

Characterization of the Water Economy of Sugarcane Transgenic Genotypes

ABSTRACT

Aims: Soil water deficit is the most yield-limiting environmental factor in drought-prone Texas. The objective of this research was to characterize the water economy of a selected group of sugarcane transgenic lines growing under rain sheltered conditions.

Study design: Randomized complete block with 3 replications.

Place and Duration of Study: The study was conducted in the Drought Tolerance Laboratory at the Texas AgriLife Research and Extension Center near Corpus Christi during 2012.

Methodology: Stem cuts of 14 transgenic lines and two non-transgenic background genotypes were hand-planted in 13.5-L pots. The study was designed in two phases. In the 1st phase, all lines were subjected to a continuous well-watered regime consisting of daily excess irrigation to produce drainage during nighttime. In the 2nd phase, one of the extra plants in each "plot" was moved to the corresponding lysimeter for continuous weight measurements and plants were exposed to water deficits by reducing the length of the daily irrigation from 2 minutes (well-watered treatment) to 1 minute. At the end of both phases, plants 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.

Results: Most of the TCP87-3388-based transgenic lines (11, 15, 132, 133, 136, 158, 171), showed lower daily whole-plant transpiration values than the genetic background, but only lines 11 and 132 showed final cumulative transpiration values significantly lower than that of their genetic background. Transgenic line 20 showed much higher daily whole-plant transpiration than its TCP87-3388 background and a higher significant final cumulative whole-plant transpiration value.

Conclusion: When transgenic lines were compared to their respective genetic backgrounds under well-watered conditions, seven of the tested lines (15, 133, 136, 158, 170, 112, 163) exhibited higher nominal water use efficiency than their backgrounds.

Keywords: Soil water deficit; water use efficiency; biomass production; soil water availability.

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 [4,5,6]. 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 and to compare their performance to their particular non-transgenic background genotypes.

2. MATERIAL AND METHODS

The study was conducted at the Drought Tolerance Laboratory at the Texas AgriLife Research and Extension Center near 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 plant water use and controlled watering with a nutrient solution.

Stem cuts of 14 transgenic sugarcane lines and two of the non-transgenic background genotypes 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 BAG4 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 the soil medium to minimize maximum soil water availability as a variable factor affecting plant growth and plant water economy. Upon planting, the soil surface was covered with aluminum foil to minimize soil evaporation but leaving a central opening to allow the seedlings 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 to produce drainage during nighttime. 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. 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. This procedure allowed for a field-like onset of water stress on the test plants. 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 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. 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. 1-4). Declining weather evaporative demands during autumn lead to decreased daily transpiration during the second half of the well-watered test period. Declining daily transpiration rates during the water-stressed test was due to both declining atmospheric evaporative demand and the effects of water deficits.

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. 1A), but only lines 11 and 132 showed final cumulative transpiration values significantly lower (66% at $P=0.0817$ and 58% at $P=0.0308$, respectively) than that of their genetic background TCP87-3388 that transpired 7.563 L (Table 1). Transgenic lines 23 and 170 showed quite similar daily whole-plant transpiration values to the TCP87-3388 background (Fig. 1B). Transgenic line 20, on the contrary, showed much higher daily whole-plant transpiration than its TCP87-3388 background (Fig. 1C) and higher significant final cumulative whole-plant transpiration value (150% at $P=0.0118$) than its genetic background (Table 1). The four CP72-1210-based transgenic lines exhibited parallel progressions of daily whole-plant transpiration (Fig. 2), but only line 112 showed significantly lower cumulative whole-plant transpiration at the end of the well-watered period (66% at $P=0.0879$) than the CP72-1210 background that transpired 7.242 L (Table 1).

