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Stability analysis of yield and capsaicinoids content in chili (*Capsicum* spp.) grown across six environments

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Abstract There is a need for identifying chili cultivars with stable amounts of capsaicinoids so that the amount of pungency in the final product can be controlled. Therefore, six cultivars were evaluated for yield and capsaicinoid contents across six environments, four in Thailand and two in Bhutan, ranging from 200 to 1,630 m above mean sea level. Combined analyses showed significant differences among cultivars, environments, and for cultivar by environment interactions for fruit yields, individual capsaicinoid and total capsaicinoid contents. A large proportion (46.1 %) of variation on yield was attributed to environments; however, for total capsaicinoid contents, genotype effect accounted for 74.2 % of variation. Variation due to environment was 5.8 %, while for cultivar by environment interaction was 15.8 %. Cultivar Dallay khorsaney had high capsaicin, dihydrocapsaicin and total capsaicinoids but was very sensitive to environmental changes, and therefore good for specific adaptation. Cultivar KKU-P-11003 with total capsaicinoid contents of 78,721 Scoville heat unit was stable for dry fruit yield, capsaicin, dihydrocapsaicin, and total capsaicinoids with regression coefficients $b = 1.06$, 1.06, 0.78, and 0.96 respectively. Therefore, KKU-P-11003 was considered suitable for diverse environments. In addition, this result indicates that it is possible to select stable cultivar for capsaicinoid contents.

Keywords Capsaicin · Cultivar · Dihydrocapsaicin · Environment · Genotype · Interaction

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Introduction

Stability of pungency in fresh chili and in processed chili products is one of the concerns in food industries. The manufacturers can label the food products as hot, medium or low if the specific pungency levels are maintained (Zewdie and Bosland 2000). Moreover, capsaicinoids use in pharmaceutical and cosmetic industries is increasing. Each of these applications requires a specific level of capsaicinoids. Therefore, there is a need for identifying chili cultivars with stable amounts of capsaicinoids so the amount of pungency in the final product can be controlled. A genotype or cultivar that shows consistent performance across different environments and years for a given trait is considered stable. Although, the pungency characteristic is affected by genotype and environmental factors (Zewdie and Bosland 2000), cultivar has a major effect (Gurung et al. 2011). Therefore, it is possible to select widely adapted stable cultivar for pungency. Plant breeders can selectively develop cultivars with certain ranges of pungency. Partitioning of growing environments to reduce genotype × environment (G × E) interaction is challenging especially in regions where climatic variation is large. Therefore, evaluation of cultivars by stability parameters across multi-environments is important to identify the consistent performing and high yielding cultivars.

There are several methods developed to assess stability of cultivars across environments. However, each method has its advantages and limitations. Combined analysis of variance (ANOVA) has been used to detect G × E interactions and their magnitude. However, this analysis does not provide the measurement of response by individual genotype to environments. Regression technique was proposed by Finlay and Wilkinson (1963) and was improved by Eberhart and Russell (1966). This is a popular method in stability analysis and has been applied in many crops. Given the limitation of information on the stability of capsaicinoid contents in chili, this study was conducted across six environments to understand the responses and to identify varietal stability on capsaicinoids.

Materials and methods

Plant materials and field experiments

Six cultivars (Table 1) from different sources and with different pungency levels were used for stability test. These cultivars were sampled among the most promising cultivars from the previous experiments. Field experiments were conducted across six environments, four in Thailand and two in Bhutan. In Thailand, the main growing season or rainy season experiments were conducted from June–October 2009 and dry season was done from October 2010–April 2011 at Khon Kaen (KK) University farm (18°51′N and 98°45′E, at 200 m a.s.l) and at Royal Project Foundation Research Centre Pangda, Chiang Mai (CM) (16°28′N and 102°48′E, at 680 m a.s.l). In Bhutan, experiments were conducted during chili growing season i.e. April–September 2010 at the College of Natural Resources farm, Lobesa (LB) (27°30′N and 89°52′E, at 1,400 m a.s.l) and a farmer's field in the Kabesa sub-district (KB), (27°38′N and 89°52′E, at 1,630 m a.s.l). A randomized complete block design with three replications was used in all experiments. The plants were spaced 60 cm between plants, and 50 cm between rows. Standard crop management practices, through nursery to harvest, were followed in all locations. Drip irrigation system was laid out in all the experiments so that soil moisture was not limiting. Environment data such as soil properties, temperature, relative humidity, rainfall and solar radiation were recorded (Table 2). Four subsequent harvests were done and fruits were sundried for 2 days and then oven dried at 80 °C for 12–24 h to obtain the constant dry weight.

