Impact of Environments on the Accumulation of Capsaicinoids in *Capsicum* spp.

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***Abstract*. The use of a chili fruit is distinguished by its capsaicinoid content, which shows many beneficial effects in food and pharmaceutical applications. However, chilies exhibit wide variations in the accumulation of capsaicinoids depending on their genotype and environmental interaction. Therefore, we conducted experiments to evaluate the capsaicinoid responses of 14 cultivars of chili across four different elevations. Experiments were conducted during the rainy season from June to Oct. 2009 at elevations of 200 m asl (Khon Kaen) and 680 m asl (Chiang Mai) in Thailand and from Apr. to Sept. 2010 at elevations of 1400 m asl (Lobesa) and 1630 m asl (Kabesa) in Bhutan. A high-performance liquid chromatography technique was used to determine capsaicin and dihydrocapsaicin. Significant differences were observed among the cultivars, the locations, and the cultivar-by-location interactions. Large variations of cultivar effects indicate that it is possible to select cultivars for capsaicinoid concentration that are adapted over a wide range of environments. Average capsaicin, dihydrocapsaicin, and total capsaicinoids were greater at higher elevations in a particular year. There was significant correlation between capsaicin and total capsaicinoid contents with elevations, but capsaicinoid yield showed negative correlation. Small-fruited cultivars with high pungency showed consistent capsaicinoid production over different environments. Dallay khorsaney, KKU-P-22006, KKU-P-31141, and KKU-P-21041 cultivars showed high stability for pungency, producing high capsaicinoids at all four locations.**

Chili pepper belongs to genus *Capsicum* in the Solanaceae family, the only plant genus known to produce capsaicinoids. Capsaicinoids are acid amides of vanillylamine and C_9 to C_{11} branched-chain fatty acids and are responsible for pungency of the chili fruits. The major analogs are capsaicin and dihydrocapsaicin, accounting for more than 90% of the total capsaicinoid content in the majority of *Capsicum* spp. (Nunez-Palenius and Ochoa-Alejo, 2005). Genetically, capsaicinoid production is inherited as a dominant trait and is controlled by the *Pun1* locus (Blum et al., 2002). In their homozygous recessive state, *pun1/pun1*, capsaicinoids are not produced by chili peppers. The degree of pungency is also regulated by environment and by genotype–environment interaction; therefore, high variation of pungency level is found between and within genotypes (Harvell and Bosland, 1997; Zewdie and Bosland, 2000). The variation is attributable to pungency being a polygenic trait (Otha, 1962). Although chili peppers are considered autogamous, high rates of cross pollination (7% to 90%) have been recorded, which could lead to genetic variation of cultivars (Bozokalfa et al., 2009) and greater heterogeneity in pungency, especially in local cultivars. Pungency increases with environmental stress such as high temperature (Otha, 1960), water stress (drought or flooding) (Bosland and Votava, 2002; Sung et al., 2005), and soil fertility imbalances (Johnson and Decoteau, 1996; Medina -Lara et al., 2008; Montforte-Gonzalez et al., 2010). Medina-Lara et al. (2008) reported that nitrogen fertilizer significantly increased plant growth and fruit yield while maintaining high levels of capsaicin and that application of potassium has no effect on growth or productivity. Iwai et al. (1979) suggested that light exposure may be an important factor in the formation and accumulation of capsaicinoids. In their survey of wild *Capsicum chacoense*, Tewksbury et al. (2006) reported that the total capsaicinoid content increased significantly with increasing elevation.

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Innovative applications of capsaicinoids in the pharmaceutical industry have stimulated interest in the use of capsaicinoids, and more information is needed on the genotype and environmental effects on this important trait. The information will aid in selecting the variety and manipulating the environment to maintain the pungency according to market demand. Therefore, the objectives of our experiments were to evaluate the effects on capsaicinoids of the environment at different elevations and to identify cultivars suitable for production of capsaicinoids.

Materials and Methods

Fourteen chili cultivars from different genetic backgrounds, based on regions of cultivation and pungency, were used as treatments (Table 1). We categorized the cultivars into low [less than 50,000 Scoville Heat Units (SHU)], medium (50,000–100,000 SHU), and high (greater than 100,000 SHU) pungency groups.

