

TRANSCRIPTOMIC ANALYSIS OF FOUR SOYBEAN GENOTYPES IN RESPONSE TO WATER STRESS.

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Drought is one of the most important environmental stresses in agriculture. The ability to produce high seed yield in drought affected fields is the ultimate indicator of drought tolerance. Genetic improvement for drought adaptation is largely addressed through the conventional approach of selecting stable, high-yielding cultivars tested at various locations and years. This approach is expensive and time consuming, therefore the development of a reliable pot method for screening phenotypic responses to water stress in soybean under controlled or semi-controlled conditions, would allow for a more rapid and cheaper way of identifying genes involved in such a response.

A water stress of -0.65 MPa during 21 days was applied at the reproductive stage (R5) to eight genotypes of soybean potted plants. Total seed yield, total seed weight, 10-seed weight and total water consumption were measured and statistically analysed. When calculating the drought susceptibility index (DSI) three of the genotypes were found to be more tolerant (NA8000, PI416937 and Munasqa) and one genotype very susceptible (TJ2049) towards water deficit. Samples from leaves were collected 72 h after water holding and total RNA was purified for transcriptome analysis using 454 pyrosequencing technologies (ROCHE). A total of 1,412,783 raw reads were obtained and assembled by GS Reference Mapper software (ROCHE) into 18,219 high quality sequences. Average sequence length was 399 bp, and all unique sequences were queried against the Phytozome 7.0 Soybean database for annotation. Functional Gene Ontology annotation was performed with sequences that produced significant similarity ($x \leq 10^{-10}$) with the MapMan Database. Informatics expression analysis identified 396 genes as typical for genotype Munasqa, 846 for NA8000, 1,099 for PI416937, while 487 were identified only in the susceptible genotype TJ2049. A total of 1,157 genes were common to the three tolerant genotypes, 38 of them up-regulated. Numerous genes were found to be over expressed in response to drought in tolerant genotypes while non-induced or even repressed in the susceptible genotype. Other groups of genes showed genotype-specific changes, suggesting that different mechanisms are involved in drought response for the genotypes tested.

Transcriptomic analysis of four soybean genotypes under drought stress using 454 pyrosequencing

BiotecSojaSur: Soybean Virtual Lab in Mercosur

*E.M. Pardo, **G.R. Vellicce**, M. G. García, C.M.L. Rocha, G. Westergaard, B. Welin and A.P. Castagnaro**

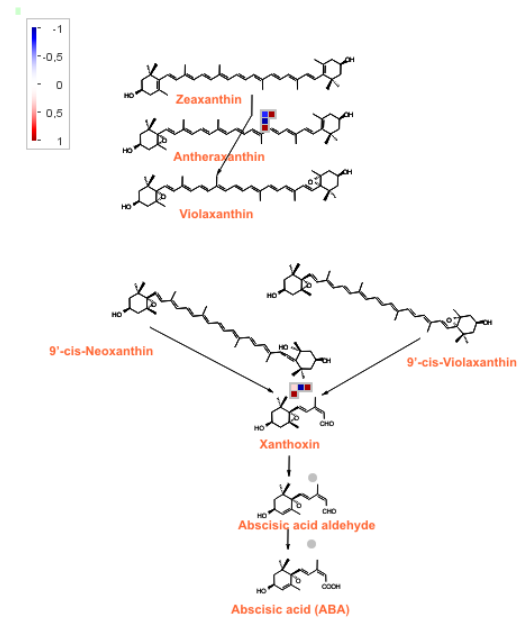
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Reliable method for drought screening



Gene detection



1. Development of method

2. Three evaluations: 2009, 2010 and 2012

3. Large scale transcriptome analysis

1^o assay

2008/2009

Developmental stage	start	finish
V7	Feb 18	Feb 28
R3	March 11	March 18
R5	March 28	April 5

Table 1. Stress duration. Beginning and end of treatment. V7: plants with seven nodes; R3: pod appearance stage; R5: pod-filling stage.

Effect of water stress on soybean seed production in different developmental stages

Treatment	total seeds		seeds weight		100 seeds weight	
control	90	A	14,29	A	19,02	A
V7+R3	84,2	A	13,65	A	18,13	A
R3	80,8	A	13,1	A	17,13	A
V7	74,8	A	12,7	A	16,95	A
V7+R5	49,4	B	8,94	B	15,92	A
R5	45,2	B	8,56	B	15,7	A

Different letters indicate statistical differences (Tukey test, $p=0.05$).

