MOLECULAR CHARACTERIZATION AND FIELD EVALUATION OF GLYPHOSATE TOLERANT SUGARCANE : A STEP TOWARDS COMMERCIAL RELEASE



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Sugarcane production in Argentina



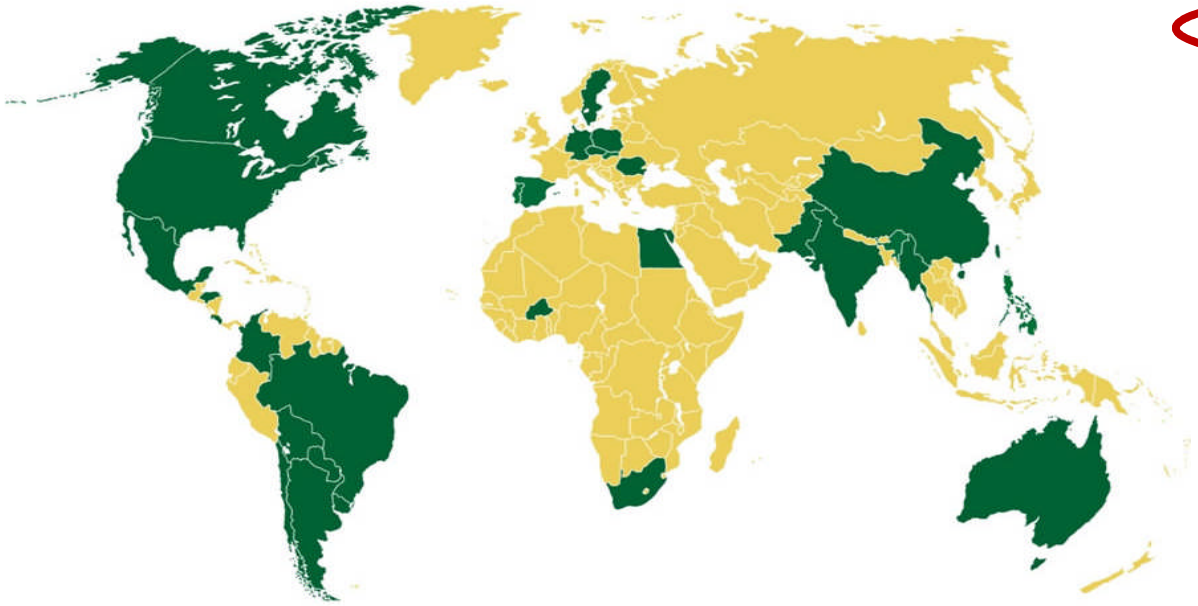




Tucumán

Three regions: Northern (Salta and Jujuy Provinces, 2 and 3 mills, respectively) (33%)
Litoral (Santa Fe and Misiones Provinces, 2 and 1 mills, respectively)(1%)
Tucumán Province (15 mills) (66%)

Transgenic Crop Adoption worldwide





	Million Ha
USA	73.1
Brazil	42.2
Argentina	24.3
India	11.6
Canada	11.6
China	3.9
Paraguay	3.9
Pakistan	2.9
South Africa	2.7
Uruguay	1.6
Bolivia	1.0
The Philippines	0.8
Australia	0.5
Burkina Faso	0.5
Myanmar	0.3
Mexico	0.2
Spain	0.1

Adapted from Clive James 2014



Glyphosate-tolerant crops

✓**Most important commercial applications of plant genetic engineering.**

✓**Easier and more effective weed control, increase in profits, less tillage and no restrictions to crop rotation.**

✓**Negative environmental and health impacts of agrochemical weed management are reduced.**

✓**Seven commercial crops: alfalfa, rape, cabbage, cotton, maize, soybean and sugar beet.**



