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Variation in Glucosinolate Contents and Quality Characteristics in the Seed of Radish (*Raphanus sativus* L.) along an Altitudinal Gradient in trans-Himalayan Ladakh

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ABSTRACT

Glucosinolates (GS) are amino acid derived secondary metabolites present specifically in Brassicaceae family. The effect of altitude (2800 m - 4000 m) on GS contents and seed quality characteristics in radish (*Raphanus sativus* L.) was investigated. The total GS in radish seed was 147.5±21.5 µmol/g DW, which included five aliphatic and four indolic GS compounds. Increasing altitude was related linearly to increase in total GS contents ($R^2 = 0.759$). Among individual GS, glucoraphanin and glucoraphenin showed consistently increasing trend with altitude. Increasing altitude is related linearly to increase in 100 seed weight ($R^2 = 0.266$) and seed yield per plant ($R^2 = 0.849$). A steady decreasing trend was observed in seed moisture content ($R^2 = 0.831$) and siliqua length ($R^2 = 786$) with increasing altitude. Altitude of plant origin was positively correlated with GS contents (r = 0.900, $p \leq 0.01$), seed weight per plant (r = 0.919, $p \leq 0.01$) and geometric mean diameter (r = 0.661, $p \leq 0.01$), and negatively correlated with siliqua length (r = -0.880, $p \leq 0.01$), moisture content (r = -0.884, $p \leq 0.01$) and seed vigour index (r = -0.547, $p \leq 0.01$). Our results indicate that the concentration of GS in radish seed and seed quality traits can be significantly increased by growing plants at higher altitude.

Keyword: Anticarcinogenic; High altitude; Himalaya; Ladakh; Seed production

1. INTRODUCTION

Glucosinolates (GS) are sulfur and nitrogen containing secondary metabolites that are widely found in Brassicaceae family. GS biosynthesis occurs from amino acid and involves three major steps viz., side chain elongation, core structure formation, and side chain modifications. Based on the precursor amino acid used, GS are mainly divided into aliphatic (derived from Met, Leu, Ile), aromatic (Phe) and indolic (Trp) GS. Till date, about 200 different GS structures have been identified, formed due to side chain elongation and extensive side chain modifications[1]. GS are chemically stable until it comes in contact with the enzyme myrosinase during the process of cutting or chewing[2]. The breakdown product, isothiocyanate, is reported to have anticarcinogenic[3] and antioxidant[4] properties.

The GS content in seeds and other plant parts are known to be influenced by several factors including biotic (herbivory, fungal, bacterial) and abiotic (metals, UV, temperature, salts, season etc) factors. Change in GS contents due to altered temperature and photoperiod[5], light quality[6], fertilizer application[7], water deficiency[8], storage treatment[9] and season[10] have been studied in few Brassicaceae species. However, there is a paucity of information describing the effect of altitude on GS contents in Brassicaceae seeds.

Radish (*Raphanus sativus* L.), a Brassicaceae vegetable, is widely cultivated in trans-Himalayan Ladakh for many decades. It is consumed mostly during winter and very little during summer season. The local cultivars, *Gya Labuk* and *Tsentay Labuk* has thick rind and is traditionally stored in underground pits for up to 5-6 months during winter[11]. Seeds are produced locally to maintain the cultivars. Different parts of radish viz. root, seed, leaf and stem are known to contain health-promoting GS[12]. The objective of the present investigation was to study the effect of altitude on seed GS contents and various seed quality traits. Our results indicate that the concentration of GS in radish seed and seed quality traits can be significantly increased by growing plants at higher altitude.

2. MATERIALS AND METHODS

2.1 Plant Materials

The study was conducted during 2014 - 2015 in trans-Himalayan Ladakh, India. Seeds of local cultivar, *Raphanus sativus* L.(*Gya Labuk)* was collected from 18 villages located at elevation ranging from 2800 m - 4000 m above sea level and sown at different altitude with triplicates. Altitude and location of study sites were established using GARMIN GPS 72, Olathe, Kansas, USA.