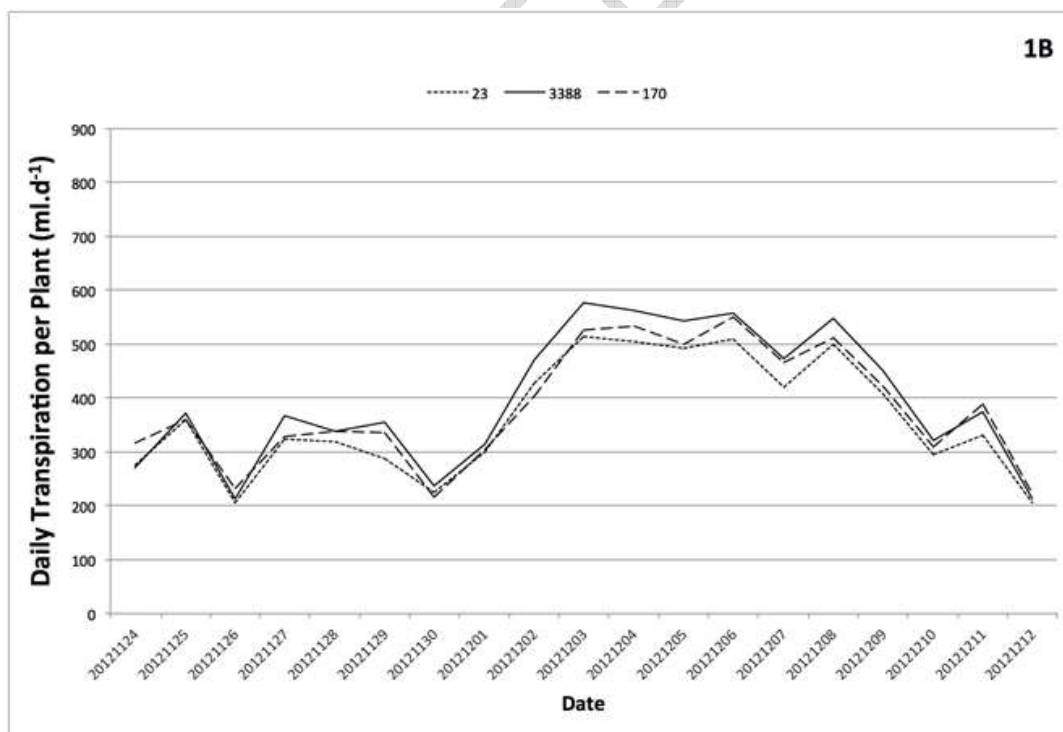
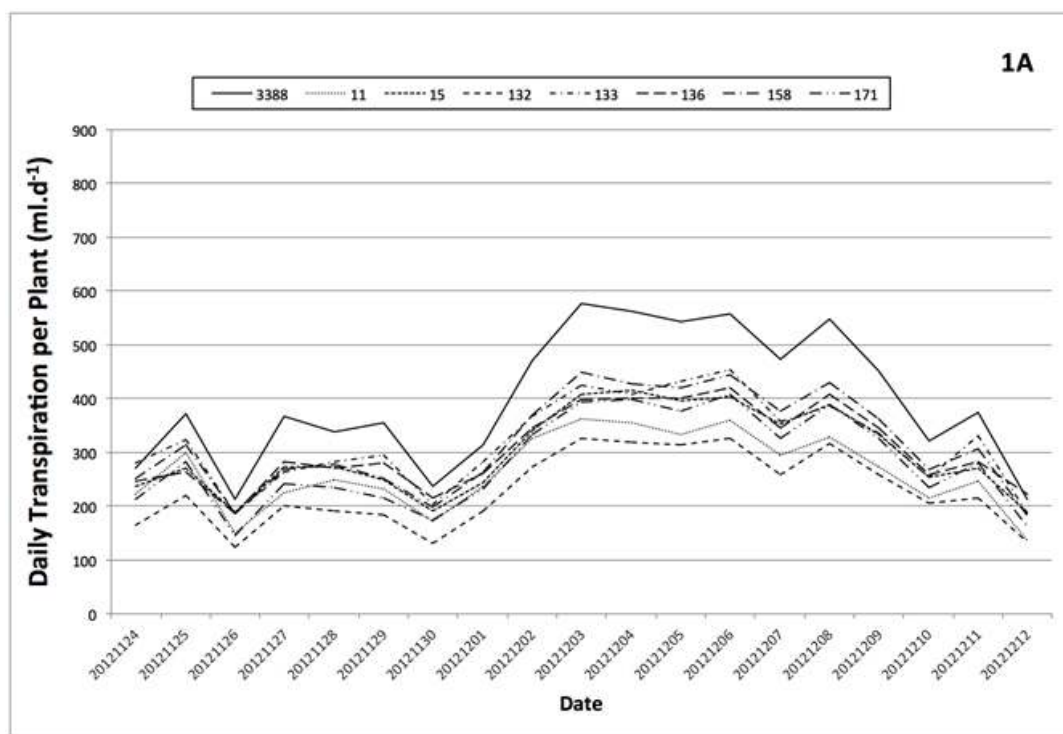
Table 1. Summary data from well-watered study comparing transgenic lines to their non-transgenic genotype background, including final cumulative values of state variables whole-plant water use (PWU), maximum daily water use per unit leaf area (MDWU/LA), whole-plant dry biomass (PDB), and water use efficiency (WUE), 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 ²)	MDWU/LA vs 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 genotype	Water Use Efficiency (WUE) (g/L)	WUE Relative to background genotype	P value of WUE vs background genotype
11	5.228	0.88	0.0027	2.124	0.88	<0.0001	37.584	2.55	0.0018	12.387	2.25	0.0020
15	5.384	0.75	0.0040	2.184	0.85	0.0704	92.975	2.41	0.0025	17.480	2.58	0.0059
20	11.356	0.90	0.0008	2.805	0.87	0.0004	79.997	2.08	0.0015	59.79	1.95	0.0055
23	8.907	0.94	0.0480	0.888	0.74	0.0001	73.648	2.03	0.0008	11.158	2.94	0.0045
133	4.858	0.58	0.0008	0.979	0.74	0.0005	50.008	1.81	0.0001	11.558	2.43	0.0094
136	5.060	0.80	0.0055	2.400	0.80	<0.0001	97.820	2.55	0.0004	15.822	3.49	0.0085
158	5.888	0.77	0.0008	1.543	0.65	0.0007	58.850	1.81	0.0002	12.932	2.57	0.0074
163	5.049	0.80	0.0001	2.115	0.89	0.0004	78.129	2.05	0.0005	18.039	2.70	0.0040
170	7.237	0.95	0.0000	0.884	0.67	0.0009	94.740	2.48	0.0008	18.480	2.90	0.0048
175	5.870	0.74	0.0005	0.985	0.74	0.0008	43.009	1.81	0.0001	9.688	2.04	0.0005
non-transgenic background 3388	7.368			1.930			38.161			4.819		
59	5.755	0.99	0.7980	1.642	0.88	0.0009	50.003	1.41	0.0002	7.271	1.52	0.0005
112	1.017	0.36	0.0079	0.905	0.55	0.0005	43.532	1.80	0.0005	11.905	2.55	0.0001
163	8.584	1.29	0.0002	1.904	0.95	0.0007	92.558	2.90	0.0002	12.140	2.70	0.0005
175	5.528	0.75	0.0004	1.552	0.79	0.0004	58.475	1.50	0.0001	11.072	2.45	0.0042
non-transgenic background 1210	7.342			0.969			35.585			4.500		

