

## Characterization of the Water Economy of Sugarcane Transgenic Genotypes

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### Authors' contributions

This work was carried out in collaboration between all authors. Authors CJF, EM and MBD designed the study, wrote the protocol of the study and the literature searches. Authors MBD, EM, MFM and MDMR performed the genetic transformations and selection of transformed lines. Author JCC managed the rain-shelter facility and performed data collection and statistical analyses. Author CJF developed and managed the computerized system to measure daily plant water use and wrote the first draft of the manuscript. Author WJG formatted the manuscript for publication. All authors read and approved the final manuscript.

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### ABSTRACT

**Aims:** Study designed to characterize water economy of a group of sugarcane transgenic lines grown in a rain-sheltered, well-watered, and water-stressed conditions.

**Study Design:** Randomized complete block with 3 replications.

**Place and Duration of Study:** The study was conducted at the Texas A&M AgriLife Research and Extension Center in Corpus Christi during 2012 and early 2013.

**Methodology:** Stem cuts of 14 transgenic lines and two non-transgenic background genotypes

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(NTBGs) were hand-planted in 13.5-L pots. The study had two phases. In the 1st phase, lines were subjected to a well-watered regime of 1.3 L d<sup>-1</sup>. In the 2nd phase, lines were exposed to water deficits by reducing irrigation to 0.65 L every other day. At the end of both phases, plants were harvested to determine biomass and leaf area.

**Results:** Under well-watered conditions the higher nominal water use efficiency (NWUE) attained by lines 15 and 133 over the NTBG was related to their higher production of biomass, which was not paralleled by an increase in whole-plant transpiration. Lines 132 and 20, which showed NWUE not different than their NTBG, exhibited lower and higher cumulative whole-plant transpirations, respectively. Under water stress, lines 11 and 163 had lower NWUE than their respective NTBGs, as a result of lower biomass production not paralleled by a lower whole-plant transpiration. Lines 132 and 112 had NWUE not different than the respective NTBGs, but they both exhibited lower cumulative whole-plant transpiration and lower biomass production.

**Conclusion:** The study helped characterize the water economy of 14 transgenic sugarcane lines. Under well-watered conditions, lines 15 and 133 exhibited higher NWUE, line 132 was more water-conservative, and line 20 was more water-prodigal than the NTBG. Under water stress, lines 11 and 163 had lower NWUE and lines 132 and 112 were less tolerant to water stress than their respective NTBGs.

*Keywords: Water economy; plant water use; biomass production; water use efficiency; drought tolerance.*

## 1. INTRODUCTION

Drought is the most dominant environmental factor limiting sugarcane yields in many areas of the world, including drought-prone Texas croplands in South Texas. To overcome this yield limitation, irrigation must be applied to sugarcane crops to maintain biomass production and accumulation of sucrose in the stalks [1,2] in combination with best agronomic practices to further increase water use efficiency [3].

Plants exposed to soil water deficits exhibit decreased expansive growth (particularly leaf area and stem elongation), decreased transpiration and photosynthesis through stomata closure, and, due to the latter, decreased biomass production [4-6]. But as McCree and Fernandez [4,5] demonstrated, exposure to soil water deficits can also increase water use efficiency (expressed as mass of carbon uptake per mass of water transpired) as transpiration is decreased more than photosynthetic carbon uptake. This is particularly notable in C4 species such as sorghum and sugarcane species. Crop species and their intra-species genotypes respond differently to soil water availability [5,7]. Substantial genotype-by-environment interactions have been repeatedly found in standard crop field tests, including sugarcane, conducted under naturally or managed variable soil water regimes. Sugarcane genotype-by-environment interactions have been reported in relation to leaf elongation rate and leaf senescence [8] and stomatal resistance [9]

and biomass production [10]. However, no reports have been found in relation to the characterization and quantification of the whole-plant water economy of sugarcane genotypes.

Irrigation use in sugarcane production could be significantly decreased and production become more economical and stable with the use of improved drought-tolerant genotypes, as this would reduce the negative impacts of water deficits on yield. A number of sugarcane transgenic lines have been developed at the Institute of Genomics and Biotechnology at Texas A&M University, which incorporate genes allegedly providing tolerance to drought, cold, heat, and salt. The magnitudes of these various environmental tolerances have not been yet quantified. The objective of this study was to characterize and quantify the whole-plant water economy of these selected sugarcane transgenic lines grown in a rain-shelter under well-watered and water-stressed conditions and to compare their performance to their particular non-transgenic background genotypes.