Glyphosate-tolerant Sugarcane

✓**Weeds principally compete for moisture, light and nutrients.**

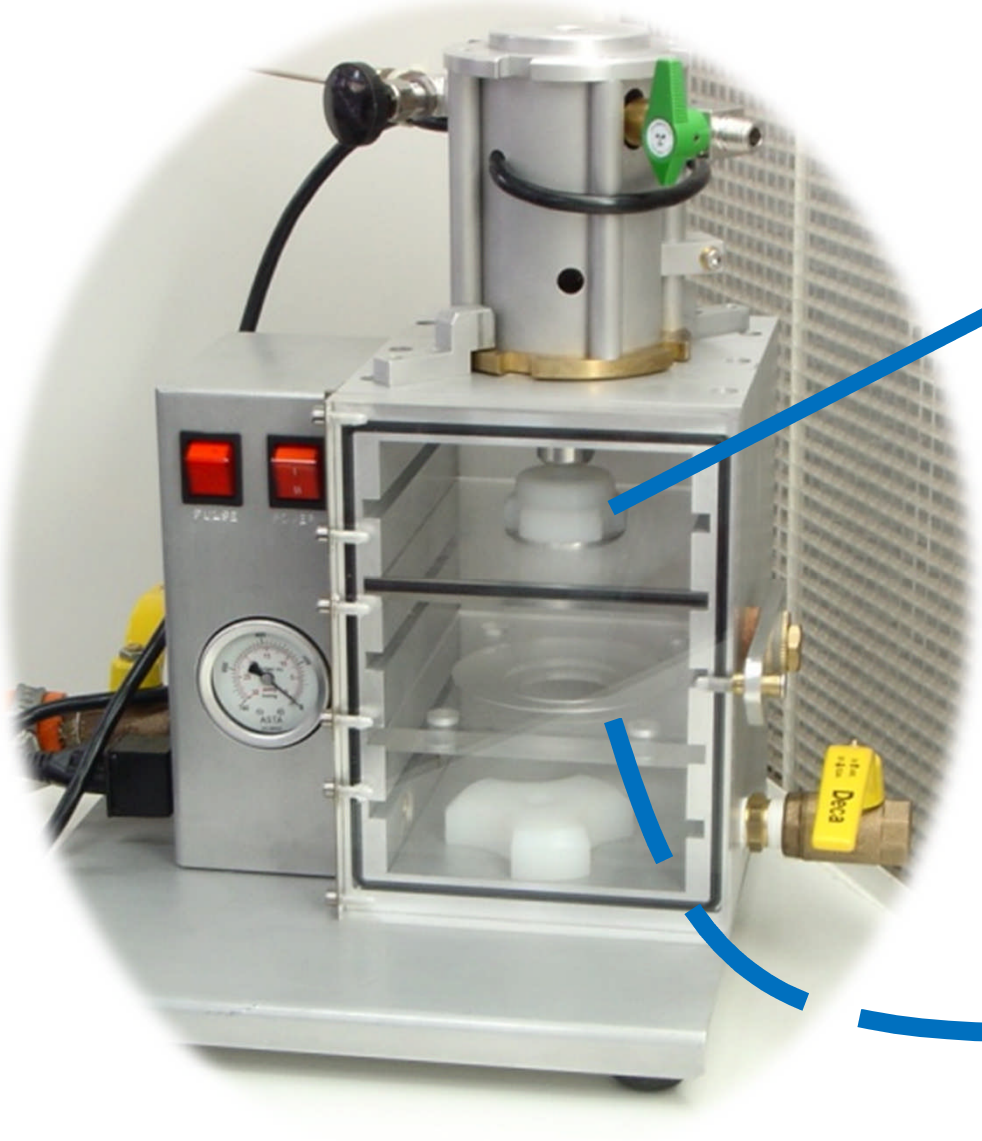
✓**Weeds can also harbour certain diseases and pests that lead to indirect losses.**

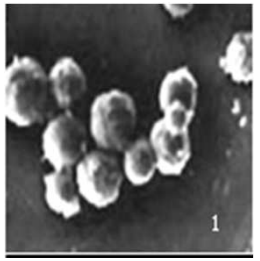
✓**In Argentina more than 300 weedy species affect sugarcane production and studies have shown that in some regions yield losses can reach up to 30%.**



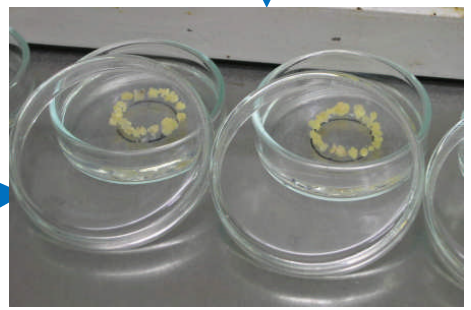
Our aim was to obtain a glyphosate tolerant commercial sugarcane variety (RA 87-3).