Lines 11, 20, and 133 showed significantly higher daily transpiration per unit leaf area (182, 137, and 181% at $P < 0.0001$, $P = 0.0314$, and $P < 0.0001$, respectively) than their genetic background TCP87-3388 whose transpiration was 1.33 L m^{-2} . Likewise, lines 112 and 163 showed significantly higher daily transpiration per unit leaf area (156 and 155% at $P = 0.0166$ and 0.0187 , respectively) than their genetic background CP72-1210 whose transpiration was 0.969 L m^{-2} . 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 backgrounds 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 0.0014 to 0.0802) than the genetic background TCP87-3388, which produced 38.161 g. Line 163 also produced significantly more biomass (260% at $P = 0.0022$) than the genetic background CP72-1210, which produced 35.585 g. Possibly, the higher production of biomass exhibited by these transgenic lines could be explained in part by higher photosynthetic rates than that of their respective genetic backgrounds. Zhao et al. [13] reported differences in leaf photosynthetic rates among sugarcane genotypes.

Accordingly with observed differences in biomass production, differences in nominal water use efficiency between transgenic lines and their genetic backgrounds were also more prevalent than differences in water use through transpiration (Table 1). Lines 15, 133, 136, 158, and 170 had significantly higher nominal water use efficiency (ranging from 257 to 363% at P values ranging from 0.0086 to 0.0874) than the genetic background TCP87-3388 that had 4.819 g L^{-1} . Likewise, lines 112 and 163 had higher nominal water use efficiency (265 and 270% at $P = 0.0931$ and 0.0836 , respectively) than the genetic background CP72-1210 that had 4.5 g L^{-1} .