## 2. MATERIALS AND METHODS

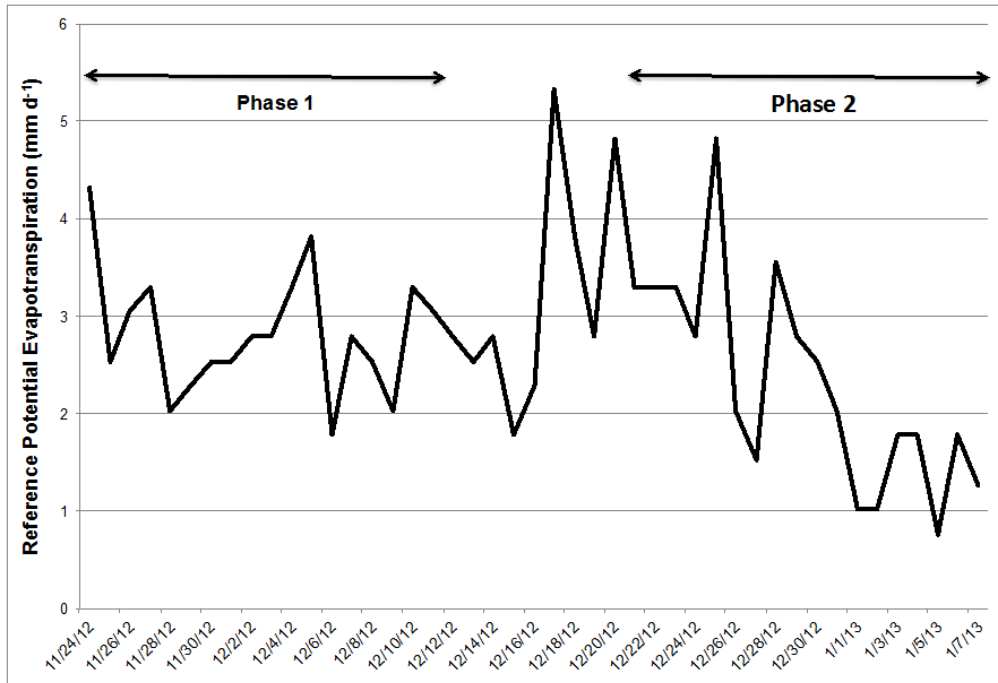
The study was conducted at the Drought Tolerance Laboratory at the Texas AgriLife Research and Extension Center in Corpus Christi during the fall of 2012. This facility consists of two joined greenhouse structures modified to operate as rain shelters housing a large number of electronic mini lysimeters capable of measuring continuous whole-plant transpiration

under controlled watering regimes. Computerized systems monitored whole-plant water use and controlled watering with a nutrient solution.

Embryogenic calli of sugarcane (*Saccharum officinarum* L.) genotypes TCP87-3388 and CP72-1210 were transformed using *Agrobacterium*-mediated transformation method, with the anti-apoptotic genes CED-9, a *C. elegans* homolog of the mammalian Bcl-2 cytoprotective gene family; and AtBAG4, an *Arabidopsis thaliana* BCL-2-associated athanogene 4. Both of the target genes had the expression driven by the constitutive promoter UBI from maize. Transformed plants were selected under *in vitro* conditions in culture medium containing Geneticin as selectable marker. Stem cuts of 14 transgenic independent sugarcane lines and two of the non-transgenic background genotypes that passed through the *in vitro* tissue culture process supplied by M. B. Dickman (TAMU's Institute of Genomics and Biotechnology) were hand-planted and allowed to germinate in large 13.5-L pots. Transgenic lines were numerically identified. Ten of these lines (11, 15, 20, 23, 132, 133, 136, 158, 170, and 171) had the common genotype TCP87-3388 carrying genetic background identified as CED-9. Four of them (69, 112, 163, and 375) had the common genotype CP72-1210, of which 69 and 375 carry genetic background AtBAG4 while 112 and 163 carry genetic background CED-9. Planting was done on 27 Sep 2012. Plantings emerged 8 days later on 5 Oct 2012. The soil medium consisted of fritted clay, which is known by its high water holding capacity [11]. Pots were uniformly filled with 11.4 L of the soil medium to minimize maximum soil water availability as a variable factor affecting plant growth and plant water economy. Drained water holding capacity of pots was 4.1 L, of which about 60% (2.46 L) was available to plants. Upon planting, the soil surface was covered with aluminum foil to minimize soil evaporation but leaving a central opening to allow the shoots to emerge through. Tiny holes were punctured in the aluminum foil to allow infiltration of irrigation water. Twelve fairly uniform plants of each transgenic line were grown and spatially arranged to conform a 3-replication randomized complete block experimental design. Each "plot" had four plants of a same transgenic line. One of these four plants was permanently hanged from a micro lysimeter for continuous measurement of plant water use. All experimental plants were individually irrigated daily to excess with a modified Hoagland solution made up with purified

city water throughout the juvenile stage and until the start of the water regime treatments.

The study was designed in two phases. In the first phase (24 Nov – 12 Dec), all transgenic lines were subjected to a continuous well-watered regime consisting of daily excess irrigation (2 minutes per day at 0.65 L min<sup>-1</sup>) to produce drainage during nighttime. Plants were 50 d old at the start of phase 1, with 8-12 green leaves in the main culm and with 1-4 tillers per plant. In the second phase (20 Dec – 7 Jan 2013), one of the extra plants in each "plot" was moved to the corresponding lysimeter for continuous weight measurements. Plants were 76 d old at the start of the phase 2 and, therefore, somewhat bigger than plants at the start of phase 1. In this second phase, plants were exposed to water deficits (starting on Dec. 21) by reducing the length of the daily irrigation from 2 minutes (well-watered treatment) to 1 minute every other day. This procedure allowed for a slow field-like onset of water stress on the test plants. Pots were individually irrigated using a spout-based distribution system to secure uniform application of irrigation treatments. At the end of both phases, the plants monitored with lysimeters were harvested to determine dry biomass distribution among main plant fractions (leaves in main tiller, main culm stem, tillers, and roots), number of tillers and plant leaf area. Leaf area was not measured or estimated in the second phase due to pronounced leaf senescence. Pot weights were measured continuously at 10-min intervals using a computerized automated system. Daily whole-plant transpiration was calculated as the 24-hr cumulative pot weight differences between consecutive hours. This method removed almost all interference of plant growth in the calculation of plant transpiration. At the end of both water regime phases, the total cumulative whole-plant transpiration was calculated as the sum of the daily whole-plant transpiration values. Because whole-plant transpiration depends on transpiration per unit leaf area and total leaf area, the experimental lines grown under well-watered conditions (first phase) were compared on the basis of the maximum daily transpiration per unit leaf area, calculated as maximum daily whole-plant transpiration and whole-plant leaf area measured at the end of the test period. In both phases of the study, transgenic lines were also compared in terms of the cumulative whole-plant transpiration, final dry biomass production, and a nominal water use efficiency value. Because this nominal water use efficiency combines



**Fig. 1. Progression of daily reference potential evapotranspiration from the start of phase 1 to the end of phase 2 of the study**

Data source: The Crop-Weather Program for South Texas available online at <http://cwp.tamu.edu>

cumulative whole-plant transpiration during the test phases (not from planting to harvest) and final dry biomass, these values overestimate the actual water use efficiency, but despite this systematic error, these nominal water use efficiency values can be useful for the sake of lines comparisons. In both phases of the study, lines were compared to their respective background non-transgenic genotypes.

