Water stress response of Tricepiro and its progenitors Triticale and Trigopiro

Ruiz MA¹,³, A Golberg¹,², ML Molas¹

Abstract. In semi-arid regions rainfall is often scarce and erratic in space and time, making valuable the exploration of new genotypes with superior behavior in dry land systems. Tricepiro (X Triticoscale Wittmack x X Agrotriticum Ciferri & Giacom) is a synthetic cereal with long cycle and high biomass production in semi-arid environments. However, its performance under controlled water stress has not yet been evaluated. The aim of this work was to investigate the effect of water stress on tricepiro genotypes in comparison with their progenitors: triticale and trigopiro. Morphophysiological traits related to forage yield were evaluated in the greenhouse and the field. In the greenhouse, water potential, stomatal resistance, root biomass and wilted/green leaves were significantly associated with forage production under severe water stress [leaf water potential (WP) = -3 MPa]. In the field, under mild-moderate stress (leaf WP = -1.23 MPa), forage yield was highly related to plant height and leaf area index. We found that under water stress tricepiro performed similar to triticale, and both were superior to trigopiro. Among tricepiros, L62 and the line RC50 were the best performing genotypes. Hence, tricepiro has potential to diversify cropping systems in semi-arid regions.

Keywords: Tricepiro; Synthetic cereal; Drought; Semi-arid environment.

Resumen. En las regiones semiáridas las precipitaciones son muy escasas y erráticas tanto desde el punto de vista espacial como temporal. Estas condiciones hacen valiosa la búsqueda de nuevos genotipos adaptados a la sequía. El Tricepiro (X Triticoscale Wittmack x X Agrotriticum Ciferri & Giacom) es un cereal sintético de ciclo largo y gran producción de biomasa en ambientes semiáridos; sin embargo, su respuesta a condiciones de estrés hídrico todavía no se ha investigado. El objetivo de este trabajo fue investigar el efecto del estrés hídrico sobre genotipos de tricepiro en comparación con sus progenitores: triticale y trigopiro. Se evaluaron algunas características morfofisiológicas de los genotipos relacionadas con la producción de forraje, en invernáculo y en campo. En invernáculo, el potencial hídrico, la resistencia estomática, la biomasa de raíz y las hojas secas/hojas verdes estuvieron significativamente asociadas con la producción de forraje bajo estrés hídrico severo (potencial hídrico foliar = -3 MPa). En el campo, bajo estrés hídrico moderado (-1,23 MPa), la producción de forraje estuvo estrechamente relacionada con la altura de la planta y el índice de área foliar. Las experiencias mostraron que bajo estrés hídrico el tricepiro se comportó de manera similar al triticale, y ambos genotipos fueron superiores al trigopiro. Entre los tricepiros, L62 y la línea RC50 tuvieron un comportamiento superior. Esto sugiere que existe la posibilidad de utilizar a tricepiro para diversificar los sistemas de cultivo en regiones semiáridas.

Palabras clave: Tricepiro; Cereal sintético; Sequía; Ambiente semiárido.
INTRODUCTION

In semiarid regions, water is the most limiting factor for crop production. For this reason, successful agricultural and grazing systems in these areas must make an efficient use of precipitation. Forage systems (e.g., corn, millet and triticale) have higher water use efficiency than high frequency rotations of small grain and/or oilseed crops (Nielsen et al., 2006). Grazing livestock systems in most semiarid regions around the world are largely based on cultivated and native pastures (Entz et al., 2002; Clapham & Fedders, 2008). In cultivated pastures, perennial forages provide some of the livestock feed. In addition, small grains cereals are used regularly as forages in many areas to extend the grazing season during winter, fall and spring, and to complement established mixed pastures in many areas to extend the grazing season during winter, fall and spring, and to complement established mixed pastures (McCoy, 1971; Brown & Almodares, 1976; Juskiw et al., 1999). Although there are associated costs with planting annual forages, spring-planted winter annuals establish readily, and the yield distribution of spring-planted winter annuals provides forage for grazing in late summer and fall in the Northern hemisphere (Jedel & Salmon, 1995), and in late winter and spring in the Southern hemisphere (Amigone et al., 2005). Moreover, frequent drought periods in semiarid regions result in a shortage of feed, increasing the demand for alternative feed sources to complement pastures that many times can not keep up the demand of cattle, which is gaining size and weight (Entz et al., 2002). In this context, exploration of new plant genotypes that may have the potential to grow as forage in dryland systems is valuable.