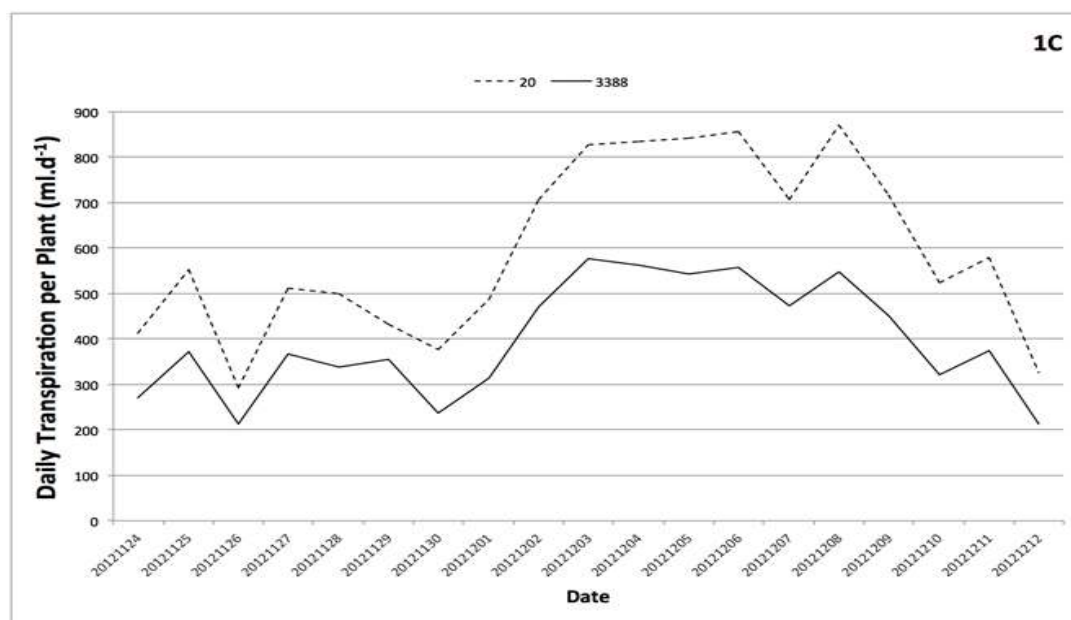


Figure 1. Progression of daily whole-plant transpiration of well-watered TCP87-3388-based transgenic lines compared to that of their genetic background. 1A: lines with daily transpiration values below background, 1B: lines with daily transpiration values similar to background, and 1C: line with daily transpiration values values above background.

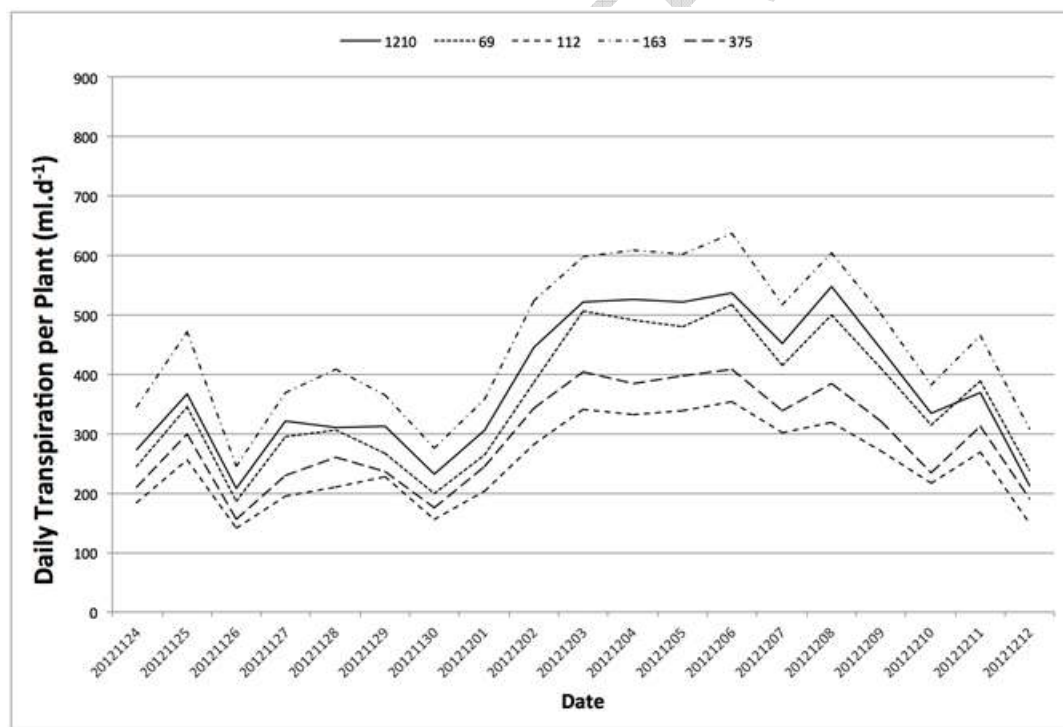


Figure 2. Progression of daily whole-plant transpiration of well-watered CP72-1210-based transgenic lines compared to that of their genetic background

