Searching for Molecular Markers for Salt Tolerance in Rhodes Grass (*Chloris gayana* Kunth)

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SEARCHING FOR MOLECULAR MARKERS FOR SALT TOLERANCE IN RHODES GRASS (*Chloris gayana* Kunth)

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Abstract

Rhodes grass *(Chloris gayana* Kunth), a C_4 forage grass, is regarded as salt-tolerant and exhibits *intra*- and *inter*-cultivar variability for this trait. Plants of cv Boma were selected for salt tolerance at the seedling and adult stages, cloned and characterized by RAPD and AFLP amplification patterns. Both techniques were equally efficient for fingerprinting these clones. More bands were obtained by AFLP but the ratio of polymorphic bands and the proportion present only in tolerant clones were the same by both methods. These bands, along with those exclusive for sensitive clones could be useful as markers for assisted selection.

Keywords: Rhodes grass, salt tolerance, RAPD, AFLP, molecular markers.

Introduction

In the Argentinean Arid Chaco, several million hectares climatically suitable for cattle production, are affected by salinity distributed in patches where pasture productivity is severely reduced (Angueira, 1986). *Chloris gayana*, a C_4 grass known for its salt tolerance (Gauch and Wadleigh, 1951; Gausman *et al.*1954; Fossati *et al.*, 1979) is well adapted to the climate prevailing in the area, where it was introduced many years ago. *Inter*- and *intra*-cultivar variability for salt tolerance has been detected in this species (Malkin and Waisel, 1986, Pérez et al., 1999, Taleisnik *et al*., 1997, Luna et al., 2000). The purpose of this work was to characterize clones of cv. Boma with contrasting salt tolerance, using RAPD (Randomly Amplified Polymorphic DNA) (Welsh y McClelland, 1990; Williams et al. 1990) and AFLP (Amplified Fragment Length Polymorphism) (Voss, et al. 1995). The resulting patterns will be used to fingerprint the clones and to develop "QTL" (quantitative trait locus) markers for salt tolerance.

Material and Methods

Chloris gayana cv Boma plants were used. Seedlings and adult plants were identified for salt tolerance in hydroponics. The procedure for identifying salt-tolerant and -sensitive adult plants was described by Luna *et al.* (2000). To select seedlings, seeds were germinated in a humid chamber at 32°C and a 14hr. light period. After two-three days, germinated seeds were transferred to a sandwich of blotting paper moistened with tap water (Myhill & Konzak, 1967) for two weeks and then treated with either 250 or 400mM NaCl. Seedlings surviving after two weeks in 400 mM NaCl, were

transferred to pots with soil. All plants were subsequently multiplied vegetatively (cloned).

The molecular characterization was performed on three salt-tolerant (IF3; 13; 15) and one sensitive (IF12) clone. DNA extraction and RAPD protocol was according to Hoisington *et al*. (1994). For RAPD, 30 primers from Operon Technology were used, 17 from series F (1; 2; 3; 4; 5; 7; 8; 9; 10; 11; 12; 13; 14; 16; 17; 18; 20) and 13 from series J (1; 4; 5; 6; 7; 10; 11; 12; 13; 16; 17; 18; 19). The final concentration of $MgCl_2$ was 1.5mM, and the amplification program was as described by Pérez *et al.* (1999). The electrophoresis separation of amplification products was performed in agarose gels (1.5%) with ethidium bromide. AFLP was performed according to Voss *et al*. (1995). Genomic DNA was digested with EcoRI and MseI restriction enzymes. The primers combinations used were: Eco31/Mse 34; 35; 36; 37; 38; 39; 40; 41; 42; 43; 44; 45; 46 and Eco32/Mse 31; 41; 43; 44; 45; 46 and final $MgCl_2$ concentration was 1.6mM. The amplification program was: 2min at 94°C, 13 cycles of 30sec at 94°C, 30sec at 65°C with a touch down of 0.7°C per cycle, 25 cycles with an annealing temperature of 56°C and an extension cycle of 10min at 72°C. Electrophoresis was performed in polyacrilamide sequencing gels (5% acrilamide and 7M urea) and gels were stained with silver nitrate using the Promega Corp. protocol. Results were analyzed by Chi square and Fisher's exact test.

Results and Discussion

A very high number of seedlings were obtained after 2 or 3 days germination under the chosen light and temperature conditions, and survival was 86-92% after four weeks in tap water. Compared to the controls, 93 plants in the 250mM NaCl treatment survived; while only 31% survived with 400mM NaCl.

In field trials, plant survival in the saline plot was 89% and 54%, for a tolerant and a sensitive clone, respectively. Productivity of the IF3 tolerant clone in saline plots was 63% of the non-saline plots, while it was only 12% in the most sensitive clone, IF12.

