# Growth and development of tomato plants Lycopersicon Esculentum Mill. under different saline conditions by fertirrigation with pretreated cheese whey wastewater

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#### ABSTRACT

Pretreated cheese whey wastewater (CWW) has been used at different salinity levels: 1.75, 2.22, 3.22, 5.02 and 10.02 dS m<sup>-1</sup> and compared with fresh water (1.44 dS m<sup>-1</sup>). Two cultivars (cv.) of the tomato plant Lycopersicon Esculentum Mill. (Roma and Rio Grande) were exposed to saline conditions for72 days. Salinitylevel (treatment) had no significant effects on the fresh weightand dry matter of the leaves, stems and roots. Similar results were found when specific leafarea, leaflet area, ramifications numberof1storder/plant, stem diameter and length, nodes number/stem and primary root length were considered. Conversely, the salinity level significantly influenced the Soil Plant Analysis Development (SPAD) index and the distance between nodes in the plant stem. In the first case, an increase of 21% was obtained in the salinity levels of 5.02 and 10.02 dS m<sup>-1</sup> for cv. Rio Grande, compared with the control run. The results showed that the pretreated CWW can be a source of nutrients for tomato plants, with reduced effects on growth and development.

Key words | agricultural reuse, cheese whey wastewater, leaf area, salinity level, SPAD index, tomato plant

#### INTRODUCTION

Due to the generated volume and organic/nutritional com- position, cheese whey wastewater (CWW) has an important role in the negative environmental impact of dairy effluents. Organic/nutritional load is responsible for oxygen depletion leading to eutrophication of the natural aquatic receptors. These negative effects occur when CWW disposal is not conducted in a controlled way. Additionally, CWW characterization varies considerably. In this context, the organic load ranges from approximately 0.8 to 77 kg m <sup>3</sup> and 0.6 to 16 kg m <sup>3</sup> for chemical oxygen demand (COD) and biological oxygen demand (BOD), respectively.

The acid pH and high salinity level are other important characteristics (Prazeres et al. 2012). In the latter case, sodium (0.9-1.7 kg m<sup>3</sup>) and chloride (2.1-2.8 kg m<sup>3</sup>) are the main cause of the salinity values. Thus, biological and chemical treatments, valorization technologies and

agricultural reuse have emerged as promising management mechanisms. Agricultural reuse after chemical precipitation has been the technology selected in this work. In the chemical precipitation step, an effluent with low content in fats and suspended solids but rich in nutrients is generated. Physicochemical pretreatment also avoids the damage of pumps or equipment downstream (Rivas et al. 2011). This pretreated wastewater has a high salinity level that permits its use in plant irrigation with moderate to high salinity tolerance. Several studies have been conducted to report the effect of salinity on the production and quality of tomatoes (Petersen et al. 1998; Sato et al. 2006). However, the use of pretreated wastewater containing intrinsic characteristics of salinity is scarce.

Use of non-conventional water resources for agricultural purposes has been the target of several research studies (Hancock 1999; Al-Lahham et al. 2007). The background of unconventional resource application is very extensive; usually it is in response to the growing need for water, and it reduces the pollution and effluent treatment costs. How- ever, irrigation water quality can severely affect the growth and development of the plant. These perturbations can be evaluated by symptoms of deficiency/toxicity, namely, fresh and dry weight, stem diameter and length, root length, leaf area, etc.

Thus, in this work the growth and development of the tomato plants Lycopersicon Esculentum Mill. (cv. Roma and cv. Rio Grande) under five different salinity conditions by fertirrigation with pretreated CWW were investigated. The biometric response of the different vegetative parts (leaves, stems and roots) of the tomato plants was assessed.

#### METHODS

Raw and pretreated cheese whey wastewater

Raw CWW was collected from a small 'Serpa Cheese industry' located in the south of Portugal. Prior to its use as irrigation water, basic precipitation was applied to raw CWW at the optimal operating conditions. Fats and suspended solids were mainly removed. Pretreated CWW (Table 1) still presents a high organic load (approximately 8 kg m<sup>3</sup> of BOD and 10 kg m<sup>3</sup> of COD) with a biodegradability index of approximately 0.82. The principal drawback of the pretreated CWW is the salinity level, monitored by the average electrical conductivity (15 dS m') and total dis- solved solids (TDS) (13 kgm<sup>3</sup>). The high salinity is, principally, due to

the sodium content, followed by chloride and potassium contents. Magnesium hardness is responsible for 64.3% of the total hardness. Additionally, pretreated CWW is rich in nutrients, such as phosphorus and Kjeldahl nitrogen. Analytical procedures can be found elsewhere (APHA 1998; Rivas et al. 2010).