Field experiments were conducted at two locations each in Thailand and Bhutan during the main growing season there. In Thailand, trials were conducted during June to Oct. 2009 at the Khon Kaen University experimental farm, Khon Kaen (KK) (lat. 18°51′ N, long 98°45′ E, 200 m asl) and the Royal Project Foundation Pangda, Chiang Mai (CM) (lat. 16°28′ N, long. 102°48′ E, 680 m asl). In Bhutan, trials were conducted during Apr. to Sept. 2010 at the research and training farm at the College of Natural Resources, Lobesa (LB) (lat. 27°30′ N, long. 89°52′ E, 1400 m asl) and a farmer's field in the Kabesa subdistrict (KB) (lat. 27°38′ N, long. 89°52′ E, 1630 m asl).

A randomized complete block design with three replications was used in all experiments. Plants were spaced at 60 cm between plants and 50 cm between rows. Irrigation and fertigation through drip was applied at all locations, ensuring that soil moisture and key plant nutrients were not limiting factors. Standard crop management practices from the nursery to harvest were applied for all locations.

Soil analysis was conducted for major nutrients, i.e., nitrogen (N), phosphorus, potassium (K), and organic matter before fertilizer application and after basal fertilizer application. Soil pH was also tested. For capsaicinoid analysis, 10 fully ripe fruits per replication from the second harvest were bulked and dried in a hot air oven at 80 °C until the weight remained constant. Capsaicinoids were extracted and quantified using high-performance liquid chromatography following the "short run" protocol (Collins et al., 1995). Capsaicin and dihydrocapsaicin were determined and pooled as total capsaicinoids. Capsaicinoid yield was calculated based on dry fruit yield by multiplying the capsaicinoid content per plant by the number of plants per hectare.

Combined analysis of variance (ANOVA) was done to estimate the main effects of cultivars, location, and their interactions on capsaicinoid content (Gomez and Gomez, 1984). Least significant difference at 0.05 levels was used to compare mean differences. The sum of squares attributed to locations was further partitioned into orthogonal comparison among capsaicinoid traits. Simple correlations among capsaicinoid traits, elevations, and weather conditions were analyzed.

Results

There was variation in physical and chemical properties of soil among the four experimental sites (Fig. 1). KK soil was sandy loam, CM soil was red clay, and LB and KB soils were sandy clay loam. Phosphorus content was very low in soils of LB and KB. KK soil had the lowest amount of organic matter and N. The nitrogen level in the LB and KB soils was considerably higher, whereas K in the KB and CM soils was considerably higher after basal fertilizer application.

Weather conditions of the two consecutive experimental years are shown in Figure 2. KK experienced mean maximum temperature higher by ≈4.5 °C compared with CM, and the mean maximum temperature at LB was higher by 2.5 °C compared with KB. Maximum temperature recorded was 34.8 °C at KK followed by 29.4 °C in LB. Solar radiation and relative humidity were similar at the two locations. Total rainfall in CM was 1087.5 mm, whereas KK received 666.9 mm; KB received 1236.7 mm of rainfall, which was double the amount received at LB.

Combined ANOVA showed significant differences for the cultivar, location, and cultivar × location (C × L) interaction for all capsaicinoid traits (Table 2). High variations resulting from cultivar were observed for capsaicin (73.3%), dihydrocapsaicin (77.5%), total capsaicinoids (76.5%), and capsaicinoid yield (79.9%). Location differences [(KK CM vs. LB KB) and (KK vs. CM)] were significant for the capsaicin, total capsaicinoids, and capsaicinoid yield and non-significant for dihydrocapsaicin (Table 2). However, LB vs. KB showed significant differences for capsaicin, dihydrocapsaicin, and capsaicinoids but not for the yield of capsaicinoids.

The mean capsaicinoid production of the two locations in Bhutan was greater than in Thailand (Table 3); however, LB, at 1400 m asl, produced the lowest mean capsaicinoids among the four locations. KB, situated at 1,630 m asl, gave the maximum capsaicinoids mean of 95,347 SHU, although the capsaicinoid yield was the lowest. CM, at 680 m asl, was found most suitable for high capsaicinoid yield because most cultivars gave higher capsaicinoids and high fruit yield (data not shown).

