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# Variability in salt tolerance of native populations of *Elymus scabrifolius* (Döll) J. H. Hunz from Argentina

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## Abstract

*Elymus scabrifolius* is a native C3 South American grass species. It is valued as forage species adapted to various environments in Argentina and is also a potential source of traits for wheat-breeding programmes. Efficient utilization of native genetic resources requires extensive collection and characterization of available material. The purpose of this study was to identify and characterize variability in salt tolerance within *E. scabrifolius* populations in Argentina. Specimens of *E. scabrifolius* were collected from a wide range of soils in Argentina, and most populations were found in saline environments with high sodium levels. Intraspecific variability in salt tolerance was estimated, and its relation to the salinity level of the populations' natural environment was assessed. A principal component analysis based on growth data distinguished lines from saline and non-saline habitats only under salt conditions. Results suggest that selecting under stressed environments is a reasonable strategy for breeding *E. scabrifolius*. Lines of saline origin had higher biomass under both control and saline conditions, suggesting that higher gains from selection would be obtained if germplasm from this origin was used, and tillering may be the most useful indirect selection criterion for improving salt tolerance. The association between salt tolerance, ion content and osmotic adjustment was also assessed. Salt-sensitive lines accumulated high sodium levels in leaves. However, osmotic adjustment did not correlate with the maintenance of leaf elongation rates under salinity in the genotypes included in this study.

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## Introduction

Soil salinity and sodicity have long been major constraints for increasing crop and beef production in Australasia, Asia and South America (Rengasamy, 2006; Taleisnik *et al.*, 2008). In addition, anthropogenic salinity caused by deforestation and overgrazing contributes to the aggravation of the problem (Hatton *et al.*, 2003; Brown *et al.*, 2005). The final report of Australia's National Dryland Salinity Program pointed out that the future success of salt-tolerant pastures will require further plant breeding, matching species to soil types and landscapes and greater understanding of mixed salt-tolerant pasture management (National Dryland Salinity Program, 2001). The introduction of salt-tolerant perennial species is one of the most promising alternatives to overcome salinity problems (Rengasamy *et al.*, 2003; Yunusa and Newton, 2003), and the development of salt-tolerant perennial forages from native species adapted to target areas can make substantial contributions to reclaiming saline areas (Aronson, 1985; Ashraf, 1994; Shannon, 1997; Flowers and Flowers, 2005; John *et al.*, 2005). In addition, native species from saline habitats could be a source of genes for salt tolerance that may potentially be transferred to crops (Rogers *et al.*, 2005; Yamaguchi and Blumwald, 2005; Munns *et al.*, 2006).

*Elymus scabrifolius* (Döll) J. H. Hunz [synonym: *Elymus breviaristatus* (Hitchc.) Á Löve subsp. *scabrifolium*] is a native South American grass species common in Argentina and Uruguay (Hunziker, 1998). It is valued as a forage (Parodi, 1940; Covas, 1978), especially in areas affected by salinity (Ranmuno, 1980; Vargas Lopez, 1982), although reports indicate moderate salt tolerance in this species (Lucero, 1970; Lavado, 1977; Priano and Pilatti, 1989). An accurate description of this species is given by Nicora (2006).

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Efficient utilization of native genetic resources requires extensive collection and characterization of available material. This had not previously been conducted for *E. scabrifolius* in Argentina, and no information was available on the physiological and genetic basis of salt tolerance in this species.

Intraspecific variability in tolerance to salt stress has been reported for perennial species within the *Triticeae* (Shannon, 1978; Marcar, 1987; Horst and Dunning, 1989). In other grass species, variability for this trait is associated with population origin (Venables and Wilkins, 1978; Ashraf, 1994). This is not surprising because specific salt-tolerance mechanisms have evolved in halophytes, plants naturally adapted to saline environments (Flowers *et al.*, 1986; Flowers and Colmer, 2008). Ion exclusion, sodium tissue tolerance (Tester and Davenport, 2003; Munns and Tester, 2008) and osmotic adjustment (Zhang *et al.*, 1999) are some physiological traits most commonly associated with salinity tolerance in the *Gramineae*. It is not known whether these traits also contribute to salt tolerance in *E. scabrifolius.* Growth parameters such as dry-matter accumulation (Munns and Tester, 2008), tillering (Ruan *et al.*, 2008) and leaf growth (Taleisnik *et al.*, 1997; De Luca *et al.*, 2001) have been used as screening criteria for salt tolerance in grasses and were used to characterize the *E. scabrifolius* populations. The relative magnitude of salt tolerance normally changes during the life cycle of a plant (Maas, 1993; Maas *et al.*, 1994; Pearen *et al.*, 1997), and perennial forage grasses are usually less tolerant at earlier stages of development (Dewey, 1962; Shannon *et al.*, 1994; Sanderson *et al.*, 1997; Taleisnik *et al.*, 1997). Therefore, this study was conducted in the first stages of vegetative development.

The purpose of this study was to identify and characterize salt-tolerance variability within *E. scabrifolius* populations in Argentina. To achieve this aim, an extensive collection of *E. scabrifolius* populations was undertaken, and variability to salt tolerance and its association with some physiological traits was assessed.

## Materials and methods

### Germplasm collection

Two collecting expeditions were conducted in 2004–2005 in Argentina. Each followed a predetermined route in the environments where this species is usually found. Information about collection sites for *E. scabrifolius* was obtained from records of herbarium specimens at the Instituto de Botánica Darwinion (SI) and Facultad de Ciencias Agrarias, Universidad Nacional del Litoral (SF). The collecting expeditions covered a wide range of habitats, soil types and management conditions (Table 1).

Seeds were collected from individual plants (lines), separated by a minimum of 3–5 m to ensure that a representative sample was obtained from each population. Herbarium specimens were collected and conserved in the SF Herbarium. Samples from those seeds have now been conserved at the Germplasm Bank of INTA (INTA, 2010). Soil samples (0–20 cm) were taken for analysis of electrical conductivity (EC) and pH. Seeds from each plant were stored in separate envelopes, at room temperature, until sowing. Multiplication was carried out at the Facultad de Ciencias Agrarias (Universidad Nacional del Litoral), Santa Fé, Argentina (31° 25′ S and 60° 56′ W). Five to ten caryopses from each plant were germinated, and only one of the resulting seedlings was transplanted to the field. *E. scabrifolius* is an autogamous species (P. Tomas, personal communication); therefore, each plant was considered a line ('line' refers to naturally inbred plants, predominantly homozygous). Caryopses were harvested at maturity and kept dry at room temperature.

