

Pepper Photosynthesis, Stomatal Conductance, Transpiration, and Water Use Efficiency Differ with Variety, Indigenous Habitat, and Species of Origin

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Abstract. The instantaneous photosynthetic rate (P_n), transpiration rate (E), and stomatal conductance (g_s) were measured for 33 outdoor-grown *Capsicum* varieties (varying in species of origin and indigenous habitat) between 29 July and 22 Aug. 2017 using a portable gas exchange meter. Cuvette leaf temperature (T_{leaf}) and relative humidity (RH) were recorded at that same time. P_n differed from 3.6 to 3.7 for ‘Malawi Piquante’ and ‘Korean Long Green’ peppers to 16.3 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ (fixed) for ‘Thai Hot’ peppers. The g_s differed from 0.01 to 0.05 among 13 varieties to 0.28 $\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$ for ‘Thai Hot’ peppers. E differed from 0.43 to 0.59 among three varieties to 4.14 to 4.20 $\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$ for ‘CGN 22091’ and ‘Peruvian Purple’ peppers. Water use efficiency (WUE; P_n/E) varied from 2.92 to 3.43 among three varieties to 5.10 to 7.20 for 16 other varieties. *C. annuum* derived varieties had higher P_n (9.4 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ fixed) than varieties derived from other species (4.5–8.6 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ fixed). Varieties originating from dry climates had higher P_n (12.5 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ fixed) than those originating from temperate or tropical climates (8.0–8.8 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ fixed). T_{leaf} (27 to 33 °C) and RH (38% to 39% and 57% to 59%) differed among varieties. P_n was positively correlated with g_s , E , and RH and was negatively correlated with WUE. We found that *Capsicum* P_n , E , and g_s varied more than has been previously reported, and our data suggested that P_n , g_s , and E data of outdoor-grown peppers should be used only when selecting parents for a breeding program (unless progeny is intended for greenhouse production).

Peppers (*Capsicum* sp.) were one of the first crops domesticated in the Western Hemisphere using several independent, geographically distinct, and regional domestication efforts (Bosland and Votava, 2012). Cultivated peppers are derived from five

species (*C. annuum* L., *C. baccatum* L., *C. chinense* Jacq., *C. frutescens* L., and *C. pubescens*) among the 32 documented species in the *Capsicum* genus (Solanaceae family) (Qin et al., 2014). These domestication efforts resulted in peppers becoming a globally important fresh vegetable (fruit) and spice crop that is now consumed by nearly one-quarter of the world’s population, with fresh and dry pepper production reaching 32.2 and 3.8 million tons annually, respectively, in 2014 (FAO, 2014). In the United States, 44,800 acres of bell peppers and 19,400 acres of chili pepper were produced in 2015, with a wholesale value of \$806 million and \$135 million, respectively (USDA NASS Report, 2016). Peppers are consumed as a fresh food and contain carbohydrates, fiber, and a variety of nutrients, including antioxidants such as vitamin C, vitamin E, β -carotene, and carotenoids (Carvalho et al., 2011; Howard et al., 2000;

Howard and Wildman, 2007; Kantar et al., 2016; Palevitch and Craker, 1995; Russo and Howard, 2002; Topuz and Ozdemir, 2007; Wahyuni et al., 2011). Other pepper products are consumed as spices, including dried pepper flakes, processed “hot” sauces, and infused/pressed oils, among other products (Bosland, 1996; Zewdie and Bosland, 2001).

Abiotic factors such as low temperatures, high temperatures, salt stress, drought (Serrano et al., 2017), and waterlogging can limit pepper yield (Ou and Zou, 2012; Zhai et al., 2016). For instance, optimal temperatures for pepper photosynthesis range from 25 to 35 °C, and temperatures outside this range can limit the yield; for example, *C. chinense* flower abortion increased 2-fold and fruit set decreased 3-fold when greenhouse temperatures were increased from 30 to 40 °C (Garruna-Hernandez et al., 2014). Erickson and Markhart (2002) showed that *Capsicum* flower abortion after pollination was particularly sensitive to high temperatures (33 °C).