For both years, the average capsaicin, dihydrocapsaicin, and capsaicinoids were greater at higher elevations. Correlations between capsaicinoid traits and elevations were positively significant for capsaicin (r = 0.57) and total capsaicinoids (r = 0.37) but negatively significant for dihydrocapsaicin (r = –0.18) and capsaicinoids yield (r = –0.62).

Mean temperature and solar radiation showed significant negative correlation for capsaicinoids (r = –0.62 and r = –0.48, respectively). However, there was a positive correlation between temperature and capsaicinoid yield (r = 0.97). This is because dry fruit yield was low (data not shown) at low temperature and the yield of capsaicinoids is calculated based on the yield of dry fruit, consequently producing a low arithmetic result for capsaicinoid yield. Hence, for capsaicinoid production, both high fruit yield and high capsaicinoid concentration should be considered when selecting a variety.

There were significant correlations among rainfall and capsaicin (r = 0.75), dihydrocapsaicin (r = 0.85), and capsaicinoids (r = 0.84), except capsaicinoid yield (Table 4). Average capsaicin, dihydrocapsaicin, capsaicinoids, and capsaicinoid yield over four locations showed high variations of the low pungency group (Table 5). Additionally, the capsaicinoid

Table 1. List of 14 chili cultivars tested and their pedigree, source, pungency category, and characteristics.

Cultivar	Species	Pedigree	Source	Pungency category[z]	Characteristics
Baegap Ema	*annuum*	—	Bhutan	Low	Large fruit, elongated, pointed end
KKU-P-11012	*annuum*	Donyang	KKU	Low	Medium fruit, elongated, pointed end
KKU-P-11015	*annuum*	Numkeawtong80	KKU	Low	Large fruit, elongated, pointed end
KKU-P-11175	*annuum*	—	KKU	Low	Large fruit, elongated, pointed end
KKU-P-21031	*annuum*	C04538	AVRDC	Low	Large fruit, elongated, pointed end
KKU-P-32024	*annuum*	PI693662	USDA	Low	Medium fruit, elongated, pointed end
Sha Ema	*annuum*	—	Bhutan	Low	Large fruit, elongated blunt end
KKU-P-11003	*annuum*	Yodson	KKU	Medium	Small fruit, elongated, pointed end
KKU-P-12010	*annuum*	Keenoo-Pama	KKU	Medium	Small fruit, elongated, pointed end
KKU-P-21003	*annuum*	C05047	AVRDC	Medium	Small fruit, elongated, pointed end
Dallay khorsaney	*chinense*	—	Bhutan	High	Small, round to oblong fruit
KKU-P-21041	*annuum*	C05680-1	AVRDC	High	Small fruits, elongated, pointed end
KKU-P-22006	*annuum*	C00307	AVRDC	High	Small fruit, elongated, pointed end
KKU-P-31141	*annuum*	PI640500	USDA	High	Small fruits, elongated, pointed end

[z]High pungency = greater than 100,000 SHU Scoville Heat Units (SHU), medium = 50,000 to 100,000 SHU, low = less than 50,000 SHU.