#### Experimental design

Pretreated CWW was diluted with fresh water (1:50; 1:22; 1:10; 1:5 and 1:2) obtaining five salinity levels ( $T_1 = 1.75$ ,  $T_2 = 2.22$ ,  $T_3 = 3.22$ ,  $T_4 = 5.02$  and  $T_5 = 10.02$  dS m '). Table 1 presents the main characteristics of the irrigation waters. Fresh water was used as control (T0) and is poor in organic matter, hardly biodegradable and with low nutrient content, such as phosphorus, nitrogen and potassium.

# Agronomy

Field experiments were conducted at the Experimental Center of the Escola Superior Agrária de Beja, Portugal (38 01°37.28"N and 7<sup>w</sup>52°10.75"W; altitude of 240 m). Tomato plants of two cultivars (cv.) Lycopersicon Esculentum Mill. (cv. Roma and cv. Rio Grande) were transplanted with a distance of 0.20 and 1 m in the line and between lines, respectively. The experimental design was bifactorial. Four tomato producing plants for each cultivar and treatment were analyzed after an exposure time of 72 days. The experiment was installed in a soil with medium texture, scarcely salty and alkaline with conductivity = 0.455 dS m<sup>-1</sup> and pH = 8.3. Additionally, this soil is poor in organic matter (1.28%) and presents the following nutrient contents (mg kg phosphorus (135), potassium (>200), magnesium (>125), sodium (77) and chloride (266.3).

# Monitoring the environmental conditions

The experimental area is influenced by the Mediterranean climate, characterized by soft winters and hot and long summers, with frequent and long-lasting periods of drought. The environmental conditions of the experiment were collected from the meteorological station of the Quinta da Saúde, in the city of Beja (COTR-Centro Operativo e de Tecnologia de Regadio). The minimum and maximum temperature during the experiment ranged from 5.80 to 21.24 <sup>W</sup>C (13.92 ± 3.30) and 19.70 to 39.34 <sup>W</sup>C (31.32 ± 5.06), respectively (values in brackets are average ± standard deviation). Reference evapotranspiration exhibited an average value of  $6.1 \pm 1.1 \text{ mm}$  (2.4-8.0 mm). Precipitation had an average value of 0.16 mm and a range of 0-8.50 mm.

# Analyzed characteristics

The stem diameter was determined using a digital paquimeter Quantum-Maschinen. Leaf area was determined by a Li-COR<sup>®</sup> Biosciences LI-3100C apparatus. The SPAD (Soil Plant Analysis Development) index was monitored by a SPAD Minolta 502 chlorophyll-meter. Dry matter was determined by the gravimetric method after drying in an oven with forced ventilation at 65-70 <sup>w</sup>C, until constant weight (Varennes 2003).

#### Statistical analysis

The results were treated by using the statistical program MSTAT-C. The Least Significant Difference (LSD) test for

Table 1 Physicochemical characterization of the pretreated CWW and irrigation waters. Results after four different collections. Parameters are expressed in ppm

Parameters	Pretreated CWW	το	τ,	<b>T</b> <sub>2</sub>	<b>T</b> <sub>3</sub>	τ.	τ <sub>s</sub>
COD	7,931-12,660	17-43	100-240	293-547	666-1,299	1,533-2,565	3,931-7,197
BOD <sub>5</sub>	6,100-10,900	2-4	80-200	200-440	600-950	1,200-2,500	3,300-6,900
BOD <sub>5</sub> /COD <sup>a</sup>	0.74-0.91	0.09-0.12	0.78-0.84	0.68-0.81	0.73-0.90	0.78-0.97	0.84-0.96
Turbidity <sup>b</sup>	7.7-244.7	1.0-2.7	9.2-15.2	17.1-21.7	19.3-32.8	23.6-55.9	31.5-125.4
pH	7.05-8.02	7.27-7.50	7.31-7.89	6.99-7.69	7.11-7.61	6.98-7.57	6.83-7.91
Temperature <sup>c</sup>	20.0-27.1	25.3-26.2	22.3-27.7	22.1-28.2	22.1-28.0	21.9-27.8	21.9-27.7
Conductivityd	12.23-16.24	1.40-1.47	1.73-1.79	2.08-2.33	3.03-3.40	4.65-5.42	9.18-11.08
Redox potentiale	-314.5-(-97.9)	188.9-235.3	55.7-217.5	-105.4 - 198.4	-257.1 - 163.9	-290.3 - 118.6	-268.4-(-79.4)
TS	12,324-16,868	816-850	874-952	1,126-1,304	1,682-2,208	2,828-4,082	6,238-9,230
TVS	3,086-6,274	312-312	252-286	320-442	398-842	772-1,686	1,630-3,822
TSS	160-440	84-152	68-96	36-176	164-236	248-304	172-276
VSS	112-336	16-28	92-120	28-196	136-188	208-252	148-260
Na	3,255.2-3,553.9	88.1-106.5	139.3-160.4	200.8-232.4	359.4-510.5	615.9-932.2	1,381.6-2,110.8
K	221.6-403.7	9.4-11.3	11.3-16.4	18.7-38.4	29.6-65.0	49.3-104.4	108.3-231.4
Р	39.9-57.3	-	-	-	-	-	3.8-18.3
Cl	1,922.0-2,945.9	237.1-237.1	255.1-280.2	294.6-323.3	391.6-485.0	564.0-797.6	1,077.8-1,706.5
N	224.9-333.9	1.3-5.2	5.2-11.3	9.7-28.7	23.6-42.1	46.6-90.7	118.1-186.9
СН	61.7-138.8	185.1-185.1	123.4-138.8	115.7-138.8	115.7-123.4	123.4-246.8	92.5-123.4
Ca	24.7-55.5	74.0-74.0	49.4-55.5	46.3-55.5	46.3-49.4	49.4-98.7	37.0-49.4
MH	154.2-208.2	161.9-181.2	169.6-208.2	131.1-154.2	146.5-223.6	123.4-215.9	138.8-262.2
Mg	37.6-50.8	39.5-44.2	41.4-50.8	32.0-37.6	35.7-54.6	30.1-52.7	33.9-64.0
TH	231.3-347.0	347.0-366.3	308.5-347.0	269.9-269.9	269.9-347.0	269.9-462.7	231.3-385.6
Ca/Mg <sup>a</sup>	0.6-1.1	1.7-1.9	1.1-1.3	1.2-1.7	0.9-1.4	1.4-2.5	0.7-1.1
CH/MH <sup>a</sup>	0.4-0.7	1.0-1.1	0.7-0.8	0.8-1.1	0.6-0.8	0.8-1.5	0.4-0.7
a dimonolonol							