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. 3A), but only the final cumulative values of lines 11, 132, and 158 were significantly lower than that of TCP87-3388 background at 6.096L; 70% at $P=0.0612$, 62% at $P=0.0181$, and 63% at $P=0.0208$, respectively (Table 2). Transgenic lines 15 and 170 showed quite similar daily whole-plant transpiration values to the TCP87-3388 background (Fig. 3B). Transgenic line 20, on the contrary, showed higher daily whole-plant transpiration values throughout the test (Fig. 3C) and a higher significant final cumulative whole-plant transpiration value (141% at $P=0.0120$) than that of genetic background TCP87-3388 (Table 2). Of the four CP72-1210-based transgenic lines, lines 163 and 375 showed daily and cumulative whole-plant transpiration values not different than the genetic background, while lines 69 and 112 showed, respectively, higher and lower daily and cumulative whole-plant transpiration values than the genetic background (Fig. 4 and Table 2). Cumulative whole-plant transpiration of line 69 was higher than that of the background (133% at $P=0.0712$), while that of line 112 was lower (52% at $P=0.0092$). Cumulative whole-plant transpiration of genetic background CP72-1210 was 5.563L.

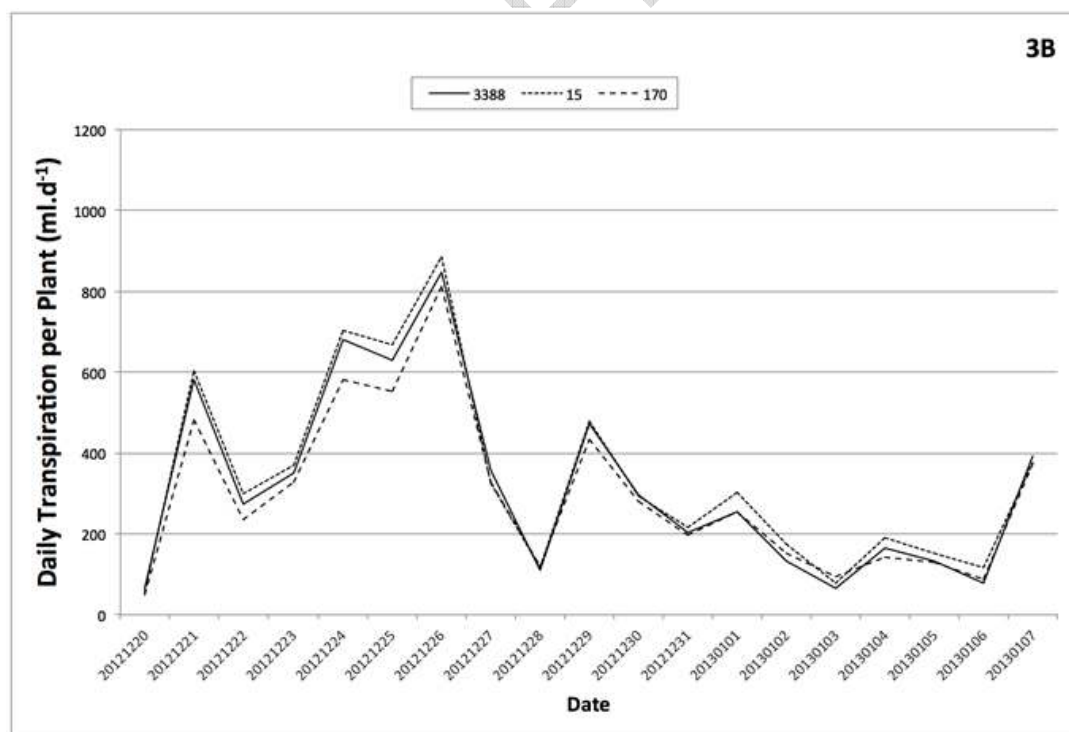
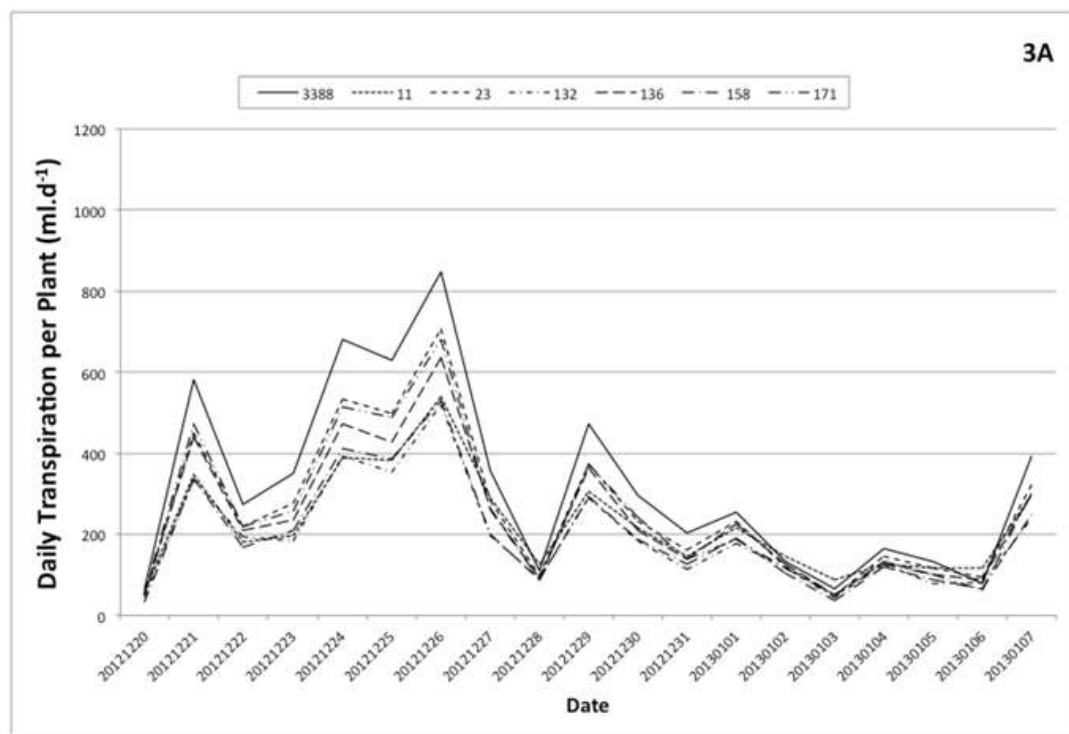
Table 2. Summary data from water-stressed study comparing transgenic lines to their non-transgenic genotype background, including final cumulative values of state variables whole-plant water use (PWU), whole-plant dry biomass (PDB), and water use efficiency (WUE), along with their ratio to the respective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