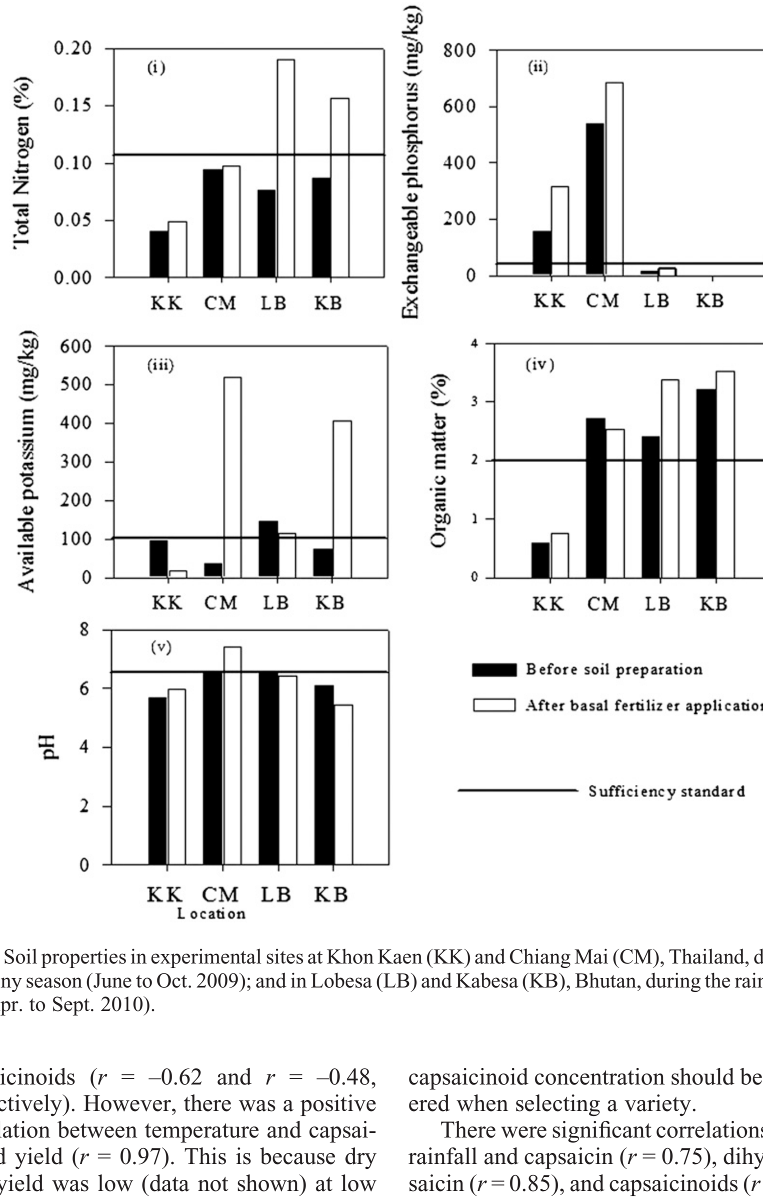

Fig. 1. Soil properties in experimental sites at Khon Kaen (KK) and Chiang Mai (CM), Thailand, during the rainy season (June to Oct. 2009); and in Lobesa (LB) and Kabesa (KB), Bhutan, during the rainy season (Apr. to Sept. 2010).

