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Evaluation of genotype, environment, and genotype-by-environment interaction for capsaicinoids in *Capsicum annuum* L.

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Summary

The response, in terms of capsaicinoid content, of chile (*Capsicum annuum* L.) genotypes to different environments was studied. Double haploid lines, an F_1 hybrid, and an open-pollinated cultivar estimated the genotype, environment, and genotype-by-environment interaction effect on the total capsaicinoids and on individual capsaicinoids. Significant differences were observed among the genotypes and among genotype-by-environment interactions over the environments. Among the genotypes in an environment, the within-genotype variances were also significantly different. The double haploid line, HDA 207, had low within-genotype variance for individual and total capsaicinoids, with the exception of the isomer of dihydrocapsaicin. Also for HDA 270, the genotype-by-environment interaction was negligible for individual and total capsaicinoids, indicating stability across environments.

Introduction

Pungency, or the organoleptic sensation of heat, is a major quality-determining factor in chile (*Capsicum annuum* L.) and is caused by the presence of one or more of the fourteen alkaloid compounds known as capsaicinoids (Kobata et al., 1998). Krajewska & Powers (1988) reported a linear relationship between the pungency and the total amount of capsaicinoids. Therefore, the total pungency value of a given sample is obtained by adding the pungency values of the individual capsaicinoids.

Variation in pungency can be attributed to genotypic and environmental differences. Capsaicinoid content ranges in chile from zero up to more than 300,000 Scoville Heat Units depending on genotype (DeWitt & Bosland, 1993). Harvell & Bosland (1997) observed a significant difference in total capsaicinoid amount among individuals of a single homozygous genotype when grown in the field. They demonstrated that the environment could have a greater effect on pungency level than genotype.

Specific pungency levels must be maintained for food manufacturers to reliably label food products as mild, medium, and hot. Therefore, identifying chile genotypes that are stable for pungency levels across environments is a goal of the chile breeder. The stability of a genotype across environments is defined as the consistent performance of a genotype across different environments and/or years for a given character. In order to identify stable genotypes, the genotype-by-environment interactions must be partitioned into stability statistics that are assignable to each genotype evaluated across a range of environments (Fernandez, 1991). Stability indices have allowed researchers to identify widely-adapted genotypes for use in breeding programs and have helped to improve recommendations to growers (Pritts & Luby, 1990). Information pertaining to genotype-by-environment interactions for capsaicinoid content in chile is limited. Therefore, the objectives of this research were to measure the response of nine chile genotypes within and across environments for individual and total capsaicinoid content and to determine stable genotypes across environments.

Materials and methods

Seven double haploid lines, HD a1, HD a6, HD a25, HDA 207, HDA 233, HV 4, and HDA 248, were evaluated in 1996. From these, four double haploid lines, HD a25, HDA 207, HV 4, and HDA 248, were randomly selected and evaluated with an F_1 hybrid, 'Tula', and an open-pollinated cultivar, 'Sandia', in 1997. The F_1 hybrid and open-pollinated cultivar were included as checks.

Field experiments

Plants were grown at the Leyendecker Plant Science Research Center (LPSRC) in 1996 and 1997 and at the Fabian Garcia Science Center (FGSC) in 1997, Las Cruces, NM, USA. The soil type of LPSRC is agua clay loam and at FGSC is glendale loam (Bulloch & Neher, 1980). The years, 1996 and 1997, were considered as separate environments.

Seeds of each genotype were sown, in mid March in 1996 and in mid February in 1997, in the greenhouse and seedlings were transplanted to the field seven weeks after sowing. Individual plants were spaced 30 cm within the row and 100 cm between rows in each year. Standard growing practices for southern New Mexico were used (Bosland et al., 1994). The LPSRC field was furrow-irrigated as needed, usually at seven-to ten-day intervals. The field at FGSC was drip-irrigated. There were ten plants per plot. Four plants were randomly chosen from each replication and the red mature succulent fruits were harvested from the first four node positions. Fruits were bulked for analysis per plant.

Sample preparation and laboratory analysis

Capsaicinoids were extracted, separated, and quantified using high-performance liquid chromatography (HPLC) following the 'long run' method (Collins et al., 1995). The raw HPLC data was transformed to parts per million (ppm) using a modification of the formula described by Collins et al. (1995). Instead of multiplying the peak area of dihydrocapsaicin by 0.82 as in the formula, it was multiplied by 1.00, thus the total peak area of dihydrocapsaicin was considered in the calculation. Each sample was injected twice and the mean of two HPLC runs was used for the data analysis.