### Variability in growth parameters (trial 1)

Forty lines from four populations collected at sites with contrasting salinity conditions were included in this study (Figure 1): (i) lines 1–10 from population 34, and lines 11–20 from population 35 came from a highly saline and alkaline habitat; and (ii) lines 21–30 from population 5, and lines 31–40 from population 7 came from non-saline and non-alkaline habitats. The lines were chosen at random from each population. Seeds were germinated in Petri dishes, on filter paper moistened with distilled water, placed in the dark, in a controlled environment chamber with an alternating temperature regime of 15°C (16 h) and 20°C (8 h). These conditions are optimal for germination (Zabala *et al.*, 2009). Three-day-old seedlings of each line were transplanted to a plastic net covered with a 1 cm layer of wet vermiculite and placed on top of a glass box with nutrient solution (Hoagland and Arnon, 1938). Salinization began when roots emerged through the net (5 d after transplantation) by adding a NaCl solution that increased salinity by 40 mm $d^{-1}$, until 160 mm NaCl was reached. This salinity level was chosen on the basis of a preliminary experiment, which indicated that 200 mm NaCl caused early leaf and seedling death in some genotypes. Controls were not salinized, and the nutrient solution was renewed once every 2 weeks in all glass boxes.

Eight seedlings per line were evaluated. Number of live leaves (LN), length of leaves 3, 4, 5 (LL3, LL4 and LL5 respectively) and tiller number (TN) were measured at 60 d after stress initiation. Dry weight of shoots (SDW) and roots (RDW) and total dry weight (TDW) were determined at the same time. The dry mass was

**Table 1** Origin of *Elymus scabrifolius* populations collected for this study.

| Pop. | Province (Department) | Latitude | Longitude | Habitat |
|---|---|---|---|---|
| 1 | Santa Fé (San Javier) | S 31° 19′ | W 60° 17′ | 'Ruderal' (roadside*) |
| 2 | Santa Fé (San Javier) | S 31° 29′ | W 60° 26′ | 'Ruderal' (roadside) |
| 3 | Santa Fé (San Javier) | S 31° 35′ | W 60° 34′ | 'Ruderal' (roadside) |
| 4 | Córdoba (Córdoba) | S 31° 20′ | W 64° 20′ | Xerophytic forest at the edge of the Primero river |
| 5 | Santa Fé (San Cristóbal) | S 30° 22′ | W 61° 10′ | 'Ruderal' (roadside)† |
| 6 | Buenos Aires (Punta Indio) | S 35° 31′ | W 57° 15′ | 'Ruderal' (roadside) |
| 7 | Santa Fé (San Cristóbal) | S 30° 15′ | W 61° 07′ | 'Algarrobal' (xerophytic forest dominated by the genus *Prosopis*)† |
| 8 | Santa Fé (Santa Fé) | S 31° 07′ | W 60° 32′ | 'Saline praire' (*Distichlis spicata* praire) |
| 9 | Santa Fé (San Justo) | S 29° 58′ | W 60° 15′ | 'Ruderal' (roadside) |
| 10 | Buenos Aires (Chascomús) | S 35° 44′ | W 57° 22′ | *Spartina spartinae* tall grasslands |
| 11 | Buenos Aires (Chascomús) | S 35° 44′ | W 57° 22′ | Edge of *Spartina spartinae* tall grasslands |
| 12 | Santa Fé (San Justo) | S 30° 19′ | W 60° 39′ | 'Ruderal' (roadside) |
| 13 | Santa Fé (San Javier) | S 31° 19′ | W 60° 17′ | 'Ruderal' (roadside) |
| 14 | Santa Fé (San Javier) | | | 'Ruderal' (roadside) |
| 15 | Santa Fé (San Cristóbal) | S 30° 32′ | W 61° 10′ | 'Ruderal' (roadside) |
| 16 | Santa Fé (San Justo) | S 29° 58′ | W 60° 16′ | 'Algarrobal' (xerophytic forest dominated by genus *Prosopis*) |
| 17 | Entre Ríos (Uruguay) | S 32° 26′ | W 58° 32′ | 'Algarrobal' (xerophytic forest dominated by genus *Prosopis*) |
| 18 | Santa Fé (Santa Fé) | S 31° 17′ | W 60° 36′. | 'Algarrobal' (xerophytic forest dominated by the genus *Prosopis*) |
| 19 | Santa Fé (San Cristóbal) | S 31° 08′ | W 60° 57′ | *Panicum prionitis* tall grasslands |
| 20 | Santa Fé (Santa Fé) | S 31° 01′ | W 60° 17′ | 'Algarrobal' (xerophytic forest dominated by the genus *Prosopis*) |
| 21 | Santa Fé (Santa Fé) | S 31° 01′ | W 60° 17′ | *Spartina spartinae* tall grasslands |
| 22 | Santa Fé (San Javier) | S 30° 27′ | W60° 05′ | 'Ruderal' (roadside) |
| 23 | Santa Fé (Santa Fé) | S 31° 17′ | W 60° 38′ | 'Algarrobal' (xerophytic forest dominated by the genus *Prosopis*) |
| 24 | Tucumán (Leales) | S 27° 10′ | W 65° 15′ | 'Saline praire' (*Distichlis spicata* praire) |
| 25 | Santa Fé (Las Colonias) | S 31° 29′ | W 60° 46′ | 'Chañaral' (xerophytic forest dominated by *Geoffroea decorticans*) |
| 26 | Santa Fé (Vera) | S 29° 28′ | W 60° 06′ | *Spartina spartinae* tall grasslands |
| 27 | Santa Fé (Santa Fé) | S 31° 17′ | W 60° 38′ | 'Degraded Quebrachal' (degraded xerophytic forest dominated by *Schinopsis balansae*) |
| 28 | Santa Fé (Santa Fé) | S 31° 18′ | W 60° 35′ | *Spartina spartinae* tall grasslands |
| 29 | Santa Fé (Vera) | S 29° 41′ | W 60° 18′ | 'Quebrachal' (xerophytic forest dominated by *Schinopsis balansae*) |
| 30 | Santa Fé (Vera) | S 29° 41′ | W 60° 18′ | 'Quebrachal' (xerophytic forest dominated by *Schinopsis balansae*) |
| 31 | Santa Fé (Vera) | S 29° 28′ | W 60° 06′ | 'Degraded Quebrachal' (degraded xerophytic forest dominated by *Schinopsis balansae*) |
| 32 | Buenos Aires (Punta Indio) | S 35° 42′ | W 57° 21′ | *Spartina densiflora* tall grasslands |
| 33 | Santa Fé (Vera) | S 29° 40′ | W 60° 33′ | *Spartina spartinae* tall grasslands |
| 34 | Santa Fé (Vera) | S 29° 17′ | W 59° 51′ | Palm grove of *Copernicia alba*† |
| 35 | Santa Fé (San Cristóbal) | S 30° 22′ | W 61° 10′ | *Spartina spartinae* tall grasslands† |