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2.2 Seed Quality Characteristics

Seed quality characteristics such as seed weight, siliqua length, number of seeds per siliqua, moisture content and seed yield per plant were recorded from each altitude. Three linear dimensions namely length (L), width (W) and thickness (T) were measured. Geometric mean diameter (Dg) was calculated using the formula $(LWT)^{1/3}$. Seed moisture content was determined using the oven drying method and expressed in percentage. Germination test was done in petri dishes (90 mm diameter) with moistened filter paper at the base at room temperature. Seedling counts were performed after every 24 h and final germination rate was recorded after seven days.

Seed vigour index (SVI) was calculated at the final count by measuring an average seedling length of 20 seedlings using the formula:

SVI = germination (percent) × seedling length (cm)/100

Reproductive yield (RY) was calculated as:

RY = Mean total seed mass per siliqua × total number of siliquae

2.3 Glucosinolate Content

The total GS content and composition of root were determined using HPLC[13]. Briefly, GS were extracted in 70 per cent methanol after adding 50 µM Sinalbin (p-hydroxybenzyl glucosinolate) as the internal standard. Samples were loaded onto DEAE Sephadex A25 columns and desulphated overnight using purified sulphatase prior to HPLC. The concentration of individual GS was calculated relative to the internal standard peak applying their relative response factors (2 for aliphatic GS and 0.5 for indolic and aromatic GS) and expressed in µmoles g^{-1} dry weight (DW). At least three independent measurements were performed to obtain the data.

2.4 Statistical Analysis

Assumptions of normality were checked for all variables with Kolmogorov-Smirnov test and variables that significantly deviate from normality were log transformed. The experimental results were expressed as mean ± standard deviation (SD). One way analysis of variance (ANOVA) and post hoc analysis with 2-sided Tukey's HSD at $p \leq 0.05$ level were performed. Pearson's correlation analysis was performed to compare the data. To, further examine the relationship between morphological characters and altitude linear regression was performed fitting data with simple linear model ($y = a+bx$). All analysis were performed in SPSS statistical analysis software.

3. RESULTS AND DISCUSSION

3.1 Glucosinolate Contents in Radish Seeds

The total GS content in radish seeds obtained across the trans-Himalayan Ladakh region was 147.5±21.5 µmol/g DW. Previously, Ciska[14], *et al.* reported 11.4 µmol/g and 188.7 µmol/g DW GS in red and white *Raphanus sativus* (radish), respectively. Matthaus[15], *et al.* reported 6-9 µmol/g in three cultivars of *Raphanus sativus* (radish). Bhandari[16], *et al.* reported 2.4 µmol/g DW. This large variation of GS in radish seeds may be because of genotypic and environmental effects. In comparison, the total GS in seeds of Brassicaceae crops are 110.8 in *Brassica oleracea* var *italic* (broccoli), 105.6 in *Brassica oleracea* var capitata (cabbage), 49.5 in *Brassica oleracea* var botrytis (cauliflower), 36.4 in *Brassica rapa* sub sp. perkinensis (Chinese cabbage), 83.0 in *Brassica oleraceae* var sabellica (kale), 82.7 in leaf of *Brassica juncea* (mustard) and 107.8 µmol/g DW in *Brassica rapa* subsp. chinensis (pakchoi)[16]. Ciska[14], *et al.* reported 349.72 µmol/g DW in mustard seeds, which erroneously seems to be very high for a cultivated Brassicaceae species. *Brassica napus* seed contain 54.7-114.7[17] while red cabbage and broccoli contain 120 µmol/g and 93 µmol/g DW GS, respectively[18]. In *Brassica juncea* (Indian mustard) oilseed crop contain seed glucosinolate ranging from 80-120 µmol/g DW[13].

Nine GS were detected in the radish seeds including five aliphatic (glucoraphanin, glucoraphenin, gluconapoleiferin, glucoerucin, glucoraphasatin) and four indolic compounds (4-hydroxyglucobrassicin, glucobrassicin, 4-methoxyglucobrassicin, unknown). In comparison, Bhandari[16], *et al.* detected only four GS (glucoraphanin, glucoraphenin, glucoerucin, glucobrassicin) in radish seeds. The most dominant GS compound was the glucoraphenin which accounts for 81.5 per cent of the total GS in the radish seeds. The second highest compound was glucoraphasatin accounting for 8.6 per cent of the total GS (Table 1). In comparison, Sarikamis[19], *et al.* reported that in radish seeds glucoraphenein and glucoraphasatin accounted for 80 per cent and 3 per cent of the total aliphatic GS, respectively.