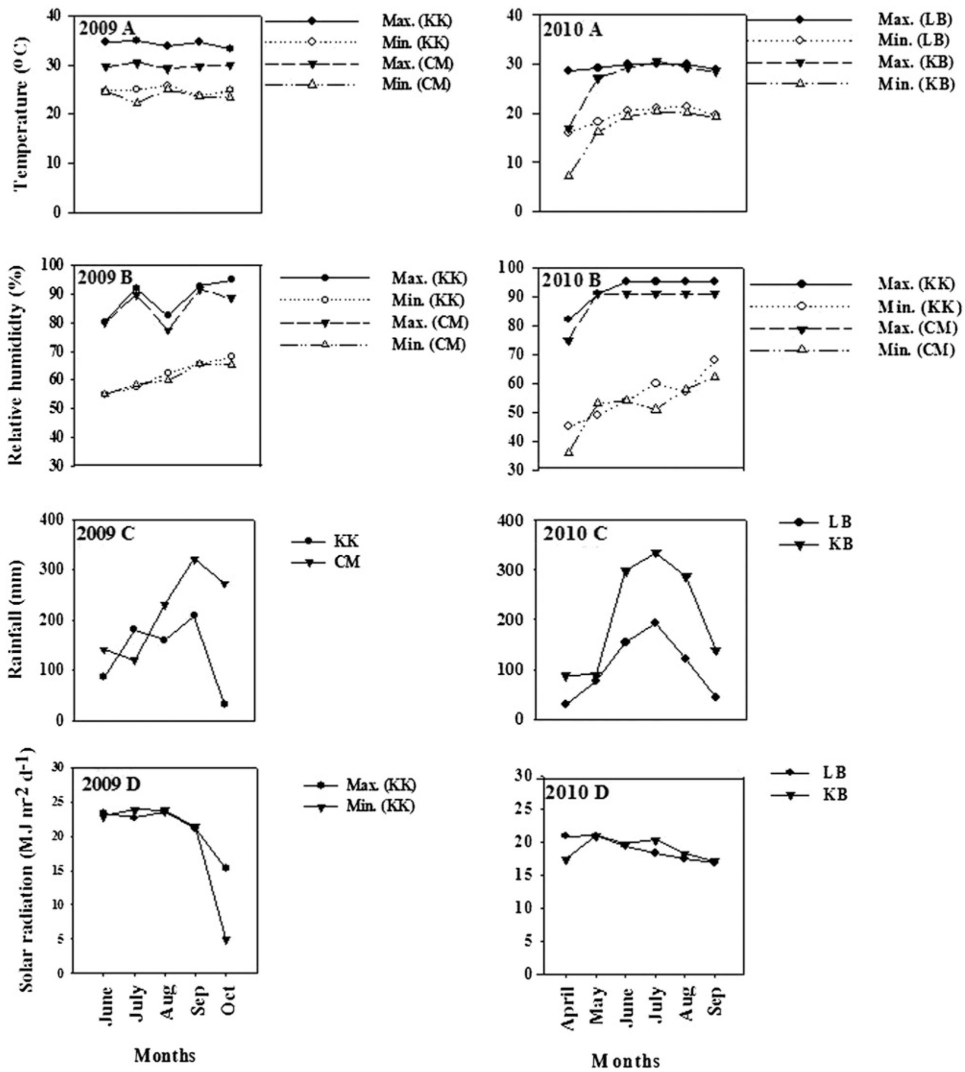

Fig. 2. Weather conditions in experimental site at Khon Kaen (KK) and Chiang Mai (CM), Thailand, during the rainy season (June to Oct. 2009); and in Lobesa (LB) and Kabesa (KB), Bhutan, during the rainy season (Apr. to Sept. 2010).

Table 2. Sources of variation for pungency characteristics in 14 chili cultivars grown during the rainy season at Khon Kaen (KK) and Chiang Mai (CM), Thailand, and Lobesa (LB) and Kabesa (KB), Bhutan.

Source of variation	DF	Capsaicin (SHU)	Dihydrocapsaicin (SHU)	Capsaicinoids (SHU)	Capsaicinoid yield (SHU)
Location (L)	3	7,381,000,000** (8.4)	3,926,000,000** (5.2)	13,380,000,000** (6.8)	101* (0.90)
KK CM vs. LB KB	1	3,361,884,152** (1.3)	554,973,109 NS (0.7)	1,936,172,207* (0.3)	10.9** (0.03)
KK vs. CM	1	1,807,409,548** (0.7)	72,007,563 NS (0.1)	2,972,359,032** (0.5)	5.4* (0.01)
LB vs. KB	1	16,970,706,300** (6.4)	3,299,019,328** (4.3)	35,241,468,761** (6.0)	1.6 NS (0.00)
Error L*R[a]	8	105,100,000 (0.3)	235,800,000 (0.3)	215,800,000 (0.3)	18 (0.41)
Cultivar (C)	13	14,920,000,000** (73.3)	58,840,000,000** (77.5)	34,860,000,000** (76.8)	2,021** (79.70)
L × C	39	943,700,000** (13.9)	8,762,000,000** (11.5)	1,766,000,000** (11.7)	108** (12.70)
Error L*R*C[b]	104	106,500,000 (4.2)	4,161,000,000 (5.5)	252,200,000 (4.4)	21 (6.6)
[a]CV %		21.99	21.22	20.56	31.22
[b]CV %		22.1	24.7	22.2	33.83

NS, *, **Nonsignificant, significant at $P \leq 0.05$ and significant at 0.01 P levels, respectively. Numbers within parentheses are percent sum squares.

content of low-pungency cultivars fluctuated as a result of elevations, whereas medium- and high-pungency groups showed consistently high capsaicinoid production as elevation increased (Fig. 3). Dallay khorsaney (*Capsicum chinense*) from Bhutan had the highest capsaicinoids of 182,672 SHU and highest capsaicinoid yield of 48.17 kg·ha^{-1}. In all locations, KKU-P-22006, KKU-P-31141, and KKU-P-21041 gave similar capsaicinoid contents of 132,459 SHU, 130,880 SHU, and 128,848 SHU, respectively. Capsaicinoid yield for these three cultivars ranged from 22.95 to 26.74 kg·ha^{-1}.