Microprojectile Bombardment

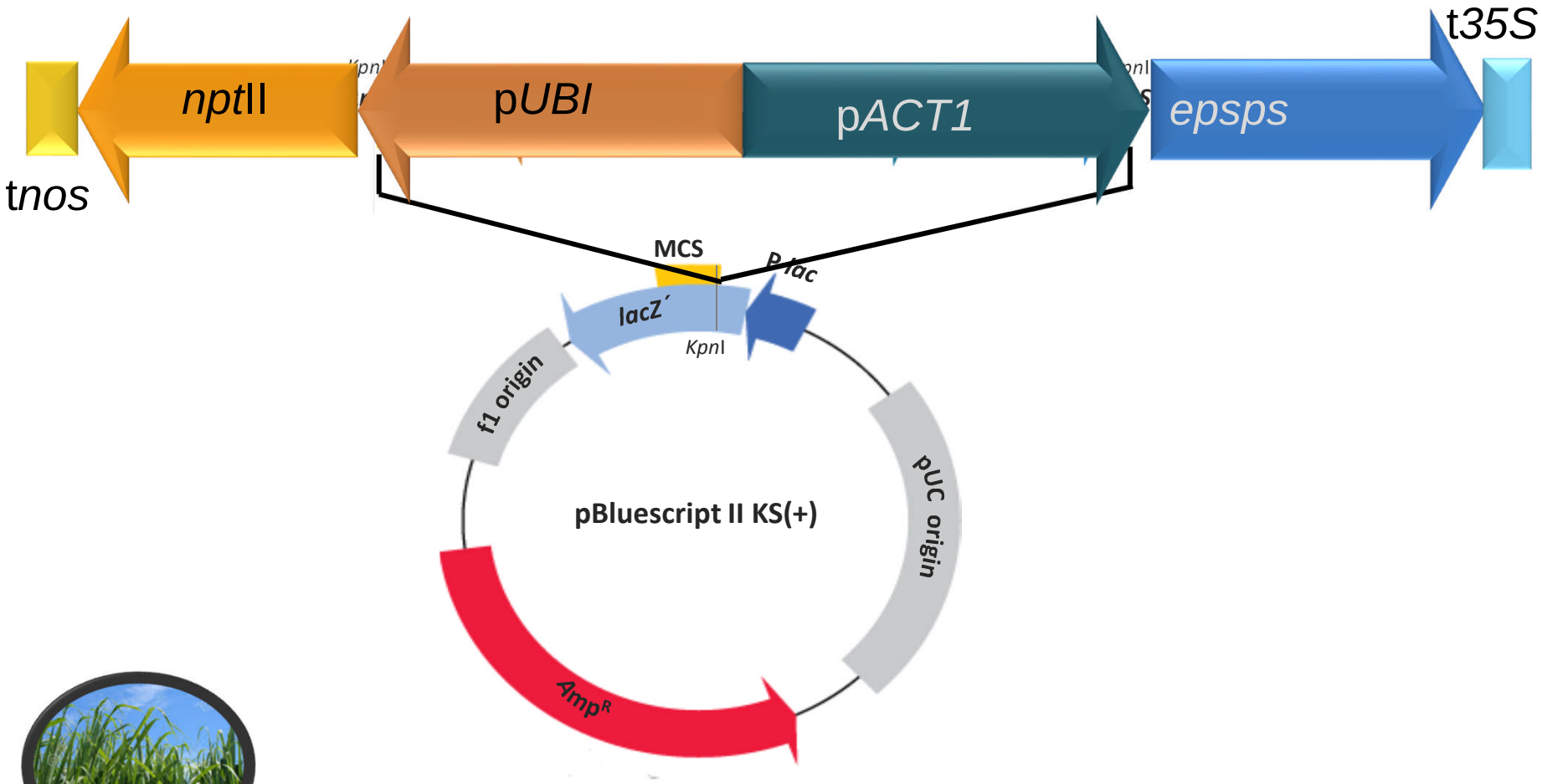


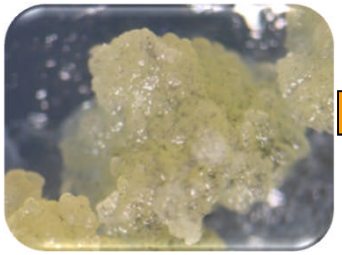






Plasmid pEA1 expressing the *epsps* gene from *Agrobacterium* strain CP4





Bombardment calli



Selection of transformed tissue



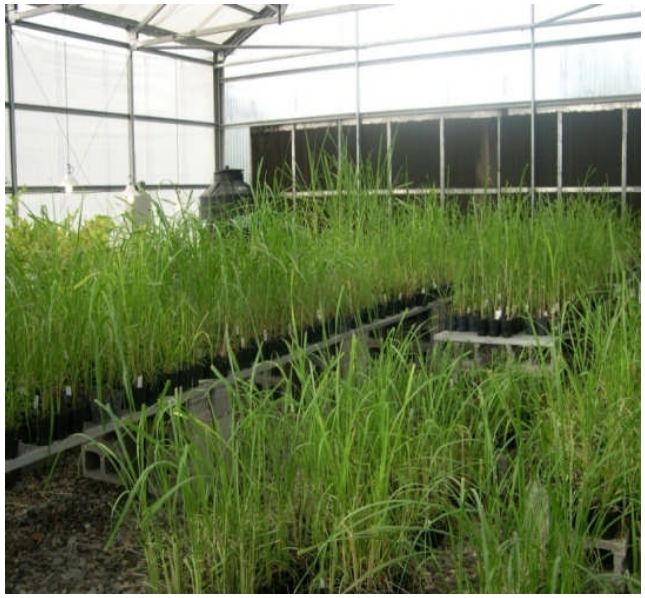
Regeneration

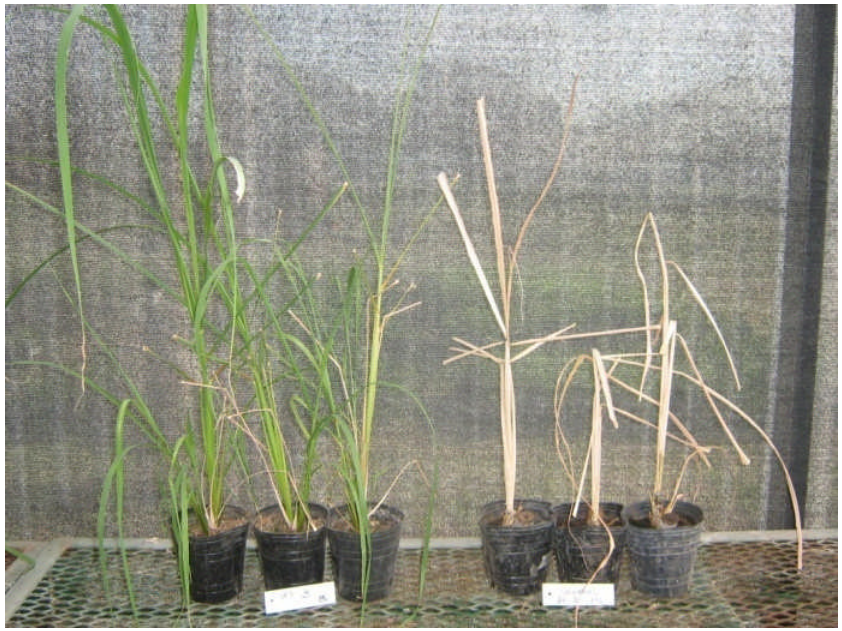


***in vitro* propagation**

Evaluation under greenhouse conditions

RA 87-3





Tolerant Susceptible

- ✓ **43 survived the *in vitro* process**
- ✓ **17 tolerant**
- ✓ **5 moderate tolerant**
- ✓ **21 susceptible**

CONABIA resolution Nº 200/2008



Evaluation under field grown conditions



Before application



After application

✓**17 tolerant**

✓**6 high phenotypic and growth resemblance to the parental line RA 87-3 (15; 18; 22; 27; 28 and 37)**

CONABIA Resolution Nº 114/2009

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Morphological characteristic determination

UPOV characters (RA 87-3 control and 15; 18; 22; 27; 28 and 37 lines)