We determined whether photosynthetic and water use attributes differed among outdoor-grown pepper varieties that differed in parental species and indigenous climates. Previous studies of *Capsicum* focused on differences in photosynthetic and water use attributes among species only or varieties within a species. Past *Capsicum* research often used greenhouse-grown or controlled environment-grown plants. Our research objectives were to: 1) determine whether instantaneous P_n , g_s , E , WUE meter cuvette T_{leaf} , and RH of 33 outdoor-grown pepper varieties differed; 2) to determine whether variety photosynthetic and water use attributes differed based on indigenous parents or habitat; and 3) to determine whether pepper photosynthetic and/or water use attributes were correlated with each other.

Materials and Methods

Thirty-three pepper varieties derived from five species (*C. annuum* L., *C. baccatum* L., *C. chinense* Jacq., *C. frutescens* L., and *C. pubescens*) from three indigenous habitats (dry, temperate, and tropical) were selected (Table 1). Seeds were sourced from different seed producers/suppliers, and the reported species of origin were confirmed by phenotyping of flowers, leaves, and fruits during the experiment (Table 1). Seeds were sown 0.6 cm deep in Master Garden Premium media (Premier Tech Horticulture, Ltd., Ontario, Canada) in 32-cell trays (one seed per cell; individual cell volume = 150 cm^3) in January and February 2017. Trays were then covered with a clear plastic lid (10 cm above media) and were placed in a greenhouse (23 \pm 2 °C air temperature). After seeds germinated and cotyledons unfolded, the lid was removed and plants were grown for 9 to 14 additional weeks under natural daylight plus 25 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ supplemental irradiance (0800–0200 HR; Sunblaze T5 fluorescent lights; Sunlight Supply, Inc., WA; +1.62 $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ daily light integral) in the same greenhouse. After five leaves unfolded,

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Table 1. Variation in the instantaneous photosynthetic rate (P_n ; $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), stomatal conductance (g_s ; $\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$), transpiration rate (E ; $\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$), cuvette leaf temperature (T_{leaf} ; $^{\circ}\text{C}$), and relative humidity after 5 min (RH; %) and water use efficiency (WUE; $\text{WUE} = P_n/E$) among 33 outdoor-grown pepper varieties that vary in indigenous habitat and species. Indigenous habitats used for analysis are shown as superscript numbers after the variety name (¹desert; ²temperate; ³tropical; [?]unknown).