3.2 Altitudinal Variation in Glucosinolate Contents

Altitude of seed origin has a significant impact on seed GS contents (ANOVA, Table 1). Increasing altitude is related linearly to the increase in total GS contents ($R^2 = 0.759$) (Fig. 1). Seeds collected from 2800 m - 3000 m altitude contained 119.8±4.2 µmol/g DW while those of 3801 m - 4000 m altitude contained 182.4±15.3 µmol/g DW. Among individual GS, glucoraphanin and glucoraphenin showed consistently increasing trend with altitude. Increase in GS content with increasing altitude could be because of enhanced environmental stress, particularly chilling, experienced by the plants. Generally, when plants are stressed, secondary metabolism may increase, because growth is often limited more than photosynthesis and carbon fixation is predominantly invested to secondary metabolites production[20]. GS concentration is reported to be linearly related to seed size[21]. We observed a positive linear relationship between

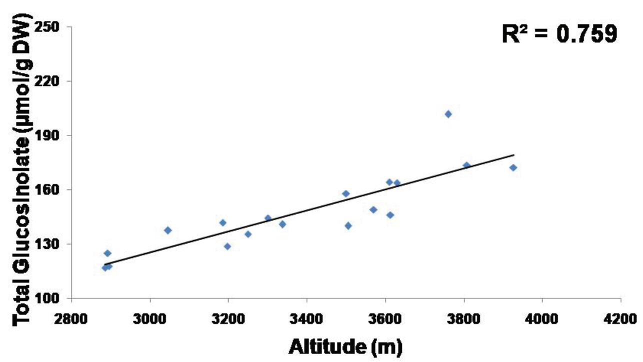

Figure 1. Change in glucosinolate contents in radish seed along an altitudinal gradient in trans-Himalaya.

Table 1. Individual and total glucosinolates (µmol/g DW) in radish seed along an altitudinal gradient in trans-Himalaya

Altitude	GRA	GRE	GNF	4HGBS	ERU	GRS	BRA	4MGBS	UNK	Total GS
2800-3000	3.0 ±0.6^{a}	97.9 ±5.0^{a}	2.5±0.3^{a}	4.0±0.5^{bc}	1.7±1.4^{a}	6.7±3.3^{a}	1.3±1.4^{a}	1.7±2.8^{a}	0.92±0.99^{b}	119.8±4.2
3001-3200	2.5 ±0.4^{a}	105.3 ±7.0^{ab}	2.6±0.3^{ab}	3.3±0.4^{ab}	1.0±0.8^{a}	17.9±8.8^{bc}	2.3±1.1^{a}	0.8±0.4^{a}	0.09±0.06^{ab}	135.9±6.7
3201-3400	3.1 ±0.4^{ab}	117.6±9.5^{bc}	2.5±0.4^{a}	2.9±0.2^{a}	1.9±1.4^{a}	8.9±4.7^{ab}	2.1±1.1^{a}	0.7±0.1^{a}	0.48±0.69^{ab}	140.1±5.2
3401-3600	3.2 ±0.4^{ab}	126.3 ±11.7^{c}	3.1±0.3^{b}	3.5±0.4^{abc}	1.4±0.8^{a}	8.7±2.8^{a}	2.1±0.5^{a}	0.5±0.2^{a}	0.04±0.05^{a}	148.8±8.9
3601-3800	4.0±0.6^{bc}	127.9 ±10.0^{c}	3.2±0.3^{b}	4.0±0.7^{bc}	1.9±1.2^{a}	14.6±1.4^{abc}	1.5±0.5^{a}	0.6±0.2^{a}	0.08±0.03^{ab}	157.9±9.6
3801-4000	4.5 ±0.6^{c}	146.3 ±8.2^{d}	3.9±0.2^{c}	4.3±0.9^{c}	1.9±1.2^{a}	19.5±6.3^{c}	1.3±0.4^{a}	0.5±0.1^{a}	0.11±0.05^{ab}	182.4±15.3
Total	**3.4 ±0.8**	**120.2±18.0**	**3.0±0.6**	**3.7±0.7**	**1.6±1.1**	**12.7±6.9**	**1.8±0.9**	**0.8±1.1**	**0.29±0.56**	**147.5±21.5**