Discussion

The properties of the soil did not show any indication of their effect on capsaicinoids. Potassium content was greater at sites that produced high capsaicinoidscontent; however, previous studies have shown that, by itself, K did not have any effect on the synthesis of capsaicinoids (Johnson and Decoteau, 1996; Medina-Lara et al., 2008). Although some studies reported that N fertilization increased capsaicin (Johnson and Decoteau, 1996; Medina-Lara et al., 2008; Montforte-Gonzalez et al., 2010), that effect is ruled out because N was highest at LB but capsaicinoids was low.

Table 3. Capsaicin, dihydrocapsaicin, capsaicinoids, and capsaicinoid yield from 14 chili cultivars over four locations.

Location	Capsaicin (SHU)	Dihydrocapsaicin (SHU)	Total capsaicinoids (SHU)	Capsaicinoid yield ($kg\cdot ha^{-1}$)
Thailand (2009)				
Khon Kaen (200 m)	37,515[c]	26,481[b]	63,996[c]	13.5[a,b]
Chiang Mai (680 m)	46,793[b]	28,319[a,b]	75,112[b]	15.6[a]
Mean	42,154	27,400	69,554	14.5
Bhutan (2010)				
Lobesa (1400 m)	36,885[c]	17,496[c]	54,381[d]	13.0[b]
Kabesa (1630 m)	65,316[a]	30,031[a]	95,347[a]	11.8[b]
Mean	51,101	23,764	74,864	12.4
F-test	**	**	**	*

*, **Significant at $P \leq 0.05$ and 0.01 P levels, respectively. Means in the same column followed by the same letter are not significantly different at ($P \leq 0.05$) by least significant difference.
SHU = Scoville Heat Units.

Table 4. Correlation coefficient among pungency characteristics, elevations and weather conditions for 14 chili cultivars over four locations in Thailand and Bhutan.

	Capsaicin (SHU)	Dihydrocapsaicin (SHU)	Capsaicinoids (SHU)	Capsaicinoid yield ($kg\cdot ha^{-1}$)
Dihydrocapsaicin (SHU)[z]	0.93**			
Capsaicinoids (SHU)[z]	0.98**	0.97**		
Capsaicinoid yield ($kg\cdot ha^{-1}$)[z]	0.79**	0.84**	0.83**	
Elevation (m)[y]	0.57**	–0.18 NS	0.37**	–0.62**
Mean temperature (°C)[y]	–0.62**	–0.13 NS	–0.51**	0.97**
Relative humidity (%)[y]	–0.96**	–0.48**	–0.87**	0.46**
Rainfall (mm)[y]	0.75**	0.85**	0.84**	0.26 NS
Solar radiation ($MJ\cdot m^{-2}\cdot d^{-1}$)[y]	–0.48**	0.16 NS	–0.31*	0.18 NS

[z]n = 168.
[y]n = 56.
NS, *, ** Non-significant, Significant at $P \leq 0.05$ and 0.01 P levels, respectively.
SHU = Scoville Heat Units.

Table 5. Capsaicin, dihydrocapsaicin, capsaicinoids, and capsaicinoid yield of 14 chili cultivars over four locations in Thailand and Bhutan.

Cultivars	Capsaicin (SHU)	Dihydrocapsaicin (SHU)	Capsaicinoids (SHU)	Capsaicinoid yield ($kg\cdot ha^{-1}$)
Low pungency				
Baegap Ema	11,934[g]	9,302[e,f]	21,236[e,f]	3.85[g,h]
KKU-P-11012	21,419[e,f]	10,790[d,e]	32,209[d,e]	6.67[e–g]
KKU-P-11015	24,954[e]	15,057[d]	40,011[d]	8.22[e,f]
KKU-P-11175	17,690[e–g]	8,819[e,f]	26,509[e]	5.07[f–h]
KKU-P-21031	17,781[e–g]	9,021[e,f]	26,802[e]	4.16[g,h]
KKU-P-32024	10,313[g]	5,248[f]	15,561[f]	1.71[h]
Sha Ema	14,776[f,g]	11,618[d,e]	26,394[e]	4.95[f–h]
Medium pungency				
KKU-P-11003	53,045[c,d]	26,486[c]	79,531[c]	13.73[d]
KKU-P-12010	49,270[d]	25,399[c]	74,669[c]	8.94[e]
KKU-P-21003	59,501[c]	26,325[c]	85,826[c]	8.61[e,f]
High pungency				
Dallay khorsaney	120,616[a]	65,788[a]	186,404[a]	48.17[a]
KKU-P-21041	81,689[b]	47,159[b]	128,848[b]	26.74[b]
KKU-P-22006	82,337[b]	50,122[b]	132,459[b]	25.34[b,c]
KKU-P-31141	87,460[b]	47,007[b]	134,467[b]	22.95[c]
F-test	**	**	**	*