Annual forages are promoted as a source of feed supply because they can extend the grazing period or increase hay and silage production. However, annual crops differ in phenology, growth habit and forage quality and their selection should be based on intended, final use. Traditionally, cool-season winter annual forage crops include wheat, rye, oats and barley. Synthetic cereals, such as triticale (X Triticosecale Wittmack) and tricepiro (X Triticosecale Wittmack x X Agrotriticum Ciferri & Giacom) can be added to this list. Triticale has outperformed traditional cereals in wide-ranging semiarid environments (McLeod et al., 1998; Stallknecht & Wiriman, 1998), being well adapted to extreme cold, drought and acidic soils (Brown & Almodares, 1976; Yagmurr & Kaydan, 2008). On the other hand, tricepiro, a new cereal original from Argentina, has been studied locally in its agronomic traits, showing a valuable performance in marginal areas (Covas, 1989, 1995).

Tricepiro was obtained with the purpose to develop a new crop adapted to marginal areas by crossing a hexaploid triticale (2n=6x=42, AABBRR) with an octoploid trigopiro ([X Arotriticum Ciferri & Giacom]; 2n=8x=56, AABBDDJJ]) (Covas, 1989; 1995; Ferrari et al., 2005). Several years of self pollination in tricepiro resulted in a line that was registered as a cultivar under the name Tricepiro “Don René INTA” (Covas, 1989, 1995). This cultivar possesses high potential as a parent line in breeding programs due to its notable disease resistance and freezing and drought tolerance, combining desirable agronomic traits from its origin species (Covas, 1989, 1995). Tricepiro has a longer growing period than triticale, and better performance for tillering, dry matter yield, resistance to grain shattering, resistance to black rust, and salt tolerance. Furthermore, in comparison with trigopiro, tricepiro has greater initial growth rate, more active re-growth, higher dry matter yield, and it is easier to thresh (Covas, 1995).

Among the forage winter cereals, tricepiro present the longest cycle of production and high biomass yield either in semi-arid (Frecentese & Covas, 1985; Ferri et al., 1995), sub-humid and humid environments (Gonella & Hernández, 1987, 1990; Brizuela & Cseh, 1996; Coraglio et al., 1998). According to Coraglio et al. (1998), tricepiro is stable in its biomass production, with a minimum yield of 3500 kg dry matter per hectare. Concomitantly, studies of biomass quality demonstrate high values of dry matter digestibility throughout its life cycle (75-80%), pointing out this cereal as a good candidate to be used as a forage reserve (Brizuela & Cseh, 1996).

Tricepiro was originally conceived as winter forage (Frecentese & Covas, 1985). However, its feature as a grain producer for human and animal feeding was also tested (García & Torresi, 1996; Gros et al., 1995). Preliminary studies of protein composition show a high level of lysine, suggesting a good value as a food, and open the avenue for further studies concerning amino acid composition and biological value of its proteins (Gros et al., 1995). Tricepiro grains have also been tested as a livestock food. When diary diet of pigs was primarily based on tricepiro grain, the dorsal fat was thinner compared to that based on diets rich in grains other than tricepiro (García & Torresi, 1996). Taken as a whole, tricepiro has a potential to be used in the food industry in addition to its current use as a forage cereal (Bertoni et al., 1995 a and b).

The use of physiological traits, in addition to agronomics ones, to evaluate the drought tolerance in winter cereals is well documented (Araus et al., 2002). Variables such as stomatal resistance (SR) (Santivelli Morata, 1993; Morant-Avice et al., 1994; Nogueira et al., 2000; Frank & Berdahl, 2001); water potential (WP) (Hanson et al., 1977 cited by García et al., 2002; Frank & Berdhal, 2001; Wery 2005); leaf area (LA) (Araus et al., 2002); growth of leaves (Shonfed et al., 1988; Van Loo, 1992; Cabuslay et al., 2002; García et al., 2002; Bennett et al., 2002), and roots (Grzesiak et al., 2001; Zhang et al., 2001; Araus et al., 2002) have been extensively used to better understand their response to water stress. For instance, genotypes of triticale have been discriminated by their response to drought using WP (Grzesiak et al., 2003, 2006), and root growth (Grzesiak et al., 2001; Grzesiak et al., 2007) among others.

In cereals, from the beginning of agriculture, drought has been the main yield-limiting factor (Araus et al., 2002). Background information in triticale and cereals in general shows...
that it is possible to find water stress tolerance in these species (Oskan et al., 1999; Barary et al., 2003; Grzesiak et al., 2003). Performance of tricepiro in the field allows us to infer a good adaptation to water deficit conditions (Covas, 1989; Dreussi, 1998). However, the performance of tricepiro under controlled water stress conditions has not been evaluated to date. Therefore, in this study, we investigated the effect of water deficit in tricepiro cultivar “Don Rene” and the experimental lines L54, L62 and LFRC50 in comparison with their progenitors (triticale and trigoipiro) in field and under greenhouse conditions using physiological (i.e. elongation and leaf area, water potential, stomatal resistance) and agronomical variables (i.e. biomass production, grain yield, and yield components).