Values represents the mean±SD, for each column different superscript indicate significantly different at $p<0.05$

GRA: glucoraphanin; GRE: glucoraphenin; GNF: gluconapoleiferin; 4HGBS: 4-Hydroxyglucobrassicin; ERU: Glucoerucin; GRS: Glucoraphasatin; BRA: Glucobrassicin, 4MGBS: 4-methoxyglucobrassicin; UNK: Unknown; GS- glucosinolate

seed size and altitude, which may contribute to increasing GS contents along the altitudinal gradient. Chilling temperature led to higher levels of glucosinolates- especially aliphatic glucosinolate in *Arabidopsis thaliana* accessions[22]. Broccoli grown at field condition during spring and summer season had higher glucosinolate content than autumn and winter associated with higher temperature, higher irradiance and longer photoperiod[23].

3.3 Altitudinal Variation in Seed Quality Characteristics

Altitude of seed origin has significant impact on seed quality characteristics (Fig. 2). Increasing altitude is related linearly to increase in 100 seed weight (R^2=0.266) and seed weight per plant (R^2=0.849). A steady decreasing trend was observed in seed moisture content (R^2=0.831) and siliqua length (R^2=0.786) with increasing altitude. Seed weight is a crucial plant life history trait, determining establishment success and dispersal ability of a species. In stressful environmental conditions, larger seeds have a better chance of giving rise to an established offspring[24]. Increase in seed weight with increasing altitude observed in the present study is in agreement with previous reports[24-26]. However, negative relations have also been reported[27,28].