Statistical analysis

For the combined analysis of variances only four genotypes, HD a25, HDA 207, HV 4, and HDA 248, tested across the three environments were used to determine genotype-by-environment interaction for each capsaicinoid using the GLM procedure of SAS computer program (SAS Institute Inc., 1996). Genotypes were considered as fixed effects while environments were random effects. Significant levels were determined as suggested by McIntosh (1983) for combined analysis. Estimated variance components were calculated for genotype, environment, and genotype-by-environment interaction. Five capsaicinoids (nordihydrocapsaicin, capsaicin, dihydrocapsaicin, isomer of dihydrocapsaicin, and homodihydrocapsaicin) and their sum (total capsaicinoids) were examined. A significant genotype-by-environment interaction was detected, therefore, stability variance statistics (Shukla, 1972) were computed to determine the contribution of each genotype for the total genotype-by-environment interaction sum of squares using the IML procedure of SAS program (Kang, 1989). Fernandez (1991) reported that Tai's method was preferred to Shukla's method when evaluating small number of genotypes. Tai's method would be considered less conservative, that is genotypes found to be stable by Tai's method may not be stable by Shukla's method. Because the purpose of this manuscript was to investigate for the first time the stability of pungency, the more conservative, Shukla's method was chosen.

Within each environment, variances between plants (within-genotype variances) were determined based on expected mean squares for individual and total capsaicinoids. Heterogeneity for within-genotype variances among any two genotypes was determined by Hartley F-max test (Hartley, 1950).

Results and discussion

Variance across environments

Analysis of variance for individual and the total capsaicinoids across environments indicated significant differences among the four genotypes and their genotype-by-environment interactions. Significant differences among environments were observed only for nordihydrocapsaicin (Table 1). Variance components for genotypes exceeded the variance components for environment and genotype-by-environment interaction for individual and total cap-

Table 1. Mean squares for five capsaicinoids and their sum from four chile genotypes tested across three environments

Source of variation	df[a]	CAPSD	CAP	DH	NDH	ISO	HD
Environment	2	83415.85^{ns}	16790.18^{ns}	18412.04^{ns}	942.92*	406.51^{ns}	35.00^{ns}
Block (Environment)	6	17552.28	3647.47	4135.29	94.46	80.01	21.60
Genotype	3	2108175.24**	233638.10**	754125.80**	23433.33**	2983.62**	3062.20**
Genotype-by-environment interaction	6	113427.84*	12996.91*	38009.25*	1376.58**	148.10*	186.96**
Error	18	29923.79	4011.28	9694.45	211.55	54.55	31.59

[a] df = degree of freedom; CAPSD = total capsaicinoids; CAP = capsaicin; DH = dihydrocapsaicin; NDH = nordihydrocapsaicin; ISO = isomer of dihydrocapsaicin; HD = homodihydrocapsaicin.
^{ns},*, and ** non-significant, significant at 5, and 1% level, respectively.

Table 2. Estimated variance components for each capsaicinoid

Source of variation	CAPSD[a]	CAP	DH	NDH	ISO	HD
Environment	–1470.04	346.42	–1169.84	–26.38	19.41	–11.52
Genotype	221638.60	24515.69	79568.51	2450.75	315.66	319.47
Genotype-by-environment interaction	27834.68	2995.21	9438.26	388.55	31.18	51.79

[a] CAPSD = total capsaicinoids; CAP = capsaicin; DH = dihydrocapsaicin; NDH = nordihydrocapsaicin; ISO = isomer of dihydrocapsaicin; HD = homodihydrocapsaicin.

saicinoids (Table 2). The magnitude of the genotype-by-environment interaction component, when compared to the genotype variance component suggests that obtaining broadly adapted genotypes for pungency level may be possible, because the small genotype-by-environment interaction variance component should facilitate identifying broadly adapted genotypes (Delacy et al., 1996).

When the nature of the genotype-by-environment interaction was examined, changes in genotype rank and differences in magnitude were observed (data not shown). Rasamivelona et al. (1995) reported that change in a genotype rank across environments would lessen the usefulness of using genotype means as a way to select a stable cultivar. Stability variances provide useful information to assist in selecting stable genotypes. Stability variance defined as the contribution of a genotype to the genotype-by-environment interaction sum of squares after adjusting for the average genotypic contribution (Fernandez, 1991).

The genotypes varied in their average capsaicinoid content and stability variances across environments for individual and total capsaicinoids (Table 3). The contribution of HDA 207 to the total genotype-by-environment interaction sum of squares was non-significant for the capsaicinoids measured. In contrast, the contribution of HV 4 to the total genotype-by-environment interaction sum of squares was significant for individual and total capsaicinoids. The stability variances were significant for only some of the capsaicinoids in HDA 248 and HD a25 (Table 3).