*Seeds collected from roadside were not found outside, in the field.

†Seeds of lines of these populations were utilized in experiments.

obtained after drying in an oven at 60°C for 48 h. Relative dry weight (ReDW) was expressed as percentage of dry weight relative to control [(mean of dry weight in each line under salt stress/control value) × 100].

### Ion content and osmotic adjustment (trial 2)

Five contrasting lines from trial 1 were used for further analysis; lines 1, 14 and 19 from saline environments (the first two with high TDW, the last one with low TDW) and lines 21 and 23 from non-saline environments, with high and low TDW respectively. A new seed sample was germinated, and the control and salt stress treatments were imposed as in trial 1, and growth parameters were measured at 40 d after stress initiation. Sodium ($Na^+$) and potassium ($K^+$) concentration were determined by flame photometry in leaves 3, 4, 5 and in tillers. Samples for measuring osmotic potential were taken from expanding tissues of leaves 3 and 4. Samples were rehydrated in a humid chamber and allowed to take up water until constant

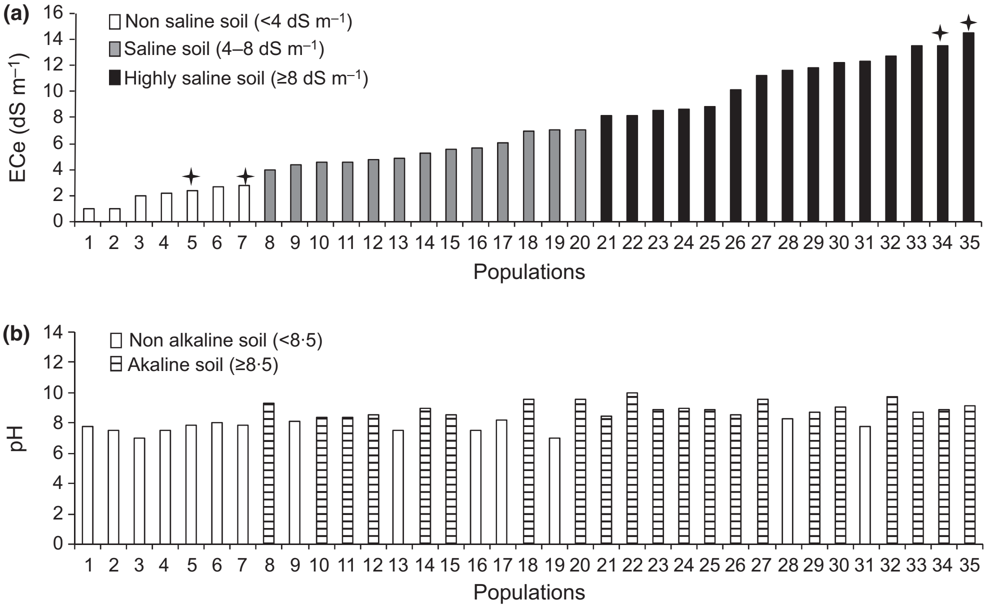

**Figure 1** Salinity characteristics of soils where populations were collected: (a) Electrical conductivity (EC) of the soil saturation extract (classification according to M.A.G., 1982). (b) Soil extract pH (✦) selected populations for analysis of salt tolerance.

weight (full turgor) and were then inserted into the barrel of a 1-ml syringe, immediately frozen and thawed just prior to the measurement. A drop of sap was extracted onto a disc of filter paper by pressing the plunger of the syringe, the disc was then placed into the chamber of a Wescor HR 33T psychrometer (Turner, 1981), and readings were taken at the dew point setting after a 3-min equilibration period. Osmotic adjustment for the salinity treatment was calculated as osmotic potential difference between control and salinized samples.

**Table 2** Analysis of variance and estimate of genetic parameters on line basis.

| Source of variation | DF (degrees of freedom) | MS (mean squares) |
|---|---|---|
| Line | 19 | LMS |
| Error | 140 | EMS |
| Total | 159 | |

Genotypic variability ($\Phi_G$) = LMS-EMS ⁄ 8 (number of replications).
Phenotypic variance ($\sigma^2_p$) = $\Phi_G$ + EMS.
Coefficient of genotypic determination (CGD) = $\Phi_G / \sigma^2_p$.
Coefficient of genotypic variation (CGV) = $\sqrt{\Phi_G}/m \times 100$ (where m is the general mean).
Variation index (VI) = CGV ⁄ CEV (where CEV is the coefficient of experimental variation) (CEV is calculated as $\sqrt{(EMS/8)}/m \times 100$).

## Data analysis

The data were analysed using INFOSTAT statistical package (InfoStat, 2009. InfoStat versión 2009. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.). In trial 1, growth parameters were subjected to a Two-sample *t*-test to determine whether means by origin (saline or non-saline) were statistically different.

Relationships between variables and variability in lines were examined using multivariate analysis. Means of variables in forty lines were used in a principal component analysis (PCA). Two separate PCA were performed for control and saline treatment.

In order to analyse the variability of biomass accumulation under salt stress in lines coming from saline habitats and non-saline habitats, genetic parameters were estimated according to Nyquist and Baker (1991) and Cock *et al.* (2002). The coefficient of genotypic determination (CGD), coefficient of genotypic variation (CGV) and variation index (VI) on a line basis were calculated from ANOVA (Table 2). In each origin ($n = 20$), the statistical model was:

$$Y_{ij} = \mu + G_i + E_{ij},$$

where $Y_{ij}$, observation on the $i$th genotype in the $j$th replication; $G_i$, random effect attributed to $i$th genotype ($i = 1, 2, 3, \ldots, 20$); and $E_{ij}$, experimental error attributed to $Y_{ij}$ observation.