Variety	Country of Origin	Species	P_n	g_s	E	T_{leaf}	RH	WUE
Malawi Piquante ¹	Malawi	<i>baccatum</i>	3.6 a ^z	0.02 a	0.47 a	31 ab	44 abcdef	5.9 b
Korean Long Green ²	Korea	<i>annuum</i>	3.7 a	0.01 a	0.43 a	32 ab	40 abcd	6.8 b
Antohi Romanian ²	Romania	<i>annuum</i>	4.3 ab	0.05 a	1.40 abc	32 ab	42 abcdef	4.4 ab
Pakistan ¹	Pakistan	<i>annuum</i>	4.3 ab	0.02 a	0.59 a	33 b	39 abc	6.3 b
Fatali ²	Africa	<i>chinense</i>	4.4 ab	0.02 a	0.69 ab	32 ab	41 abcd	6.5 b
Tabasco Red ³	Mexico	<i>frutescens</i>	4.5 ab	0.03 a	1.03 abc	32 ab	42 abcdef	4.6 ab
Hawaiian Sweet Hot ³	Hawaii	<i>annuum</i>	4.8 abc	0.03 a	0.90 ab	32 ab	38 a	5.8 b
Hungarian Sunshine	Hungary	<i>annuum</i>	4.8 abc	0.07 a	1.75 abc	30 ab	44 abcdef	3.7 ab
II Sweet ²								
Black Pearl ²	USA	<i>annuum</i>	5.9 abcd	0.08 abc	1.39 abc	28 a	51 abcdef	4.7 ab
Red Rocoto ³	Ecuador	<i>pubescens</i>	6.0 abcd	0.04 a	1.23 abc	32 ab	41 abcd	4.9 ab
Shishitou ²	Japan	<i>annuum</i>	6.1 abcd	0.08 abc	2.03 abc	32 ab	45 abcdef	3.1 a
Moses Orange ³	Kenya	<i>annuum</i>	6.3 abcde	0.04 a	1.13 abc	33 b	39 ab	6.0 b
Trinidad Moruga								
Scorpion ³	Trinidad	<i>chinense</i>	7.7 abcdef	0.05 a	1.27 abc	32 ab	42 abcdef	7.2 b
Jamaican Red Hot ³	Jamaica	<i>chinense</i>	7.7 abcdef	0.03 a	1.05 abc	33 b	40 abcd	6.8 b
Apple Hungarian ²	Hungary	<i>annuum</i>	7.9 abcdef	0.05 a	1.50 abc	32 ab	42 abcdef	5.7 b
Brazilian Star Fruit								
Yellow ³	Brazil	<i>baccatum</i>	8.1 abcdef	0.08 ab	1.69 abc	32 ab	45 abcdef	6.3 b
Explosive Ember ²		<i>annuum</i>	8.2 abcdef	0.09 abc	1.58 abc	28 a	54 abcdef	5.7 b
Aji Lemon [?]		<i>baccatum</i>	8.3 abcdef	0.07 ab	1.30 abc	32 ab	45 abcdef	6.7 b
Mako Akokosrade ³	Ghana	<i>annuum</i>	9.3 abcdef	0.12 abc	2.19 abc	28 a	54 abcdef	5.9 b
Giant Mexican								
Rocoto	Mexico	<i>pubescens</i>	10.4 abcdef	0.16 abc	2.73 abc	28 a	54 abcdef	5.5 b
Bebere Ethiopian								
Brown ¹	Ethiopia	<i>annuum</i>	10.5 abcdefg	0.17 abc	3.60 abc	32 ab	45 abcdef	3.71 a
Czechoslovakian								
Black ²	Czech Rep.	<i>annuum</i>	10.7 bcdefg	0.10 abc	2.21 abc	28 ab	54 abcdef	5.17 ab
Zimbabwe Bird ³	Zimbabwe	<i>annuum</i>	10.8 bcdefg	0.14 abc	3.32 abc	32 ab	45 abcdef	3.43 a
Goronong ³	Malaysia	<i>chinense</i>	10.9 bcdefg	0.19 abc	2.85 abc	28 ab	55 abcdef	4.48 ab
Bolivian Rainbow ¹	Bolivia	<i>annuum</i>	11.1 bcdefg	0.13 abc	2.27 abc	28 ab	57 def	4.61 ab
CGN 22091 ³	Costa Rica	<i>chinense</i>	11.5 cdefg	0.21 abc	4.14 c	31 ab	49 abcdef	2.92 a
Pepperoncini ²	Italy	<i>annuum</i>	12.6 defg	0.19 abc	3.09 abc	27 a	56 bcdef	4.84 ab
Numex Sunset Chili ²	USA	<i>annuum</i>	13.1 efg	0.20 abc	3.11 abc	28 ab	57 def	4.69 ab
Aleppo ¹	Syria	<i>annuum</i>	13.3 fg	0.20 abc	3.13 abc	29 ab	57 ef	4.53 ab
Bulgarian Carrot ²	Bulgaria	<i>annuum</i>	13.5 fg	0.18 abc	3.13 abc	29 ab	56 cdef	4.76 ab
Haiti Cluster ³	Haiti	<i>annuum</i>	14.1 fg	0.20 abc	3.30 abc	28 ab	56 bcdef	5.90 b
Peruvian Purple ³	Peru	<i>annuum</i>	14.6 fg	0.27 bc	4.21 c	29 ab	55 abcdef	4.83 ab
Thai Hot ³	Thailand	<i>annuum</i>	16.3 g	0.28 c	3.87 bc	27 a	59 f	5.10 b
ANOVA								
Species			*y	NS	NS	NS	NS	NS
Variety			***	***	***	***	***	**
Region			*	NS	NS	NS	*	NS
Species:			P_n		Climate		P_n	RH
<i>annuum</i>			9.4 c		Desert		12.5 b	56 b
<i>baccatum</i>			6.4 ab		Temperate		8.0 a	47 ab
<i>chinense</i>			8.6 ab		Tropical		8.8 ab	46 a
<i>frutescens</i>			4.5 a					
<i>pubescens</i>			8.2 ab					

^zMeans followed by different lowercase letters within each column are significantly different based on Tukey's honestly significant difference test ($P \leq 0.05$) for mean separation based on the analysis of variance (ANOVA).

^yStatistical significance as determined by analysis.

NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

plants were transplanted into 18.9-L plastic pots with the same media. Potted plants were placed outdoors (St. Paul, MN) on 1 June and were watered with a drip irrigation system as needed. Nutrients were provided by incorporating granular Sustane Natural Fertilizer (4–6–4; Cannon Falls, MN) with media at a rate of 12 g/L to provide ≈ 100 ppm of N (3.2N–6P₂O₅–4K₂O).

P_n , g_s , and E data were collected from the third unfolded mature leaf (expansion completed) below the shoot tip using a LI-COR LI6400XT portable gas exchange meter (LI-COR, Inc., Lincoln, NE) with an external

cuvette with a built-in LED light source (cuvette dimensions = $3 \times 2 \text{ cm}^2$) on 29 July, 3 Aug., 5 Aug., 10 Aug., 17 Aug., and 22 Aug. 2017 (similar weather conditions). Because data were collected at different times, a different leaf was used each time as the plant grew. Data were recorded 5 min after placing the cuvette around a leaf (P_n had stabilized). Cuvette T_{leaf} and RH were also recorded at that time. Cuvette irradiance, carbon dioxide concentration, and atmospheric flow rate were set to $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 400 ppm (approximately outdoor ambient), and $500 \mu\text{L}\cdot\text{min}^{-1}$, respectively. The outdoor air tem-

perature, RH, and photoperiod on collection dates varied from 22.4 to 27.9 $^{\circ}\text{C}$, 40% to 70%, and 13.25 to 15.5 h, respectively. Garruna-Hernandez et al. (2014) reported that *C. chinense* g_s varied during the day and was highest between 1100 and 1300 HR; therefore, we only collected data between 1100 and 1300 HR. WUE was calculated by dividing P_n by E (Ou and Zou, 2012).

The experiment was organized in a completely randomized statistical design. P_n , E , g_s , and WUE were dependent variables, and variety (33 levels), species of origin (5 levels), and indigenous habitat (3 levels)

were independent variables (Table 1). Indigenous habitats were subjectively categorized as dry/desert, temperate, and tropical based on the climate in the country of origin (Table 1). Data were collected at six different times from a different leaf each time (198 total values each for P_n , E , g_s , and WUE). Data were analyzed using an analysis of variance followed by mean separation (Tukey's honestly significant difference and least significant difference; $\alpha < 0.05$) using the SPSS statistical software package (IBM SPSS Statistics, version 24; IBM Corp., Armonk, NY). Pearson correlations among dependent variables were also determined.

Results

P_n varied from 3.6 to 3.7 for 'Malawi Piquante' and 'Korean Long Green' peppers to 16.3 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ (fixed) for 'Thai Hot' peppers (Table 1). The g_s varied from 0.01 to 0.59 for most varieties (Malawi, Korean Long Green, Antohi Romanian, Pakistan, Fatali, Tabasco Red, Hlan Sweet Hot, Hungarian Sunshine, Red Roccoto, Moses Orange, Trinidad Moruga Scorpion, Jamaican Red Hot, and Apple Hungarian) to 0.28 $\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$ for Thai Hot pepper (Table 1). E varied from 0.43 to 0.59 for 'Malawi Piquante', 'Korean Long Green', and 'Pakistan' peppers to 4.14 and 4.21 $\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$ for 'CGN 22091' and 'Peruvian Purple' peppers, respectively (Table 1). WUE differed from 2.92 to 3.43 among three varieties to 5.10 to 7.20 for 16 other pepper varieties (Table 1). T_{leaf} varied from 27 °C for 'Thai Hot' and 'Pepperoncini' peppers to 33 °C for 'Pakistan', 'Moses Orange' and 'Jamaican Red Hot' peppers (Table 1). Cuvette RH differed from 38% for 'Hawaiian Sweet' peppers to 59% for 'Thai Hot' peppers (Table 1).

C. frutescens, *C. baccatum*, *C. chinense*, and *C. pubescens* derived varieties had lower P_n (4.5–8.6 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ fixed) than *C. annuum* derived varieties (9.4 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ fixed) (Table 1). There were no differences in g_s , E , T_{leaf} , RH, or WUE among varieties based on parental species (Table 1). Varieties originating from temperate climates had lower P_n (8.0 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ fixed) than those originating from dry regions (12.5 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ fixed) (Table 1). Varieties

from tropical regions had a lower cuvette RH (46% to 47%) than those from dry regions (56%) (Table 1) when tested in our experiment.