RAPD and AFLP amplification patterns are shown in Figure 1. Both techniques were equally efficient in characterizing and identifying selected clones. Though the number of bands per primer was greater with AFLP than with RAPD, the proportion of polymorphic bands and bands present only in tolerant clones was the same (P=0.23). Bands present only in sensitive or in tolerant clones are putative markers for assisted selection. To determine a possible association between them we are now analyzing progenies from the IF3 x IF12 crosses. Using a similar procedure, QTL's for salt tolerance were found in *Hordeum spontaneum* (Pakniyat et al. 1997).

Summarizing, clones with high salt tolerance were isolated from *Chloris gayana* cv. Boma. These clones were characterized by RAPD's and AFLP patterns. Both techniques revealed putative markers for salt tolerance.

References

Angueira M.C. (1986). Geomorfología de la provincia de Santiago del Estero. In: Curso Taller Internacional "Desmonte y habilitación de tierras en zonas semiáridas" Red de Cooperación Técnica en uso de Recursos Naturales- FAO **1**: 32-54.

Fosatti, J.L., Bruno O.A., Panigatti J.L. and Gambaudo S.P. (1979). Comportamiento de forrajeras estivales en los bajos submeridionales. Informe técnico N*1. Convenio Fundación José María Aragón-INTA- Gobierno de la Pcia. de Santa Fé. Estación Experimental Regional Agropecuaria Rafaela.

Gauch, H.G. and Wadleigh C.H. (1951). Salt tolerance and chemical composition of Rhodes and Dallis grasses, grown in sand culture. Botanical Gazette **112**: 259-261.

Gausman, H.W., Cowley W.R. and Barton J.H. (1954). Reaction of some grasses to artificial salinization. Agronomy Journal **46**: 412-414.

Hoinsington, D., Kairallah M. and González de León D. (1994). Laboratory protocols: CIMMYT applied molecular genetics laboratory. Second Edition. Mexico, D.F.:CIMMYT.

Luna, C., García Seffino L., Arias C. and Taleisnik E. (2000). Oxidative stress indicators as selection tools for salt tolerance in *Chloris gayana*. (Plant Breeding, in press).

Malkin, E. and Waisel Y. (1986). Selection for salt resistance in Rhodes grass (*Chloris gayana*) Physiologia Plantarum **66**: 443-446.

Myhill, R.R. and Konzak C.F. (1967). A new technique for culturing and measuring barley seedlings. Crop Science **7**: 275-276.

Pakniyat, H., W. Powel, E. Baird, L.L. Handley, D. Robinson, C.M. Scrimgeour E. Nevo, C.A. Hackett, P.D.S. Caligari and B.P. Foster (1997). AFLP variation in wild barley (*Hordeum spontaneum* C. Koch) with reference to salt tolerance and associated ecogeography. Genome **40**: 332-341.

Pérez, H., Bravo S., Ongaro V, Castagnaro A., García Seffino L. and Taleisnik E. **(**1999). *Chloris gayana* cultivars: RAPD polymorphism and field performance under salinity. Grass and Forage Science **54**: 289-296.

Taleisnik, E., Pérez H., Castagnaro A., Díaz D., García Seffino L., Arias C., Grunberg K., Córdoba A., Bravo S., Orellana D., Vellicce G., Ongaro V. and Maíz E. (1997). Variabilidad intraespecifica para tolerancia a la salinidad en Grama Rhodes(*Chloris gayana* Kunth). Revista Argentina de Producción Animal, **17**: 118-119.

Voss, P., Hogers R., Bleeker M., Reijans M., De Lee T.V., Hones M., Frijters A., Pot J., Peleman J., Kuiper M. and Zabeau M. (1995). AFLP: a new technique for DNA fingerprinting. Nucleic Acid Res., **23** : 4407-4414.

Welsh, J. and McClelland M. (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acid Research **18:** 7213-7218

Williams, J.G.K., Kubelik A.R., Livak K.J., Rafalski J.A. and Tingey S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Research., **18** : 6531-6535.

Table 1 - Comparison between RAPD and AFLP polymorphisms.