In trial 2, variables in each line were subjected to an ANOVA to determine the significance in salinity tolerance. In these analyses, differences between means values were tested for significance using a Tukey test ($P < 0·05$).

## Results

### Naturally occurring *E. scabrifolius* populations in Argentina

Seeds of *E. scabrifolius* were collected from thirty-five sites in Argentina, and most populations were found in saline (37%) and very high saline (43%) environments (Figure 1a). In Argentina, alkaline soils with pH above 8–8·5 have sodium problems (i.e. sodic soils) and 60% of the populations were found on soils of this kind (Figure 1b). Within each origin (saline, very high saline or non-saline), populations were also associated with different vegetation types (Table 1). The range of distribution of populations in our collection occurs between annual rainfall isohyets of 800–1200 mm.

### Growth variability in response to salinity in *E. scabrifolius* populations of different origin (trial 1)

Lines from saline habitats had higher average TDW than those from non-saline environments under salt stress (Figure 2a). Salt stress decreased the dry weight of shoots and roots in most of the lines, but effects were significantly lower in those lines from saline habitats ($P = 0·002$, 0·0026 and 0·0033 for shoot, root and total RDW respectively) (Figures 2b and 3).

Leaf number was similar in plants from both populations in the absence of stress (Table 3). However, it

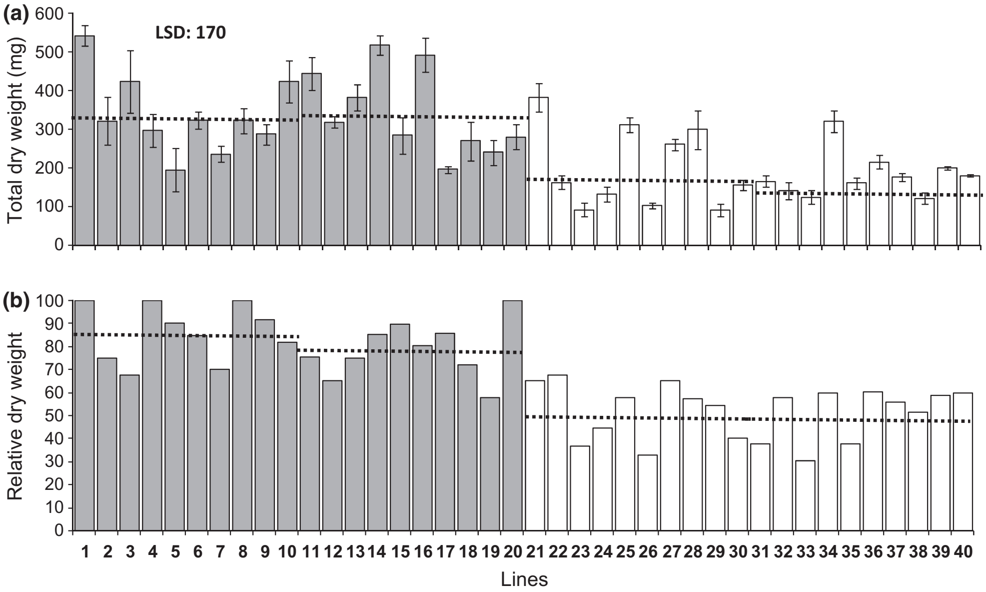

**Figure 2** (a) Total dry weight of plants in lines from different habitats under salt stress (means and standard deviations). (b) Relative dry weight (% of control). LSD, least significant difference at $P < 0·05$ (Tukey's test). Dotted lines indicate populations means. Dark columns: lines from saline habitats, light columns: lines from non-saline habitats.

**Table 3** Leaf number (LN), percentage of plants with one or more tillers (%TN) and leaf length (cm) of leaves 3, 4 and 5 (LL3, LL4 and LL5) of *Elymus scabrifolius* lines coming from different habitats under control (C) and saline conditions (S), 60 d after treatment initiation.

| | LN | | %TN | | LL3 | | LL4 | | LL5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Origin** | **C** | **S** | **C** | **S** | **C** | **S** | **C** | **S** | **C** | **S** |
| Saline | 5·9a | 4·1b | 70·1 | 40·1 | 21·1a | 20·5a | 23·8a | 24·3a | 20·0a | 15·3a |
| Non-saline | 5·5a | 2·8a | 30·3 | 9·6 | 26·6b | 23·2b | 27·8b | 24·8a | 26·3b | 14·2a |

Different letters indicate significant differences between origins at $P < 0·05$ (Tukey's Test).

was higher in genotypes originating from saline habitats under saline conditions. Tillers per plant were higher in lines from the latter population, and the effect of salinity depressed it less than in lines from non-saline origin. Leaf length of leaves 3, 4 and 5 was greater in lines from non-saline habitats.

Principal component analysis for growth data obtained under non-stress conditions showed that 79% of the total variability could be explained by three main components (PC) (Figure 4a). LN, SDW and RDW had the highest influence in PC1. These traits could not distinguish lines from saline and non-saline habitats. SDW was correlated with RDW ($r = 0{\cdot}9$; $P \leq 0{\cdot}01$), LN ($r = 0{\cdot}51$; $P < 0{\cdot}01$), TN ($r = 0{\cdot}39$; $P = 0{\cdot}013$) and LL5 ($r = 0{\cdot}42$, $P \leq 0{\cdot}01$). LL3 and LL4 were characters with higher weights in PC2, and the highest LL3 and LL4 values were recorded in lines from non-saline habitats. In the control, SDW was correlated with RDW ($r = 0{\cdot}9$; $P \leq 0{\cdot}01$), LN ($r = 0{\cdot}51$; $P < 0{\cdot}01$), TN ($r = 0{\cdot}39$; $P = 0{\cdot}013$) and LL5 ($r = 0{\cdot}42$, $P \leq 0{\cdot}01$).