Genotypes with a non-significant or negative stability variance would be considered stable across environments (Shukla, 1972), therefore, HDA 207 was stable for individual and total capsaicinoids. In contrast, HDA 248 was stable for the total capsaicinoids, but was not stable for the individual capsaicinoids; capsaicin, isomer of dihydrocapsaicin, and homodihydrocapsaicin. From our results, stability in total capsaicinoids does not imply stability for individual capsaicinoids. In addition, stability for several individual capsaicinoids does not guarantee stability in total capsaicinoids as demonstrated by HV a25 that was stable for capsaicin, an isomer of dihydrocapsaicin, and homodihydrocapsaicin, but was not stable for total capsaicinoids (Table 3).

Low capsaicinoid content and a high stability variance or conversely, high capsaicinoid content and low stability variance were observed. For example, HD a25 had higher capsaicin content than HDA 248, but the stability variance for HD a25 was about half the variance of HDA 248. HDA 248 had a lower isomer of dihydrocapsaicin content than HDA 270, but the stability variance for HDA 248 was about twice as high as the variance of HDA 270. The double haploid, HDA 207, had low capsaicinoid content and

Table 3. Average capsaicinoid content and stability variance for four chile genotypes

Genotype	CAPSD[a]		CAP		DH		NDH		ISO		HD	
	Mean[b]	Stability variance	Mean	Stability variance	Mean	Stability variance	Mean	Stability variance	Mean	Stability variance	Mean	Stability variance
HDA 207	303.70 c[c]	–33071.45^{ns}	83.42 c	–3563.69^{ns}	147.86 c	–13323.03^{ns}	7.70 c	–67.33^{ns}	58.36 a	121.46^{ns}	6.36 b	109.38^{ns}
HDA 248	664.30 bc	76081.80^{ns}	266.20 b	27511.74**	272.33 bc	645.39^{ns}	57.74 cb	360.24^{ns}	29.10 b	262.63*	39.46 a	284.08**
HV 4	973.20 b	190577.49**	419.62 a	14964.69*	421.53 b	68417.51**	77.27 b	2825.31**	15.65 b	223.42*	39.10 a	303.05**
HD a25	1445.50 a	220123.81**	424.63 a	13073.06^{ns}	814.52 a	96296.13**	131.13 a	2521.11**	27.01 b	–15.08^{ns}	48.25 a	50.94^{ns}

[a] CAPSD = total capsaicinoids; CAP = capsaicin; DH = dihydrocapsaicin; NDH = nordihydrocapsaicin; ISO = isomer of dihydrocapsaicin; HD = homodihydrocapsaicin.
[b] average of three environments and three replications in parts per million (ppm).
[c] means followed with the same letter in a column are not statistically significant at 5% level according to Duncan's new multiple range test. ^{ns}, *, and **non-significant, significant at 5, and 1% level, respectively.

low stability variance as compared to other genotypes. HD a25 is an example where a genotype exhibited high capsaicinoid content and high stability variance for dihydrocapsaicin, nordihydrocapsaicin, and total capsaicinoid (Table 3).

Within-genotype variance

The within-genotype variances of the genotypes were heterogeneous according to the F-max test at an upper 5% level (Tables 4, 5, and 6). When tested at LPSRC in 1996, the average total capsaicinoids content of HDA 248 was about three times larger than the content of HD a6; however, HDA 248 had a smaller within-genotype variance than HD a6 (Table 4). In comparison, HD a25 and 'Tula' had higher averages of capsaicinoid content and higher within-genotype variances than the other genotypes when they were tested in 1997 (Tables 5 and 6).

Genotypes with high averages tend to have high deviations or variances (Pritts & Luby, 1990); however, this relationship was not consistent for capsaicinoid content. Some genotypes with high capsaicinoid content had lower within-genotype variance than genotypes with low capsaicinoid content. Therefore, the magnitude of variance differences observed among genotypes could be due to genotypic effects rather than the magnitude of trait means. The within-genotype variance is often assumed to be high for open-pollinated cultivars because of their heterogeneous nature. However, some double haploid lines and 'Tula', the F_1 hybrid, exhibited higher within-genotype variances than the open-pollinated cultivar, 'Sandia', for most of the capsaicinoids.

The results indicated that a significant difference in the response of chile genotypes within and across environments for capsaicinoids. HDA 207 had the lowest within-genotype variance, except for isomer of dihydrocapsaicin, and was stable across environments for all capsaicinoids. The objective of the plant breeder is to develop a uniform and stable cultivar with a specific pungency level. Our results illustrate that selection for a stable genotype is possible.