*, **Significant at $P \leq 0.05$ and 0.01 P levels, respectively. Means in the same column followed by the same letter(s) are not significantly different at ($P \leq 0.05$) by least significant difference.
SHU = Scoville Heat Units.

Low soil nutrients at KK could be the result of the leaching effect of sandy loam soil.

High temperature was reported to increase the accumulation of capsaicinoids; capsaicinoids were considerably higher in plants grown at 30 °C day and night temperature than those grown in the field temperature of 21 to 24 °C; higher night temperatures, in particular, are responsible for the higher capsaicinoid contents (Otha, 1960). However, in our result, capsaicinoids showed negative correlations with temperature and solar radiation. Capsaicinoid content was high at low temperature in CM and low at high temperature in KK. At higher temperature, higher respiration used most photosynthates for growth of the plant (as indicated by the bigger canopy; data not shown) and the rest of the photosynthates were shared among other biosynthetic pathways. Van Soest (1987) reported that high temperature increased lignifications of the plant cell wall and promoted rapid metabolic activity, which decreased other metabolites. Because capsaicin is a product of a metabolic pathway, competition between capsaicinoids and other compounds might have resulted in less capsaicinoids at higher temperatures.

High capsaicinoids at a high rainfall site such as CM and KB might be explained as was observed in New Mexico (Bosland and Votava, 2002), where pungency in chili pods increased after water was applied by furrow irrigation. This phenomenon may be ascribed here because although drip irrigation was appropriately applied at all locations, plants at CM and KB might have been subjected to excess water supply compared with other locations. Subsequently, plants suffered and increased capsaicinoid accumulation.

Based on the C × L interaction, mean capsaicinoid content was higher in Bhutan than in Thailand. However, considered by location, it was lowest in Lobesa, Bhutan. In addition, capsaicinoid content was greater at high-elevation areas of each country during its particular growing season. Our results concur with the finding by Tewksbury et al. (2006) that capsaicinoids increased at higher elevation, which they attributed to the higher levels of abiotic and biotic selective pressures at high elevation that influenced the chemical components of chili fruit. In addition, there is more ultraviolet B radiation, low total atmospheric pressure, and partial pressures of atmospheric gases such as oxygen and carbon dioxide at high altitudes (Körner, 2007); these could have influenced higher capsaicinoid production at higher altitude. Moreover, McLeod et al. (1982) theorized that pungency in *Capsicum* originated as a protective response to radiation from arid, high-elevation valleys of Bolivia. Although capsaicinoid content at LB was not as high as at KB, the difference in capsaicinoid yield was nonsignificant. This is attributed to higher fruit yield at LB. Furthermore, it is observed that the small-fruited cultivars with medium and high pungency showed a consistent increase in capsaicinoids at all locations. However, the low-pungency cultivars that did not perform consistently in capsaicinoid production at different elevations also varied in plant type and fruit size. Thus, it can be concluded that the small-fruited cultivars are less affected by environment compared with the medium- and large-fruited cultivars.

The highly significant differences of variances resulting from cultivar, compared with those of other sources of variation, indicated that it is possible to select the suitable cultivars with optimum capsaicinoid contents for particular locations (Zewdie and Bosland, 2000). Dallay khorsaney is a good chili cultivar for all the locations we examined; KKU-P-31141 is suitable for Kabesa, Bhutan, and KKU-P-21041 and KKU-P-22006 are good for Chiang Mai, Thailand, as a result of their high capsaicinoids at that particular location.