Effect of water stress on soybean seed production in different developmental stages

Treatment	total seeds		seeds weight		100 seeds weight	
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Effect of water stress on soybean seed production in different developmental stages



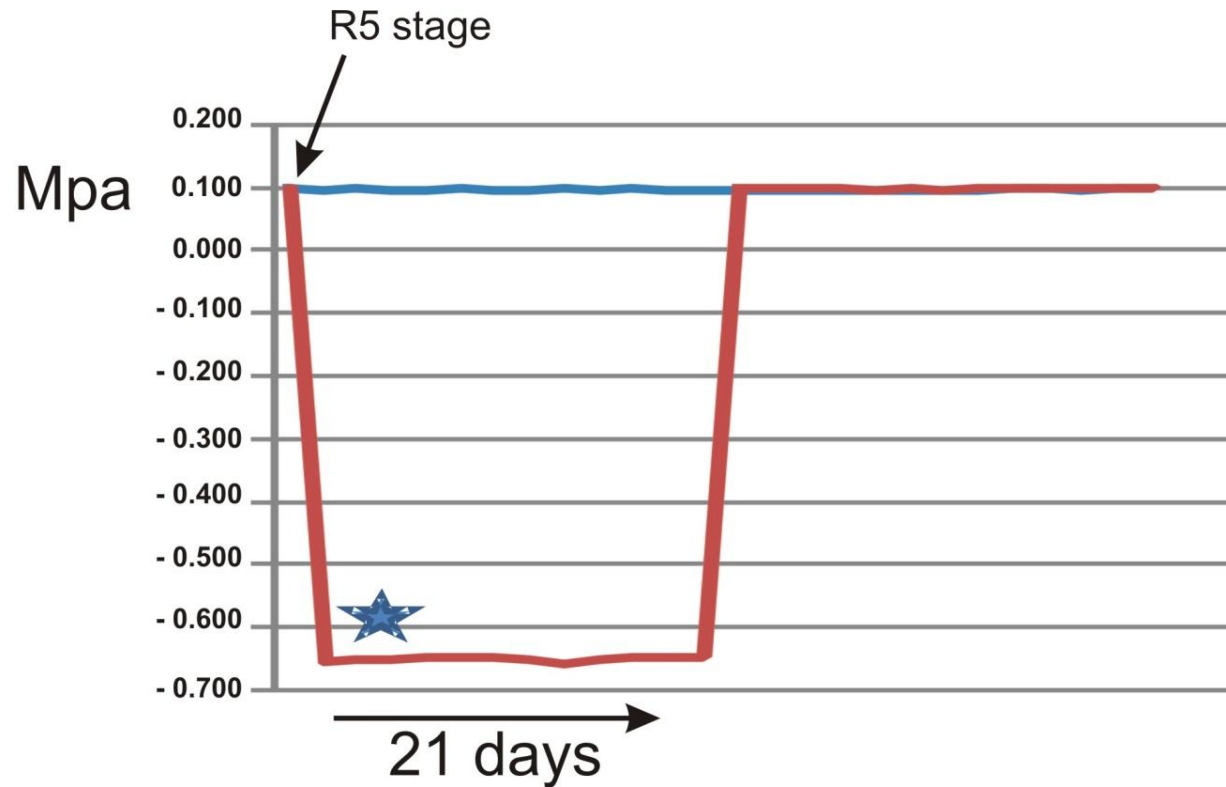
Treatment	total seeds		seeds weight		100 seeds weight	
control	90	A	14,29	A	19,02	A
V7+R3	84,2	A	13,65	A	18,13	A
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V7	74,8	A	12,7	A	16,95	A
V7+R5	49,4	B	8,94	B	15,92	A
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Genotypes evaluated

- A8000
- Munasqa
- PI416937
- BR16
- Conquista
- TJ 2049
- N7001
- PI res (no-id)

Experimental design



Started in R5

Pod Filling

Throughout 21 days

Control: -0.1 MPa – stressed: - 0.65 MPa

Tissue sampling (★)