Weather conditions during the experimental period are best summarized by the daily variation in reference evapotranspiration measured by an automated field weather station located approximately 100 m east of the Drought Tolerance Laboratory (Fig. 1 above). Experimental data (sums, averages, standard deviations, and coefficients of variation) were summarized using Excel (Microsoft Corporation, Redmond, WA) and statistical analyses including ANOVA, mean separations, and contrasts were performed using JMP (SAS Institute, Cary, NC).

### 3. RESULTS AND DISCUSSION

Day-to-day variation of whole-plant transpiration in both studies, phase 1 under well-watered conditions and phase 2 under water deficits,

resulted from daily variations in weather conditions and progressive variations in leaf area per plant, including leaf expansive growth, production of new leaves, and leaf senescence (Figs. 2-5). The increase in daily whole-plant transpiration from the start of phase 1 to December 3rd followed primarily the increase in leaf area production, while its subsequent decline was caused by a decrease in weather evaporative demands occurring towards the end of autumn as shown in Fig. 1. In phase 2, despite the drastic reduction of irrigation, the daily whole-plant transpiration continued to increase for about seven days as plant leaf area continued to increase while soil water storage was being depleted, but thereafter, daily whole-plant transpiration began to decrease as a combined result of the onset of water stress and the declining atmospheric evaporative demand (Figs. 1, 4, and 5).

#### 3.1 Well-watered Study

Most of the TCP87-3388-based transgenic lines, namely 11, 15, 132, 133, 136, 158 and 171, showed lower daily whole-plant transpiration values than the genetic background (Fig. 2A), but only line 132 showed final cumulative

transpiration value significantly lower (58% at  $P=0.03$ ) than that of the genetic background TCP87-3388 that transpired 7.56 L (Table 1). Transgenic lines 23 and 170 showed quite similar daily whole-plant transpiration values to the TCP87-3388 background (Fig. 2B). Transgenic line 20, on the contrary, showed much higher daily whole-plant transpiration than its TCP87-3388 background (Fig. 2C) and higher significant final cumulative whole-plant transpiration value (150% at  $P=0.01$ ) than its genetic background (Table 1). The four CP72-1210-based transgenic lines exhibited parallel progressions of daily whole-plant transpiration (Fig. 3), but none showed significantly lower cumulative whole-plant transpiration at the end of the well-watered period than the CP72-1210 background that transpired 7.24 L (Table 1). The lower whole-plant transpiration shown by line 132 can be mostly related to its lower plant leaf area (average plant leaf areas of 0.334 and 0.434 m<sup>2</sup> for line 132 and TCP87-3388, respectively). The higher whole-transpiration shown by line 20 can be mostly related to its higher maximum transpiration per unit leaf area (average plant leaf area of 0.480 m<sup>2</sup> for line 20), which, as explained below, it could have likely resulted from a higher stomatal conductance [8,12].

Lines 11, 20, and 133 showed significantly higher daily transpiration per unit leaf area (182, 137, and 181% at  $P<0.001$ ,  $P=0.03$ , and  $P<0.001$ , respectively) than their genetic background TCP87-3388 whose transpiration was 1.33 L m<sup>-2</sup>. Likewise, lines 112 and 163 showed significantly higher daily transpiration per unit leaf area (156 and 155% at  $P=0.02$  and 0.02, respectively) than their genetic background CP72-1210 whose transpiration was 0.97 L m<sup>-2</sup>. Higher daily transpiration per unit leaf area in these transgenic lines likely resulted from higher stomatal conductance. Inman and De Jager [8] and Zhao et al. [12] reported differences in stomatal conductance among sugarcane genotypes.

Differences in biomass production under well-watered conditions between transgenic lines and their genetic background genotypes were more prevalent than differences in water use through transpiration (Table 1). Lines 15, 20, 23, 133, 136, 158, and 170 produced significantly more biomass (ranging from 181 to 256% at  $P$  values ranging from 0.001 to 0.03) than the genetic background TCP87-3388, which produced 38.2 g. Line 163 also produced significantly more biomass (260% at  $P=0.002$ ) than the genetic

background CP72-1210, which produced 35.6 g. Possibly, the higher production of biomass exhibited by these transgenic lines could be explained in part by higher photosynthetic rates derived from higher leaf and/or stomatal conductances and/or higher photosynthetic capacity than that of their respective non-transgenic background genotypes. As discussed above regarding cumulative whole-plant transpiration and transpiration per unit leaf area, a higher leaf or stomatal conductance could be the main factor involved in line 20. Inman and De Jager [8] and Zhao et al. [12] reported differences in stomatal conductance among sugarcane genotypes. Zhao et al. [13] reported differences in leaf photosynthetic rates among sugarcane genotypes.