<sup>b</sup>(NTU).

f(°C).

d(dS m-1).

°(mV).

To represents the fresh water (control run). T1, T2, T3, T4, and T5 represent the pretreated CWW diluted with fresh water in the following ratios 1:50; 1:22; 1:10; 1:5 and 1:2, respectively.

CWW – Cheese whey wastewater, COD – Chemical oxygen demand, BOD – Biological oxygen demand, TS – Total solids, TVS – Total volatile solids, TSS – Total suspended solids, VSS – Volatile suspended solids, CH – Calcium hardness (CaCO<sub>3</sub>), MH – Magnesium hardness (CaCO<sub>3</sub>), TH – Total hardness (CaCO<sub>3</sub>).

a confidence range of 95% was applied for the differences among the means.

# **RESULTS AND DISCUSSION**

Plant fresh weight and dry matter: leaves, stems and roots

The effect of the pretreated CWW reuse on the plant fresh weight is shown in Table 2. No significant differences were found in the plant fresh weight. Salinity can severely affect the dry matter content of different vegetative parts of the plant. The tomato cultivars' response to salinity has been quite diversified. Reductions in the dry matter of 61% in leaves, 40% in stems and 44% in roots have been reported when a solution of 200 mM in NaCl was used as irrigation water (Zribi et al. 2009). Conversely, a root dry matter increase of about 40% was reported by Maggio et al. (2007) when a tomato exposition to a solution of 13.2 dS m<sup>-1</sup> conductivity was accomplished. Dry matter reduction results from the osmotic effect and toxicity of certain ions, like sodium and chloride (Hajiboland et al. 2010).

Thus, in this work the effect of the pretreated CWW reuse on the tissues' dry matter was analyzed (Table 3). From the experimental data obtained, it is inferred that the cultivar effect was statistically significant for the dry matter content of the leaves for the test day after the

Table 2 | Effect of the pretreated cheese whey wastewater reuse on the fresh weights of the tomato plants Lycopersicon Esculentum Mill.