Under salt stress, the three main principal components accounted for 82% of the total variation. SDW, RDW, LN and TN were the variables with the highest load in PC1 and the highest values for these traits were observed in lines from saline habitats (Figure 4b). LL3 and LL4 explain variability in PC2. PC3 discriminates lines based on LL5 but none of these traits discriminated lines by origin. In saline conditions, SDW was correlated with RDW ($r = 0{\cdot}85$; $P \leq 0{\cdot}01$), LN ($r = 0{\cdot}72$; $P < 0{\cdot}01$), TN ($r = 0{\cdot}67$; $P \leq 0{\cdot}01$) and LL5 ($r = 0{\cdot}42$, $P \leq 0{\cdot}01$).

As can be seen in Table 4, the estimated CGD and VI were >0·4 and 1 for TDW and LN60, respectively, in lines from saline and non-saline habitat. TN was

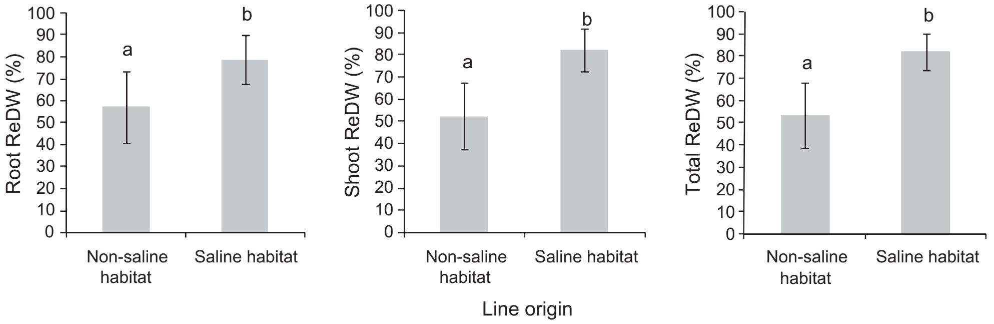

**Figure 3** Dry weight (as percentage of dry weight under non-saline conditions, ReDW) of *Elymus scabrifolius* lines coming from different habitats (columns are means and standard deviations). Means with different letters are different at $P < 0{\cdot}05$ (Tukey's Test).

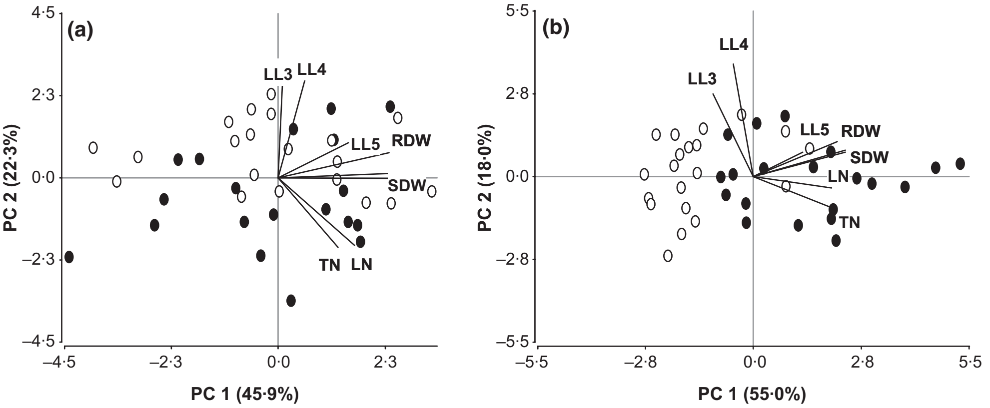

**Figure 4** Biplots of principal component analysis. (a) Biplot of two principal components from control plants and (b) biplot of two principal components of plants from saline treatment. Data in both figures obtained at 60 d after treatment initiation. Points represent lines (empty circles, lines from non-saline habitat; full circles, lines from saline habitat). Vectors represent variables (LL3, LL4 and LL5 leaf length; LN, leaf number; TN tiller number; RDW, root dry weight; SDW, shoot dry weight). Cophenetic correlation coefficient (CCC): 0·945 and 0·968 for control and saline treatments respectively.

**Table 4** Coefficient of genotypic determination (CGD), coefficient of genotypic variation (CGV) and variation index (VI) in lines from different habitat under salt stress for total dry weight (TDW), leaf number (LN) and tiller number (TN) at 60 d after treatment initiation.

| | **Saline habitat** | | |
|---|---|---|---|
| | **Trait** | | |
| **Parameter** | **TDW** | **LN** | **TN** |
| CGD | 0·49 (0·06*) | 0·45 (0·11) | 0·43 (0·08) |
| CGV | 32·9 | 25·8 | 72·7 |
| VI | 2·6 | 2·7 | 2·7 |
| | **Non-saline habitat** | | |
| | **Trait** | | |
| **Parameter** | **TDW** | **LN60** | **TN60[†]** |
| CGD | 0·46 (0·06) | 0·49 (0·09) | – |
| CGV | 36·5 | 27·7 | – |
| VI | 2·8 | 2·5 | – |

*Standard errors estimated according to Nyquist (1991).

[†] TN60 was not analysed (see text).

analysed only in lines from saline habitat because TN60 in lines from non-saline habitat were 0 in several lines, and <0·2 in other lines. In lines from saline habitat CGV was much higher in TN than in LN and TDW.

### Ion content and osmotic adjustment (trial 2)

Growth results obtained in trial 2 were consistent with those of trial 1 (Table 5). TDW in lines 1 and 14 was higher than in line 19, and in line 21 it was higher than in line 33. High TDW in lines 1 and 14 was related to tiller presence. RDW at 40 d was higher in lines from the saline environment, but at 60 d differences were evident only between lines 1 and 14 compared with the rest.

Under salt stress, the Na$^+$ concentration of sheaths and blades was lower in lines from saline habitats (Table 6). In leaf 3 of lines 14 and 19, Na$^+$ concentrations in sheaths were much higher than in blades. Total Na content in leaves 3 and 4 is shown in Figure 5. It can be seen that sheaths did not buffer Na accumulation in blades, it built up even before blades were developed, especially in lines 21 and 33 from non-saline habitats. K$^+$ concentration in leaves was similar in plants from the five lines, and it was decreased by salinity in leaves 3 and 4 (Figure 6).