Leaves

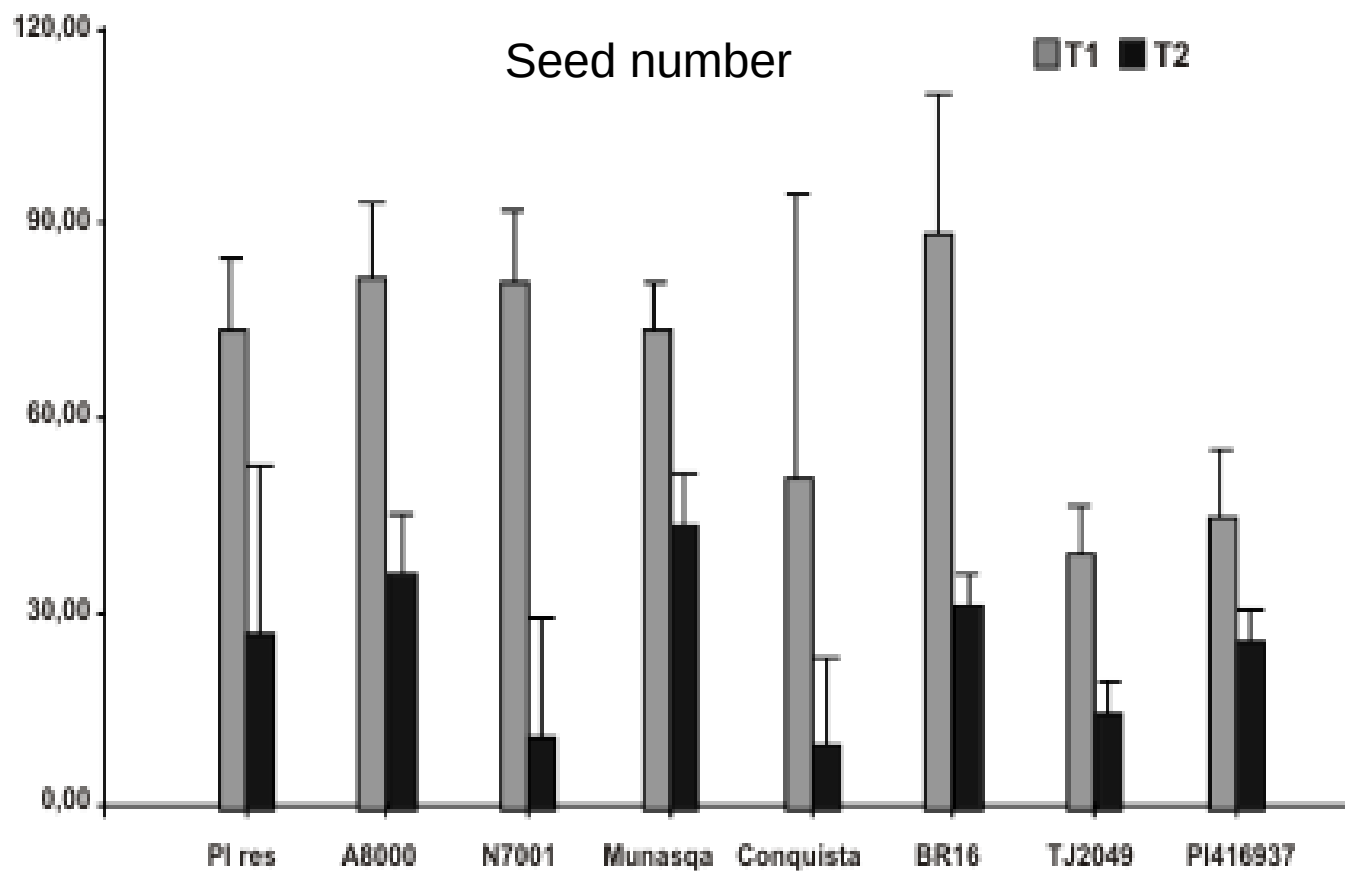


Figure 1. Total seed yield, total **seed** weight, ten **seed** weight, and total water used under well watered and **water stress** conditions at R5-R8 stages.