Capsaicinoids analysis

Capsaicinoids were extracted and quantified according to the 'short run' method with high performance liquid chromatography (HPLC), and the data was converted to Scoville heat unit (SHU), as described by Collins et al. (1995).

Table 1 Descriptions of chili cultivars used in experiments

Cultivar	Pedigree name	Source	Fruit type	Pungency[a]	Species	Fruit characteristics
KKU-P-11015	Num keaw tong 80	Thailand	Long cayenne	Low	*C. annuum*	Medium, elongated, pointed end
KKU-P-11175	–	Thailand	Long cayenne	Low	*C. annuum*	Large, elongated, pointed end
KKU-P-11003	Yodson khem 80	Thailand	Chili	Medium	*C. annuum*	Small, elongated, pointed end
Dallay Khorsaney	–	Bhutan	Chili	High	*C. chinense*	Small, round to oblong
KKU-P-21041	C05680-1 (Punjab Lal)	India	Chili	High	*C. annuum*	Small, elongated, pointed end
KKU-P-22006	C00307	Taiwan	Bird chili	High	*C. annuum*	Small, elongated, pointed end

[a] <50,000 SHU is considered as low pungency; 50,000–100,000 SHU as medium pungency; >100,000 SHU as high pungency

Table 2 Descriptions of environments where trials were conducted during 2009–2011

Environments	Planting date	Geographical coordinates	Altitude (m asl)	Temperature (°C)		Relative Humidity (%)		Rainfall (mm)	Solar radiation (MJ m^{-2} day^{-1})	Soil type
				Max	Min	Max	Min			
Khon Kaen (KK1)	June 2009	16°28′N 102°48′E	200	34.8	23.6	88.4	61.7	133.3	23.7	Sandy loam
Chiang Mai (CM1)	June 2009	18°51′N 98°45′ E	680	30.4	22.1	85.4	60.8	219.6	20.6	Red clay
Lobesa (LB)	April 2010	27°30′N 89°52′E	1,400	32.2	16.4	92.1	55.0	103.2	18.1	Sandy clay loam
Kabesa (KB)	April 2010	27°38′N 89°52′E	1,630	30.5	13.6	88.0	52.0	206.1	18.9	Sandy clay loam
Khon Kaen (KK2)	October 2010	16°28′N 102°48′E	200	31.3	19.5	83.1	56.3	0.8	10.3	Loamy sand
Chiang Mai (CM2)	October 2010	18°51′N 98°45′E	680	28.0	17.6	88.6	54.5	1.1	16.4	Silty loam

Data analysis

Yield and capsaicinoid traits were statistically analyzed for each environment. Error variances were tested for homogeneity with Bartlett's test as described by Gomez and Gomez (1984). Duncan multiple range test (DMRT) was used to calculate to compare mean differences for significant cultivar and environment effect by using MSTAT-C software (Russel 1994). Combined analysis of variance was done for six environments according to a statistical model explained by Freeman and Dowker (1973). Since there was significant interactions between G × E, stability parameters were calculated as suggested by Eberhart and Russell (1966). Means across environments, linear regression coefficient (*b*), deviation from regression (Sd^2) of genotypes means over environment index were calculated. Regression coefficient *(b)* value was tested for its difference from 0 and if significant it was tested from 1.

Results

Cultivar by environment interaction

The results of combined analysis of variance for yield and capsaicinoid traits are presented in Table 3. There were significant differences among cultivars, environments, and for cultivar by environment interactions for all traits. A large proportion (47.9 %) of variation on yield in dry weight per plant was attributed to environments. Source of variation on yield by Cultivar (G) × environment (E) and cultivar accounted, respectively, for 19.5 and 19.5 % of the total variation. However, for capsaicinoids high variations due to cultivar were observed for individual and total capsaicinoid contents. Variations due to cultivar were 71.9, 68.7, and 74.2 % for capsaicin, dihydrocapsaicin, and total capsaicinoid contents, respectively.