Variable/Entry	Whole-Plant Water Use (PWU) (L)	Relative to background genotype	P-value of PWU vs background genotype	Whole-Plant Dry Biomass (PDB) (g)	Relative to background genotype	P-value of PDB vs background genotype	Water Use Efficiency (WUE) (g/L)	Relative to background genotype	P-value of WUE vs background genotype
11	4.183	0.69	0.0612	86.833	0.36	0.0002	16.933	0.35	0.0450
15	5.403	0.90	0.7400	217.037	0.91	0.8010	27.233	0.94	0.7889
20	6.983	1.14	0.0000	274.837	1.14	0.0000	35.840	0.99	0.8803
23	4.993	0.81	0.0003	109.837	0.46	0.0073	43.733	0.70	0.0043
132	3.783	0.62	0.0000	159.800	0.66	0.0000	44.233	0.78	0.0007
133	3.983	0.65	0.0000	139.837	0.58	0.0000	27.277	0.59	0.0000
136	4.983	0.79	0.0000	139.837	0.58	0.0000	28.877	0.60	0.0000
158	3.803	0.62	0.0000	187.833	0.80	0.0000	30.833	0.58	0.0000
171	3.987	0.65	0.0000	204.833	0.85	0.0000	27.873	0.58	0.0043
170	4.743	0.78	0.0070	289.833	0.99	0.8803	31.240	0.78	0.0000
Non-transgenic background 3388	6.096			239.767			39.713		
69	7.107	1.16	0.0712	288.837	1.19	0.0077	41.880	1.05	0.4000
112	2.783	0.45	0.0000	129.837	0.53	0.0000	27.813	0.70	0.0000
163	4.983	0.81	0.8803	139.837	0.58	0.0000	28.873	0.72	0.0000
375	4.983	0.81	0.8803	139.837	0.58	0.0073	40.000	0.84	0.0000
Non-transgenic background 1210	5.563			252.633			47.844		