P_n was positively correlated with g_s (Pearson correlation = 0.88), E (0.86), and RH (0.62), and it was negatively correlated with WUE (−0.35) (Table 2). The g_s was positively correlated with E (0.95) and RH (0.56), and it was negatively correlated with T_{leaf} (−0.18) and WUE (−0.56) (Table 2). E was positively correlated with RH (0.44) and negatively correlated with WUE (−0.67) (Table 2). T_{leaf} was negatively correlated with g_s (−0.18) and RH (−0.21) (Table 2). RH was negatively correlated with T_{leaf} (−0.21) (Table 2).

Discussion

Capsicum P_n values varied more in our experiment (3.6–3.7 to 16.3 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ fixed) than they did in other experiments reported by others; for example, Ou and Zou (2012) reported that the P_n of five *Capsicum* species varied from 15.8 to 21.8 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ (fixed). Among pepper varieties within a species, P_n also varied more in our experiment than it did in others (Ghasemi et al., 2016; Rosado-Souza et al., 2015). Borisev et al. (2012) reported that the P_n of 10 *C. annuum* varieties differed from 14.5 to 16.6 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ (fixed); the *C. annuum* variety P_n (22 varieties) in our work ranged from 3.7 to 16.3 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ (fixed) (Table 1). Ridzuan et al. (2018) reported that the *C. annuum* variety/accession P_n differed from 11.5 to 19.1 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ (fixed). In contrast, Hassan et al. (2014) reported that the *C. annuum* variety P_n ranged from 2.5 to 5.3 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ (fixed; irradiance not reported). Pérez-Grajales et al. (2004) reported similar P_n rates (at a lower irradiance of 500 $\mu\text{mol}/\text{m}^2/\text{s}$) for *C. pubescens*. Rosado-Souza et al. (2015) reported that the *C. chinense* accession P_n varied from 17 to 25 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ (fixed).

Differences between our P_n values and others may have been related to the irradiance levels when P_n was measured, where plants were grown, or the number of varieties studied. For instance, irradiance in our work was at saturating photosynthetic levels (1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), whereas irradiance in other

studies was undocumented or at 300 to 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Because peppers are typically grown in the field, where they are routinely exposed to saturating irradiance levels, we believe that our data more accurately reflected actual field P_n . Furthermore, P_n can vary more for field-grown than for greenhouse-grown/controlled environment-grown *Capsicum*. Borisev et al. (2012) reported that the P_n of nine *Capsicum* varieties differed from 14.5 to 16.6 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ (fixed), but that P_n differed from 12.8 to 18.7 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ (fixed) when those same varieties were grown in the field. Furthermore, the greater variation in P_n in this study compared with that in some other studies may have occurred because we evaluated a greater number of varieties that were intentionally selected to represent a broad array of genetic and indigenous backgrounds.

Our data regarding the varieties derived from *C. annuum* included collectively higher P_n than the data regarding the varieties derived from other species. However, broad conclusions regarding which species have higher P_n should be made cautiously because abiotic factors can interact with species and affect P_n . For instance, Ou and Zou (2012) reported that the P_n of *C. frutescens* was higher than that of four other *Capsicum* species (21.8 vs. 15.8–17.7 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ fixed) when plants were grown at 35 °C, but the P_n of *C. pubescens* was highest when plants were grown at 15 °C. In the same study, the P_n of *C. baccatum* and the P_n of *C. pubescens* were higher than that of three other *Capsicum* species when plants were drought-stressed. In contrast, Okunlola et al. (2017) reported that the *C. chinense* P_n was more drought-tolerant than the P_n of *C. annuum* or *C. frutescens*. The environment where a plant is grown or the water status of a plant when P_n data are collected can lead to different conclusions about which *Capsicum* species has higher P_n . Although varieties are reportedly derived from a species, many are interspecific hybrids (often unreported) (Petkova et al., 2014) and genetically related (Amarul J'union et al., 2005). That hybridization alone may impact the photosynthetic or water use data. Petkova et al. (2014) showed that the P_n of four F_1 pepper hybrids was more tolerant to high temperatures (33 to 35 °C) than that of parental species.