Environment evaluation

Due to highly significant differences among cultivar by environment interactions, the mean of six cultivars for yield and capsaicinoid traits from each environment was used to rank the environmental effects on each trait as suggested by Finlay and Wilkinson (1963). Among varieties, KKU-P-11015 produced high mean yield of 174.8 g/plant across six environments, however, it was not significantly different from KKU-11175, Dallay khorsaney and KKU-P-21041 (Table 4). KK1 in rainy season was the most favorable environment with mean yield of 196 g/plant. Mean yield at Kabesa Bhutan, during rainy season was not

Table 3 Combined analysis of variance for yield and capsaicinoid traits of six chili cultivars evaluated in six environments during 2009–2011

Source of variation	df	Mean Square			
		Dry weight (g/plant)	Capsaicin (SHU)	Dihydrocapsaicin (SHU)	Total capsaicinoid (SHU)
Cultivars (G)	5	12,966.2 (19.5)**	2.77×10^{10} (71.9)**	1.19×10^{10} (68.7)**	7.56×10^{10} (74.2)**
Environment (E)	5	31797.5 (47.9)**	3.32×10^{9} (8.6)**	1.21×10^{9} (7.0)**	5.92×10^{9} (5.8)**
Error (E x Rep)[a]	5	810.0 (2.9)	1.07×10^{8} (0.7)	6.84×10^{7} (0.9)	3.1×10^{8} (0.7)
G x E	12	2576.4 (19.5)**	1.15×10^{9} (14.9)**	6.81×10^{8} (19.7)**	3.21×10^{9} (15.8)**
Error (E × Rep × G)[b]	25	563.4 (10.2)	1.24×10^{8} (3.9)	5.29×10^{7} (3.7)	2.97×10^{8} (3.5)
CV (%)[a]		19.4	17.0	21.4	17.9
CV (%)[b]		16.2	18.2	18.9	17.3

Numbers in parentheses are % sum of squares which shows the % of variation

[a] and [b] shows coefficient of variation due to error (E × Rep) and error (E × Rep × G), respectively

** Significant at $P \leq 0.01$ probability level

significantly different from yield during dry season at Khon Kaen and Chaing Mai. The variation on individual yield ranged from 44.4 g/plant for KKU-P-11003 at Chiang Mai dry season to 270.7 g/plant for KKU-P-11015 at Khon Kaen during rainy season.

Capsaicin, dihydrocapsaicin, and total capsaicinoids showed similar responses among cultivars across six environments. Dallay khorsaney produced the highest capsaicin (115532.5 SHU), dihydrocapsaicin (74212.1 SHU), and total capsaicinoids (187100.7 SHU) among cultivars, across all environments (Table 5). Environmental mean for capsaicin (79,048 SHU) was highest at Kabesa during rainy season. For dihydrocapsaicin (46257.3 SHU) it was highest at Khon Kaen during dry season. For total capsaicinoids the highest value (120,394 SHU) was obtained at Chiang Mai during rainy season. In addition, among individual cultivars, Dallay khorsaney produced the maximum capsaicin (160471.3 SHU) at Chaing Mai during rainy season. However, dihydrocapsaicin

Table 4 Dry fruit weight (g/plant) of six chili cultivars grown at six environments during 2009–2011

Cultivars (G)	Environments (E)						
	KK1	CM1	LB	KB	KK2	CM2	Mean
KKU-P-11015	270.7 [a]	170.0	221.3	136.6 [b]	130.3[a]	119.5 [b]	174.8 [A]
KKU-P-11175	192.7 [b]	191.3	207.1	126.2 [bc]	142.4[a]	153.0 [a]	168.8 [A]
KKU-P-11003	173.0 [b]	132.3	157.7	72.2 [c]	91.9 [b]	44.4 [d]	112.0 [C]
Dallay khorsaney	177.5 [b]	195.2	218.7	198.8 [a]	86.1 [c]	71.2 [cd]	158.6 [AB]
KKU-P-21041	210.8 [b]	191.8	152.7	109.3 [bc]	93.6 [b]	137.1 [ab]	149.3 [AB]
KKU-P-22006	153.0 [b]	188.4	138.3	70.4 [c]	92.9 [b]	81.2 [c]	120.7 [BC]
Mean	196.0 [A]	178.2 [A]	182 [A]	119.0 [B]	106.3 [B]	101.1 [B]	147.4
F test	**	ns	ns	**	**	**	
CV%	10.4	16.3	20.4	18.4	13.8	11.8	

