ABSTRACT: 359

PLEIOTROPIC EFFECTS OF THE ALLELE FOR SULFONYLUREA TOLERANCE, Als1, ON SOYBEAN (Glycine max (L) Merr.)

SANTONE W D 1, CAIRO C 2, SALA C.A 3, ROSSI R 1

1 Soybean Breeding Program, NIDERA S.A.; Route N° 8 Km 376.5, Venado Tuerto, 2600, Santa Fe, Argentina. 2 Plant Physiology Department, Faculty of Agricultural Sciences, Rosario National University, Santa Fe, Argentina. 3 Biotechnology Department, NIDERA S.A. E-mail: wsantone@nidera.com.ar

Soybean is naturally susceptible to the family of sulfonylurea herbicides. A sulfonylurea (SU) tolerant mutant allele (Als1) was developed in 1989 and, since then, it was introgressed in many soybean breeding programs all over the world. In Argentina, this source of germplasm was introduced into commercial cultivars providing an efficient way to control glyphosate resistant weeds. There is evidence that the introduction of mutant or transgenic genes can result in yield reduction and other adverse effects for the recipient crop. The aim of this study was to determine the possible pleiotropic effects of Als1. Three F2 populations segregating for SU tolerance were obtained by crossing different SU-tolerant cultivars with SU-susceptible genotypes. These populations were inbred by single seed descent until the F5. In this generation, individual plants were derived in progeny rows for the subsequent selection of tolerant (T, Als1/Als1) and susceptible (S, als1/als1) isolines. The selected T and S isolines were planted together with their parents used as checks, in 7 locations in Argentina during 2009 and 2010. Days from sowing to beginning of flowering, maturity, height, grain yield and its components, protein and oil content, and fatty acid profile were evaluated on each plot. Members of each pair of T and S isolines did not differ from each other with respect to their cycle to flowering and maturity, and their height. The genotypes carrying the Als1 allele, however, differed significantly from their als1 isogenic counterparts for grain yield, oil content, and protein and oil yield per hectare. These results are discussed in the framework of soybean breeding and resistant weed management.
Pleiotropic effects of the allele for sulfonylurea tolerance, $Als1$, on soybean [$Glycine\ max\ (L)\ Merr.$]

Agr. Eng., Ms. Sc. Walter D. Santone
Soybean breeding program, NIDER A S.A.
Argentina

wsantone@nidera.com.ar
Soybean is the main crop in Argentina. Glyphosate tolerance has created a new management scenario, leading to the development of weed biotypes resistant to Glyphosate. The selection of new herbicides is required for "chemical fallow".
Sulfonylurea herbicides

- High herbicide activity and significant soil residual
- Low mammals toxicity
- Broad spectrum weed control

“Soybean is susceptible to this herbicide”
“Development of SU tolerant varieties (STS)”
Some mutations that confer tolerance to herbicides have further effects on the functionality of the AHAS enzyme (reduced\(^1\), increased\(^2\), unchanged\(^3\)).

Genetic mutations have adverse pleiotropic effects on plant growth and reproductive capacity\(^4\).

In 2000, Reddy & Whiting reported STS cultivars had lower performance.

\(^1\)(Ashigh & Tardiff, 2007), \(^2\)(Yu et al., 2003), \(^3\)(Preston et al., 2006), \(^4\)(Vila-Aiub et al., 2009)
OUR HYPOTHESIS

✓ The mutant allele $A/s1$, does not have pleiotropic effects on the composition and grain yield of soybean.
Objectives

General objectives

✓ Determine if Als1 gene has pleiotropic effects on the composition and grain yield of soybean.

Especific objectives

✓ Analize the Als1 gene effect on the developmental stages and the morphological features of a set of soybean isolines obtained from different genetic backgrounds.

✓ Assess the Als1 gene effect on the protein and oil content of the isolines selected.

✓ Evaluate the Als1 gene effect on the fatty acids composition of the oil grain.
Isolines obtaining procedure (SSD)

Materials and methods

**Susceptible** × **Tolerant**

- **F**<sub>1</sub>
- **F**<sub>2</sub>
- **F**<sub>4</sub>
- **F**<sub>5</sub>
- **F**<sub>6</sub>
- **F**<sub>7</sub>

**Grain increase and phenotypic selection of isolines**

**Characterization of individuals according to the presence or absence of *Als1* gen**

**Phenotypic selection of individuals plants**

**Identify segregants**

**Selected isolines**

NA 4209RG
NA 4613RG
NA 5009RG

NA 3933RG
A 3302RG
NA 4413RG

Arising from W20
Performance trials

Experimental design:
- Completely randomized blocks
- 3 replications

Experimental units:
- 4 rows plots spaced 0.52m
- 5m length

Material tested:
- 20 varieties
- 7 isolines pairs
- 6 parents (checks)

Evaluation environments:
- 14 locations
  - 7 locations 2009-10
  - 7 locations 2010-11
We measured

✓ Days to R1
✓ Days to R8
✓ Plant height (at R8)
✓ Number of fruiting nodes
✓ Total pods number
✓ Pods number with 2 grains
✓ Pods number with 3 grains
✓ Thousand grain weight