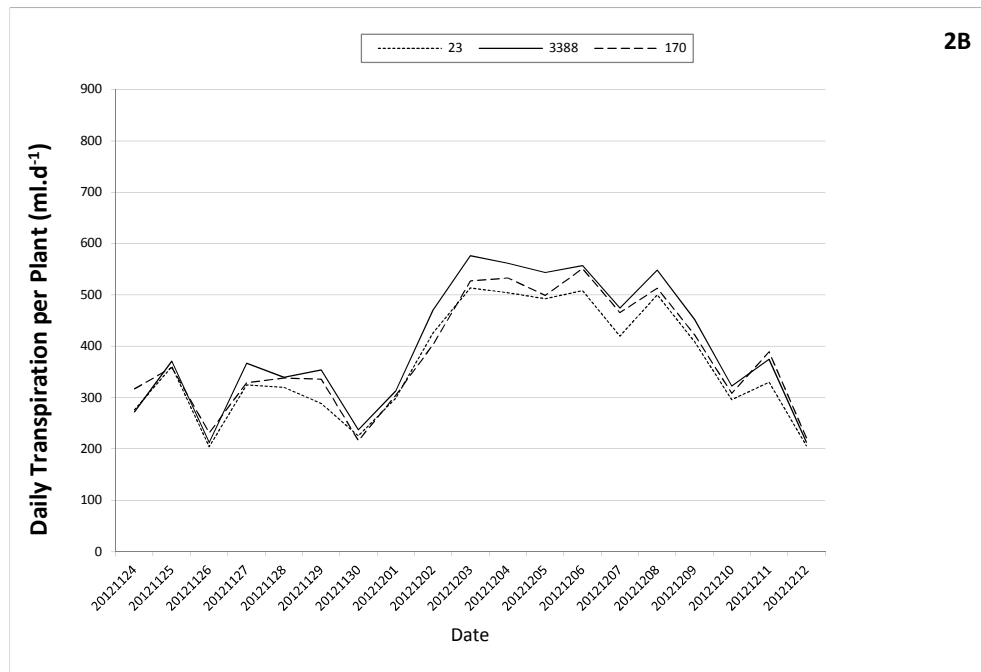
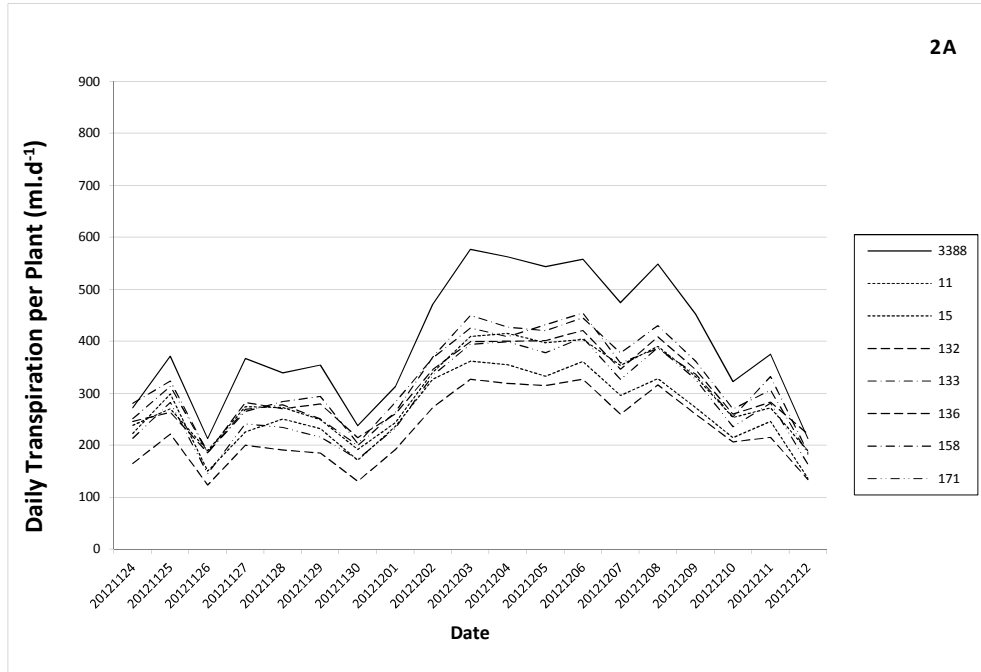
Nominal water use efficiency was significantly higher in lines 15 and 133 (363 and 349% at  $P=0.006$  and  $P=0.009$ , respectively) than the 4.82 g L<sup>-1</sup> observed in the non-transgenic background genotype TCP87-3388 (Table 1). The higher nominal water use efficiency attained by lines 15 and 133 can be related to their higher production of biomass, which was not paralleled by a concomitant increase in whole-plant transpiration. This outcome might indicate that lines 15 and 133 could have a higher photosynthetic capacity than TCP87-3388, as reported by Zhao et al. [13] for other sugarcane genotypes. The nominal water use efficiency of all other transgenic lines were not significantly different than their respective non-transgenic background genotypes.