KK1 Khon Kaen rainy season 2009, *CM1* Chiang Mai rainy season 2009, *LB* Lobesa rainy season 2010, *KB* Kabesa rainy season 2010, *KK2* Khon Kaen dry season 2010–2011, *CM2* Chiang Mai dry season 2010–2011

** Significant at $P \leq 0.01$ probability level. Mean in the same column and row followed by a common letter are not significantly different at $P \leq 0.01$ by DMRT. Different capital letter(s) indicate significant difference between environments and between cultivars

(137,077 SHU) and total capsaicinoids (266,405 SHU) during dry season was highest at Khon Kaen. In summary, all capsaicinoid traits among cultivars followed this ranking: Dallay khorsaney > KKU-P-21041 > KKU-P-22006 > KKU-P-11003 > KKU-P-11015 > KKU-P-11175 (Table 5). However, there was no significant difference between KKU-P-21041 and KKU-P-22006.

Stability for yield and capsaicinoids

Stability parameters for yield and capsaicinoid traits are shown in Table 6. KKU-P-11003 showed good stability for all traits with $b = 1.06$, 1.06, 0.78, and 0.96 for dry fruit yield, capsaicin, dihydrocapsaicin, and total capsaicinoids respectively (Table 6). It indicates general adaptability in all traits. KKU-P-

Table 5 Capsaicin, dihydrocapsaicin and total capsaicinoid contents (SHU) of six chili cultivars grown at six environments during 2009–2011

Cultivars (G)	Environments (E)						Mean
	KK1	CM1	LB	KB	KK2	CM2	
Capsaicin contents (SHU)							
KKU-P-11015	21794.4 cd	26201.1 d	20828.0 cd	30992.4 d	25401.0 d	7249.4 c	22077.7 D
KKU-P-11175	20565.9 d	8043.7 d	3752.2 d	38397.2 cd	8011.3 e	5513.4 c	14047.3 E
KKU-P-11003	35001.5 cd	63727.5 c	41159.9 bc	72290.4 bc	52297.6 c	40514.7 b	50831.9 C
Dallay khorsaney	104688.8 a	160471.3 a	108945.6 a	108356.7 a	145198.5 a	65535.7 b	115532.8 A
KKU-P-21041	40958.7 c	93429.6 bc	60974.1 b	131392.2 a	73858.1 b	102209.9 a	83803.7 B
KKU-P-22006	69201.5 b	106572.3 b	60707.9 b	92865.0 ab	57941.9 c	94226.4 a	80252.5 B
Mean	48701.8 CD	76407.6 A	49394.6 D	79049.0 A	60451.4 AB	52541.6 BC	61091.0
F test	**	**	**	**	**	**	
CV%	16.1	18.5	20.0	20.0	7.5	20.2	
Dihydrocapsaicin contents (SHU)							
KKU-P-11015	17207.4 d	15175.0 bc	12246.8 c	15600.5 b	17877.6 de	10983.9 d	14848.5 D
KKU-P-11175	11072.0 d	2410.0 c	2379.5 bc	19415.5 b	4963.6 e	679.6 d	6820.0 E
KKU-P-11003	29755.8 c	34194.7 b	14689.9 bc	27302.3 b	25234.9 cd	31414.7 c	27098.7 C
Dallay khorsaney	76476.6 a	79458.3 a	49429.1 a	57787.7 a	137077.4 a	45043.5 b	74212.1 A
KKU-P-21041	38768.2 c	63170.9 a	34376.9 a	52321.0 a	52493.4 b	86500.6 a	54605.1 B
KKU-P-22006	53189.5 b	69506.9 a	30972.9 ab	46820.4 a	39896.5 bc	76768.6 a	52859.1 B
Mean	37744.9 B	43985.9 AB	24015.9 C	36541.2 B	46257.2 A	41898.5 AB	8407.3
F test	**	**	**	**	**	**	
CV%	12.2	24.0	31.4	19.1	15.3	12.4	
Total capsaicinoid contents (SHU)							
KKU-P-11015	39001.8 de	41376.1 de	33075.8 cd	46593.9 c	45566.1 bc	18106.2 d	37286.3 D
KKU-P-11175	31637.9 e	10454.7 e	6132.6 d	57813.8 c	12975.3 c	6193.0 d	20867.4 E
KKU-P-11003	64757.3 cd	97922.2 cd	55850.8 bc	99593.6 b	82276.4 b	71929.4 c	78721.3 C
Dallay khorsaney	181165.4 a	239930.6 a	158375. 7 a	166144.4 a	266405.0 a	110579.2 b	187100.7 A
KKU-P-21041	79727.0 c	15660.5 c	9535.0 b	183713.1 a	136836.6 b	188711.5 a	140156.3 B
KKU-P-22006	12239.9 b	176079. 2 b	9168. 8 b	139685.4 a	103802.9 b	170995.9 a	134106.5 B
Mean	86447.0 CD	120394.0 A	73410.0 D	115590.0 A	107977.0 AB	94419.0 BC	99705.5
F test	**	**	**	**	**	**	
CV%	13.9	19.2	24.1	23.6	8.0	15.6	