Characteristics	Total g plant <sup>-1</sup>	Leaves g plant <sup>-1</sup>	Stems g plant <sup>-1</sup>	Roots g plant <sup>-1</sup>
CULTIVAR	n.s.	n.s.	n.s.	n.s.
cv. Roma	$397.76 \pm 36.50$	$177.68 \pm 38.77$	$213.38 \pm 13.78$	$6.70 \pm 1.38$
cv. Rio Grande	$345.45 \pm 72.72$	$158.85 \pm 53.70$	$180.09 \pm 34.27$	$6.51 \pm 1.07$
TREATMENT	n.s.	n.s.	n.s.	n.s.
$T_0$	$312.56 \pm 128.92$	$120.49 \pm 56.98$	$185.13 \pm 69.61$	$6.95\pm2.33$
$T_1$	$360.34 \pm 37.53$	$173.63 \pm 11.81$	$181.44 \pm 49.69$	$5.28\pm0.35$
<i>T</i> <sub>2</sub>	$435.84 \pm 16.39$	$219.39 \pm 28.44$	$208.71 \pm 12.04$	$7.74\pm0.02$
T <sub>3</sub>	$399.95 \pm 67.99$	$210.46 \pm 49.52$	$183.16 \pm 17.84$	$6.33 \pm 0.64$
$T_4$	$373.38 \pm 10.15$	$150.38\pm10.50$	$216.01 \pm 0.05$	$6.99 \pm 0.30$
T <sub>5</sub>	$347.59 \pm 6.28$	$135.24 \pm 3.13$	$205.98 \pm 7.88$	$6.38 \pm 1.52$
CV×TREAT	n.s.	n.s.	n.s.	n.s.
cv. Roma				
T <sub>0</sub>	$403.73 \pm 211.53$	$160.78 \pm 71.24$	$234.35 \pm 140.64$	$8.60\pm0.35$
$T_1$	$386.88 \pm 115.36$	$165.28 \pm 73.36$	$216.58 \pm 41.61$	$5.03 \pm 0.39$
T <sub>2</sub>	$424.25 \pm 114.34$	$199.28 \pm 79.37$	$217.23 \pm 33.69$	$7.75 \pm 1.27$
T <sub>3</sub>	$448.03 \pm 125.33$	$245.48 \pm 71.95$	$195.78 \pm 51.30$	$6.78\pm2.09$
$T_4$	$380.55 \pm 133.93$	$157.80 \pm 55.15$	$215.98 \pm 79.23$	$6.78\pm0.46$
T <sub>5</sub>	$343.15 \pm 88.53$	$137.45 \pm 44.69$	$200.40 \pm 42.99$	$5.30\pm0.85$
cv. Rio Grande				
$T_0$	$221.40 \pm 132.16$	$80.20 \pm 73.19$	$135.90 \pm 58.27$	$5.30\pm0.71$
$T_1$	$333.80 \pm 180.03$	$181.98 \pm 100.52$	$146.30 \pm 79.41$	$5.53\pm0.11$
T <sub>2</sub>	$447.43 \pm 298.58$	$239.50 \pm 142.34$	$200.20 \pm 152.74$	$7.73\pm3.50$
<i>T</i> <sub>3</sub>	$351.88 \pm 26.34$	$175.45 \pm 22.20$	$170.55 \pm 5.73$	$5.88 \pm 1.59$
$T_4$	$366.20 \pm 22.20$	$142.95 \pm 10.47$	$216.05 \pm 11.81$	$7.20\pm0.07$
<i>T</i> <sub>5</sub>	$352.03 \pm 40.48$	$133.03 \pm 4.99$	$211.55 \pm 37.76$	$7.45\pm2.26$
Coeff. Variat. (%)	37.65	42.33	36.30	22.52

n.s. - not significant, Coeff. Variat. - Coefficient of variation.

Table 3 | Effect of the pretreated cheese whey wastewater reuse on the dry matter, leaf area and SPAD index of the tomato plant Lycopersicon Esculentum Mill.