	RAPD	AFLP
Band number	566[(1)]	995[(2)]
Polymorphic Bands	297	523
Bands present only in tolerant clones IF3; IF13; IF15	4	8[§]
Bands present only in sensitive clone IF12	45	17
Number of primers used	30	19
Polymorphic bands per primer	9.9	27.3
% of polymorphic bands	52.5	52.5
% of shared bands by the tolerant clones	1.3	1.5

[(1)] Scored on clones IF3; 7; 12; 13; 15. [(2)] Scored on clones IF3; 12; 13;14; 15

[§] P=0.23

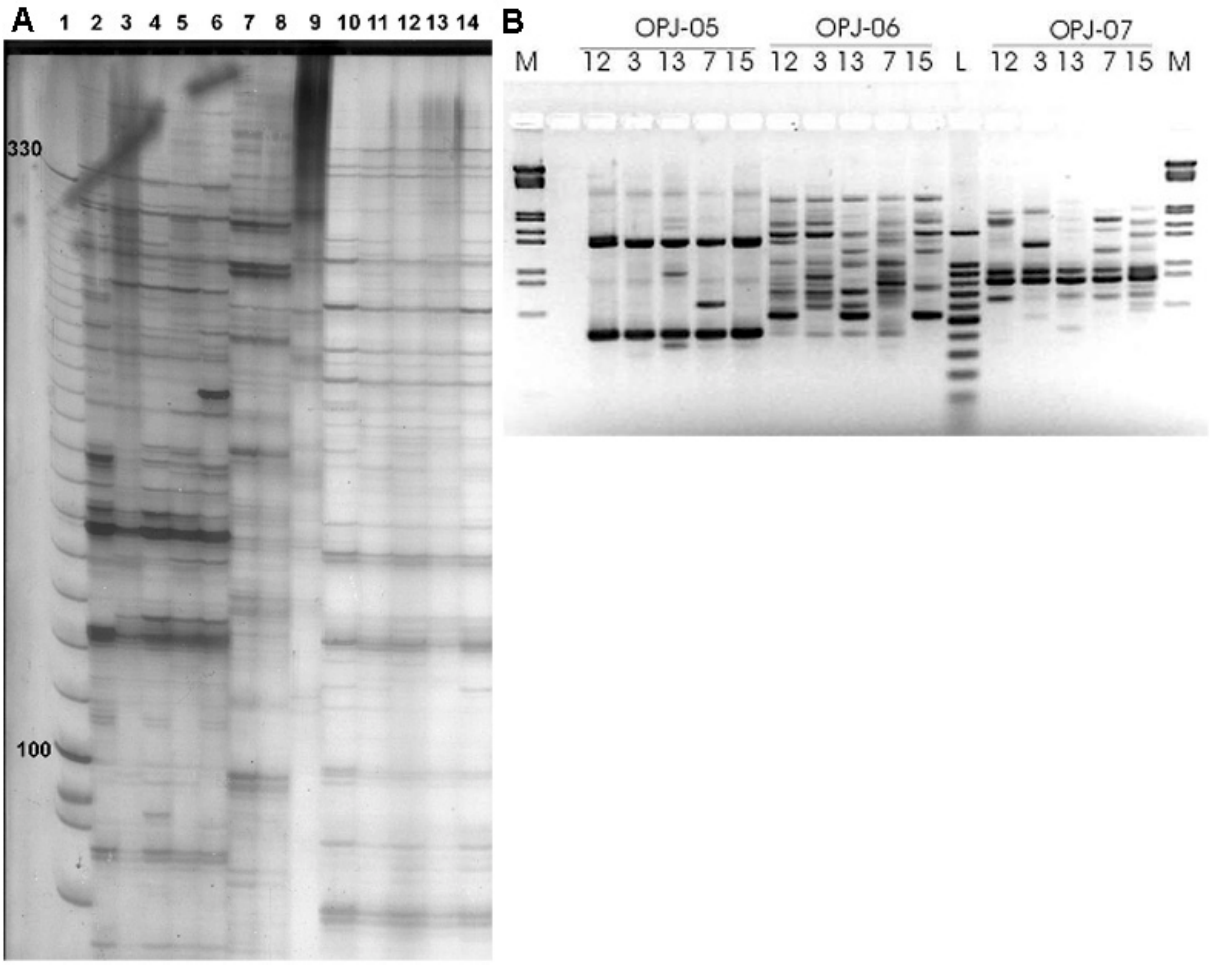

Figure 1 - A AFLP amplification on clones from *C. gayana* cv Boma (2 & 10 IF3; 3 & 11 IF12; 4 & 12 IF13; 5 & 13 IF15; 6 & 14 IF14; 7,8,9 barley samples) with primers combination Eco31/Mse41 (1-8) and Eco32 Mse42(9-14). PAGE (5%, urea 7M). Lane1 leader (GIBCO). **B** RAPD's amplifications from clones IF12; IF3; IF13; IF7 and IF15. Electrophoresis on agarose gel (1.5%). M Lambda EcoRI/HindIII, L leader (PROMEGA).