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Table 4. Average and within-genotype variance for capsaicinoid content of chile genotypes at LPSRC in 1996

Genotype	CAPSD[a]		CAP		DH		NDH		ISO		HD	
	Mean[b]	Variance	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance
HDA 207	264.10	1494.66 d[c]	72.08	447.76 cd	127.28	59.26 d	3.33	0.00 d	58.07	90.01 ab	3.33	0.00 b
HDA 233	181.00	4855.47 cd	40.22	174.63 d	73.32	1351.91 c	16.25	56.82 c	31.61	171.46 a	19.61	128.39 a
HD a1	162.78	14638.13 bc	55.72	2379.21 bc	80.56	3642.53 bc	12.00	112.41 c	7.64	50.43 ab	61.11	75.10 a
HDA 248	654.72	38184.46 abc	286.33	7533.06 ab	271.42	13009.26 ab	44.45	66.11 c	17.38	12.40 bc	36.65	51.08 a
HD a6	213.77	39507.88 ab	72.14	5251.34 ab	100.34	11361.20 ab	18.55	184.49 bc	9.02	35.81 abc	13.72	116.10 a
HV 4	950.95	172526.93 a	366.49	25338.68 a	441.81	48397.09 a	88.75	1440.37 ab	8.33	5.07 c	45.55	159.10 a
HD a25	1131.84	203091.26 a	322.02	19931.98 a	649.01	67664.27 a	98.57	1849.43 a	20.59	82.12 ab	41.65	221.18 a
F-max$_{0.05,7,9}$		8.10										

[a] CAPSD = total capsaicinoids, CAP = capsaicin; DH = dihydrocapsaicin; NDH = nordihydrocapsaicin; ISO = isomer of dihydrocapsaicin; HD = homodihydrocapsaicin.
[b] average of three replications in parts per million (ppm).
[c] variances followed with same letter in a column are not heterogenous according to Hartley F-max test at upper 5% level.

Table 5. Average and within-genotype variance for capsaicinoid content of chile genotypes at LPSRC in 1997

Genotype	CAPSD[a]		CAP		DH		NDH		ISO		HD	
	Mean[b]	Variance	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance
HDA 207	361.54	6499.24 c[c]	106.09	764.97 c	167.69	1416.45 d	16.45	119.76 c	58.89	123.65 a	12.42	119.24 b
HDA 248	539.48	61026.27 b	218.95	10619.20 b	212.54	10510.29 cd	48.75	435.34 bc	27.35	112.78 a	31.90	155.19 b
Sandia	499.53	76637.91 b	207.48	17250.39 b	253.63	19702.45 bc	31.75	233.63 c	3.33	0.00 b	3.33	0.00 c
HV 4	1175.78	130007.79 b	524.02	30227.47 b	504.31	26573.74 bc	83.74	513.58 bc	24.62	–2.09 b	39.09	56.68 b
HD a25	1497.90	413325.36 ab	497.17	60488.23 ab	794.22	110469.10 ab	132.80	3239.67 ab	25.40	97.42 a	48.31	302.74 ab
Tula	4365.03	1700888.94 a	2015.11	406024.39 a	1872.02	321495.13 a	307.49	11093.08 a	49.57	144.79 a	120.83	1469.82 a
F-max$_{0.05,6,9}$		7.55										

[a] CAPSD = total capsaicinoids, CAP = capsaicin; DH = dihydrocapsaicin; NDH = nordihydrocapsaicin; ISO = isomer of dihydrocapsaicin; HD = homodihydrocapsaicin.
[b] average of three replications in parts per million (ppm).
[c] variances followed with same letter in a column are not heterogenous according to Hartley F-max test at upper 5% level.

Table 6. Average and within-genotype variance for capsaicinoid content of chile genotypes at FGSC in 1997

Genotype	CAPSD[a]		CAP		DH		NDH		ISO		HD	
	Mean[b]	Variance	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance
HDA 207	285.48	3778.87 c[c]	72.10	406.40 c	148.60	1340.21 d	3.33	0.00 d	58.12	64.69 b	3.33	0.00 d
HDA 248	798.56	2879.20 c	293.09	2261.77 c	333.04	3464.15 cd	80.03	65.19 c	42.59	–7.09 c	49.03	12.03 c
Sandia	583.52	296567.49 ab	236.05	44590.50 b	287.92	82550.42 ab	43.48	1313.40 ab	6.30	52.81 b	9.76	173.63 b
HV 4	792.78	99109.41 b	368.34	17337.19 b	318.48	20203.70 bc	59.32	700.88 b	13.98	125.48 ab	32.66	231.66 b
HD a25	1706.86	231892.30 b	454.69	22129.15 b	1000.33	78651.40 ab	162.02	1796.83 ab	35.03	37.66 b	54.79	187.16 b
Tula	5236.53	1784890.19 a	2526.65	365882.75 a	2234.68	376474.28 a	302.95	7522.67 a	49.74	597.03 a	122.51	1815.04 a
F-max$_{0.05,6,9}$		7.55										

[a] CAPSD = total capsaicinoids, CAP = capsaicin; DH = dihydrocapsaicin; NDH = nordihydrocapsaicin; ISO = isomer of dihydrocapsaicin; HD = homodihydrocapsaicin.
[b] average of three replications in parts per million (ppm).
[c] variances followed with same letter in a column are not heterogenous according to Hartley F-max test at upper 5% level.

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