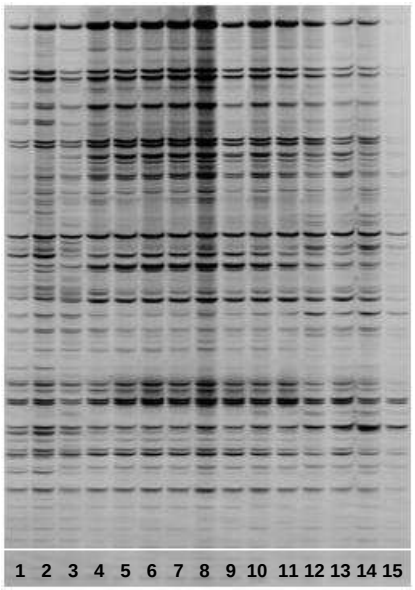
- ✓**Leaf sheath adherence**
- ✓**Stem diameter**
- ✓**Stem shape**
- ✓**Color where exposed to sun**
- ✓**Color where not exposed to sun**
- ✓**Zigzag alignment expresion**
- ✓**Bud shape**
- ✓**Necklace color leaf sheath**
- ✓**Width at the longitudinal mid-point leaf**

✓**Transformed lines 28 and 37 only differed in color characters when compared to the parental variety.**