MATERIALS AND METHODS

Tricepiro cultivar Don Rene INTA (DR), and the experimental lines 62, 54 and LF RC50 (L62, L54 and RC50), were tested in comparison with their progenitor’s species trigoipiro cultivar Don Noe INTA (DN) and triticale cultivar Don Santiago INTA (DS). Genotypes were provided by the Universidad Nacional de La Pampa (UNLPam), Argentina (L62, L54, DR, DN) and the Universidad Nacional de Rio Cuarto (UNRC), Argentina (RC50). Two different trials were conducted, one in the greenhouse and the other in the field.

Greenhouse trial. Cultivars and experimental lines were grown in PVC pots (50 cm deep and 16 cm diameter with 9 kg of soil) in the greenhouse of the Faculty of Agronomy of UNL Pam, Santa Rosa, La Pampa, Argentina (36˚ 34´ S, 64˚ 16´, 210 m.a.s.l.) from March 2004 to October 2004. Soil was composed by 59% sand, 34% silt and 7% clay, and it had 1.4% organic matter, 19.7 ppm phosphorous, pH 6.00, 12.1% of field capacity and 7.83% permanent wilting point (PWP). Figure 1 shows the relative humidity and average temperature values registered in the greenhouse during the study period. The photosynthetic photon flux density ranged from 500 to 1200 μm/m²/s.

A water stress treatment was imposed. Watering was withheld at the beginning of stem elongation (57 days after emergence) until water stress symptoms were evident (average leaf water potentials were -3 MPa and -0.4 MPa in the water stress and control treatments, respectively). After reaching this point, irrigated pots were re-watered to measure plant recovery to water stress. Controls were watered to field capacity every day. Pots were randomly arranged into a 2 x 6 (water x genotype) factorial design with 5 replicates.

After withholding water, stomatal resistance (SR; Delta T Porometer, AP4-UM-2, 2.28 Version, 1991) and leaf water potential (LWP; Scholander pressure chamber) were measured twice a week until the end of the experiment. At the same time, leaf growth on the youngest leaf of the main stem (L) from every plant was registered. L was used to calculate the average relative growth rate (RGR), $RGR = [(\ln L_2 - \ln L_1) / (t_2 - t_1)]$, where $L_1$ and $L_2$ were leaf length at times 1 and 2 ($t_1$ and $t_2$) (Fitter & Hay, 1983). At the end of the experiment [55 days after withholding water (DAWW)], leaf area was measured using a LICOR LI 3000 leaf area meter. Shoot and root dry weights were determined and used to calculate specific leaf area (SLA, leaf area/leaf weight), root/shoot ratio and wilted/green leaf weight (W/G).

Field trial. The study was conducted in the Agricultural Experiment Station Anguil “Ing. Agr. Guillermo Covas”, La Pampa Argentina (EEA Anguil, 36˚ 30´ S, 63˚ 59´ W), during 2005 and 2006; the soil was an entic haplustol with 41.86 ppm phosphorous and 0.12% nitrogen, field capacity 15.7% and PWP 11.6%. Weed control was applied both years (500 cc/ha 2.4D + 600 cc/ha Brominal). The experimental design was 2 x 6 (water x genotype) factorial design with 5 blocks in a split plot arrangement, where water treatment was the main plot and the genotypes the subplots. Sowing was performed on May 10, 2005 and April 21, 2006 at a density of 93 kg/ha. Plots (5 x 1.4 m) had row intervals of 0.2 m. Plots had two water conditions, rain-feed and water irrigation (in addition to rainfall). Irrigation (35.7 mm each) was applied every 15 days; a total of 285.6 mm and 214.2 mm were added in 2005 and 2006, respectively. Rainfall during 2004 was 814 mm/year; monthly averages during 2005 and 2006 are shown in Table 1 (Meteorological Service, INTA EEA Anguil). Rainfall during the experimental period (highlighted in Table 1) was 199 and 178 mm in 2005 and 2006, respectively.

Measurements of plant height, SR and WP were made during the experiment as described earlier. Leaf area was determined (LICOR LI 3000) on leaves of plants from 0.5 m² subplots during stem elongation. Measurements were conducted on October 11, 2005 (1544 DD) and September 27 (1453 DD in 2006), and leaf area index was determined.
Table 1. Historical and 2005-2006 rainfall, number of days with frost (5 cm), mean and minimum temperatures during 2005 and 2006 in the site under study: National Institute of Agricultural Technology - Agricultural Experiment Station Anguil “Ing. Agr. Guillermo Covas”, La Pampa, Argentina.