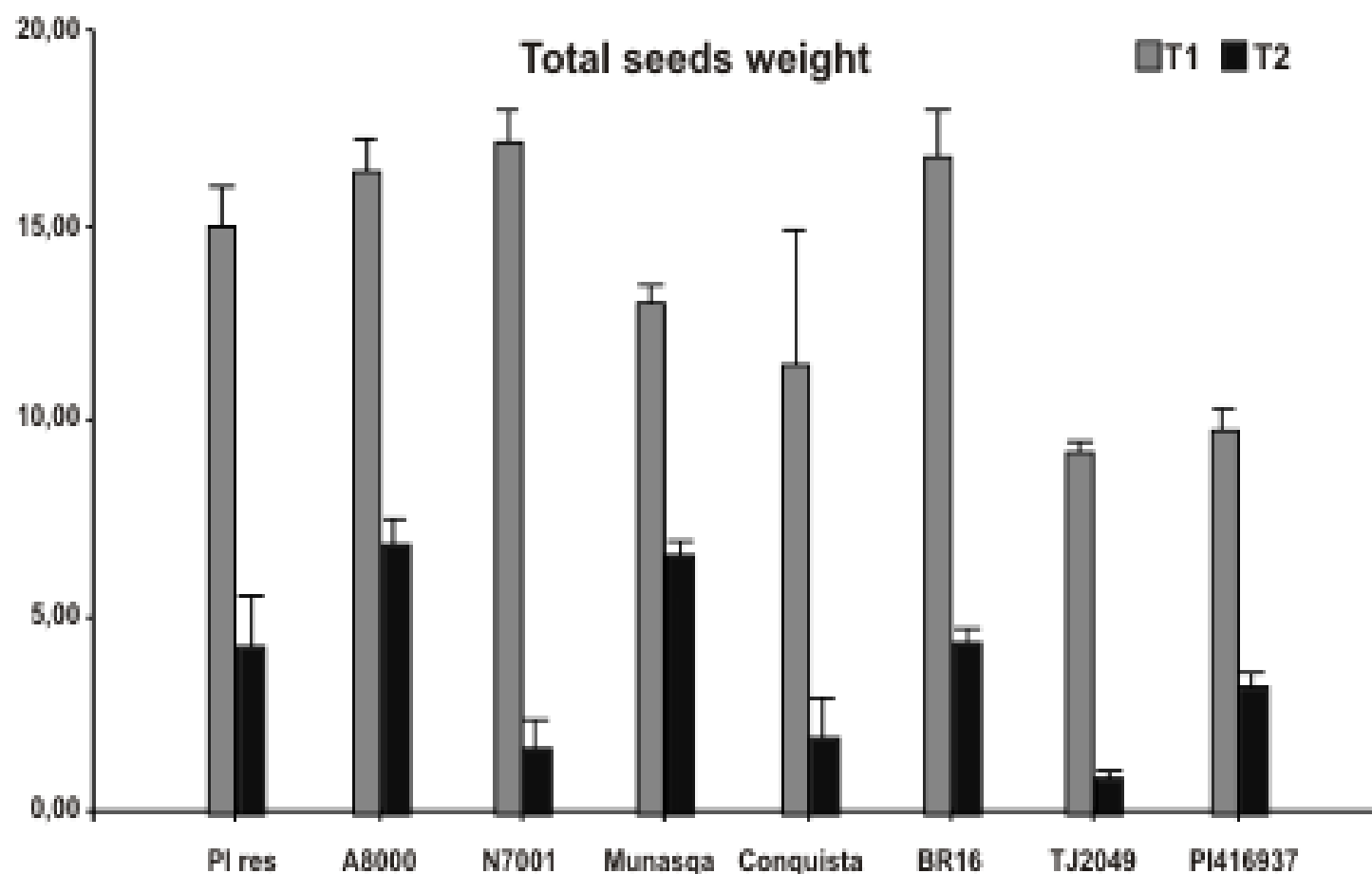


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