And also

✓ Grain yield (kg.ha⁻¹ 13% H°)
✓ Protein content
✓ Protein yield (Kg.ha-1)
✓ Oil content
✓ Oil yield (Kg.ha-1)
✓ Fatty acid composition
No phytotoxicity symptoms were observed in any of the evaluated environments that might relate to the presence of SU in the soil.
Comparison of developmental stages between Susceptibles and Tolerant SU isolines
Tolerant isolines and their susceptible counterpart, are indistinguishable.
**Results and discussion**

**Total pods number**

Als1 — Lineal (Als1)

Variation source | Pr > F
--- | ---
Environments | 0.0001
Rep (env) | 0.0001
Genotype | 0.0001
Background | 0.0001
Gen.Back | 0.0028
Gen.Back.Env | 0.0001
Media | 35,29
CV | 12,91
R² | 0,81

**Pods with 2 grains**

Als1 — Lineal (Als1)

Variation source | Pr > F
--- | ---
Environments | 0.0001
Rep (env) | 0.0001
Genotype | 0.0001
Background | 0.0001
Gen.Back | 0.0001
Gen.Back.Env | 0.0001
Media | 9,53
CV | 32,35
R² | 0,69

**Pods with 3 grains**

Als1 — Lineal (Als1)

Variation source | Pr > F
--- | ---
Environments | 0.0001
Rep (env) | 0.0001
Genotype | 0.0001
Background | 0.0001
Gen.Back | 0.0028
Gen.Back.Env | 0.0001
Media | 25,76
CV | 15,52
R² | 0,80

**Thousand grain weight**

Als1 — Lineal (Als1)

Variation source | Pr > F
--- | ---
Environments | 0.0001
Rep (env) | 0.0001
Genotype | 0.0001
Background | 0.0001
Gen.Back | 0.0001
Gen.Back.Env | 0.0001
Media | 154.83
CV | 6.93
R² | 0.94
Total pods number and pods with 3 grains

The highest values of tolerant genotypes showed that they set a greater number of pods and grains than susceptible genotypes.

Thousand grain weight

Significant \((p<0,01)\) highest values of the susceptible isolines.

As compensatory effect of the increased number of grain per plant, they have a lower thousand grain weight.
Results and discussion

Variation source | Pr > F
--- | ---
Environments | 0.0001
Rep (env) | 0.0006
Genotype | 0.0001
Background | 0.0001
Gen.Back | 0.0418
Gen.Back.Env | 0.0004
Media | 3733.49
CV | 10.45
R² | 0.87

Correlation
Grain yield-total pods number | 0.32**
Grain yield-pods with 3 grains | 0.20**

Grain yield (Kg.ha⁻¹)

Als1 – Lineal (Als1)

Grain yield (Kg.ha⁻¹)

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Correlation
Grain yield-total pods number | 0.32**
Grain yield-pods with 3 grains | 0.20**

Grain yield (Kg.ha⁻¹)
Average yield of susceptible and tolerant isolines across 14 environments

Grain Yield (Kg.ha⁻¹)
Environments without SU residual (12)

Results and discussion
Grain yield

Higher yield of tolerant lines (173kg.ha⁻¹, 4.7%).

Highest number of pods setting per plant

[yield - total pods number (r=0.31; p<0.01)] and [yield - number of pods with 3 grains (r=0.20; p<0.01)].

Not only associated with the presence of SU residual in the soil

Yield significant differences in the environments without SU in the soil profile are not expected (cost associated with the resistance)¹.

Pleiotropic effect of A/s1 gene  

Close linkage with QTLs

SU tolerant gene is associated with increased yield

¹(Vila-Aiub et al., 2009)
Greater production of branched amino acids

Increase production - translocation of assimilates to growing points and reproductive targets

Greater AHAS activity of tolerant isolines

High yield of tolerant isolines based in largest setting pods per plant

Relationship $A/l{s}1$ gene - cytokinins activity

Cytokinins availability limits the potential seed production

1(Reese et al., 1995)
Results and discussion

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Stearic acid (%)

Results and discussion

Variation source | Pr > F
--- | ---
Environments | 0.0001
Rep (env) | 0.0001
Genotype | 0.0001
Background | 0.0001
Gen.Back | 0.0001
Gen.Back.Env | 0.0001
Media | 4.31
CV | 5.25
$R^2$ | 0.90
Grain protein content

Significantly higher \((p<0.01)\) mean values for susceptible genotypes.

For protein yield \((\text{kg.ha}^{-1})\), considered as a new variable, higher mean values were observed for tolerant isolines.

Grain Oil content

Tolerant isolines showed significantly \((p<0.01)\) greater values than their susceptible counterparts.

Fatty acid profile

Tolerant isolines showed significantly higher values \((p<0.01)\) for stearic acid content than the susceptible counterparts.
Conclusions

The *Als1* mutant gene may have a pleiotropic effect on the composition and grain yield of the evaluated isolines, rejecting the hypothesis of our work.

Cited pleiotropic effects have been positive on most of the analyzed variables, except for protein content of the grain.

The detrimental effect of the mutant gene on grain protein content, is compensated by the higher protein yield per unit area.

Tolerant isolines showed an increase in the oil content of the grain.

Tolerant genotypes had a significant higher yield than susceptible genotypes.

This higher yield of tolerant isolines is produced by an increased number of setting pods, indicating a possible effect of the gene on the production, transport or cytokinins activity.
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Dr. Carlos Sala
Agr. Eng. Rodolfo Rossi
NIDERA Soybean Breeding team
NIDERA Biotech team
Advisors
My family

Thank you for your attention