The range and g_s values reported in our study (0.01–0.28 $\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$) were less than those reported by others. Ridzuan et al. (2018) reported that the g_s of the *C. annuum* variety (14) varied from 0.25 to 0.68 $\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$. Hassan et al. (2014) reported that the g_s of the *C. annuum* variety varied from 0.50 to 0.60 $\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$. Differences between our g_s data and that of others may have been related to the environments where plants were grown. For instance, plants grown at temperatures below 15 °C or above the optimal temperature for pepper P_n can decrease the *Capsicum* g_s (Jaimez and Rada, 2016). Because the T_{leaf} data were collected on some days when the temperature was above the optimal temperature reported for

Table 2. Pearson correlation between instantaneous photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (E), cuvette leaf temperature (T_{leaf}), and humidity after 5 min (RH) and water use efficiency (WUE; $WUE = P_n/T$).

Factor	P_n	g_s	E	T_{leaf}	RH	WUE
P_n	—	0.88**z	0.86**	−0.09	0.62**	−0.35**
(2-tailed)	—	***	***	NS	***	***
g_s	—	—	0.95**	−0.18*	0.56**	−0.56**
(2-tailed)	—	—	***	*	***	***
E	—	—	—	−0.02	0.44**	−0.67**
(2-tailed)	—	—	—	NS	***	***
T_{leaf}	—	—	—	—	−0.21 **	−0.010
(2-tailed)	—	—	—	—	**	NS
RH	—	—	—	—	—	−0.010
(2-tailed)	—	—	—	—	—	NS

^zStatistical significance as determined by the analysis of variance.

NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 3. Variation in instantaneous photosynthetic rate (P_n ; $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ fixed), stomatal conductance (g_s ; $\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$), transpiration rate (E ; $\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$), cuvette leaf temperature (T_{leaf} ; $^{\circ}\text{C}$), and relative humidity (RH; %) and water use efficiency (WUE; $\text{WUE} = P_n/E$) among 33 outdoor-grown pepper varieties.

Factor	Collection dates						Significance
	29 July Rep 1	3 Aug. Rep 2	5 Aug. Rep 3	10 Aug. Rep 4	17 Aug. Rep 5	22 Aug. Rep 6	
P_n	8.7 abc ^z	9.2 abc	11.1 c	10.3 bc	7.4 ab	6.9 a	** y
g_s	0.13 ab	0.15 b	0.13 ab	0.13 ab	0.7 ab	0.7 a	**
E	2.28 a	2.47 a	2.33 a	2.67 a	1.48 a	1.52 a	*
T_{leaf}	27.7 a	28.8 a	34.0 b	33.6 b	29.6 a	28.5 a	***
RH	43 ab	50 c	58 d	49 bc	48 bc	40 a	***
WUE	4.33 a	4.41 a	5.55 ab	4.48 ab	6.12 b	5.38 ab	**

^zMeans followed by different letters within each row are statistically different based on Tukey's honestly significant different test ($P \leq 0.05$) for mean separation.

^yStatistical significance as determined by analysis of variance.

ns, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Capsicum P_n (25 to 30 $^{\circ}\text{C}$), the g_s may have been impacted (Table 3).

Our data indicated that g_s does not vary among domesticated *Capsicum* species (Table 1). Milla et al. (2013) also found that g_s did not vary between wild-type and domesticated *C. annuum* or *C. baccatum*. Interestingly, we observed that g_s differed among varieties derived from those species. Rosado-Souza et al. (2015) reported that the *C. annuum* variety g_s differed. Furthermore, Kang et al. (2001) reported that the *C. chinense* variety g_s differed. Yet, in both of those studies, the species g_s did not differ. Similarly, Percy et al. (1996) showed that the g_s of interspecific selections of cotton (*Gossypium barbadense* L.) was higher than that of the parents or ancestral lines. Differences in the g_s of varieties observed by us and others may have been due to the integration of a mutation into breeding programs over time and/or interspecific hybridization resulting in greater variations in g_s than that observed for genetic parents. Furthermore, a recent study has shown a transgenerational effect on watercress (*Lepidium sativum*) g_s ; the maternal light environment effects on stomatal density and g_s were expressed in seed-propagated progeny, suggesting that differences in g_s in this experiment and in past experiments, to some degree, may be impacted by the environment in which seeds were produced (Vrablova et al., 2018). A similar relationship between the maternal water status and progeny WUE was also reported for *Eucalyptus* (Vivas et al., 2019).