Leaf dry matter (TDABSI = 35 days) %	Leaf d ry matter (TDABSI = 72 days) %	Stem dry matter %	Root dry matter %	Specific leaf area m² kg <sup>-1</sup> of dry matter	Leaflet area cm² leaflet <sup>-1</sup>	SPAD index -
n.s.	*	n.s.	n.s.	n.s.	*	*
$17.3\pm0.9$	$25.1\pm3.0$	$16.4\pm1.8$	$83.4\pm2.7$	$17.258 \pm 0.904$	$8.774 \pm 1.821$	$51.5 \pm 4.7$
$17.6 \pm 1.7$	$25.6\pm3.5$	$17.7\pm0.9$	$81.7\pm5.4$	$16.551 \pm 1.074$	$7.419 \pm 1.192$	$48.1\pm5.5$
n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**
-	-	-	-	-	-	4.873
$18.0\pm0.5$	$26.6\pm0.7$	$19.1\pm1.2$	$82.4\pm3.8$	$17.930 \pm 0.198$	$8.290 \pm 0.514$	$47.3 \pm 6.0 bc$
$16.0\pm0.9$	$23.8\pm3.2$	$17.4\pm2.0$	$83.6\pm0.9$	$16.917 \pm 0.180$	$8.488 \pm 0.727$	$42.4\pm0.7c$
$17.8\pm0.3$	$21.3\pm0.3$	$17.0\pm2.1$	$77.9\pm5.1$	$16.179\pm0.370$	$7.667 \pm 1.296$	$48.9 \pm 1.8 \text{b}$
$16.8\pm0.8$	$24.6\pm4.8$	$16.7\pm0.5$	$84.2\pm1.4$	$16.299 \pm 0.514$	$7.141\pm0.717$	$50.2 \pm 5.2$ ab
$19.1\pm1.6$	$28.1 \pm 1.7$	$16.1\pm0.5$	$85.8\pm0.4$	$16.079 \pm 1.336$	$7.073 \pm 2.252$	$55.1 \pm 0.5a$
$16.9 \pm 1.4$	$28.0\pm0.3$	$16.1\pm1.4$	$81.1\pm8.5$	$18.023 \pm 1.157$	$9.920 \pm 3.134$	$54.8 \pm 0.2a$
n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
-	-	-	-	-	-	-
8.76	22.07	18.19	5.04	9.18	17.29	6.29
	Leaf dry matter (TDABSI = 35 days) % n.s. $17.3 \pm 0.9$ $17.6 \pm 1.7$ n.s. - $18.0 \pm 0.5$ $16.0 \pm 0.9$ $17.8 \pm 0.3$ $16.8 \pm 0.8$ $19.1 \pm 1.6$ $16.9 \pm 1.4$ n.s. - 8.76	Leaf dry matter (TDABSI = 35 days) $\%$ Leaf dry matter (TDABSI = 72 days) $\%$ n.s.*17.3 $\pm$ 0.925.1 $\pm$ 3.017.6 $\pm$ 1.725.6 $\pm$ 3.5n.s.n.s18.0 $\pm$ 0.526.6 $\pm$ 0.716.0 $\pm$ 0.923.8 $\pm$ 3.217.8 $\pm$ 0.321.3 $\pm$ 0.316.8 $\pm$ 0.824.6 $\pm$ 4.819.1 $\pm$ 1.628.1 $\pm$ 1.716.9 $\pm$ 1.428.0 $\pm$ 0.3n.s.n.s8.7622.07	Leaf dry matter (TDABSI = 35 days) $\%$ Leaf dry matter (TDABSI = 72 days) $\%$ Stem dry matter $\%$ n.s.*n.s.17.3 $\pm$ 0.925.1 $\pm$ 3.016.4 $\pm$ 1.817.6 $\pm$ 1.725.6 $\pm$ 3.517.7 $\pm$ 0.9n.s.n.s.n.s.17.6 $\pm$ 1.725.6 $\pm$ 3.517.7 $\pm$ 0.9n.s.n.s.n.s18.0 $\pm$ 0.526.6 $\pm$ 0.719.1 $\pm$ 1.216.0 $\pm$ 0.923.8 $\pm$ 3.217.4 $\pm$ 2.017.8 $\pm$ 0.321.3 $\pm$ 0.317.0 $\pm$ 2.116.8 $\pm$ 0.824.6 $\pm$ 4.816.7 $\pm$ 0.519.1 $\pm$ 1.628.1 $\pm$ 1.716.1 $\pm$ 0.516.9 $\pm$ 1.428.0 $\pm$ 0.316.1 $\pm$ 1.4n.s.n.s.n.s8.7622.0718.19	Leaf dry matter (TDABSI = 35 days) $\%$ Leaf dry matter (TDABSI = 72 days) $\%$ Stem dry matter $\%$ Root dry matter $\%$ n.s.*n.s.n.s.n.s.17.3 $\pm$ 0.925.1 $\pm$ 3.016.4 $\pm$ 1.883.4 $\pm$ 2.717.6 $\pm$ 1.725.6 $\pm$ 3.517.7 $\pm$ 0.981.7 $\pm$ 5.4n.s.n.s.n.s.n.s.n.s18.0 $\pm$ 0.526.6 $\pm$ 0.719.1 $\pm$ 1.282.4 $\pm$ 3.816.0 $\pm$ 0.923.8 $\pm$ 3.217.4 $\pm$ 2.083.6 $\pm$ 0.917.8 $\pm$ 0.321.3 $\pm$ 0.317.0 $\pm$ 2.177.9 $\pm$ 5.116.8 $\pm$ 0.824.6 $\pm$ 4.816.7 $\pm$ 0.584.2 $\pm$ 1.419.1 $\pm$ 1.628.1 $\pm$ 1.716.1 $\pm$ 0.585.8 $\pm$ 0.416.9 $\pm$ 1.428.0 $\pm$ 0.316.1 $\pm$ 1.481.1 $\pm$ 8.5n.s.n.s.n.s.n.s.n.s8.7622.0718.195.04	Leaf dry matter (TDABSI = 35 days)Leaf dry matter dry matter $%$ Root dry matter $%$ Root dry matter $%$ Specific leaf area $m^2  kg^{-1}$ of dry mattern.s.*n.s.n.s.n.s.specific leaf area $m^2  kg^{-1}$ of dry mattern.s.*n.s.n.s.n.s.n.s.17.3 $\pm$ 0.925.1 $\pm$ 3.016.4 $\pm$ 1.883.4 $\pm$ 2.717.258 $\pm$ 0.90417.6 $\pm$ 1.725.6 $\pm$ 3.517.7 $\pm$ 0.981.7 $\pm$ 5.416.551 $\pm$ 1.074n.s.n.s.n.s.n.s.n.s.n.s18.0 $\pm$ 0.526.6 $\pm$ 0.719.1 $\pm$ 1.282.4 $\pm$ 3.817.930 $\pm$ 0.19816.0 $\pm$ 0.923.8 $\pm$ 3.217.4 $\pm$ 2.083.6 $\pm$ 0.916.917 $\pm$ 0.18017.8 $\pm$ 0.321.3 $\pm$ 0.317.0 $\pm$ 2.177.9 $\pm$ 5.116.179 $\pm$ 0.37016.8 $\pm$ 0.824.6 $\pm$ 4.816.7 $\pm$ 0.584.2 $\pm$ 1.416.299 $\pm$ 0.51419.1 $\pm$ 1.628.1 $\pm$ 1.716.1 $\pm$ 0.585.8 $\pm$ 0.416.079 $\pm$ 1.33616.9 $\pm$ 1.428.0 $\pm$ 0.316.1 $\pm$ 1.481.1 $\pm$ 8.518.023 $\pm$ 1.157n.s.n.s.n.s.n.s.n.s.n.s8.7622.0718.195.049.18	Leaf dry matter (TDABSI = 35 days)Leaf dry matter vStem dry matter %Root dry matterSpecific leaf area m² kg²¹ of dry matterLeaflet area cm² leaflet⁻¹n.s.*n.s.n.s.n.s.n.s.specific leaf area m² kg²¹ of dry matterLeaflet area cm² leaflet⁻¹n.s.*n.s.n.s.n.s.n.s.specific leaf area m² kg²¹ of dry matterLeaflet area cm² leaflet⁻¹n.s.*n.s.n.s.n.s.n.s.specific leaf area m² kg²¹ of dry matterLeaflet area cm² leaflet⁻¹17.3 ± 0.925.1 ± 3.016.4 ± 1.883.4 ± 2.717.258 ± 0.9048.774 ± 1.82117.6 ± 1.725.6 ± 3.517.7 ± 0.981.7 ± 5.416.551 ± 1.0747.419 ± 1.192n.s.n.s.n.s.n.s.n.s.n.s.n.s18.0 ± 0.526.6 ± 0.719.1 ± 1.282.4 ± 3.817.930 ± 0.1988.290 ± 0.51416.0 ± 0.923.8 ± 3.217.4 ± 2.083.6 ± 0.916.917 ± 0.1808.488 ± 0.72717.8 ± 0.321.3 ± 0.317.0 ± 2.177.9 ± 5.116.179 ± 0.3707.667 ± 1.29616.8 ± 0.824.6 ± 4.816.7 ± 0.584.2 ± 1.416.299 ± 0.5147.141 ± 0.71719.1 ± 1.628.1 ± 1.716.1 ± 0.585.8 ± 0.416.079 ± 1.3367.073 ± 2.25216.9 ± 1.428.0 ± 0.316.1 ± 1.481.1 ± 8.518.023 ± 1.1579.920 ± 3.134n.s.n.s.n.s.n.s.