The relative water content (RWC) in the expansion zone of leaf blades 3 and 4 was above 88% in the salt-treated plants, and it was lowest in line 21 from a non-saline habitat (Table 7). Blade elongation rate under saline conditions was similar in all lines, although in general, the decrease in relation to controls was more marked in lines from non-saline habitats (21 and 33). Osmotic adjustment in the blade expansion zone was observed in all five lines in both leaf 3 and 4; however, there was no consistent association between the magnitude of the osmotic adjustment and either growth maintenance or line origin.

## Discussion

*Elymus scabrifolius* is a perennial *Triticeae* species, endemic to South America where it is valued as forage, especially in salt-affected areas. *Elymus* species have been used as sources of salt tolerance in wheat (McGuire and Dvorak, 1981; Franke *et al.*, 1992; Choukr-Allah *et al.*, 1996; Rogers *et al.*, 2005), as these species are interfertile. The potential salt-tolerance variability within native Argentine *E. scabrifolius* genotypes had not been evaluated before, and this paper reports the first extensive collection of *E. scabrifolius* germplasm made in Argentina. Our plant collection expeditions showed that

**Table 5** Growth parameters in different *Elymus scabrifolius* lines at 40 and 60 d under salt stress in Experiment 2.

| | **SDW (mg)** | | **RDW (mg)** | | **TDW (mg)** | | **RelDW (%)** | | **TN** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Line and origin** | **40 d** | **60 d** | **40 d** | **60 d** | **40 d** | **60 d** | **40 d** | **60 d** | **40 d** | **60 d** |
| 1 (Saline high TDW*) | 90·6c | 341·2d | 24·3b | 70·0c | 114·9c | 411·2d | 87·2 | 84·9 (100[†]) | 1·1a | 1·54a |
| 14 (Saline high TDW) | 101·5c | 259·9c | 25·7b | 92·3d | 127·2c | 352·2c | 81·2 | 85·2 (85·5) | 0·5b | 1·05b |
| 19 (Saline low TDW) | 63·9b | 103·0b | 29·1b | 54·4b | 93·0b | 157·4b | 86·8 | 54·6 (56·1) | 0 | 0 |
| 21 (NSal high TDW) | 56·4ab | 128·1b | 19·6a | 68·0b | 76·1ab | 196·1b | 65·5 | 45·2 (65·9) | 0 | 0 |
| 33 (NSal low TDW) | 38·2a | 27·0a | 7·99a | 8·1a | 46·2a | 35·1a | 45·5 | 5·7 (45·2) | 0 | 0 |

Shoot dry weight (SDW), root dry weight (RDW), total dry weight (TDW), relative dry weight (RelDW) and tiller number (TN). Means with different letters are different at the 5% level according to Tukey's Test (Saline: saline origin; NSal: non-saline origin).

*Total dry weight in Experiment 1.

[†] In parenthesis, relative dry weight at 60 d in Experiment 1.

**Table 6** Total fresh weight (TFW), Na concentration and Na ⁄ K ratio in sheaths and blades of leaves 3, 4 and 5 and tillers in *Elymus scabrifolius* lines. Ions were measured after 40 d under salt stress.

| | TFW (mg) | | Na (mmol kgFW$^{-1}$) | | Na ⁄ K | |
|---|---|---|---|---|---|---|
| **Line and origin** | **Sheath 3** | **Blade 3** | **Sheath 3** | **Blade 3** | **Sheath 3** | **Blade 3** |
| 1 (Saline high TDW[†]) | 40·2b | 27·3b | 7·6a | 17·6a | 0·14a | 0·35a |
| 14 (Saline high TDW) | 52bc | 30·5b | 38·4b | 14·6a | 0·64b | 0·22a |
| 19 (Saline low TDW) | 39b | 21·3ab | 53·4c | 13·8a | 0·78b | 0·2a |
| 21 (NSal high TDW) | 49·8bc | 25·4b | 89·2d | 75·6b | 1·18c | 1·05b |
| 33 (NSal low TDW) | 30·2a | 17·2a | 92d | 85·8b | 1·22c | 1·42b |
| | **Sheath 4** | **Blade 4** | **Sheath 4** | **Blade 4** | **Sheath 4** | **Blade 4** |
| 1 (Saline high TDW) | 56·1a | 142·4b | 5·7a | 14·6a | 0·08a | 0·16a |
| 14 (Saline high TDW) | 63·1a | 166·2b | 9·2b | 12·4a | 0·11a | 0·17a |
| 19 (Saline high TDW) | 60·2a | 128·4ab | 19·4c | 17a | 0·31b | 0·21a |
| 21 (NSal high TDW) | –[‡] | 154·4b | – | 39·8b | – | 0·54b |
| 33 (NSal low TDW) | – | 95·5a | – | 79·2c | – | 0·94c |
| | **Sheath 5** | **Blade 5** | **Sheath 5** | **Blade 5** | **Sheath 5** | **Blade 5** |
| 1 (Saline high TDW[†]) | – | 96·6b | – | 11·5 | – | 0·11 |
| 14 (Saline high TDW) | – | 132·8c | – | 15·6 | – | 0·15 |
| 19 (Saline low TDW) | – | 63·2a | – | 9·05 | – | 0·08 |
| 21 (NSal high TDW) | – | – | – | – | – | – |
| 33 (NSal low TDW) | – | – | – | – | – | – |
| | | | | **Tiller** | | |
| 1 (Saline high TDW[†]) | 94·5b | 7·3a | 0·07a | | | |
| 14 (Saline high TDW) | 63·6a | 6·6a | 0·06a | | | |
| 19 (Saline low TDW) | –[§] | – | – | | | |
| 21 (NSal high TDW) | – | – | – | | | |

Means with different letters are different at $P < 0{\cdot}05$ (Tukey's test) Saline: saline origin, NSal: non-saline origin.

[†] TDW (total dry weight in Experiment 1).

[‡] Empty cells indicate that leaf sheath ⁄ blade had not emerged at 40 d.

[§] Tiller was not present at 40 d.