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Genetic evaluation



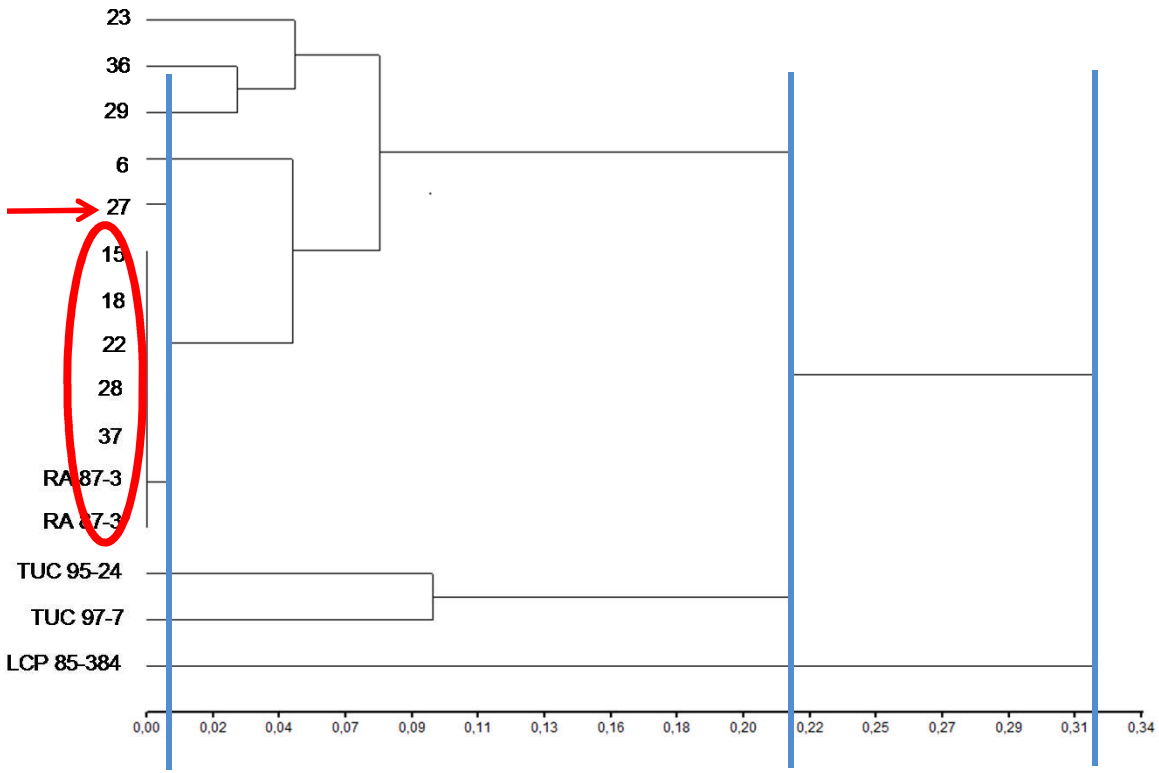


Figure: Genotypic evaluation of transformed lines using molecular markers: a) TRAP fragments generated using SuPS2 + Arbi 2 primers. **1**: LCP85-384; **2**: TUC97-7; **3**: TUC95-24; **4**: RA87-3 micropropagated control; **5-10** transgenic lines(15; 18; 22; 27; 28 and 37 respectively); **11**: RA87-3 conventional propagated control; **12-15** transformed lines (6; 29; 36 and 23 respectively) with a distinct growth phenotype. b) Dendrogram of the four sugarcane genotypes and the transgenic lines of RA87-3 based on 339 allele analysis from 9 TRAP primer combinations when using Jaccard coefficient and UPGMA clustering method with InfoStat, presented as distance (1-S, S: similarity). Lines represented similarity of 0.99; 0.78 and 0.68.

epsps expresion

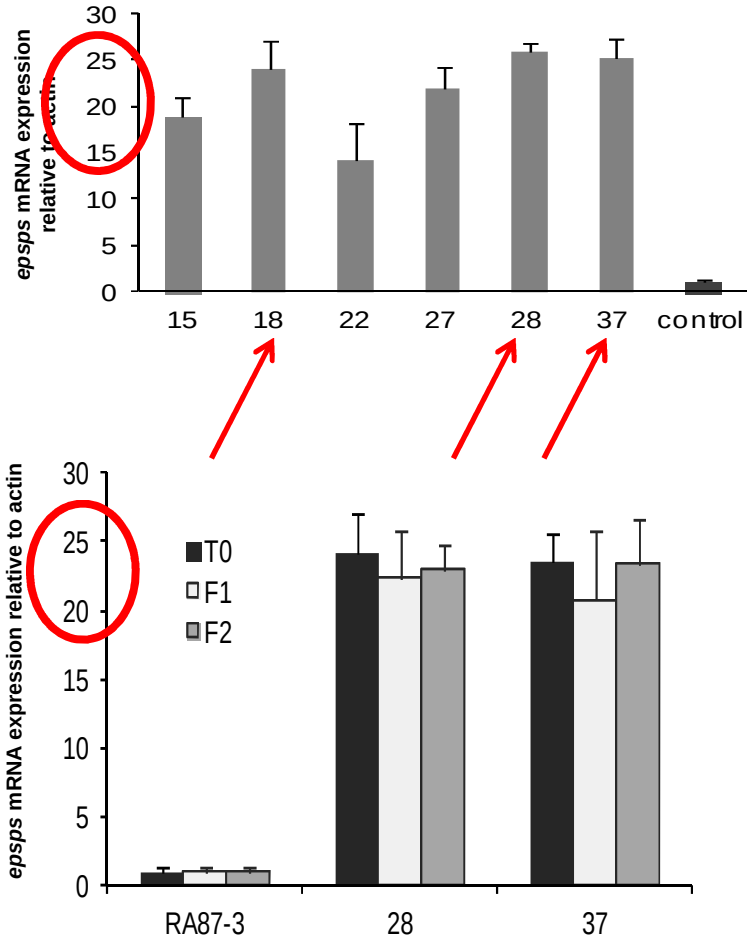


Figure. Quantitative RT-PCR analysis of transgenic lines. Average n-fold changes in epsps gene expression in six transgenic lines compared to non transformed RA87-3 plant were calculated from triplicate samples.

Figure. Stability of *epsps* gene expression in three propagation generations of by qRT-PCR. Average n-fold changes in *epsps* gene expression in two transgenic lines (28, 37) compared to non-transformed RA87-3 plant were calculated from triplicate samples.

