup>	$297.5\pm31.7^{\mathrm{abc}}$	$1.4{\pm}0.1^{\rm a-e}$
KLS	Ι	$1.4\pm0.4^{\mathrm{a-d}}$	$24.3\pm2.6^{bc}$	$0.5\pm0.1^{ m abc}$	$17.1 \pm 4.0^{bc}$	$9.7\pm1.0^{\rm abc}$	$76.9 \pm 7.1^{a-e}$	$67.0\pm7.3^{a}$	$964.3\pm508.4^{\circ}$	$301.7\pm60.6^{\mathrm{abc}}$	$1.4\pm0.2^{\mathrm{a-e}}$
	II	$1.4\pm0.2^{\mathrm{a-d}}$	$27.2\pm2.0^{b-e}$	$0.5\pm0.1^{ m abc}$	$16.2\pm0.9^{\mathrm{abc}}$	$9.8\pm0.7^{\rm abc}$	$72.6\pm 6.3^{a}$	$61.8\pm6.0^{a}$	827.0±90.5 <sup>abc</sup>	$302.2\pm41.4^{\rm abc}$	$1.5 \pm 0.3^{b-f}$
	III	$1.5\pm0.1^{bcd}$	$31.8\pm1.7^{cde}$	$0.5\pm0.1^{\rm bc}$	$16.5\pm0.7^{\rm abc}$	$10.0\pm0.8^{ m bc}$	$80.1\pm2.3^{b-e}$	$64.1\pm 2.8^{a}$	856.5±72.3 <sup>abc</sup>	$317.4\pm47.4^{\rm bc}$	$1.3\pm0.2^{\rm abc}$
NUR	Ι	$1.3\pm0.3^{a-d}$	$25.0\pm1.2^{bcd}$	$0.5\pm0.1^{ m abc}$	15.9±1.5 <sup>abc</sup>	$9.8\pm0.5^{\mathrm{abc}}$	$79.1\pm5.5^{b-e}$	68.8±7.9ª	803.9±158.6 <sup>abc</sup>	$301.4\pm28.8^{\mathrm{abc}}$	$1.6\pm0.4^{c-f}$
	II	$1.3\pm0.2^{\rm a-d}$	$27.4\pm1.7^{\mathrm{b}\cdot\mathrm{e}}$	$0.5\pm0.1^{ m abc}$	$15.7\pm0.8^{abc}$	$9.8\pm0.7^{\rm abc}$	$78.4\pm 8.5^{a-e}$	$69.2\pm7.3^{a}$	$772.3\pm 83.8^{abc}$	$300.0\pm42.7^{\mathrm{abc}}$	$1.3 \pm 0.2^{a-d}$
	III	$1.1 {\pm} 0.1^{a}$	$31.9\pm1.2^{\rm cde}$	$0.4\pm0.0^{\mathrm{ab}}$	$15.2\pm0.3^{ab}$	$9.7\pm0.4^{\mathrm{abc}}$	$81.5\pm3.2^{cde}$	69.2±5.5ª	$727.5\pm 28.6^{ab}$	$295.2\pm 26.8^{\rm abc}$	$1.2 \pm 0.1^{a}$
SPL	Ι	$1.4{\pm}0.3^{\rm a-d}$	$24.5\pm2.6^{bc}$	$0.4\pm0.1^{ab}$	15.8±1.3 <sup>abc</sup>	$9.6\pm1.0^{\mathrm{ab}}$	$76.1\pm2.8^{\rm a-d}$	$65.9 \pm 4.6^{a}$	$786.9\pm137.5^{abc}$	$294.0\pm61.4^{\mathrm{abc}}$	$1.4\pm0.3^{\mathrm{a-f}}$
	II	$1.3 \pm 0.2^{a-d}$	$25.9\pm2.0^{bcd}$	$0.4\pm0.1^{ab}$	$15.7\pm0.7^{\rm abc}$	$9.6\pm0.6^{\mathrm{ab}}$	79.7±3.7 <sup>b-e</sup>	66.0±2.5ª	775.9±68.7 <sup>abc</sup>	$288.7{\pm}36.4^{\rm ab}$	$1.6\pm0.2^{\rm def}$
	III	$1.5\pm0.1^{bcd}$	$30.5\pm1.6^{b-e}$	$0.5\pm0.1^{ m abc}$	$15.9\pm0.4^{ m abc}$	$9.7\pm0.6^{\mathrm{abc}}$	$81.4\pm1.4^{cde}$	$67.4\pm5.0^{a}$	798.3±36.2 <sup>abc</sup>	$297.7\pm35.8^{\mathrm{abc}}$	$1.4\pm0.1^{\mathrm{a-e}}$
NMU	Ι	$1.2\pm0.3^{\rm abc}$	$25.8\pm3.3^{bcd}$	$0.4\pm0.1^{ab}$	$15.9\pm1.0^{\rm abc}$	$9.2\pm0.8^{ab}$	$77.3 \pm 4.0^{a-e}$	$65.4\pm3.1^{a}$	801.5±93.7 <sup>abc</sup>	$269.0 \pm 45.1^{ab}$	$1.7\pm0.4^{\rm ef}$
	II	$1.3 \pm 0.4^{\rm a-d}$	$25.0\pm1.2^{bcd}$	$0.4\pm0.0^{\mathrm{ab}}$	$15.0\pm 1.4^{a}$	$9.8\pm0.6^{\mathrm{abc}}$	$75.6\pm5.3^{\rm abc}$	67.5±6.7ª	$714.2\pm 142.9^{a}$	$300.3\pm35.8^{\rm abc}$	$1.5 \pm 0.2^{b-f}$
	III	$1.3\pm0.2^{\rm a-d}$	$29.5\pm1.9^{b-e}$	$0.5\pm0.1^{ m abc}$	$15.8\pm0.9^{\mathrm{abc}}$	$10.0\pm0.8^{\rm bc}$	$81.4\pm4.2^{cde}$	$67.1 \pm 3.0^{a}$	$790.1\pm91.5^{abc}$	314.9±46.9 <sup>bc</sup>	$1.3\pm0.1^{\mathrm{ab}}$
TIA	Ι	$1.4{\pm}0.3^{\rm a-d}$	$23.5\pm 2.8^{b}$	$0.4\pm0.1^{a}$	$14.8 \pm 1.3^{a}$	$9.0\pm1.2^{ab}$	$76.1\pm7.0^{a-d}$	$67.5\pm9.4^{a}$	$691.3\pm128.4^{a}$	$260.7\pm69.2^{ab}$	$1.4\pm0.1^{\mathrm{a-f}}$