<table>
<thead>
<tr>
<th>Rainfall (mm)</th>
<th>J</th>
<th>F</th>
<th>M</th>
<th>A</th>
<th>M</th>
<th>J</th>
<th>A</th>
<th>S</th>
<th>O</th>
<th>N</th>
<th>D</th>
<th>Total Cycle</th>
<th>Total Annual</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>147.5</td>
<td>23.1</td>
<td>106.7</td>
<td>0.7</td>
<td>16.7</td>
<td>18.2</td>
<td>9.0</td>
<td>27.7</td>
<td>62.3</td>
<td>40.8</td>
<td>23.8</td>
<td>67.0</td>
<td>266.2</td>
</tr>
<tr>
<td>2006</td>
<td>99.2</td>
<td>87.7</td>
<td>104.0</td>
<td>25.5</td>
<td>0.0</td>
<td>3.6</td>
<td>5.8</td>
<td>30.5</td>
<td>12.5</td>
<td>106.3</td>
<td>19.4</td>
<td>68.8</td>
<td>272.4</td>
</tr>
<tr>
<td>1921-2005</td>
<td>75.3</td>
<td>73.1</td>
<td>93.3</td>
<td>55.8</td>
<td>31.6</td>
<td>21.6</td>
<td>18.8</td>
<td>22.8</td>
<td>38.9</td>
<td>71.7</td>
<td>74.4</td>
<td>88.1</td>
<td>423.7</td>
</tr>
</tbody>
</table>

Mean Temp. (˚C) Average Cycle Average Annual

| 2005                | 22  | 22.4 | 19.1 | 14  | 10.7 | 8.5 | 8.3 | 9.4 | 11.8 | 15  | 20.5 | 21.3 | 13.3 | 15.3 |
| 2006                | 23  | 22   | 18.4 | 16.1 | 9.5  | 9.6 | 9   | 9.1 | 12.8 | 17.2 | 19   | 23.1 | 14.0 | 15.7 |
| 1973-2007           | 22.8 | 21.5 | 19.3 | 15.1 | 11   | 7.9 | 7.3 | 9.3 | 12.1 | 15.6 | 18.6 | 21.7 | 11.0 | 15.2 |

Frost (Nº) Total Cycle Total Annual

| 2005                | 1   | 0   | 2   | 15  | 17  | 11  | 20  | 14  | 12  | 11  | 1    | 0    | 101  | 104 |
| 2006                | 0   | 0   | 3   | 5   | 17  | 14  | 17  | 26  | 17  | 3   | 2    | 0    | 101  | 104 |
| 1973-2005           | 0   | 0   | 1   | 4   | 11  | 17  | 20  | 17  | 12  | 4   | 1    | 0.2  | 86   | 87  |

Min. Temp.(˚C) Average Cycle Average Annual

| 2005                | 14.3 | 14.8 | 11.7 | 5   | 3.6  | 3.5 | 1.6 | 2.9 | 4.1 | 7.3 | 12.4 | 13.5 | 5.0  | 7.9 |
| 2006                | 15.6 | 14.2 | 10.8 | 8.5 | 1.5  | 3   | 1.4 | -0.5 | 3.3 | 10  | 10.5 | 14.8 | 6.5  | 7.8 |
| 1973-2005           | 15   | 13.8 | 12.1 | 8   | 4.3  | 1.4 | 0.4 | 1.6 | 4.1 | 1.7 | 10.6 | 13.8 | 8.0  | 7.8 |

Distance between meteorological station and experimental site was 1 km. Months corresponding to the growing period of the experiment are shown in bold.

La distancia entre la estación meteorológica y el sitio experimental fue de 1 km. Los meses correspondientes al período de crecimiento en este estudio se muestran en negrita.

RESULTS

In this study we evaluated morphological, physiological and agronomical variables under water stress in tricepiro cultivar “Don Rene” and the experimental lines L54, L62 and RC50 in comparison with their progenitors (triticale and trigopilro) under greenhouse and field conditions (2005 and 2006). In this section, results are organized per variable, and results from both greenhouse and field experiments are presented jointly. In field experiments, variance analysis was performed. Triple and double interactions are presented in Table 2.

Leaf water potential. In the greenhouse, differences in water potential (i.e., irrigation vs. water stress) were evident 19 days after withholding water. However, differences among genotypes during water stress and after recovering were not...
evident. Figure 2 shows LWP mean values for all genotypes exposed to each water condition.

Stomatal resistance. In the greenhouse experiment, well watered plants showed significant differences compared to stressed plants from 14 DAWW onwards in all the evaluated genotypes. The interaction W x G was significant (p<0.05) and differences among genotypes were also significant between 30 and 45 days. In this period, Trigopiro DN showed the greatest stomatal resistance (p<0.05), while the other cultivars did not differ (Table 3 shows results at 35 DAWW). Significant differences among cultivars were found the first day after re-watering plants (Table 3). On the first day after re-watering, there was a G x W interaction and differences among genotypes were registered. Trigopiro DN showed the greatest values of stomatal resistance, and tricepiro DR the lowest (p<0.05). Besides, RC50 showed the slower recovering after withholding water. On the second day after re-watering,

Table 2. Analysis of Variance in field experiments (2005 and 2006).

<table>
<thead>
<tr>
<th>HxGxW</th>
<th>YxG</th>
<th>WxG</th>
<th>YxW</th>
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</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05, (-) Not significant. H: plant height; SR: Stomatal Resistance; LAI: Leaf area index; SLA: Specific leaf area. Y: year; G: genotype; W: water condition.