Transgene copy number

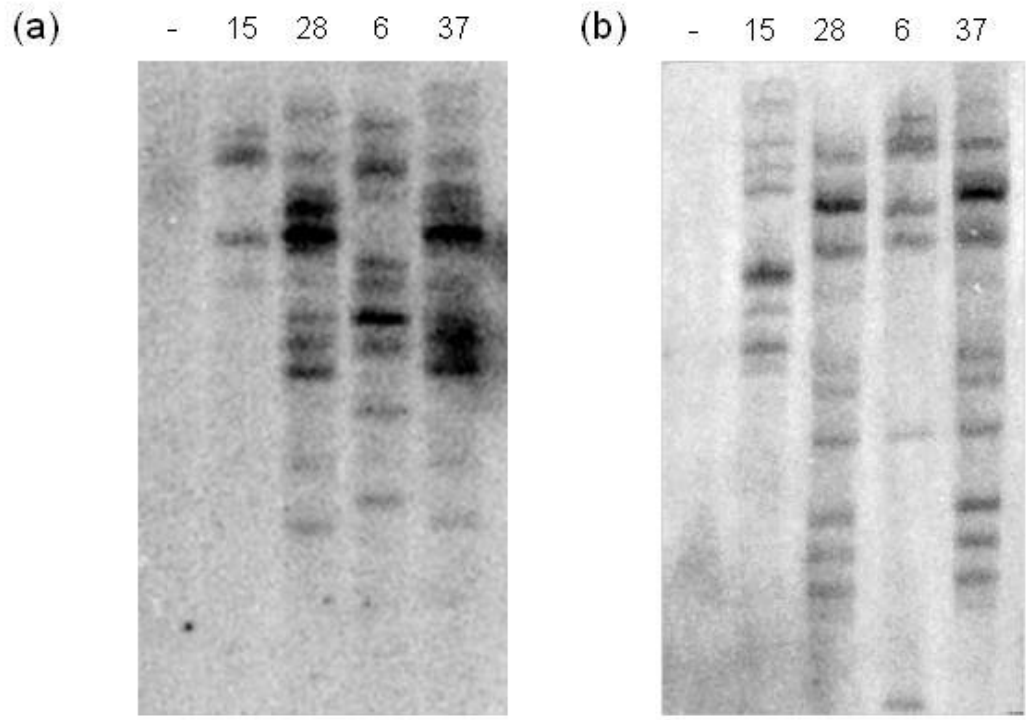
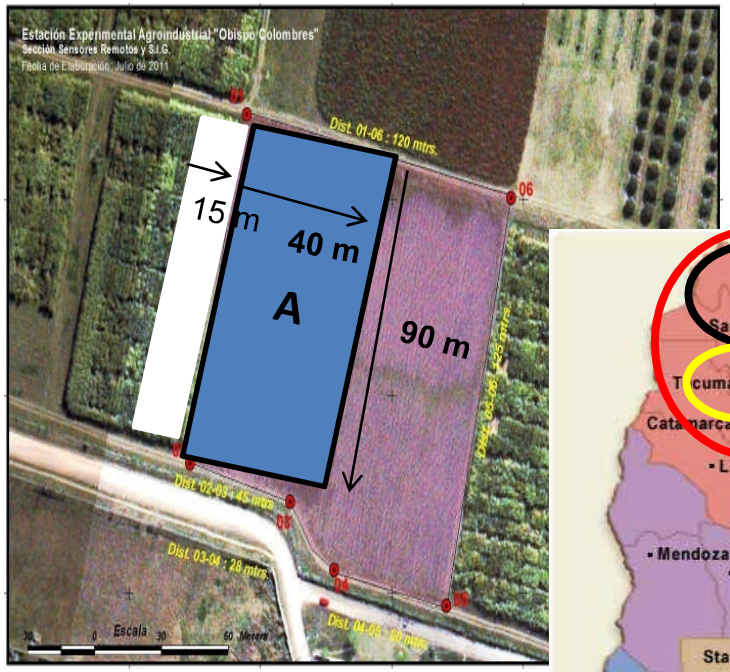


