Novel Genotypes of the Subtropical Grass *Eragrostis Curvula* for the Analysis of Apomixis (Diplospory)

S. Cardone
Universidad de Buenos Aires, Argentina

P. Polci
Universidad Nacional del Sur, Argentina

J. P. Selva
Universidad Nacional del Sur, Argentina

M. Mecchia
Universidad Nacional de Rosario, Argentina

S. C. Pessino
Universidad Nacional de Rosario, Argentina

See next page for additional authors

The XX International Grassland Congress took place in Ireland and the UK in June-July 2005. The main congress took place in Dublin from 26 June to 1 July and was followed by post congress satellite workshops in Aberystwyth, Belfast, Cork, Glasgow and Oxford. The meeting was hosted by the Irish Grassland Association and the British Grassland Society.

Proceedings Editor: D. A. McGilloway

Publisher: Wageningen Academic Publishers, The Netherlands

Presenter Information
S. Cardone, P. Polci, J. P. Selva, M. Mecchia, S. C. Pessino, P. Voigt, G. C. Spangenberg, and V. Echenique

Novel genotypes of the subtropical grass *Eragrostis curvula* for the analysis of apomixis (diplospory)

S. Cardone[1,2], P. Polci[1], J.P. Selva[1], M. Mecchia[3], S. Pessino[3], P. Voigt[4], G.C. Spangenberg[5] and V. Echenique[1,6]
[1]Depto. de Agronomía, Universidad Nacional del Sur, San Andrés 800, 8000 Bahía Blanca, Argentina [2]Facultad de Agronomía, Universidad de Buenos Aires, Argentina [3]Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, Rosario, Argentina [4]USDA, Agricultural Research Service, Appalachian Farming Systems Research Center, Beaver, USA [5]Primary Industries Research Victoria, Plant Biotechnology Centre, La Trobe University, Bundoora, Victoria 3086, Australia [6]CERZOS (CONICET), Bahía Blanca, Argentina
Email: echeniq@criba.edu.ar

Keywords: *Eragrostis curvula*, diplosporous apomixis, novel genotypes, apomixis gene(s)

Introduction *Eragrostis curvula* (Schrad.) Nees is a variable grass native to Southern Africa. Its several forms, known as lovegrasses, were introduced to Australia, USA and Argentina as forage perennial grasses. Apomixis is a common trait in the genus *Eragrostis*, with diplospory being the most frequent type. Sexual reproduction also occurs in *Eragrostis*, although not frequently. Since most tetraploid *Eragrostis* lines are apomictic, the generation of a sexual tetraploid strain is a requirement for linkage analysis of the gene(s) governing the apomictic character. Furthermore, isogenic lines of the same ploidy, reproducing alternatively by sexuality or apomixes, represent an ideal system for comparative transcriptome analysis. The aim of this work was the generation and characterization of two novel genotypes of *E. curvula*: a dihaploid strain obtained *in vitro* from an apomictic cultivar and a tetraploid plant derived from the dihaploid after chromosome duplication.

Materials and methods Plants were obtained by *in vitro* culture of immature inflorescences of the apomictic *E. curvula* cultivar Tanganyka (2n = 4x = 40) on Murashige and Skoog medium supplemented with 2,4-D and BAP. Morphological and phenological traits of the regenerated plants and the original tetraploid cultivar were comparatively assessed. Chromosome number was determined in root tips by Feulgen staining. The reproductive mode was assessed by the study of megasporogenesis and embryo sac development and by the evaluation of progeny plants using RAPD amplification. Duplication of chromosome number of the dihaploid plant was done by 0.05% colchicine treatment. Progeny tests were performed by RAPD amplification on 8 individuals originated by open pollination from the dihaploid plant.

Results One out of 23 regenerated plants showed 20 chromosomes in root tip cells instead of the normal 40 chromosomes in the original tetraploid cultivar. This plant (named UNST1122) produced wider and shorter leaves than those of the other regenerated plants and the original cultivar. Other unusual characteristics of this plant were a typical pubescence on the base of the adaxial surface of leaves, a clear tendency to produce aerial tillers, a very small ligula (0.2 mm) and panicles exhibiting variation in shape and size, with rachillas radially arranged forming an angle of approximately 90° with the rachis. Anthers were pale yellow and seeds were ellipsoidal with a light brown coat, smaller and lighter than those produced by control plants (weight of 100 seeds: 23 mgs vs 40 mgs). Under greenhouse conditions, UNST1122 flowered almost all year round, except in the colder months of June and July (Southern hemisphere). However, the number of seeds per inflorescence was lower than that of the original cultivar. Studies of megasporogenesis and megagametophyte development, as well as progeny tests using RAPD amplification, showed that this dihaploid plant was sexual and self-incompatible. Colchicine treatment of seeds from the dihaploid gave rise to two plants with 40 chromosomes, UNST1112 and UNST1131. Comparison of the amplification profiles obtained for UNST1122 with those of 8 progeny individuals obtained by open pollination revealed polymorphism that ranged from 4.6% to 40% for all analyzed plants. The lack of offspring showing a maternal profile is evidence of sexual reproduction in UNST1112.

Conclusions The molecular basis underlying mechanisms of diplosporous reproduction remains largely unknown. The novel genotypes described here will allow the future establishment of mapping populations of *Eragrostis curvula* that can be used in strategies of forward genetics to map and positionally clone the determinants of the apomictic trait. They also represent an excellent system for the identification of genes involved in diplospory and/or ploidy level gene regulation by using transcriptional profiling techniques such as differential display, cDNA-AFLP or ESTs discovery.