Table 3. Stomatal resistance (SR, s/cm) of one cultivar (DR) and experimental lines of tricepiro (L62, L54 and RC 50), triticale (DS) and trigopiro (DN) on irrigated (C) and water stress (WS) plants.

<table>
<thead>
<tr>
<th>Cultivars and experimental lines</th>
<th>Greenhouse</th>
<th>Stomatal resistance (SR)</th>
<th>Field</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water stress*</td>
<td>Recovering</td>
<td>2005</td>
</tr>
<tr>
<td>C (ns)</td>
<td>WS</td>
<td>C (ns)</td>
<td>WS</td>
</tr>
<tr>
<td>DN 8.0</td>
<td>93.5 a</td>
<td>3.0</td>
<td>58.2 a</td>
</tr>
<tr>
<td>DS 2.5</td>
<td>46.3 b</td>
<td>4.4</td>
<td>23.6 bc</td>
</tr>
<tr>
<td>DR 2.4</td>
<td>53.5 b</td>
<td>5.6</td>
<td>12.6 c</td>
</tr>
<tr>
<td>L62 7.1</td>
<td>40.8 b</td>
<td>6.0</td>
<td>25.8 bc</td>
</tr>
<tr>
<td>L54 5.3</td>
<td>40.7 b</td>
<td>6.0</td>
<td>21.8 bc</td>
</tr>
<tr>
<td>RC50 2.8</td>
<td>42.3 b</td>
<td>4.4</td>
<td>36 ab</td>
</tr>
<tr>
<td>Average 4.6</td>
<td>52.8 a</td>
<td>4.9</td>
<td>29.6</td>
</tr>
</tbody>
</table>

*Data collected at 35 days DAWW.
In a column, means with the same letter are not statistically different (LSD, p<0.05). ns: not significant.
*Datos recolectados 35 días después de haber suspendido el suministro de agua. Promedios con la misma letra en una columna no son significativamente diferentes (LSD, p<0.05). ns: no significativo.
we could not detect any significant differences among cultivars (data not shown).

In field experiments under irrigation, genotypes did not show differences among them either 2005 or 2006. However, in rainfed plots, trigopiro and tricepiro RC50 showed the highest SR value compared with the other cultivars (p<0.05; Table 3).

**Leaf area, leaf area index and leaf relative growth rate.**

In the greenhouse experiment, water stress reduced leaf area (p<0.01; Table 4) and differences among genotypes were also evident (p<0.05). However, a significant interaction W x G was detected. This reduction was quantified as leaf area reduction (LAR: Leaf area water stress / Leaf area irrigation; Table 4), and it showed significant differences among genotypes. Trigopiro DN showed the highest reduction, as well as triticale and tricepiro L54. Remaining tricepiro genotypes showed higher leaf area than their progenitors under water stress.

Water deficit significantly reduced the specific leaf area (SLA; p<0.01; Table 4) and differences were found among cultivars (p<0.01); differences were not evident in watered plants. Under water stress DR showed the greatest SLA but did not differ from L62, L54 and DN.

Water stress induced a significant increase in senescence, measured as wilted/green (W/G) leaves proportion (p<0.01). This effect was more evident in Trigopiro DN and Triticale DS, while tricepiro L62 was the less affected (Table 4). In addition, the interaction W x G was not significant.

Regarding the leaf relative growth rate (LRGR), cultivars decreased this parameter after 42 days under water stress. Triticale and trigopiro highly decreased LRGR under water stress (i.e. WS/C = 37 and 51%, respectively) in comparison with tricepiro (Fig. 3).

In field experiments (2005 and 2006), leaf area index (LAI) did not show significant differences in rainfed versus irrigated cultivars (WS/C), indicating that genotypes were affected by water stress to a similar extent. The decrease of LAI, as an average of genotypes, was 36 in 2005 and 39% in 2006. In 2005, the specific leaf area was significantly reduced between water levels (p<0.05, mean values were 186 and 173 cm$^2$/g for C and WS, respectively), although no differences were found among genotypes; the interaction W x G was not significant. In 2006, no significant differences were detected either between water levels or among genotypes.
Plant height. In field experiments, Trigopiro DN showed small plant height ($p<0.05$) in both years and water conditions. Analyzing plant height for each cultivar compared with the control (WS/C, %), no significant differences were found in 2005, suggesting a similar response of all genotypes under rainfeed conditions. However, significant differences were found in 2006 ($p<0.05$) where plant height of tricepiros RC50, DR and trigopiro DN were less affected compared to the control ($p<0.05$, Fig. 4).