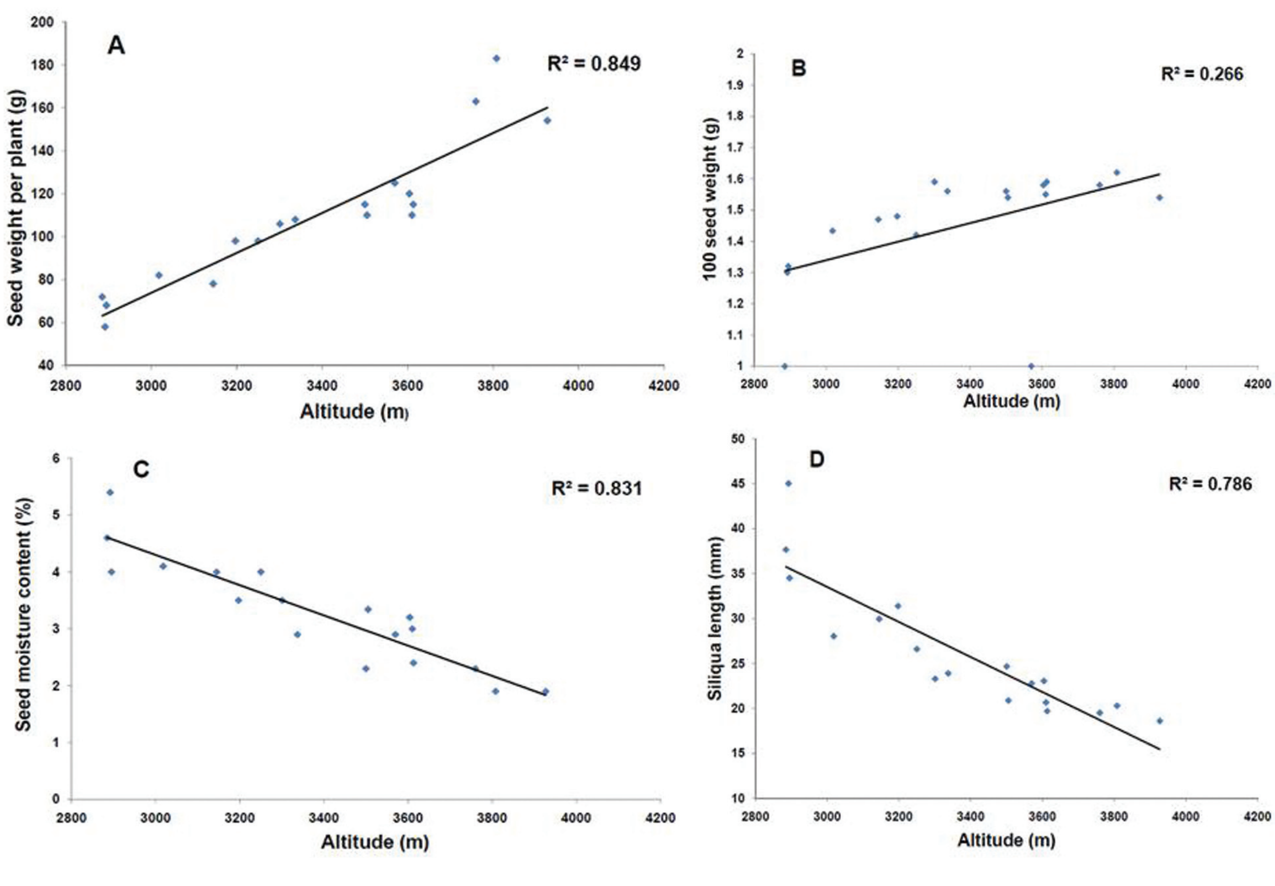

Figure 2. Altitudinal variation in radish seed quality characteristics : (a) Seed weight per plant, (b) 100 seed weight, (c) seed moisture, and (d) Siliqua length

Table 2. Pearson's correlation coefficients of altitude, seed quality characteristics and glucosinolate contents in radish seed

Variable	Altitude	1S Length	2Seeds/S	3SW/plant	4Moisture	5Dg	6SVI	7RY	8GS
Altitude	1								
1S Length	-.880**	1							
2Seeds/S	-.419	.570*	1						
3SW/plant	.919**	-.803**	-.418	1					
4Moisture	-.884**	.876**	.414	-.894**	1				
5Dg	.661**	-.423	-.289	.628**	-.440	1			
6SVI	-.547*	.442	.186	-.539*	.607**	-.151	1		
7RY	-.167	.069	.565*	-.32	.131	-.125	.060	1	
8GS	.900**	-.742**	-.473*	.870**	-.776**	.759**	-.424	-.221	1

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

1S Length: Siliqua length; 2Seeds/S: number of seeds per siliqua; 3SW/plant: seed weight per plant; 4Moisture: seed moisture percent; 5Dg: geometric mean diameter; 6SVI: seed vigour index; 7RY: reproductive yield; 8GS: total glucosinolate contents

3.4 Pearson Correlation Analysis

Table 2 shows the correlation between altitude, GS contents and seed quality characteristics. Altitude was positively correlated with total GS content (r = 0.900, $p \leq 0.01$), seed weight per plant (r = 0.919, $p \leq 0.01$) and geometric mean diameter (r = 0.661, $p \leq 0.01$), whereas negatively correlated with siliqua length (r = -0.880, $p \leq 0.01$), moisture content (r = -0.884, $p \leq 0.01$) and seed vigour index (r = -0.547, $p \leq 0.01$). GS content was negatively correlated with siliqua length (r = -0.742, $p \leq 0.01$), number of seeds per siliqua (r = -0.473, $p \leq 0.01$), and seed moisture content (r = -0.776, $p \leq 0.01$). Negative correlation between moisture content and GS might be because of the dilution factor in seeds[29].

4. CONCLUSION

In the current study, we reported identification of nine GS in radish seeds including five aliphatic and four indolic GS. Total GS, glucoraphanin and glucoraphenin increased with increasing altitude. Among the tested seed quality traits, seed weight and seed weight per plant was found to be positively correlated with increasing altitude whereas a negative trend was observed for seed moisture content and siliqua length. Overall, our results indicated that the concentration of seed GS and seed quality traits in radish can be significantly increased by growing plants at higher altitude.

Conflict of interest: None

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