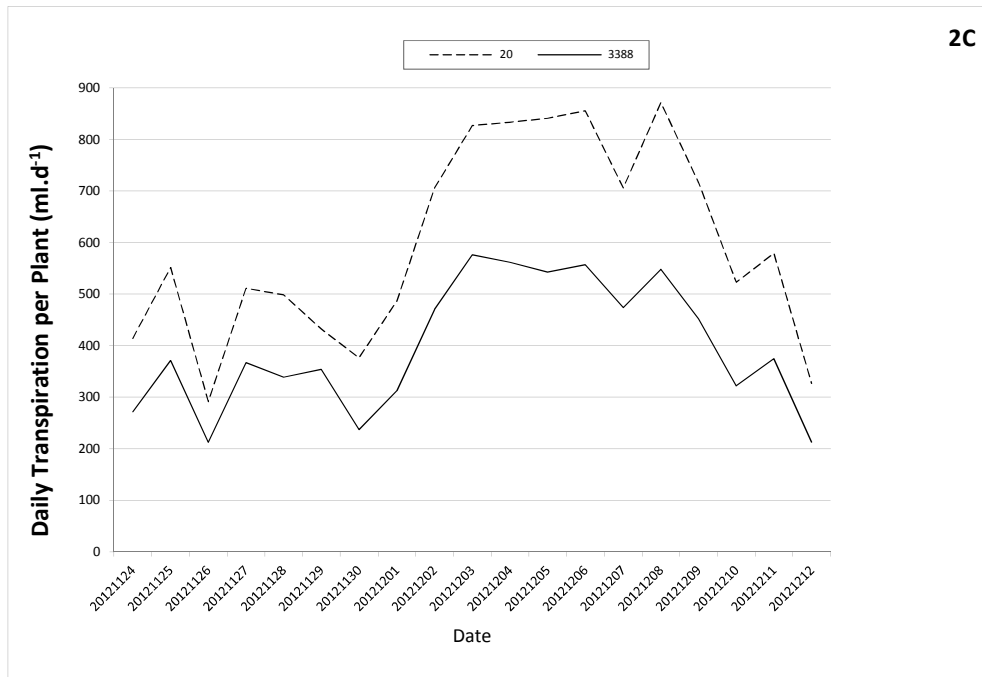
### 3.2 Watered-stressed Study

Most of the TCP87-3388-based transgenic lines, namely 11, 23, 132, 136, 158 and 171, showed lower daily whole-plant transpiration values than the genetic background (Fig. 4A), but only the final cumulative values of lines 132 and 158 were significantly lower than that of TCP87-3388 background at 6.10 L; 62% at  $P=0.02$  and 63% at  $P=0.02$ , respectively (Table 2). Transgenic lines 15 and 170 showed quite similar daily whole-plant transpiration values to the TCP87-3388 background (Fig. 4B). Transgenic line 20, on the contrary, showed higher daily whole-plant transpiration values throughout the test (Fig. 4C) and a higher significant final cumulative whole-plant transpiration value (141% at  $P=0.01$ ) than that of genetic background TCP87-3388 (Table 2). Of the four CP72-1210-based transgenic lines, only line 112 exhibited lower

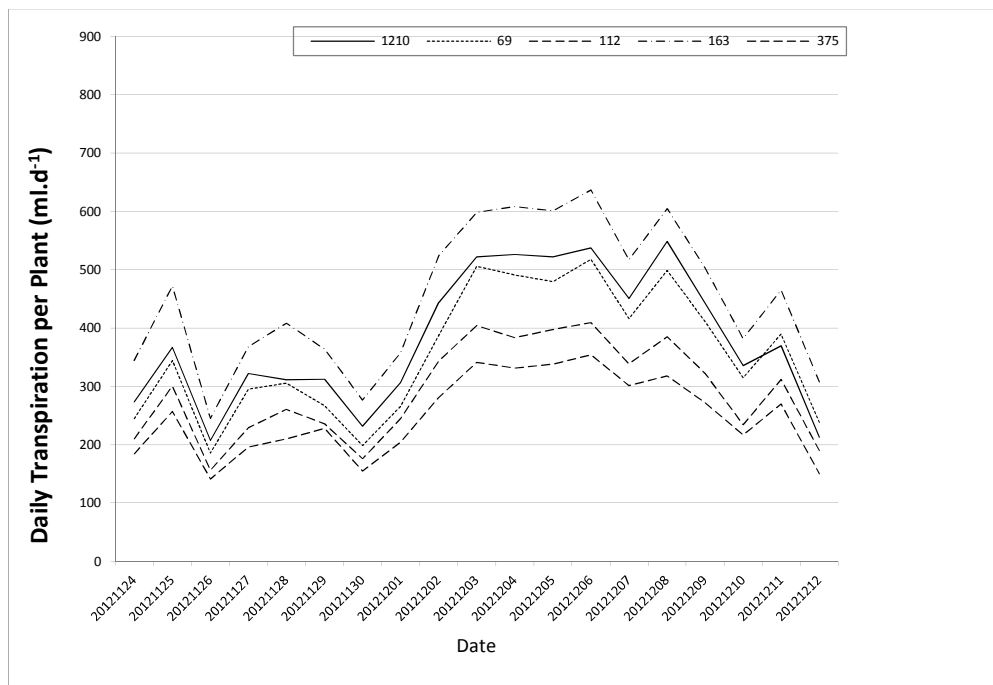
daily and cumulative whole-plant transpiration values than the non-transgenic background genotype; 52% at  $P=0.009$  (Fig. 5 and Table 2). Since, as will be discussed below, the biomass production of lines 132 and 112 was also lower

than their non-transgenic genotype backgrounds, it can be assumed that the lower cumulative whole-plant transpiration of these two transgenic was possibly related to a lower leaf or stomatal conductance [8,12].





**Fig. 2. Progression of daily whole-plant transpiration (average of three replicates) of well-watered TCP87-3388-based transgenic lines compared to that of their genetic background. 2A: lines with daily transpiration values below background, 2B: lines with daily transpiration values similar to background, and 2C: line with daily transpiration values above background. TCP87-3388 is named 3388 in the legend**



**Fig. 3. Progression of daily whole-plant transpiration (average of three replicates) of well-watered CP72-1210-based transgenic lines compared to that of their genetic background. CP72-1210 is named 1210 in the legend**

**Table 1. Summary data from well-watered study comparing transgenic lines to their non-transgenic genotype background, including final cumulative values of state variables whole-plant water use (PWU), maximum daily water use per unit leaf area (MDWU/LA), whole-plant dry biomass (PDB), and nominal water use efficiency (NWUE), along with their ratio to the respective non-transgenic genotype backgrounds and their statistical P values**