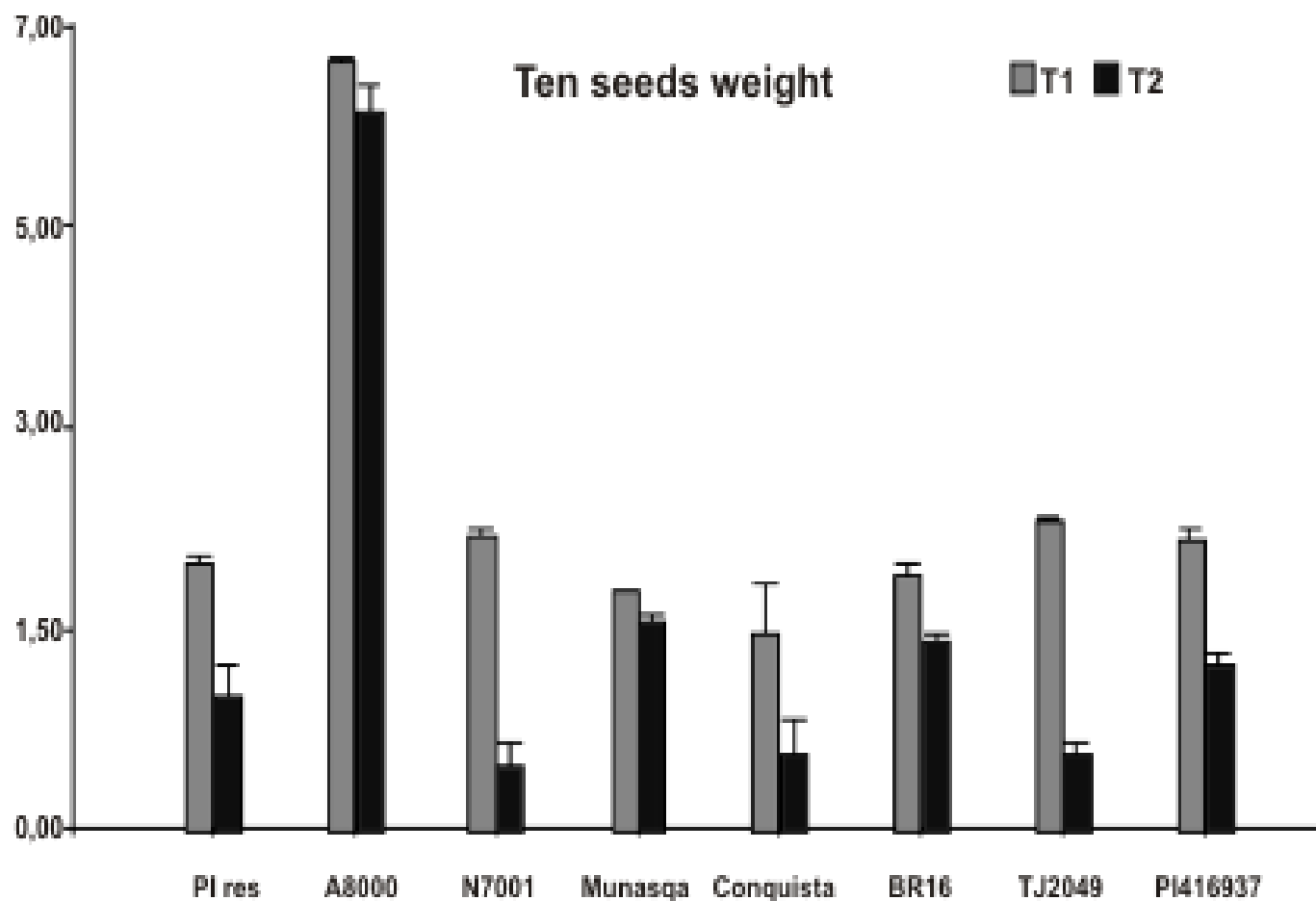