Aerial, underground biomass, aerial/underground ratio.
In greenhouse experiments, water stress significantly reduced ($p<0.01$) biomass. The interaction W x G was not significant. Genotypes did not differ among them either under water stress or under well watered conditions (Table 5). However, biomass yield (i.e. WS/C, %) showed significant differences among cultivars under water stress in comparison with controls ($p<0.05$). Tricepiro RC50 and L62 showed a small decrease in aerial biomass due to water stress while tricepiro DR was the most affected cultivar. In field experiments, trigopiro showed the lowest biomass production under irrigation and rainfeed conditions in 2005 and 2006. In contrast, triticale showed the greatest biomass yield, while tricepiro lines had an intermediate performance (Table 5). Reduction in dry matter production due to water stress was, on average, 31 and 49% in 2005 and 2006 respectively, but there were no differences among cultivars.
Regarding root biomass, the interaction W x G and the genotype effect were both significant ($p<0.05$ and 0.01, respectively). Stressed plants had a lower root biomass than

**Table 5.** Above and underground biomass in greenhouse and field experiments of one cultivar (DR) and experimental lines of tricepiro (L62, L54 and RC50), triticale (DS) and trigopiro (DN), exposed to irrigation (C) and water stress conditions (WS).

<table>
<thead>
<tr>
<th>Cultivars and experimental lines</th>
<th>Root</th>
<th>Aboveground</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biomass (kg/ha)</td>
<td>Biomass (g/plant)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>WS</td>
</tr>
<tr>
<td>DN</td>
<td>6.2 b</td>
<td>3.5 c</td>
</tr>
<tr>
<td>DS</td>
<td>7.3 b</td>
<td>3.8 bc</td>
</tr>
<tr>
<td>DR</td>
<td>8.9 b</td>
<td>5.5 abc</td>
</tr>
<tr>
<td>L62</td>
<td>9.5 b</td>
<td>7.4 ab</td>
</tr>
<tr>
<td>L54</td>
<td>15.8 a</td>
<td>5.8 abc</td>
</tr>
<tr>
<td>RC50</td>
<td>8.4 b</td>
<td>8.7 a 104 a</td>
</tr>
</tbody>
</table>

* Plots were rainfed.
Within columns, means with the same letter are not statistically different (LSD, p<0.05).
* Las parcelas fueron expuestas a lluvia natural.
Dentro de las columnas, promedios con la misma letra no son estadísticamente diferentes (LSD, p<0.05).
control plants, except for tricepiro RC50 which did not show differences (p<0.01). Under the evaluated conditions, parental genotypes triticale DS and trigopiro DN had smaller root dry matter and WS/C than tricepiro materials (Table 5).

Forage quality. In field experiments, water stress did not affect forage quality variables; mean values are presented in Table 6. In turn, differences in crude protein (p<0.05) among genotypes were detected. Tricepiro RC50 had the highest protein percentage under watering and rainfeed conditions (i.e. 14.32 and 12.97%, respectively), while triticale had the lowest (i.e., C = 9.33 and WS = 8.52%). Tricepiro DR had higher protein content under rainfeed than watering conditions (i.e., 12.57 vs 9.52%); this could be the result of a dilution effect that did not show up in other genotypes.

Grain yield. Seed production in field plots did not show a W x G interaction either in 2005 or 2006. Water stress had a significant effect, showing on average a yield reduction of 37% and 48% in 2006 and 2005, respectively (p<0.01). In 2005, Triticale had a superior seed production (Table 7). In 2006, although variance analysis did not show significant differences among varieties, triticale had a superior seed production.

Correlation between forage yield and morpho-physiological variables. Pearson correlation analysis was performed between biomass and the morpho-physiological variables, both in the greenhouse and at field, to identify variables which showed a close relationship with yield. Biomass was associated significantly to water potential (r = -0.46; p<0.01), stomatal resistance (r = 0.42; p<0.02), root biomass (r = 0.40; p<0.03) and wilted/green ratio (r = 0.41; p<0.02). Under field conditions, forage yield was highly correlated to leaf area (r = 0.64 and 0.68; p<0.001) and plant height (r = 0.37; p<0.05) in the two study years. This last variable is of relatively simple determination; hence this could be useful for screening genotipes in breeding programs.

DISCUSSION

We studied the water stress response of one cultivar and three experimental lines of tricepiro in comparison with their progenitors triticale and trigopiro. We found that genotypes of tricepiro and triticale performed similarly under drought both in the greenhouse and at the field; however, tricepiro was superior to trigopiro. Overall, among tricepiros, L62 and the line RC50 were the best performing genotypes. We evaluated morpho-physiological variables that might be related to yield under water deficit, which could be useful to select superior genotypes in breeding programs (Araus et al., 2002, 2008). In the greenhouse, forage yield had a high correlation with root biomass (p<0.001) both under control and water-stress conditions. Under water stress, WP and RE significantly correlated with forage yield (p<0.01 and 0.02, respectively) as well as the ratio wilted/green (p<0.05). In the field, forage yield had a fine association (p<0.05) with plant height both under control and water-stress conditions in 2005 and 2006. Leaf area, on the other hand, was highly related to yield during stress (p<0.01) but not under well irrigated conditions.