Variable/Entry	Whole-Plant Water Use (PWU) (L)	PWU relative to background genotype	P value of PWU vs background genotype	Maximum Daily Water Use per unit Leaf Area (MDWU/LA) (L/m <sup>2</sup> )	MDWU/LA relative to background genotype	P value of MDWU/LA vs background genotype	Whole-Plant Dry Biomass (PDB) (g)	PDB relative to background genotype	P value of PDB vs background	Nominal Water Use Efficiency (NWUE) (g/L)	NWUE relative to background genotype	P value of NWUE vs background genotype
11	5.01	0.66	0.08	2.42	1.82	<.0001	47.6	1.25	0.58	10.4	2.16	0.20
15	5.68	0.75	0.19	1.13	0.85	0.37	92.0	2.41	0.004	17.5	3.63	0.006
20	11.4	1.50	0.01	1.82	1.37	0.03	79.4	2.08	0.02	6.98	1.45	0.62
23	6.91	0.91	0.65	0.99	0.74	0.12	76.9	2.02	0.03	11.1	2.31	0.15
132	4.36	0.58	0.03	0.98	0.74	0.11	50.0	1.31	0.49	11.7	2.42	0.12
133	6.06	0.80	0.30	2.41	1.81	<.0001	97.8	2.56	0.001	16.8	3.49	0.009
136	5.83	0.77	0.23	1.54	1.16	0.33	69.0	1.81	0.08	12.4	2.57	0.09
158	6.05	0.80	0.29	1.44	1.09	0.60	78.1	2.05	0.03	13.0	2.70	0.06
170	7.27	0.96	0.84	1.28	0.97	0.83	94.7	2.48	0.002	13.5	2.80	0.05
171	5.37	0.71	0.13	0.99	0.74	0.12	46.1	1.21	0.64	9.68	2.01	0.26
<b>non-transgenic background TCP87-3388</b>	<b>7.56</b>			<b>1.33</b>			<b>38.2</b>			<b>4.82</b>		
69	6.75	0.93	0.73	1.24	1.28	0.21	50.1	1.41	0.40	7.27	1.62	0.52
112	4.75	0.66	0.09	1.51	1.56	0.02	46.6	1.31	0.52	11.9	2.65	0.09
163	8.58	1.19	0.35	1.50	1.55	0.02	92.7	2.60	0.002	12.1	2.70	0.08
375	5.53	0.76	0.24	1.15	1.19	0.40	53.5	1.50	0.30	11.1	2.46	0.13
<b>non-transgenic background CP72-1210</b>	<b>7.24</b>			<b>0.97</b>			<b>35.6</b>			<b>4.50</b>		

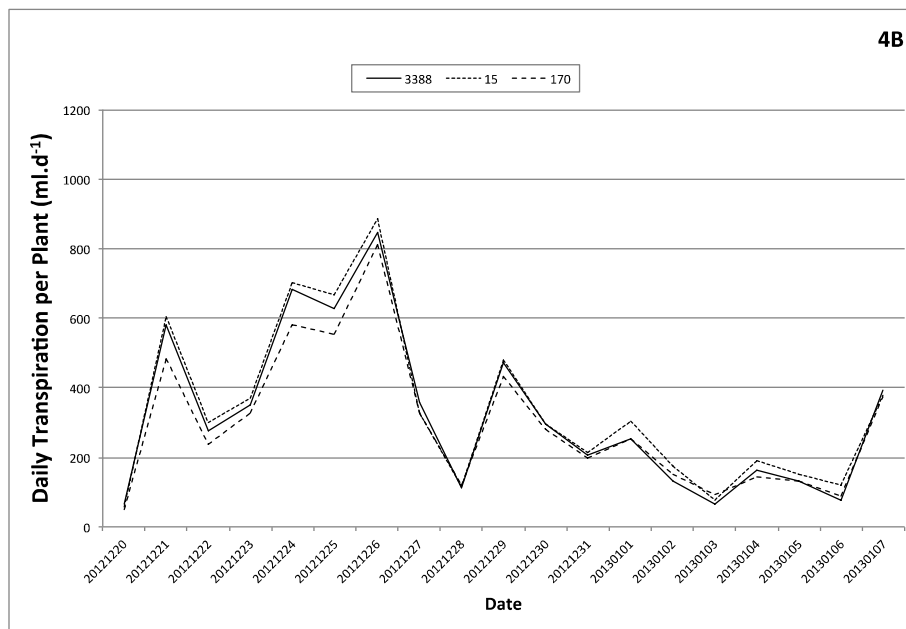
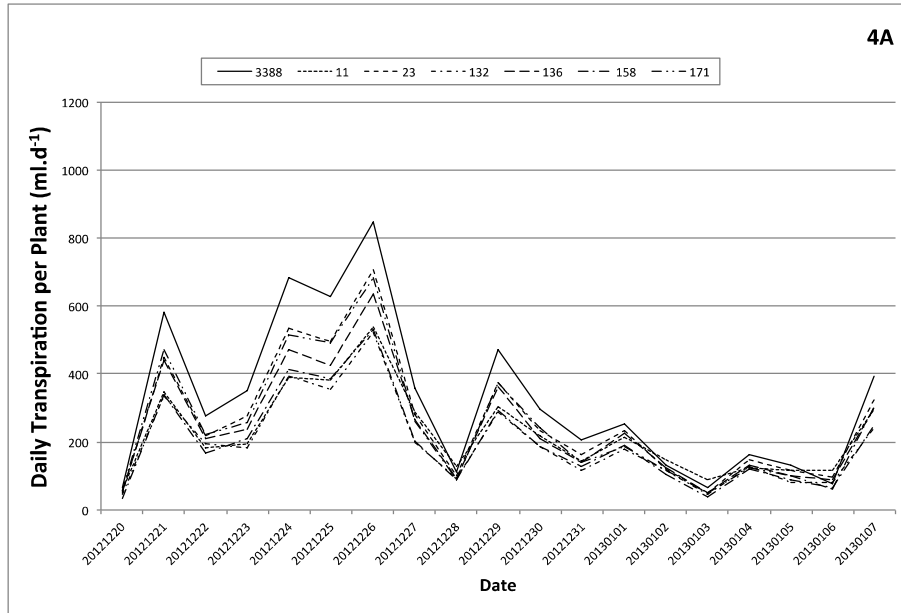