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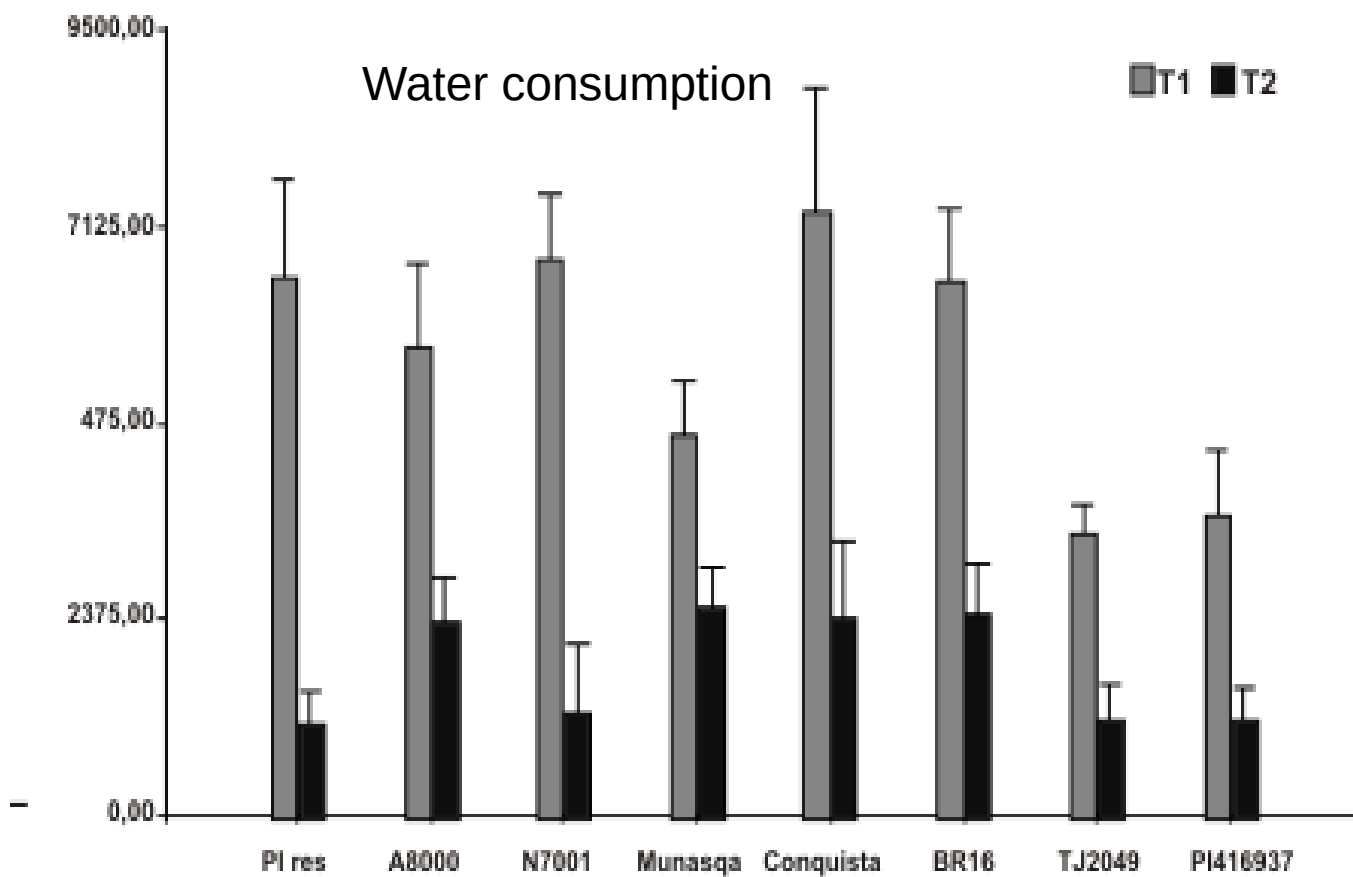


Figure 1. Total seed yield, total seed weight, ten seed weight, and total water used under well watered and water stress conditions at R5-R8 stages.

Results of greenhouse evaluation of 8 soybean genotypes under water stress

	genotype	Total seed	total seed weight	total water consumption	Total seed/total water consumption	total seed weight /total water consumption	*DSI	**% stress vs control
control	TJ2049	39.20	9.13	3267.80	0.01200	0.00279	0.920	
stress	TJ2049	14.46	0.87	1106.90	0.01306	0.00079		36.89%
control	N7001	81.20	17.10	6821.00	0.01190	0.00251	3.491	
stress	N7001	10.84	1.58	2056.80	0.00527	0.00077		13.35%
control	A8000	81.66	16.42	5221.90	0.01564	0.00314	0.682	
stress	A8000	36.00	6.87	2349.50	0.01532	0.00292		44.09%
control	MUNASQA	73.77	13.01	4645.30	0.01588	0.00280	0.376	
stress	MUNASQA	43.42	6.54	2566.10	0.01692	0.00255		58.86%
Control	CONQUISTA	81.60	18.35	8676.00	0.00941	0.00212	4.166	
stress	CONQUISTA	9.33	1.91	3575.20	0.00261	0.00053		11.43%
control	BR16	88.44	16.75	6175.90	0.01432	0.00271	1.001	
stress	BR16	30.91	4.33	2060.80	0.01500	0.00210		34.95%
control	PI res seq	73.66	14.97	6348.80	0.01160	0.00236	0.953	
stress	PI res seq	26.58	4.23	3446.40	0.00771	0.00123		36.08%
control	PI416937	44.55	9.74	3567.30	0.01249	0.00273	0.393	
stress	PI416937	25.75	3.18	1276.60	0.02017	0.00249		57.80%