\* and \*\* significance for  $p \le 0.05$  and  $p \le 0.01$ , respectively; n.s. – not significant. Different lowercase letters indicate differences with  $p \le 0.05$ , according to LSD test. Coeff. Variat. – Coefficient of variation. TDABSI – Test day after the beginning of the saline irrigation.

beginning of the saline irrigation (TDABSI) 72 (p < 0.05). The leaf dry matter according to the irrigation water salinity was monitored 35 days after the beginning of the saline irrigation and at the end of the experiment (72 days) (Figure 1, top). It was found that the exposure time to salts affects the dry matter of the leaves. Thus, a longer exposure to the salt content leads to solutes accumulation in the leaves, and consequently increases the dry matter in the tissues. In the treatment with conductivity level of 10.02 dS m<sup>-1</sup> increments of 43.6 and 35.6% were obtained for cv. Roma and cv. Rio Grande, respectively, when exposed to a period of 72 days compared to 35 days exposure. Additionally, the dry matter of the stems and roots is also depicted in Figure 1 (bottom) for both cultivars.

#### Leaf area and SPAD index

The effect of the pretreated CWW reuse on the leaf area and SPAD index is presented in Table 3. From the experimental data obtained, the cultivar effect was statistically significant for the leaflet area and SPAD index (p < 0.05). The salinity level (treatment) significantly influenced the SPAD index (p < 0.01). The relationships of the leaf area and the SPAD index with the salinity level are displayed in Figure 2, for both cultivars. The expansion rate reduction of leaf surface is the immediate response of the plants to salt stress (Wang & Nil 2000). This effect influences the carbon assimilation with the consequent reduction in photosynthetic rate (Munns 1993). However, this reduction can be presented as a strategy mechanism to increase the solute content in leaves (Fernandes et al. 2003) by keeping the osmotic potential in the dual system plant - soil. In the work carried out by Gao et al. (1998) leaf area reductions of about 41 and 66% were experienced when the tomato plants were exposed to saline conditions of 50 and 100 mM NaCl, respectively.