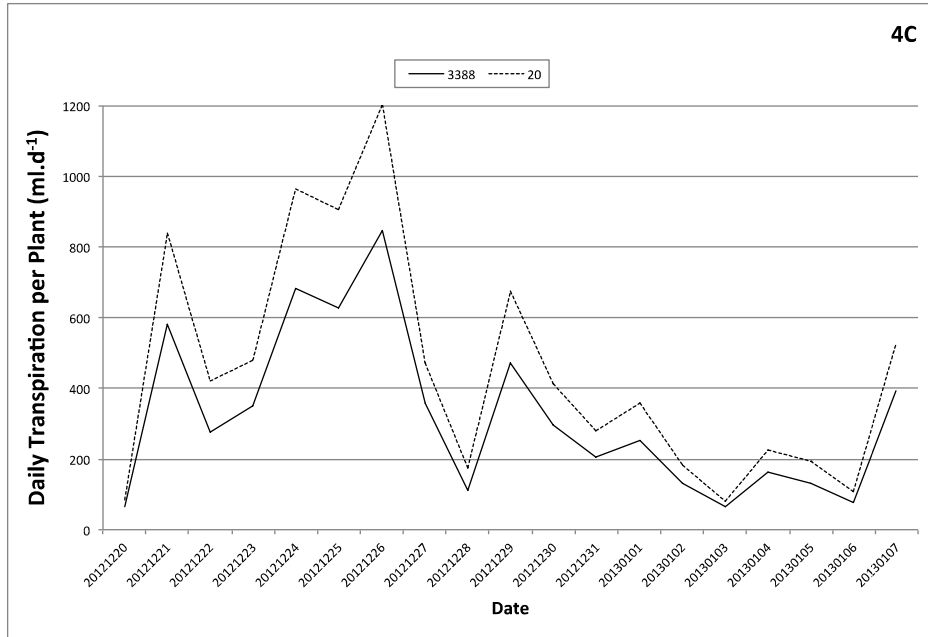
**Table 2. Summary data from water-stressed study comparing transgenic lines to their non-transgenic genotype background, including final cumulative values of state variables whole-plant water use (PWU), whole-plant dry biomass (PDB), and nominal water use efficiency (NWUE), along with their ratio to the respective non-transgenic genotype backgrounds and their statistical P values**

Variable/Entry	Whole-Plant Water Use (PWU) (L)	PWU Relative to background genotype	P value of PWU vs background genotype	Whole-Plant Dry Biomass (PDB) (g)	PDB Relative to background genotype	P value of PDB vs background	Nominal Water Use Efficiency (NWUE) (g/L)	NWUE Relative to background genotype	P value of NWUE vs background genotype
11	4.28	0.70	0.06	98.2	0.41	<0.001	23.5	0.59	0.045
15	6.41	1.05	0.74	217	0.91	0.51	37.3	0.94	0.75
20	8.59	1.41	0.01	274	1.14	0.32	32.5	0.82	0.36
23	4.97	0.81	0.23	216	0.90	0.49	43.8	1.10	0.60
132	3.77	0.62	0.02	156	0.65	0.02	42.2	1.06	0.75
133	5.96	0.98	0.89	163	0.68	0.03	27.3	0.69	0.12
136	4.56	0.75	0.11	159	0.66	0.02	35.9	0.90	0.62
158	3.82	0.63	0.02	197	0.82	0.22	50.6	1.28	0.17
170	5.64	0.92	0.63	205	0.85	0.31	38.0	0.96	0.82
171	4.74	0.78	0.16	234	0.98	0.86	51.1	1.29	0.15
<b>non-transgenic background TCP87-3388</b>	<b>6.10</b>			<b>240</b>			<b>39.7</b>		
69	7.11	1.33	0.07	294	1.16	0.24	41.4	0.86	0.41
112	2.77	0.52	0.009	106	0.42	<0.001	37.3	0.78	0.18
163	4.95	0.92	0.66	152	0.60	0.006	29.6	0.62	0.03
375	4.82	0.90	0.57	189	0.75	0.07	40.0	0.84	0.32
<b>non-transgenic background CP72-1210</b>	<b>5.36</b>			<b>253</b>			<b>47.8</b>		