KK1 Khon Kaen rainy season 2009, *CM1* Chiang Mai rainy season 2009, *LB* Lobesa rainy season 2010, *KB* Kabesa rainy season 2010, *KK2* Khon Kaen dry season 2010–2011, *CM2* Chiang Mai dry season 2010–2011

** Significant at $P \leq 0.01$ probability level. Mean in the same column followed by a common letter are not significantly different at $P \leq 0.01$ by DMRT. Different capital letter(s) indicates significant difference between environments and between cultivars at $P \leq 0.01$

Table 6 Stability analyses for yield and capsaicinoids of six chili cultivars grown at six environments during 2009-2011

Cultivars	Dry fruit weight (g/plant)			Capsaicin (SHU)			Dihydrocapsaicin (SHU)			Total capsaicinoid (SHU)		
	Mean	*b*	Sd^2	Mean	*b*	Sd^2	Mean	*b*	Sd^2	Mean	*b*	Sd^2
KKU-P-11015	174.8	1.26	28.0	22,078	0.39**	6,774	14,849	0.14**	2,750	37,286	0.30**	10,176
KKU-P-11175	168.8	0.67	16.4	14,047	0.50**	12,978	6,820	−0.06**	7,990	20,867	0.38**	21,470
KKU-P-11003	112.0	1.06	17.7	50,832	1.06	3,078	27,099	0.78	4,129	78,721	0.96	3,917
Dallay khorsaney	158.6	1.08**	47.0	115,533	1.28**	36,455	74,212	2.28**	30,248	187,100	1.65**	54,655
KKU-P-21041	149.3	0.91	26.2	83,804	1.79**	23,450	54,605	1.60**	16,763	140,156	1.61**	37,651
KKU-P-22006	120.7	0.9 4	25.6	80,252	0.96**	18,289	52,859	1.55**	16,004	134,106	1.08**	33,824
Mean	147.4			61,091			38,407			99,706		

** Significant from 1.0 at $P \leq 0.01$ probability level indicates unstable cultivars

21041 with regression coefficient of $b = 0.91$ and with above average dry fruit yield (location mean yield = 149.3 g/plant) indicated general adaptability for fruit yield. Although, KKU-P-11003 and KKU-P-22006 had regression coefficient for yield of $b = 1.06$ and 0.94 respectively, their average yield was low, therefore more stable in diverse environments but in general low yielding. KKU-P-11015 gave high yield but had a higher regression coefficient of $b = 1.26$ indicating that this cultivar performed well under favorable conditions. Dallay khorsaney produced high yield and showed stability *(b* = 1.08**), but significantly different from 1.0 and high deviation from regression (47.0). This showed that this cultivar is very sensitive to changes in environment.