In summary, although the interaction of genotype and environment for capsaicinoids is a complex phenomenon, genotype plays

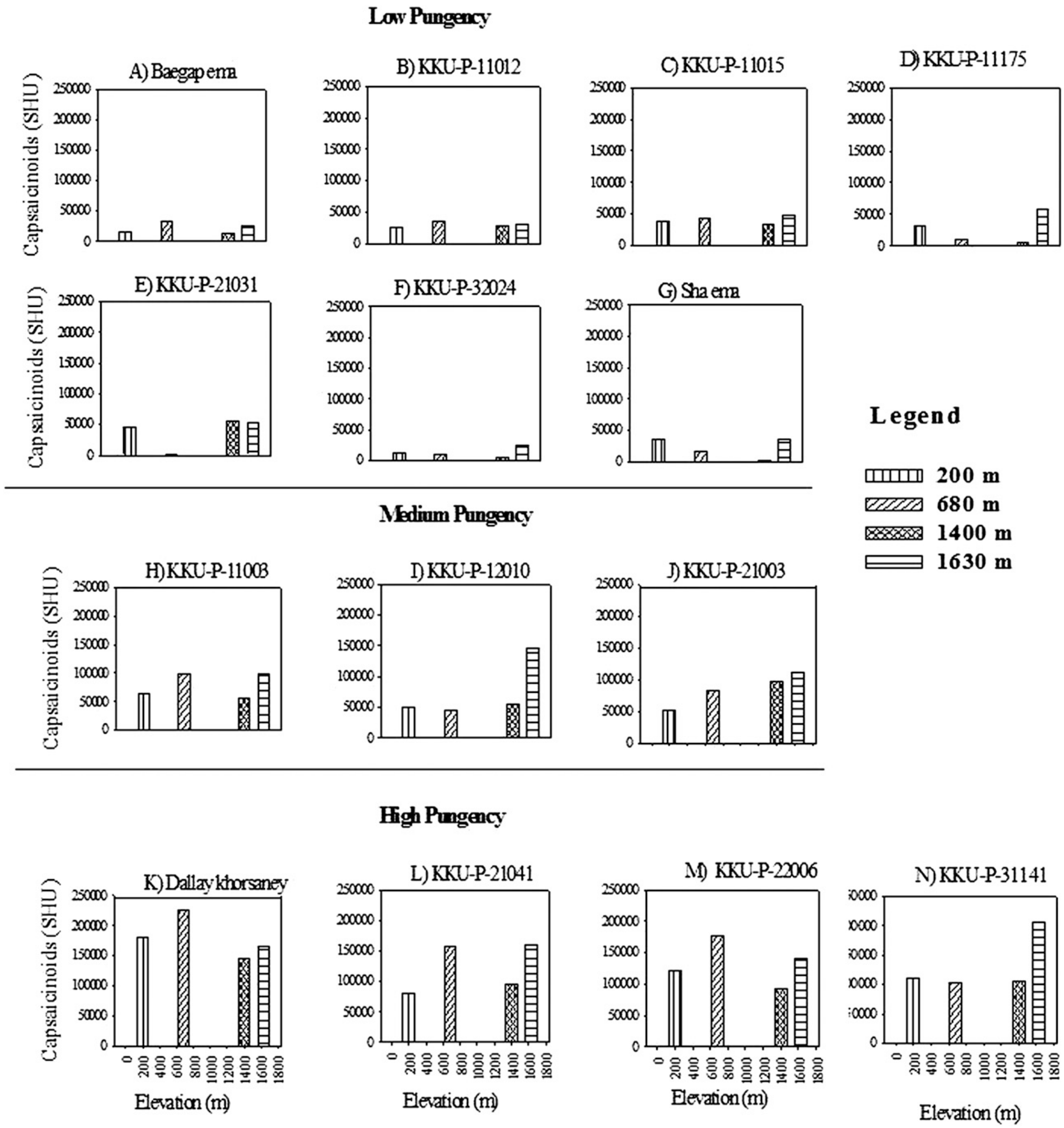

Fig. 3. Capsaicinoid contents of 14 chili cultivars grown over four elevations: 200 m and 680 m asl in Thailand; 1400 m and 1600 m asl in Bhutan. Bars show standard error.

a major role in the accumulation and content of capsaicinoids. Therefore, by selecting an appropriate cultivar and manipulating the stress level, growers can produce chili with the pungency required to meet the standards of the food and pharmaceutical industries.

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