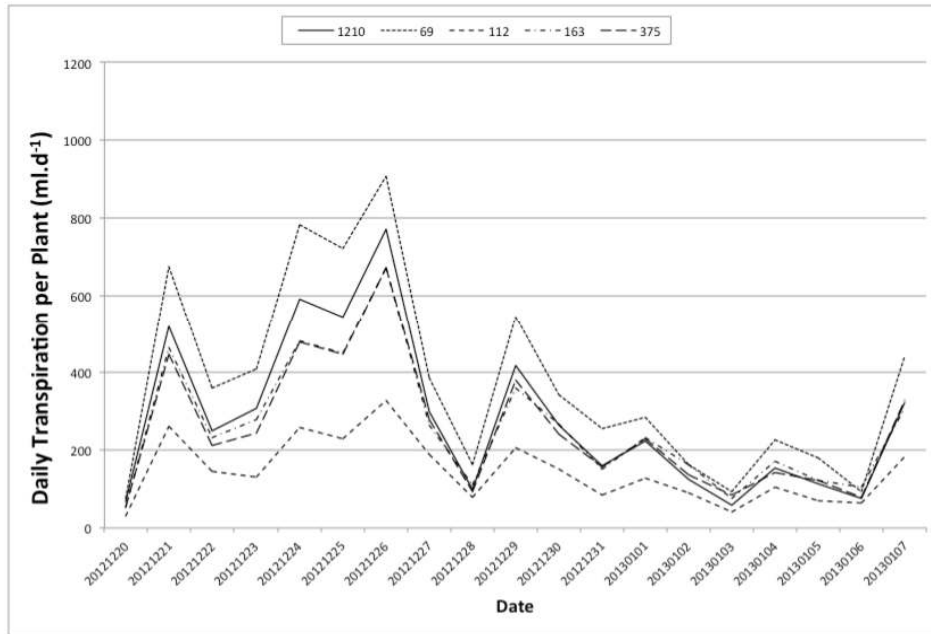
There were differences in biomass production under water stress conditions between some of the transgenic lines and their genetic backgrounds (Table 2). Lines 11, 132, 133, and 136 produced significantly less biomass (ranging from 41 to 68% at  $P$  values ranging from  $<0.001$  to 0.03) than the genetic background TCP87-3388, which produced 239 g. Lines 112 and 163 also produced significantly less biomass (42 and 60% at  $P<0.001$  and 0.006, respectively) than

the genetic background CP72-1210, which produced 252 g. The lower production of biomass exhibited by lines 132 and 112 when compared to their respective non-transgenic background genotypes could possibly be related to a lower leaf or stomatal conductance [8,12], as discussed above regarding their also lower cumulative whole-plant transpiration. In the case of lines 11 and 163, their lower biomass production was not accompanied by a lower





**Fig. 4. Progression of daily whole-plant transpiration (average of three replicates) of water-stressed TCP87-3388-based transgenic lines compared to that of their genetic background. 4A: lines with daily transpiration values below background, 4B: lines with daily transpiration values similar to background, and 4C: line with daily transpiration values above background. TCP87-3388 is named 3388 in the legend**



**Fig. 5. Progression of daily whole-plant transpiration (average of three replicates) of water-stressed CP72-1210-based transgenic lines compared to that of their genetic background. CP72-1210 is named 1210 in the legend**

cumulative whole-plant transpiration and, therefore, it could be attributed to a lower photosynthetic capacity. Zhao et al. [13] reported photosynthetic rates likely resulting from a lower photosynthetic capacity.

differences in leaf photosynthetic rates among sugarcane genotypes.

Differences in nominal water use efficiency between transgenic lines and their respective non-transgenic backgrounds exposed to water stress were only significant for lines 11 and 163 (Table 2). The nominal water use efficiency for both lines was lower than that of their respective non-transgenic backgrounds; 59% at  $P=0.045$  for line 11 and 62% at  $P=0.03$  for line 163. In both cases, the dominant factor decreasing the nominal water use efficiency was the lower production of biomass relative to their respective non-transgenic backgrounds. As discussed above, the fact that the lower biomass production in lines 11 and 163 was not paralleled by a lower whole-plant transpiration, thus decreasing their nominal water use efficiency, is an indication that their photosynthetic capacity is lower than that of their non-transgenic background genotypes.

#### 4. CONCLUSIONS

These studies made it possible to characterize and discriminate the water economy of 14 transgenic sugarcane lines growing under well-watered and water stress conditions based on their growth and water use performances relative to their respective non-transgenic background genotypes. Under well-watered conditions lines 15 and 133 exhibited higher nominal water use efficiency than the non-transgenic background genotype TCP87-3388. The higher nominal water use efficiency attained by these lines was related to their higher production of biomass, which was not paralleled by a concomitant increase in whole-plant transpiration. This outcome might indicate that lines 15 and 133 could have a higher photosynthetic capacity than TCP87-3388. Lines 132 and 20, however, showed nominal water use efficiencies not different than their background genotype TCP87-3388, which indicates that cumulative transpiration and biomass production changed in the same direction. But, line 132 exhibited lower and line 20 exhibited higher cumulative whole-plant transpirations than their background genotype TCP87-3388. The lower cumulative transpiration of line 132 appeared to be related to a lower plant leaf area, while the higher cumulative transpiration of line 20, appeared to be related to a higher leaf or stomatal conductance. Consequently, line 132 could be considered more water-conservative and line 20 more water-prodigious than the non-transgenic

background genotype TCP87-3388 when grown under well-watered conditions.

Under water stress conditions, lines 11 and 163 had lower nominal water use efficiency than that of their respective non-transgenic background genotypes, as a result of both having a lower biomass production which was not paralleled by a lower whole-plant transpiration. This outcome is a likely indication that the photosynthetic capacity of these two transgenic lines was lower than that of their non-transgenic background genotypes. Since the performance of lines 11 and 163 under well-watered conditions did not indicate a lower photosynthetic capacity, it can be assumed that the lower photosynthetic capacity of these two lines was decreased by water stress. Additionally, while the nominal water use efficiency of lines 132 and 112 was not different than that of the respective non-transgenic background genotypes, they both exhibited lower cumulative whole-plant transpiration and lower biomass production, which is an indication that their leaf or stomatal conductance was likely lower than that of their non-transgenic backgrounds. Since the well-watered study did not indicate that lines 132 and 112 had lower leaf or stomatal conductance than the respective non-transgenic background genotypes, it can be assumed that the lower conductance in these two transgenic lines was in response to water stress. Consequently, from this water stress study, it can be concluded that transgenic lines 11, 163, 132, and 112 were less tolerant to water stress than their respective non-transgenic background genotypes.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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