*DSI (Drought susceptibility index)= 1-sem ctr/sem est/1-sm tot ctr/ sm tot est

** Yield percentages of plants under water stress vs yield of control plants.



TJ2049



NA8000RG



MUNASQA



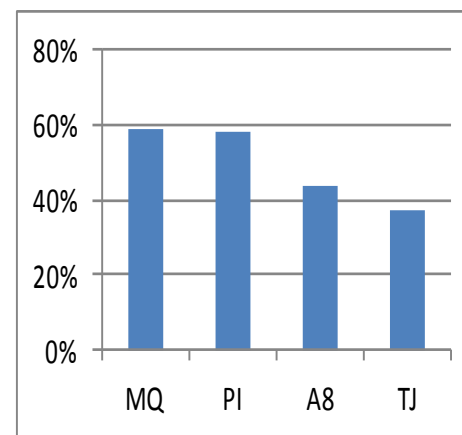
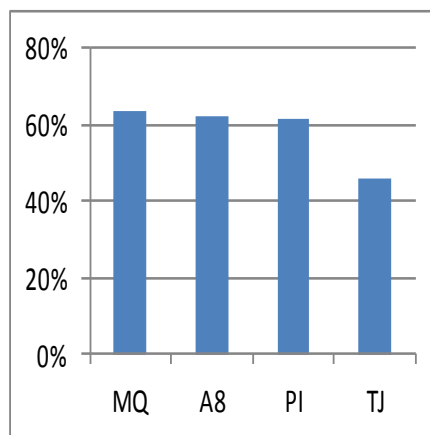
PI416937

2010

1º	MQ	0.38	59%
2º	PI	0.39	58%
3º	A8	0.68	44%
4º	TJ	0.92	37%

2012

1º	MQ	0.80	64%
2º	A8	0.84	62%
3º	PI	0.87	61%
4º	TJ	1.63	46%



Gene expression on soybean subjected to water stress

Functional Genomics

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Control Vs Stress

MUNASQA	tD	cD	tR	cR
NA8000RG	tD	cD	tR	cR
PI416937	tD	cD	tR	cR
TJ2049	tD	cD	tR	cR
Duplicates			tR	cR
Altogether	tD	cD	tR	cR

t: mapped to Transcript;
c: mapped to CDS;
D: analyzed with Inhouse
made script;
R: analyzed with edgeR.

[DELIVERABLES](#)

PROJECT DETAILS

Stats

Project Name	Soybean Comparative Transcriptomics
Number Of Runs	1 FLX Titanium
Passed Filter Wells	1,412,783
Total Bases	557,090,712bp
Average Length	399.41bp
Longest Reads Length	964
Shortest Reads Length	40

About the Project

This is a Transcriptomic run and assembly project of *Glycine max* (soja). The aim of this project is the identification of differential gene expression between genotypes in two different conditions (control and hydric stress).



Functional Genomics

Gene expression in soybean subjected to water stress

Metrics	A8 control	A8 stress	TJ CONTROL	TJ STRESS	PI CONTROL	PI STRESS	MQ CONTROL	MQ STRESS
TOTAL SEQ	110623	250325	91890	203954	185816	197848	140796	210951
TOTAL BASES	41632018	97300014	35515329	79337392	71390218	77253841	52842458	81454871
Mean (Average):	376.34	388.69	386.5	389	384.2	390.47	375.31	386.13

BIOINFORMATIC ANALYSIS

Data Base: *Glycine max* by Phytozome Version 7.0



Assembling was performed with GSMapper (ROCHE). Further comparison was based on assigned unique reads.

Relative abundance was calculated with package R, edgeR, normalization script.