The E values reported here also varied more than those reported by others, although the maximum E values were similar (3.21–4.05 $\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$) (Ou and Zou, 2012). Ridzuan et al. (2018) reported that the E of the *C. annuum* variety differed from 4.61 to 7.11 $\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$ (indoor-grown). Furthermore, differences in E values in our work and that of others may have been related to where plants were grown. Borisev et al. (2012) reported that the E of *Capsicum* variety (nine) differed more for outdoor-grown than indoor-grown peppers (4.4–6.1 vs. 4.7–5.0 $\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$, respectively).

In contrast to the P_n , E , and g_s data presented here, the WUE values we observed (3.1–7.2) were similar to those reported by

others for peppers grown in a controlled environment (3.9–6.8) (Ou and Zou, 2012). However, our values were generally higher than those reported by Borisev et al. (2012) who also reported that the WUE of indoor-grown *C. annuum* variety (nine) varied less than that of field-grown plants (3.2–3.5 and 2.3–3.9, respectively). The WUE of *Capsicum* is reportedly impacted by irradiance; WUE was higher when plants were grown under high irradiance (400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; 2.2) compared to low irradiance (200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; 1.8) (Fu et al., 2010). However, irradiance in our study was 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; therefore, it would be considered high. Moreover, the negative correlation between *Capsicum* WUE and P_n that we observed agreed with the observations of Antony and Singandhupe (2004).

Ridzuan et al. (2018) reported that the *C. annuum* variety E and g_s were correlated with P_n , as we observed (0.55 and 0.87, respectively). However, P_n is not always correlated with g_s . Yun and Ahn (2009) reported that increasing temperature and atmospheric CO_2 concentrations increased the pepper P_n , but not the pepper g_s . The strong correlation between pepper g_s and P_n reported here (0.95) (Table 2) supports the assertion that g_s limitations may reduce pepper P_n (Serrano et al., 2017). However, the observations of Yun and Ahn (2009) suggested that g_s limitations on P_n may occur less if CO_2 concentrations are increased, as is common for greenhouse-produced bell peppers (Dorais, 2003; Erwin and Gesick, 2017).

A negative correlation between g_s and T_{leaf} was previously reported ($r^2 = 0.76$) for cotton (*G. barbadense* L.–derived) (Radin et al., 1994), although that correlation was greater than what we observed here for pepper (Table 2). Interestingly, some have suggested that g_s measurements obtained later in the afternoon (after 1300 HR) may be important when evaluating plants for higher yield based on g_s ; for instance, Rebetzke et al., (2003) recommended that wheat breeding projects should involve selected progeny with higher g_s collected later in the day because g_s collected later in the day correlated more with yield than g_s collected earlier. Similarly, Radin et al. (1994) found that the cotton yield (related

to P_n) was associated more with g_s collected in the afternoon than in the morning, and that varieties with high yield under hot temperatures had higher g_s in the afternoon, but not in the morning (Lu et al., 1997). We collected data when P_n and g_s were highest during the day; whether *Capsicum* yield is associated more with g_s collected at certain times of the day is not known.

Differences in outdoor environmental conditions for data collection dates during our experiment may have reduced the resolution of our experiment. T_{leaf} was higher on collection dates 3 and 4 (33.6 to 34 $^{\circ}\text{C}$; over-reported P_n optima) than on other dates (27.7 to 29.6 $^{\circ}\text{C}$) (Table 3). Furthermore, cuvette RH was higher on collection dates 2, 3, 4, and 5 (48% to 58%) than on collection dates 1 and 6 (40% to 43%) (Table 3). Because T_{leaf} and RH are correlated with P_n , g_s , and E (Table 2), the environmental differences among collection dates may have impacted values.

Care should be taken when determining conclusions about plant photosynthesis or yield based on instantaneous P_n or water use data based on the leaf area per unit. Collecting data based on the leaf area per unit does not account for differences in the individual leaf area, plant leaf number, whole-plant leaf area, or whole-plant photosynthesis. Furthermore, instantaneous P_n data do not account for plant acclimation to changing environmental conditions (Hikosaka et al., 2006). Therefore, our study is of value because it compared photosynthetic and water use attributes of a larger number of pepper varieties at the same time of day and under the same environmental conditions simultaneously. Our study also demonstrated that outdoor-grown pepper variety photosynthetic and water use attributes vary more than previously reported, and that the reported parental species and indigenous habitat are associated with some photosynthetic and water use attributes.

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