The irrigation with saline water containing high nitro- gen concentration can lead to the development of leaves with a more intense green color due to an increase in chlorophyll content. The SPAD index, closely related to the chlorophyll content in the plant leaf, was significantly increased in saline wastewater treatments T3, T4 and T5, benefiting the photosynthesis process. The cv. Rio Grande recorded the highest increase in the SPAD index with the salinity level. Treatments T4 and T5 showed SPAD index values 21% higher than treatment  $T_0$  for cv. Rio Grande. Romero-Aranda et al. (2001) also obtained an increase in the chlorophyll content with increasing salinity.



Figure 1 | Effect of the pretreated cheese whey wastewater reuse on the tissues' dry matter of the two cultivars. Top - leaf dry matter at two different exposure times: o 35 days; T72 days. Bottom - dry matter of stems and roots: o cv. Roma; • cv. Rio Grande.



Figure 2 | Effect of the pretreated cheese whey wastewater reuse on the leaf area and SPAD index of the two cultivars. o cv. Roma; • cv. Rio Grande.

Growth and development of stems and roots: plant height and primary root length

The effect of the pretreated CWW reuse on the growth and development of stems and roots is presented in Table 4. The salinity level (treatment) significantly influenced the distance between nodes in the plant stems (p < 0.01), with maximum value in the control run (12.4 cm). Plant height reductions were found by Mohammad et al. (1998) and Romero-Aranda et al. (2001). The plant growth is inhibited firstly by osmotic effect and subsequently by the excessive accumulation of salts in the leaves that causes a toxic effect (Munns 1993; Neumann 1997).

Table 4 Effect of the pretreated cheese whey wastewater reuse on the growth and development of stems and roots of the tomato plant Lycopersicon Esculentum Mill.

Plant fraction	Stem		Primary root				
Characteristics	Ramifications number of 1st order/plant –	Diameter (TDABSI = 37 days) mm	Diameter (TDABSI = 72 days) mm	Length cm	Nodes number/stem -	Distance internode cm	Length cm
CULTIVAR	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
cv. Roma	$4 \pm 1$	$17.74 \pm 1.53$	$17.71 \pm 1.71$	$75.1 \pm 6.6$	$9\pm 2$	$9.1 \pm 1.8$	$32.9\pm4.9$
cv. Rio Grande	$3 \pm 1$	$19.68\pm3.77$	$17.29 \pm 1.99$	$66.6\pm6.1$	$8 \pm 2$	$9.3 \pm 1.8$	$27.3\pm4.7$
TREATMENT	n.s.	*	n.s.	n.s.	n.s.	**	n.s.
LSD value	-	3.447	-	-	-	2.165	-
$T_0$	$3\pm0$	$16.36 \pm 1.94 c$	$15.63\pm2.14$	$70.7 \pm 16.5$	$6 \pm 2$	$12.4 \pm 0.1a$	$26.8\pm3.6$
$T_1$	$3 \pm 1$	$17.36\pm0.05 bc$	$19.06\pm2.73$	$69.9\pm9.5$	$8 \pm 2$	$9.1 \pm 0.1b$	$31.4\pm1.9$
T <sub>2</sub>	$4 \pm 1$	$22.03 \pm 3.78a$	$18.73 \pm 1.48$	$72.8 \pm 6.4$	$9 \pm 1$	$8.8 \pm 0.5b$	$29.8 \pm 13.8$
T <sub>3</sub>	$4\pm0$	$18.97\pm0.18 abc$	$18.03 \pm 1.37$	$71.6\pm8.0$	$11 \pm 1$	$7.2 \pm 0.4b$	$33.8\pm5.3$
$T_4$	$5\pm0$	$20.78 \pm 4.15 ab$	$16.98\pm0.60$	$74.2 \pm 8.6$	$9\pm 2$	$8.9 \pm 1.2b$	$28.9\pm2.3$
T <sub>5</sub>	$4 \pm 1$	$16.77 \pm 2.37c$	$16.57\pm0.86$	$66.1\pm0.4$	$8 \pm 1$	$8.8\pm0.7b$	$30.2\pm5.4$
CV ×TREAT	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
LSD value	-	-	-	-	-	-	-
cv. Roma							
To	$3 \pm 1$	$17.73\pm4.74$	$17.14 \pm 4.24$	$82.4 \pm 11.1$	$7 \pm 3$	$12.5 \pm 3.4$	$29.4\pm3.7$
$T_1$	$4 \pm 1$	$17.33 \pm 1.27$	$20.99 \pm 4.31$	$76.6 \pm 5.1$	$9 \pm 2$	$9.0 \pm 1.8$	$30.0\pm7.5$
<i>T</i> <sub>2</sub>	$5\pm0$	$19.36 \pm 1.52$	$17.68 \pm 2.91$	$68.3 \pm 6.3$	$8 \pm 0$	$9.1 \pm 0.9$	$39.5\pm2.1$
T <sub>3</sub>	4 ± 1	$19.10\pm0.56$	$17.07 \pm 5.75$	$77.3 \pm 19.1$	$11 \pm 0$	$7.5 \pm 1.1$	$37.5 \pm 12.0$
$T_4$	$4 \pm 1$	$17.84 \pm 5.34$	$17.41\pm0.09$	$80.3\pm13.4$	$10 \pm 1$	$8.0 \pm 0.2$	$27.3 \pm 1.1$
T <sub>5</sub>	$5 \pm 2$	$15.09\pm0.20$	$15.97 \pm 1.14$	$65.8\pm0.4$	$9 \pm 0$	$8.2 \pm 0.8$	$34.0\pm2.1$
cv. Rio Grande							
To	$3 \pm 1$	$14.99\pm0.40$	$14.12\pm0.57$	$59.0 \pm 17.7$	$5 \pm 1$	$12.3 \pm 1.0$	$24.3\pm8.1$
$T_1$	$3 \pm 1$	$17.40 \pm 1.57$	$17.13\pm2.75$	$63.1\pm22.1$	$7\pm 2$	$9.2 \pm 0.9$	$32.8 \pm 15.2$
<i>T</i> <sub>2</sub>	$3 \pm 0$	$24.70\pm2.43$	$19.78\pm10.29$	$77.4 \pm 21.0$	$10 \pm 4$	$8.4 \pm 0.8$	$20.0\pm5.7$
T <sub>3</sub>	$4\pm0$	$18.84 \pm 1.47$	$19.00 \pm 3.55$	$65.9 \pm 5.8$	$10 \pm 0$	$6.8 \pm 0.5$	$30.0\pm6.4$
$T_4$	5 ± 0	$23.71 \pm 1.76$	$16.56 \pm 1.01$	$68.1 \pm 24.2$	$7 \pm 1$	9.7 ± 1.7	$30.5 \pm 2.1$
$T_5$	4 ± 1	$18.44 \pm 1.64$	$17.18 \pm 3.45$	$66.4\pm0.9$	$7\pm0$	$9.3 \pm 0.7$	$26.4\pm3.4$
Coeff. Variat.	25.92	11.84	24.34	21.68	20.68	15.15	24.67