There is currently a strong support toward an approach that the understanding of the crop at the physiological and molecular biology level might help discover the key traits that are currently limiting yield. Such approach may therefore complement the conventional breeding programs and accelerate yield improvements. Analyzing physiological determinants of yield responses to water availability may help in breeding for higher yield and stability under drought conditions (Araus et al., 2002). The identification of one or more physiological parameters as indices of drought resistance has been the subject of current research.
of extensive research works (Karamanos, 1984; Karamanos & Papatheohari, 1999). Today, several variables have been proposed to screening genotypes (Araus et al., 2008).

The relevance of water to maintain turgor in plant tissues is well known, and, in many genotypes, a high water potential in leaves is indicative of a higher drought tolerance. This is the case in cereals such as triticale, barley, rice, wheat and other grasses (Frank & Berdhal, 2001; García et al., 2002; Grzesiak et al., 2003; Grzesiak et al., 2006). In tricepiro and its progenitors, differences in WP of plants under water stress and controls were found both in the greenhouse and field experiments, although differences among genotypes were not significant. Recovering after watering of stressed plants was similar for all genotypes even after two days of re-watering (Table 3). In wheat, Shonfeld et al. (1988) found a decrease in WP in plants under stress but they did not find differences among genotypes. In triticale accessions, Santivelli Morata (1993) did not find differences either in WP or relative water content. In triticale cultivars, Grezsiak et al. (2003) reported that, under drought, changes of WP, fluorescence and leaf injury, which might be suitable for screening of drought tolerance.

Stomatal resistance has been used as a criterion in different species to select drought tolerant plants (Morant-Avice et al., 1994; Nogueira et al., 2000; Frank & Berdhal, 2001; Araus et al., 2002; Wery, 2005). In our study, we found similar results in the evaluated genotypes both in field and greenhouse experiments. Trigopiro DN showed the greatest increase in stomatal resistance under water stress and lower recovering in stomatal conductance after re-watering. Divergence in stomatal conductance during recovering was found in other cereals such as wheat. In wild and cultivated genotypes of Triticum, Shimshi et al. (1982) observed that recovery was fastest in T. longissimum, an annual species of semi-arid to sub-humid habitats, somewhat slower in T. kotsch and much slower in bread wheat. Fischer et al. (1998) in bread wheat found that high stomatal conductance was associated with higher yield. This plant response could explain the lower yield in trigopiro compared to the other accessions.

Leaf area index in field experiments showed that a limitation in water content affected tricepiro lines and its progenitors to similar extent. However, at the greenhouse, tricepiro showed a greater leaf area compared to trigopiro and triticale, and a lower reduction of leaf area in comparison with controls. The divergence between field and greenhouse experiments could be explained as a result of the more severe water stress at the greenhouse than at the field (i.e., -3 MPa and -1.23 MPa under greenhouse and field conditions, respectively).

A higher leaf area per unit of mass has resulted in a high rate of potential growth under non-stress conditions, but, in turn, it has been a disadvantage under stressful conditions.

Fig. 5. Biomass index of one cultivar and experimental lines of tricepiro (DR, L62, L54 and RC50), triticale (DS) and trigopiro (DN) in Water Stress versus Control, during 2005 and 2006.

Fig. 5. Indice de biomasa de un cultivar y líneas experimentales (DR, L62, L54 y RC 50), triticale (DS) y trigopiro (DN) expuestas a Estrés hídrico versus Control, durante 2005 y 2006.
Under water stress, the specific leaf area was reduced both at the field and the greenhouse \((p<0.05)\) with no significant differences among genotypes, indicating a similar response to water stress in the evaluated materials.

One of the plant responses to water deficit is the increase in leaf senescence to reduce the transpiration rate (Araus et al., 2002). However, this feature can compromise plant yield. In this study, significant differences in leaf senescence between well-watered and stressed plants were found, similar to other reports in the literature (Maroco et al., 2000; Cabuslay et al., 2002). Water stress did not affect all genotypes to the same extent. While triticale was the most affected \((\text{WS/C}= 362\%)\), L62 was the less affected \((\text{WS/C}= 92\%)\).

Reduction in leaf growth as a result of water stress is well documented in grasses (Van Loo, 1992; Cabuslay et al., 2002; García et al., 2002). In our study, leaf growth rate (LGR) was severely reduced under water stress conditions in triticale and trigopiro \((\text{WS/C}= 37 \text{ and } 51\%, \text{respectively})\) under greenhouse conditions. In tricepiro genotypes, in turn, leaf growth rate decreased more slightly under water stress \((\text{ranging from } 60 \text{ to } 95\% \text{ WS/C})\). This could be associated with the higher leaf area found in tricepiro materials under water stress (Table 4).