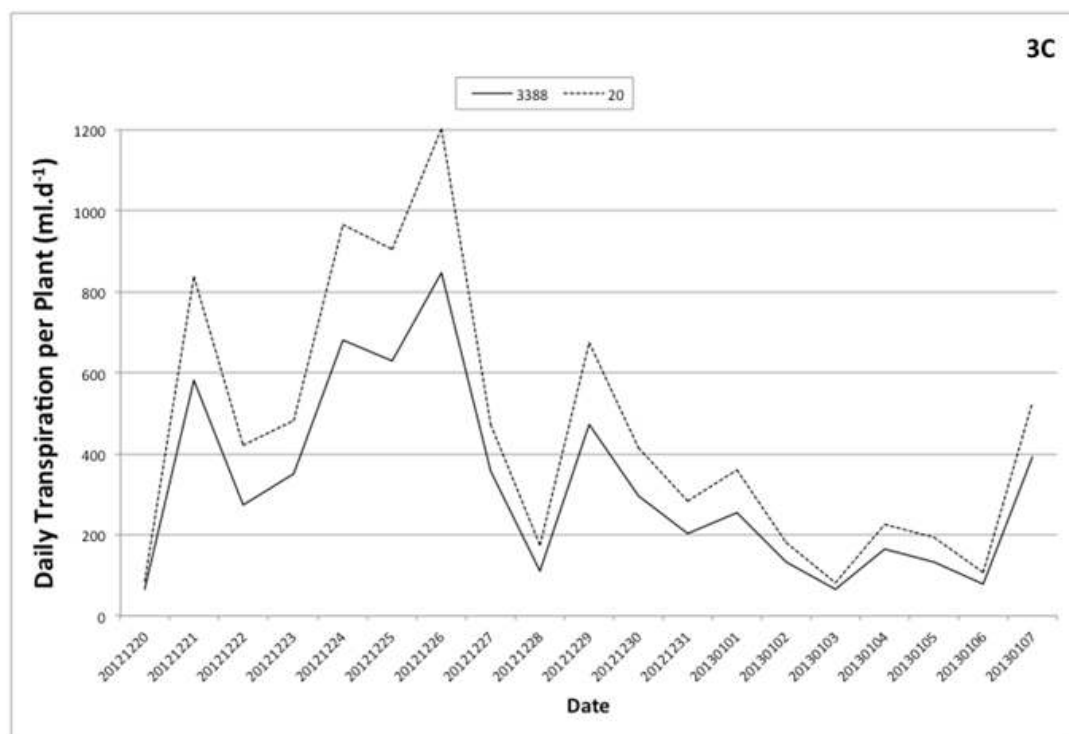
There were differences in biomass production under water stress conditions between some of the transgenic lines and their genetic backgrounds (Table 2). Lines 11, 132, 133, and 136 produced significantly less biomass (ranging from 41 to 68% at P values ranging from 0.0002 to 0.0310) than the genetic background TCP87-3388, which produced 239.767g. Lines 112, 163, and 375 also produced significantly less biomass (ranging from 42 to 75% at P values ranging from 0.0002 to 0.0720) than the genetic background CP72-1210, which produced 252.633g. Possibly, the lower production of biomass exhibited by some of these transgenic lines (11, 132, 136, and 112) when compared to their genetic backgrounds could be explained in part by lower photosynthetic rates resulting from lower stomatal conductance. This interpretation is supported by the fact that these lines showed significant lower cumulative whole-plant transpiration than their genetic backgrounds, with the exception of line 136, which was only marginally lower than the background (75% at $P=0.1103$).

Differences in nominal water use efficiency between transgenic lines and their genetic 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 genetic backgrounds; 59% at $P=0.0450$ for

188 line 11 and 62% at $P=0.0252$ for line 163. In both cases, the dominant factor decreasing the nominal
 189 water use efficiency was the lower production of biomass relative to their respective genetic backgrounds.
 190 In the case of line 11, its lower cumulative whole-plant transpiration was not enough to counterbalance its
 191 lower production of biomass.



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Figure 3. Progression of daily whole-plant transpiration of water-stressed TCP87-3388-based transgenic lines compared to that of their genetic background. 3A: lines with daily transpiration values values below background, 3B: lines with daily transpiration values values similar to background, and 3C: line with daily transpiration values values above background.