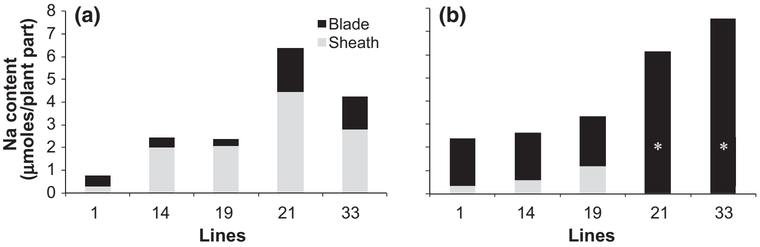

**Figure 5** Na content in leaves 3 (a) and 4 (b) measured 40 d after stress initiation in lines from saline (1, 14 and 19) and non-saline habitats (21 and 33): *Leaf sheath had not emerged.

the occurrence of this species is widespread and therefore it may not be in danger of extinction, as had been suggested previously by Andrés (1986). Nevertheless, in overgrazed lands, populations comprised a few individuals, and this species does not occur naturally in agricultural lands. *E. scabrifolius* is a forage that is highly

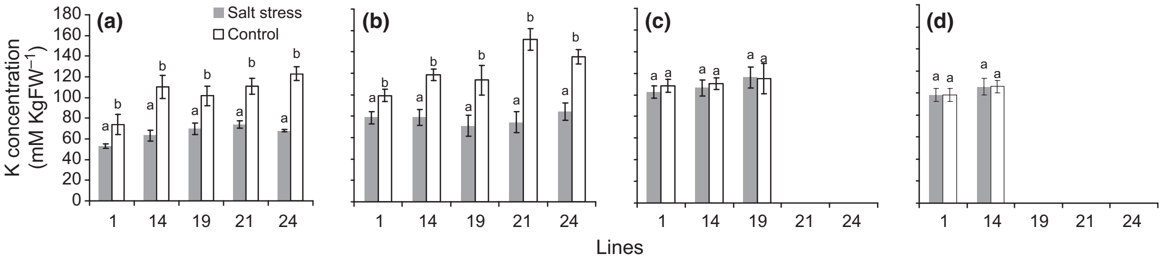

**Figure 6** K concentration in leaves and tillers of *Elymus scabrifolius* grown under control (white bars) or saline (dark bars) conditions, measured 40 d after stress initiation. (a) leaf 3; (b) leaf 4; (c) leaf 5; (d) tiller. Means with different letters are different at $P < 0{\cdot}05$, Tukey's test.

**Table 7** Osmotic adjustment (OA), relative water content (RWC), blade elongation rate (BER) and relative blade elongation rate (RBER) as percentage of BER under control conditions in blades of leaves 3 and 4 of different *Elymus scabrifolius* lines under salt conditions. Variables measured at 40 DASI.

| | Blade 3 | | | |
|---|---|---|---|---|
| **Line** | **OA (MPa)** | **RWC** | **BER (cm d$^{-1}$)** | **RBER (%)** |
| 1 (Saline habitat, high TDW*) | 0·36a | 92·2b | 0·50a | 73·9 |
| 14 (Saline habitat, high TDW) | 0·39a | 91·7ab | 0·56a | 71·1 |
| 19 (Saline habitat, low TDW) | 1·04c | 89·6ab | 0·46a | 66·6 |
| 21 (Non-saline habitat, high TDW) | 2·10c | 88·7a | 0·52a | 61·0 |
| 33 (Non-saline habitat, low TDW) | 0·69ab | 90·7ab | 0·44a | 56·2 |
| | **Blade 4** | | | |
| 1 (Saline habitat, high TDW) | 0·15a | 92·8b | 0·63a | 78·2 |
| 14 (Saline habitat, high TDW) | 0·15a | 93·1b | 0·73a | 73·6 |
| 19 (Saline habitat, low TDW) | 0·74b | 90·6ab | 0·64a | 62·1 |
| 21 (Non-saline habitat, high TDW) | 0·79b | 89·4a | 0·69a | 56·0 |
| 33 (Non-saline habitat, low TDW) | 0·66b | 90·7ab | 0·57a | 51·0 |

*TDW (Total dry weight in Experiment 1).

acceptable to grazing livestock, and our observations would indicate that poor grazing management, deforestation and expansion of agricultural lands will lead to a loss of diversity of *E. scabrifolius* in Argentinean grasslands.

Rehabilitation of saline environments in Argentina through the use of C3 forage species such as *Thinophyron ponticum* enabled the productive potential of these lands to be reclassified after a few years (Priano and Orellana, 1964; Sauberán and Molina, 1964; Molina, 1969). Unfortunately, these studies have been discontinued. It is conceivable that the introduction of *E. scabrifolius* would also allow for the productive rehabilitation of salt-affected soils. High biomass accumulation at early development stages is a key aspect for the establishment of perennial forage species (Sanderson *et al.*, 1997). We observed that lines coming from saline habitats had higher biomass accumulation, and they showed less reduction under salt stress than lines from non-saline habitats, indicating salt-tolerance traits in lines coming from saline habitats.

Multivariate analyses have evolved as powerful tools to examine phenotypic variations and interactions of growth parameters with the environment in large data sets (Godshalk and Timothy, 1988; Mohammadi and Prasanna, 2003) and have also been applied in breeding programmes for salinity tolerance (Jaradat *et al.*, 2004; Zeng, 2005). A PCA based on growth data distinguished lines from saline and non-saline habitats only under salt conditions. Moderate CGD values for DW, TN and LN were found in lines from both non-saline and saline origins. This calculated broad-sense heritability includes all genetic factors that influence phenotypic expression (not only additives) and therefore it is not a precise indicator of gains for selection. Nevertheless, together with high values obtained in CGV and VI (scale-independent genetic parameters), it suggests that biomass accumulation can be improved by selection (Vencovsky and Barriga, 1992). Lines of saline origin showed the highest biomass accumulation (i.e. highest mean value), and it is probable that higher gains from selection would be obtained if germplasm from saline environments were to be used, similar to results that have been reported for barley (Jaradat *et al*., 2004). Nevertheless, whether selection for salt tolerance should be made under optimal or stressed conditions is still an unresolved matter, and evidence for either argument is inconclusive (Richards, 1983, 1992; Ceccarelli *et al*., 1998; Kirigwi *et al*., 2004).