	II	$1.2\pm0.4^{\mathrm{abc}}$	$24.8\pm1.6^{bcd}$	$0.4\pm0.1^{ m abc}$	$14.9\pm 1.6^{a}$	$9.7\pm1.0^{\rm abc}$	$74.5\pm1.9^{ab}$	$65.9\pm3.5^{a}$	$703.1\pm155.0^{a}$	$296.4\pm60.0^{\mathrm{abc}}$	$1.3\pm0.1^{\rm abc}$
	III	$1.6\pm0.2^{d}$	$28.0\pm4.0^{\mathrm{b}\cdot\mathrm{e}}$	$0.5\pm0.1^{ m abc}$	$16.2\pm0.5^{\mathrm{abc}}$	$9.7\pm0.5^{\rm abc}$	$80.5\pm1.2^{b-e}$	$63.0\pm 2.8^{a}$	$831.2\pm54.2^{\mathrm{abc}}$	$298.4\pm32.4^{\rm abc}$	$1.3\pm0.1^{\rm abc}$
LEH	Ι	$1.2 \pm 0.3^{b}$	35.0±20.7°	$0.4\pm0.1^{ m abc}$	16.2±1.3 <sup>abc</sup>	$9.5\pm0.5^{\mathrm{ab}}$	$76.6\pm3.1^{\rm a-e}$	65.7±3.9ª	832.9±141.7 <sup>abc</sup>	$284.4\pm 28.2^{ab}$	$1.5 \pm 0.3^{b-f}$
	II	$1.3\pm0.2^{\rm a-d}$	$13.3\pm7.1^{a}$	$0.4\pm0.1^{ab}$	$15.2\pm0.8^{ab}$	$9.7\pm0.8^{\rm abc}$	$76.3 \pm 4.8^{\rm a-d}$	$67.3\pm 6.1^{a}$	$726.0\pm76.5^{ab}$	$294.9\pm52.7^{\mathrm{abc}}$	$1.4{\pm}0.1^{\rm a-e}$
	III	$1.6\pm0.3^{cd}$	$26.1\pm9.6^{bcd}$	$0.6\pm0.1^{\circ}$	$17.2\pm 1.4^{\circ}$	$10.7 \pm 0.4^{\circ}$	82.3±3.3 <sup>de</sup>	$68.6\pm7.2^{a}$	$941.0\pm157.1^{bc}$	$356.4\pm 25.1^{\circ}$	$1.5 {\pm} 0.2^{\rm a-f}$
Average	Ι	$1.3\pm0.3^{\mathrm{ab}}$	$26.3\pm8.5^{a}$	$0.4{\pm}0.1^{a}$	$16.0\pm1.9^{b}$	$9.5{\pm}0.8^{a}$	$77.0\pm4.9^{a}$	66.5±5.7ª	$821.7\pm 225.1^{b}$	$284.7\pm49.0^{a}$	$1.5\pm0.3^{b}$
	II	$1.3 \pm 0.3^{a}$	$24.5\pm5.7^{a}$	$0.4\pm0.1^{ab}$	$15.3\pm1.2^{a}$	$9.5{\pm}0.8^{a}$	$76.0\pm5.7^{a}$	$65.9\pm 6.3^{a}$	$745.2\pm119.1^{a}$	$288.9\pm46.7^{a}$	$1.4{\pm}0.3^{\rm b}$
	III	$1.4{\pm}0.2^{\rm b}$	$30.1 \pm 4.6^{b}$	$0.5 {\pm} 0.1^{\rm b}$	$16.1\pm0.9^{b}$	$9.9\pm0.6^{\mathrm{b}}$	$81.5\pm 2.7^{b}$	$66.5\pm 4.6^{a}$	$823.1\pm98.1^{b}$	$311.1\pm 39.2^{b}$	$1.3 \pm 0.1^{a}$
Overall ,	Average	$1.3 \pm 0.3$	27.0±6.8	$0.4 {\pm} 0.1$	$15.8 \pm 1.4$	9.7±0.8	78.1±5.1	66.3±5.5	797.7±160.2	$295.3 \pm 46.3$	$1.4 \pm 0.2$
Note: Values re	•nresented me	an + SD: for each o	column different ui	mercase letters ind	icate significantly o	different at $n < 0.0$	ſ				

~~~ / the ar *p* each SU; IOF Note: Values represented

SW: stone weight (g); SM: seed moisture content (%); KW: kernel weight (g); SD<sub>8</sub>: seed geometric mean diameter (mm); KD<sub>8</sub>: kernel geometric mean diameter (mm); S0: seed sphericity (%); K0: kernel sphericity (%); SS: seed surface area (mm); SS: seed surface area (mm); SCT: seed coat thickness (mm). \*Group-I: brown stone coat with bitter kernel; Group-II: brown stone coat with sweet kernel; Group-III: white stone coat with sweet kernel

|      | AMY | Alti | SW  | SM   | KW     | SDg    | KDg    | SΦ   | КΦ     | <b>S</b> <i>S</i> | KS     | SCT    |
|------|-----|------|-----|------|--------|--------|--------|------|--------|-------------------|--------|--------|
| AMY  | 1   | .041 | 019 | 018  | 028    | .019   | 067    | 098  | 181    | 014               | 058    | .279   |
| Alti |     | 1    | 187 | .230 | 148    | 226    | 188    | 092  | 032    | 216               | 194    | 067    |
| SW   |     |      | 1   | 284  | .517** | .534** | .595** | 286  | 224    | .490**            | .598** | .317*  |
| SM   |     |      |     | 1    | 028    | .095   | .021   | .041 | 096    | .058              | .002   | .204   |