An important root growth is a feature to be considered in semiarid environments, since plants roots can reach deeper layers of soils to contribute to plant yield stability (Pugnaire et al., 1994). In our greenhouse experiments, tricepiro RC50 showed the highest underground biomass under stress while trigopiro and triticale had the lowest root biomasses \((p<0.05)\). Grzesiak et al. (2001, 2007) observed that, in triticale, root biomass was more affected in sensitive genotypes under water stress. The same observation was made by Araus et al. (2002) in cereals. In this matter, it is important to note the limitation of greenhouse experiments since root growth in pots does not strictly compare with root growth under field conditions.

When water stress is imposed slowly, as is generally the case under field conditions, a reduction in C assimilation and utilization may occur along with restrictions in CO₂ assimilation due to stomatal closure when drought progresses. Concomitantly, there is a proportional reduction in the activity of various enzymes of the Calvin cycle (Maroco et al., 2002). Under water stress conditions, forage production is also affected because the leaf middle life is shorter and pastures are less dense, with a reduced numbers of tillers and green leaves per tiller (Colabelli et al., 1998). In our field experiments, water stress affected forage production, being trigopiro DN the cultivar with lowest forage yield both in 2005 and 2006. However, differences among cultivars under water stress with respect to the control were no evident in both years. Hence, divergences among cultivars seem to be inherent to each genotype instead of an effect of water stress. Water limitation in field experiments was moderate \((i.e. \ -1.23 \text{ MPa})\), and this could be the reason why differences were not evident among genotypes. In greenhouse experiments, where the water stress was severe \((i.e., \ -3 \text{ MPa})\), divergences among genotypes were evident.

To better explain the performance of the genotypes, we calculated a standardized index \((\text{higher values indicate better performance})\) in both years and watering conditions (Badiali, 2008), and plotted control vs. stress (Fig. 5). In this plot, trigopiro located in the lower left quadrant, an indication of its poor performance under both control and stress conditions in 2005 and 2006. Contrary to this, triticale and tricepiro L62 positions in the upper right quadrant showed a superior performance in all situations; among the other tricepiro genotypes the index was intermediate.

Analyzing the effect of water stress on biomass reduction in the greenhouse experiment, we observed that it was smaller in RC50 and L62 compared to the other tricepiro genotypes (Table 5). RC50 did not show a reduction in root biomass, which could contribute to a better water capture. On the other hand, L62 showed the lower leaf senescence under water stress, maintaining a longer photosynthetic capacity. These two variables \((i.e. \text{ root biomass and W/G})\) had a high correlation with biomass; hence they could be major contributors to yield. In the case of RC50, the biomass yield in the control was low; however, under water stress it showed a good performance. Thus, this line could be suitable for environments with frequent drought situations. On the other hand, L62 showed a good performance both under control and stress conditions, appearing as promissory among the tricepiro lines for both water availability conditions. Overall, it is noticeable the high variability of biomass reduction among tricepiro lines, opening an avenue for future selection.

Forage quality \((\text{NDF, ADF, DMD, CP, ME})\) of well-watered and rainfed plants did not differ, except for crude protein \((\text{CP})\). In this case, divergences in the genotypes were evident both under watered and rainfed conditions. Tricepiro RC50 had the highest values and triticale had the lowest. Regarding ADF, NDF, DMD and ME, our results differ from other authors. In some perennial summer grasses, it has been shown that water stress increased DMD and nitrogen levels in leaves (Guenni et al., 2002). In Russian rye \((\text{Psathyrostachys juncea})\), water stress increased protein levels and decreased ADF and NDF (Karn et al., 2004). However, this increase in digestibility could be the effect of delaying the stem elongation, flowering and leaf ontogenic changes (Wilson & Ng, 1975).

Grain production of tricepiro lines was intermediate in comparison with their progenitors. Triticale had the highest crop index and grain yield only during 2005. Low production of tricepiro lines could be related to a low fertility natural from interspecific crosses and, additionally, to the lower seed weight. Decreasing in grain yield as a consequence of water stress was similar among cultivars.

Interspecific and intergeneric crosses have been shown to be successful in some aspects - such as seed production, quali-
Water stress response of Tricepiro

In terms of water stress tolerance, the aim of this work, tricepiro had a good performance, similar to triticale. In addition, we found morphophysiological variables that highly correlated with biomass production. In the greenhouse, water potential, stomatal resistance, root biomass and wilted/green were close associated with forage, under a considerable water stress (~ 3 MPa). In the field (mild-moderate stress: -1.23 MPa), forage yield was highly related to plant height and leaf area in the two year trials. These physiological traits could be evaluated as potential indicators that might be useful for screening tricepiro genotypes in breeding programs. There is considerable variability among the evaluated tricepiro genotypes to allow the selection of drought tolerant materials. Besides, future tricepiro breeding plans might consider the use of parental lines with drought tolerance background in order to improve water stress tolerance.

REFERENCES