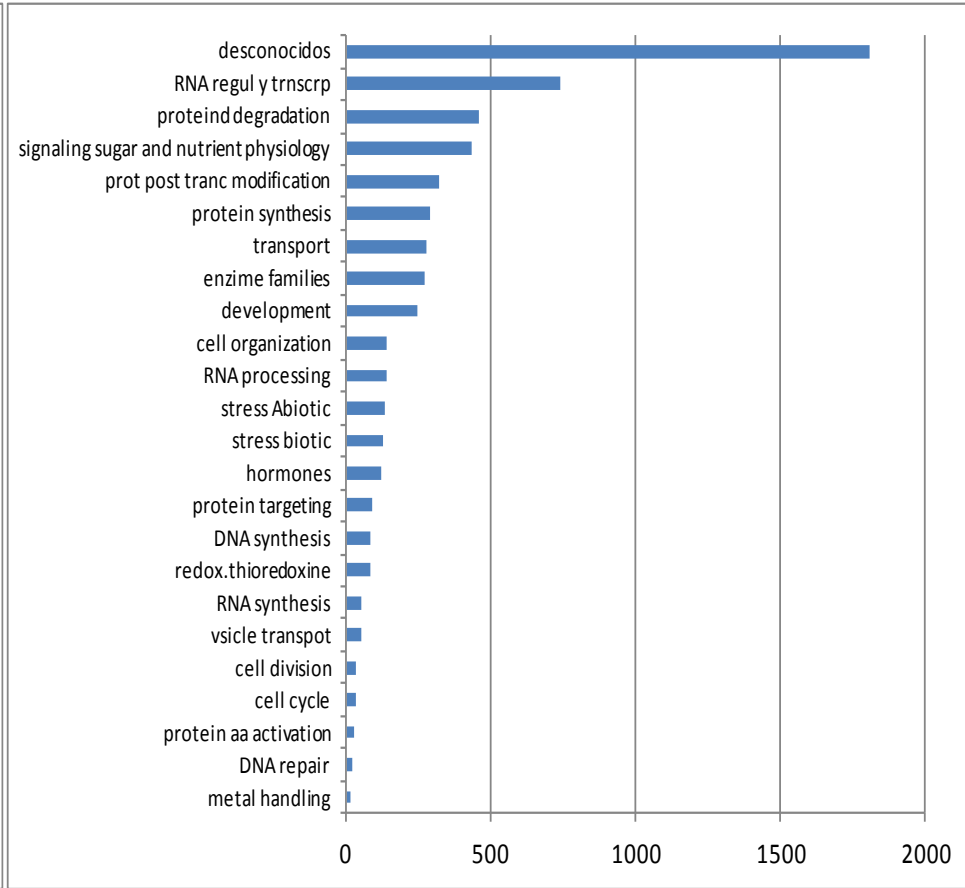
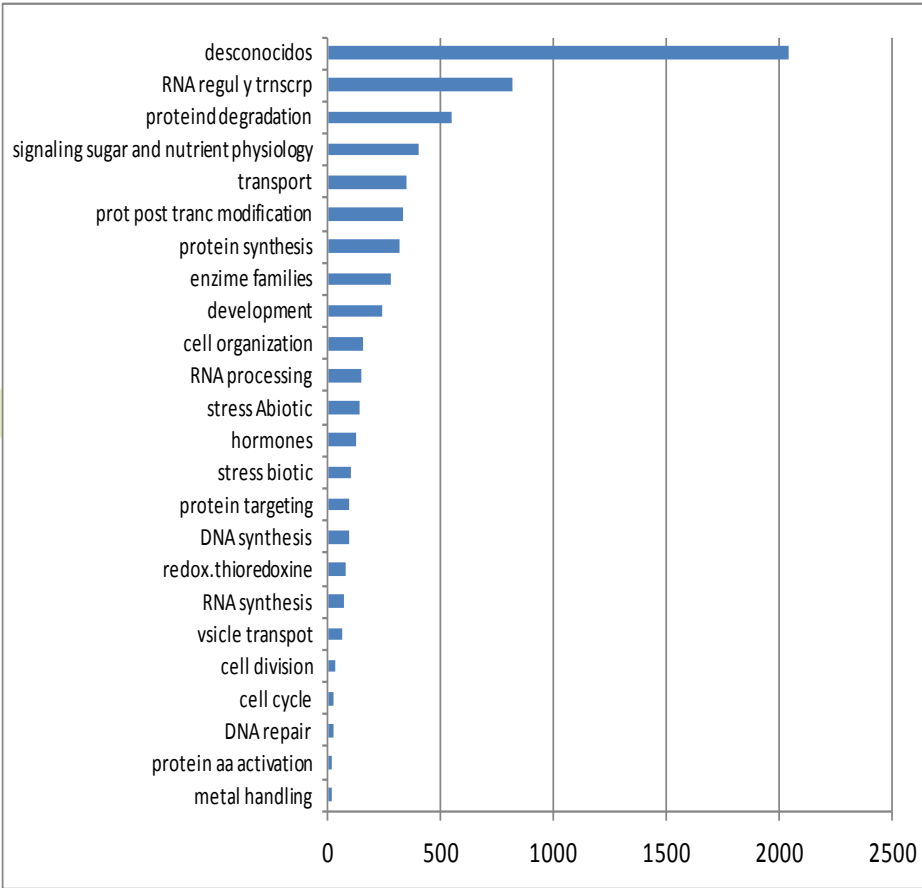
Data obtained were ordered with different criteria for a preliminary screening.

Functional Genomic

MapMan 3.0.

TJ2049

MUNASQA



Functional Genomic

MapMan 3.0.

NA8000RG

PI416937