KKU-P-11003 was the only cultivar that showed stability for capsaicinoids. All other cultivars showed *b* value significantly different from 1.0 and high deviation from regression, indicating high fluctuation in capsaicinoid contents across environments. In KKU-P-22006, capsaicin and total capsaicinoid content were stable i.e. *b* = 0.96** and 1.08**, respectively. However, it was sensitive for dihydrocapsaicin (*b* = 1.55**). Dallay khorsaney had the highest of all capsaicinoid traits, but $b > 1$ and high deviation from regression. Therefore, this cultivar is considered to be good only for specific locations. KKU-P-11015 showed low coefficient of regression value for capsaicin (*b* = 0.39**) and dihydrocapsaicin (*b* = 0.14**). Similarly, KKU-P-11175 showed low regression values for capsaicin (*b* = 0.50**) and for dihydrocapsaicin (−0.06**). These indicate less responsiveness to changes in environments for capsaicinoids in these two cultivars.

Discussion

Significant G × E effects indicated that cultivar responded differently to changes in environments. High proportion of variation on yield was found for the environment effect, therefore more testing sites are needed or the environments in locations need to be controlled (Gill et al. 1984). Many studies reported that capsaicinoids content is affected by genetic and environment conditions (Iwai et al. 1979; Contreras-Padilla and Yahia 1998; Estrada et al. 1999; Zewdie and Bosland 2000). Moreover, Harvell and Bosland (1997) reported environment has stronger effect on capsaicinoids. In contrast, we found that cultivar plays a major role in capsaicinoid contents as more than 70 % of the variation was due to cultivar effect although G × E were significant. A large source of variation due to genotype was also reported by Zewdie and Bosland (2000) in their study of haploid, F1 hybrid and open pollinated genotypes, in three different environments. However, our cultivars were local varieties from several countries and were grown across six environments. Therefore, even with diverse environments, cultivars had more effect on capsaicinoids than environments.

Since there was significant cultivar by environment interaction it will lessen the usefulness of cultivar mean as single parameter to measure stability (Rasamivelona et al. 1995; Pritts and Luby 1990). Therefore, stability analyses were done. According to Eberhart and Russell (1966) model, a genotype is considered stable in performance if it has high mean performance, unit regression coefficient, and least deviation from regression. Cultivar with a regression value above one is considered unstable with higher sensitivity to environment change. It is good for specific adaptation in high yielding environment. Regression coefficient below one indicates that the cultivar is relatively stable with greater resistance to environment change. KKU-P-21041 was the most stable for fruit yield with above average fruit yields. This cultivar performed uniformly in all environments. Earlier studies by Zewdie and Poulos (1996) on KKU-P-21041 (Punjab Lal) assessed across seven environments of different countries showed that Punjab Lal was stable but with low yield. Although direct comparisons of results are considered irrelevant as the environments are different (Pritts and Luby 1990), our results are comparable on stability. Therefore, KKU-P-21041 is a good cultivar on yield stability. KKU-P-11015 and Dallay khorsaney is good for specific locations as the yield was high and was highly responsive to favorable environments. However, Dallay khorsaney was sensitive to dry season environments.

Considering our results on capsaicinoids, three groups of stability was observed for capsaicinoids which corresponds to the pungency level. KKU-P-11003, a medium pungency cultivar with regression values almost equal to one and low deviation from regression performed consistently for fruit yield, individual, and total capsaicinoids across six environments. Therefore, this was the most stable cultivar for capsaicinoids and could be selected for stability of capsaicinoid production. High pungency cultivars; Dallay khorsaney, KKU-P-21041 and KKU-P-22006 showed regression values more than one in most traits and high deviation from regression indicated its adaptation to specific locations. The low pungency varieties; KKU-P-11015 and KKU-P-21041 were least responsive with low regression values indicating more stability although deviations from regression were high. Zewdie and Bosland (2000) also reported high stability in genotypes with low capsaicinoid content and low stability in high capsaicinoid content which corresponds to our results. The result also showed a general tendency that a relatively stable capsaicin contents corresponds to the stable total capsaicinoids. This could be due to higher capsaicin concentration than dihydrocapsaicin. The stability in total capsaicinoids did not mean stability in individual capsaicinoids and vice versa as was observed in KKU-P-22006. This could be because of different gene actions and the inheritance studies reports that different genes are controlling the synthesis of each capsaicinoid (Garcés-Claver et al. 2007). However, because of the significant difference from unity and the large deviation from regression we could not conclude stability of other cultivars except KKU-P-11003 which was stable for all characters, across all locations. Since KKU-P-11003 showed good responses and was stable for stability for fruit yield, individual and total capsaicinoids across six environments, it could be a good genetic source for stability in breeding programs for capsaicinoids.

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