A primary effect of salt stress is a reduction of leaf growth, similar to the effect that occurs under water stress (Munns and Tester, 2008). Additionally, in grass species, tillering is reduced under salt stress and this is an important component of biomass accumulation (Wu, 1981; Maas *et al*., 1994). Biomass accumulation in early vegetative stages was closely related to TN and LN. Leaf growth and tillering capacity were more affected in lines from non-saline origin than lines from saline origin. On the other hand, lines from saline provenances had shorter leaves and more tillers than those from non-saline environments. It is possible that lines from saline habitats may have some mechanism, which results in decreased leaf growth at the expense of the appearance of new leaves and tillers. Coefficient of genetic variation is a useful genetic parameter for comparative assessment between different traits. Tiller number (TN) had a higher CGV value than TDW and LN. Therefore, tillering may be the most useful indirect selection criterion for improving salt tolerance at the early stages of development in *E. scabrifolius*. Differences in salt tolerance have been related to tillering capacity in grass forage species such as *Holcus lanatus*, *Lolium perenne*, *Dactylis glomerata* and *Festuca rubra* (Ashraf *et al*., 1986) and in crop species such as rice (Zeng *et al*., 2002), barley (Katerji *et al*., 2006) and wheat (Husain *et al*., 2003; Ruan *et al*., 2008).

Variability in growth parameters was the same for lines from both origins. However, Ashraf (1994) found that populations of *F. rubra* and *Agrostis stolonifera* from non-saline environments had higher genetic variability for salinity tolerance in comparison with populations from saline habitats, because salinity is not a selection factor in non-saline environments. According to Geber and Griffen (2003), ‘heritability is inversely related to the magnitude of environmental effects on functional traits expression’. Salinity levels in this study induced moderate stress. Genetic variation may be different at higher stress levels as was found in maize under salt stress (Rao and McNeilly, 1999) and lucerne (*Medicago sativa*) and wheatgrass hybrid [*Elytrigia repens* (L) Nevski x *E.spicata* (Pursh) D.R. Dewey] under drought stress (Rumbaugh *et al*., 1984). Thus, it cannot be ruled out that variability expression may have been different under higher salinity.

The association between *E. scabrifolius* salt tolerance and some physiological responses usually associated with this trait in other *Triticeae* was assessed next. Plant breeders usually focus in $Na^+$ exclusion and osmotic adjustment as strong physiological traits in breeding programmes (Munns and Tester, 2008). Sensitive lines of *E. scabrifolius* accumulated high $Na^+$ levels in leaves and had a stronger reduction in biomass when compared to the salt-tolerant lines, as has been seen in durum wheat (trait transferred from *T. monococcum*) (Munns *et al*., 2000), *Lophopyrum elongatum* (Shannon, 1978), rice (Yeo and Flowers, 1982), bread wheat (Gorham *et al*., 1987) and *Pennisetum glaucum* (Krishnamurthy *et al*., 2007). Shoot sodium level is one of the most useful traits in breeding for salinity tolerance in some cereals (Colmer *et al*., 2005; Munns and Tester, 2008), and it has been strengthened by the identification of major genes for $Na^+$ exclusion as in durum wheat (Munns *et al*., 2003), *L. elongatum* (Deal *et al*., 1999), rice (Gregorio *et al*., 2002; Lin *et al*., 2004) and bread wheat (Dubcovsky *et al*., 1996). These genes codify for $Na^+$ and $Na^+/K^+$ transporters in durum wheat (Huang *et al*., 2006), rice (Ren *et al*., 2005) and bread wheat (Byrt *et al*., 2007). Although $Na^+$ exclusion may be energetically expensive, we identified Na-excluding genotypes with different biomass accumulation. This means that both traits could be inherited independently, and selection for higher biomass could be possible within genotypes that exclude $Na^+$.

In growing tissues, cell growth is controlled by osmolyte-driven water uptake and the rheological properties of the cell walls (as reviewed by Fricke, 2002). Organic solutes (Munns *et al*., 1982; Hu *et al*., 2000; De Luca *et al*., 2001) accumulated in the leaf elongation zone under saline conditions contribute to osmotic adjustment. Nevertheless, osmotic adjustment in the elongation zone of leaf blades did not correlate with the maintenance of elongation rates under salinity in the genotypes included in this study. It seems that sustained growth under saline conditions in the genotypes evaluated in this study was associated with other causes, such as ion exclusion or cell wall characteristics (Munns, 1988).

The maintenance of $K^+$ homeostasis also contributes to salt tolerance (Taleisnik and Grunberg, 1994; Shabala and Cuin, 2008). High $K^+$ uptake was associated with salinity tolerance in *L. elongatum* (Shannon, 1978) and bread wheat (Ashraf and Khanum, 1997). However, in this study, $K^+$ concentration in old leaves (i.e. leaves 3 and 4) was decreased by saline stress in all lines analysed, independently of their salt tolerance or $Na^+$ accumulation, so this trait may not be useful for distinguishing among lines with different salt susceptibility.

Our results showed that *E. scabrifolius* exhibits genetic variability for salt tolerance at the early development stages. *Lophopyrum and Thinopyrum* species are the primary source of salt tolerance in wheat-breeding programmes (Colmer *et al.*, 2005). These species have E ($J^c$) y J ($E^j$) genomes, whereas the genome in *E. scabrifolius* is SSHH (Wang *et al.*, 1994). Mano and Takeda (1997), Forster *et al.* (1990) and Garthwaite *et al.* (2003, 2005) analysed the salinity tolerance of several *Hordeum* species and found that the H genome is a source of genes for tolerance to salinity and waterlogging. Thus, *E. scabrifolius* could become an alternative source of salt-tolerant genes for wheat-breeding programmes, and populations established in saline areas have higher biomass and salt tolerance than those from non-saline areas. Tillering may be the most useful indirect selection criteria for improving salt tolerance at the early stages of development in *E. scabrifolius*, and Na exclusion is an additional physiological trait to be considered.

The salt-tolerant lines identified in this study were all growing in saline–alkaline soils. It has been widely documented that negative salinity effects on plants are associated with the water-deficit resulting from the low water potential in saline soils and salt-associated ion imbalances (Munns and Tester, 2008). These imbalances may be aggravated under alkaline conditions (Marschner, 1995). Comparative studies performed in *Suaeda glauca* (Yang *et al.*, 2008) and *Hordeum vulgare* (Yang *et al.*, 2009) involving saline and saline–alkaline conditions indicate the latter cause greater growth reductions. While our work does not allow for a distinction between salt and alkaline-salt tolerance, as the *E. scabrifolius* populations were adapted to grow in saline–alkaline soils where they were collected, they can, therefore, be also considered as potential sources of tolerance to saline–alkaline conditions.

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