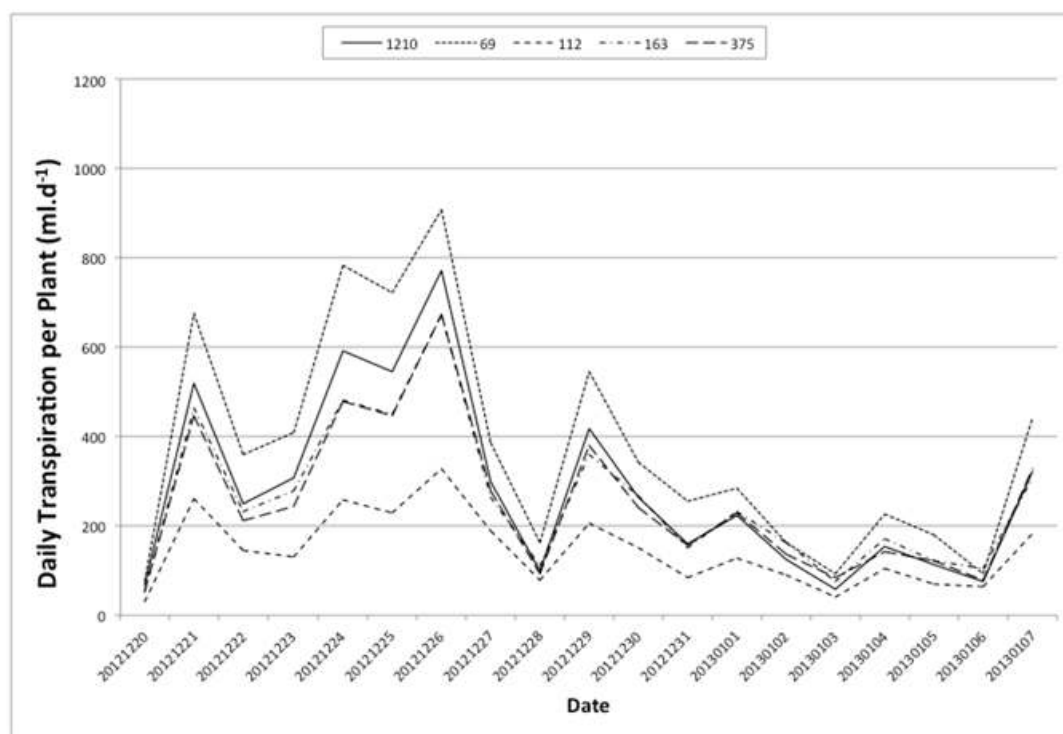


Figure 4. Progression of daily whole-plant transpiration of water-stressed CP72-1210-based transgenic lines compared to that of their genetic background.

4. CONCLUSIONS

When transgenic lines were compared to their respective genetic backgrounds under well-watered conditions, seven of the tested lines exhibited higher nominal water use efficiency than their backgrounds, namely, lines 15, 133, 136, 158, 170, 112, and 163. Except for line 112, the higher water use efficiency attained by all the other lines was related to their higher production of biomass, which was not paralleled by a concomitant increase in whole-plant transpiration. The higher water use efficiency attained by line 112 was related to a lower cumulative whole-plant transpiration, which was not paralleled by a concomitant decrease in biomass production. Data collected in this study can relate its lower cumulative whole-plant transpiration to a lower transpiration per unit leaf area. Consequently, line 112 can be categorized as a water-conservative line when grown under well-watered conditions. Conversely, although line 20 showed higher biomass production than its genetic background, it should be categorized as water-prodigal because it also increased its cumulative water use.

When transgenic lines were compared to their respective genetic backgrounds under water stress conditions, most of the lines did not exhibit differences in nominal water use efficiency. This means that most transgenic lines either were not different in cumulative water use and biomass production than their respective genetic backgrounds or that if they were different the differences in these two state variables were in the same direction, thus resulting in similar nominal water use efficiencies. The latter was the case of lines 132, 136, and 112, which exhibited both lower cumulative whole-plant transpiration and biomass production. Unlike most of the transgenic lines included in this study, lines 11 and 163 had lower nominal water use efficiency than that of their respective genetic backgrounds. In both cases, the dominant factor decreasing the nominal water use efficiency was the lower production of biomass relative to their respective genetic backgrounds and, therefore, these two lines ought to be considered less tolerant to water stress than their genetic backgrounds. Line 11 also showed lower cumulative whole-plant transpiration than the genetic background, but this did not counter-compensate the lower production

of biomass. In summary, four of the transgenic lines exhibited water conservation relative to their respective genetic backgrounds, namely 11, 132, 158, and 112. However, while lines 132, 158, and 112 did not sacrifice nominal water use efficiency, line 11 did. Two other lines, namely 20 and 69, exhibited increased cumulative whole-plant transpiration and similar nominal water use efficiency relative to their respective backgrounds without sacrificing nominal water use efficiency and, therefore, can be considered as water prodigal.

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