| KW   |     |      |     |      | 1      | .422** | .858** | 113  | .094   | .376*             | .861** | 005    |
| SDg  |     |      |     |      |        | 1      | .369*  | .027 | 343*   | .992**            | .358*  | .394** |
| KDg  |     |      |     |      |        |        | 1      | 063  | .278   | .315*             | .997** | 017    |
| SΦ   |     |      |     |      |        |        |        | 1    | .642** | .038              | 055    | 030    |
| КΦ   |     |      |     |      |        |        |        |      | 1      | 332*              | .278   | 357*   |
| SS   |     |      |     |      |        |        |        |      |        | 1                 | .304   | .349*  |
| KS   |     |      |     |      |        |        |        |      |        |                   | 1      | 028    |
| SCT  |     |      |     |      |        |        |        |      |        |                   |        | 1      |

Table 4: Pearson's correlation coefficients of altitude, seed physical characters and amygdalin content of bitter apricot kernels of trans-Himalayan Ladakh.

Note: <sup>\*</sup>Significant at  $p \le 0.05$ ; <sup>\*\*</sup>Significant at  $p \le 0.01$  levels,

AMY: amygdalin; Alti: altitude; SW: stone weight (DW); SM: seed moisture content; KW: kernel weight (DW); SDg: seed geometric mean diameter; KDg: kernel sphericity; SS: seed surface area; KS: kernel surface area; SCT: seed coat thickness

have no significant effect on amygdalin content in apricot kernel. Similarly, altitude did not showed significant correlation with amygdalin content.

## CONCLUSION

The geographical elevation had no marked influence on kernel amygdalin content. Similarly, seed and kernel physical characters have no significant effect on amygdalin content in 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 seed coat phenotype confirmed our earlier findings that white seed coat phenotypic marker is associated with sweet kernel. Therefore, white seed coat phenotype can be taken as a marker for low amygdalin content in future studies.

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## **CONFLICT OF INTEREST**

There is no conflict of interest.

## **ABBREVIATIONS**

**ANOVA:** Analysis of variance; **DW:** Dry weight; **HPLC:** High-performance liquid chromatography; **SD:** Standard deviation; **SPSS:** Statistical Program for Social Sciences.

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#### **GRAPHICAL ABSTRACT**



#### **SUMMARY**

- Fruits from 126 genotypes differing in kernel taste and seed coat colour were collected from seven locations
- Amygdalin content in bitter kernel was significantly higher (44.6±9.0 mg.g<sup>-1</sup>) than that of sweet kernel (3.1±1.8 mg.g<sup>-1</sup>) with brown seed coat
- White seed coat phenotype can be taken as a marker for low amygdalin content in future studies.
- The geographical elevation had no influence on kernel amygdalin content.

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