\* and \*\* significance for  $p \le 0.05$  and  $p \le 0.01$ , respectively, n.s. – not significant. Different lowercase letters indicate differences with  $p \le 0.05$ , according to LSD test. Coeff. Variat. – Coefficient of variation. TDABSI – Test day after the beginning of the saline irrigation.

Mohammad et al. (1998) found a root length reduction when the cv. Rio Grande was exposed under saline conditions (50-150 mM NaCl). The difference observed in the present study may be related to the water used, rich in nutrients such as phosphorus, potassium and nitrogen. Some authors have observed that the presence of phosphorus can stimulate the development and growth of the root system even in dry/salinity conditions (Mohammad et al. 1998).

# CONCLUSIONS

Fertirrigation with pretreated CWW can be a viable, promising and encouraging alternative, with two main advantages:

- 1. CWW management: this effluent is one of the major contaminants of the dairy industry, together with cheese whey, ice-cream, butter and whey effluents. The new presented strategy constitutes a solution to small and medium cheese factories: basic precipitation + reutilization. In the first case, organic load, nutrients, fats and solids contents are reduced to enable the land application, preventing the clogging and water/gas impermeabilization. Second stage consists of the pre- treated cheese whey valorization by land application in crops with moderate to high salinity tolerance.
- 2. Growth and development of tomato plants: under con- trolled conditions, leaves/stems/root fresh weight/dry matter, specific leaf area, leaflet area, ramifications number of 1st order/plant, stem diameter, stem length, nodes number/stem and primary root length were not influenced significantly (statistically) by treatment (salinity level of pretreated CWW) and interaction CV x TREAT. The treatment (salinity level) only affected significantly:
- SPAD index, obtaining the highest values of 50.2, 55.1 and 54.8 for salinity levels of 3.22, 5.02 and 10.02 dS m ', respectively;
- distance between nodes in the main stem, where a reduction was obtained of around 42% in the treatment T<sub>3</sub> (3.22 dS m '), compared to the control (with an average distance between nodes = 12.4 cm).
  On the other hand, the cultivar significantly influenced the following biometric characteristics: leaflet area, SPAD index and leaves dry matter.

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