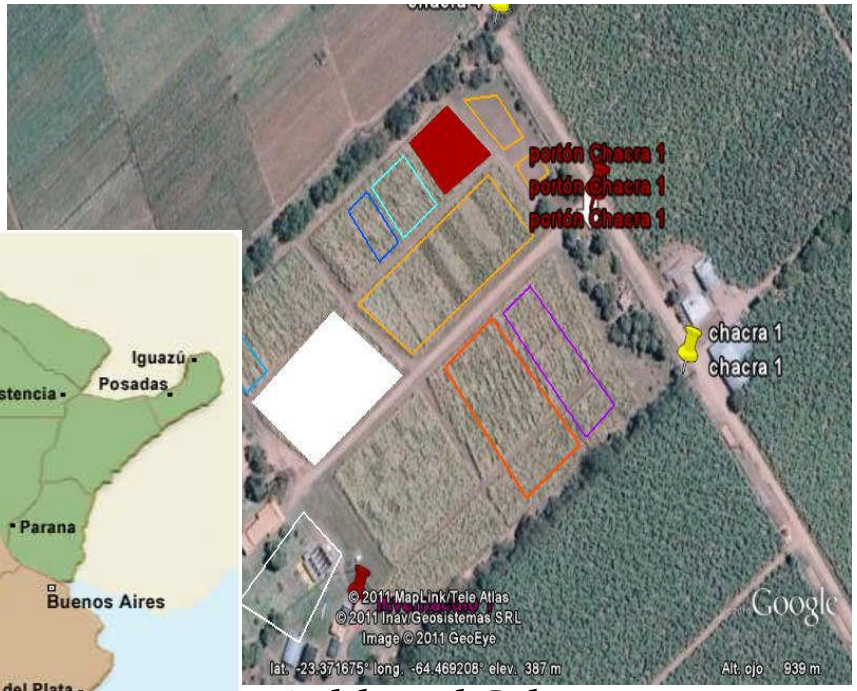


Figure Analysis of number copies in transgenic lines by means of Southern blot using *nptII and epsps* probe, respectively.

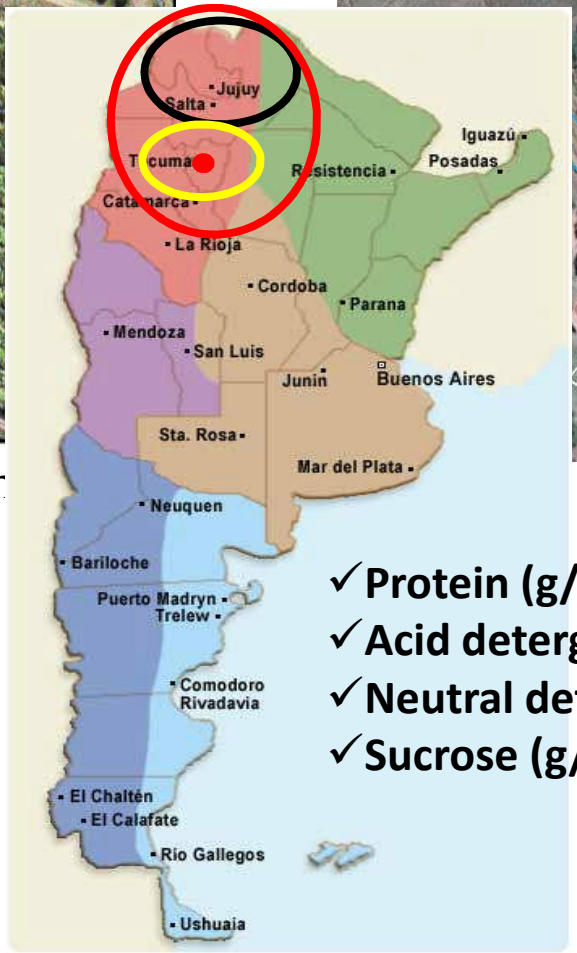
Agronomic performance and Chemical composition



Field trial Tucum



Field trial Salta



- ✓Height (cm)
- ✓Stalk weight (kg)
- ✓Stalk diameter (cm)
- ✓Stalk number
- ✓Yield (kg/plot)

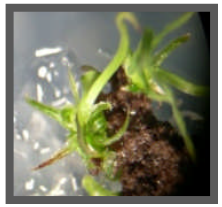
- ✓Protein (g/100 g)
- ✓Acid detergent fiber (g/100 g)
- ✓Neutral detergent fiber (g/100 g)
- ✓Sucrose (g/100 g)

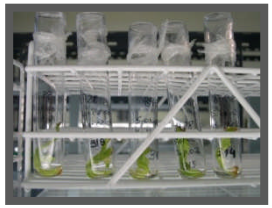
Genetic trasnformation of sugarcane varieties

2006 2007 2008 2009 2010 2011 2012 2013 2014

Process optimization and obtention of transgenic plants







Transgenic lines at greenhouse (Res.200/2008)



43 transgenic lines

Field propagation (Res.114/2009)



Fiedl trial, glyphoste applicattion (Res.114/2009)





Trials in Tucumán and Salta (Res. 621/2011)





6 transgenic lines


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