

Identification of candidate genes involved in the response to different abiotic stresses in potato (*Solanum tuberosum* L.)**L. Barandalla¹, A. Álvarez¹, J. I. Ruiz de Galarreta^{1/*}, E. Ritter¹***Received: 06/11/2018**Accepted: 15/11/2018**Accessible on line: December 2018***Summary**

Plants growing in natural habitats are exposed to multiple environmental stresses resulting from abiotic factors such as heat, drought, and cold, which have a significant impact on cultivated potato. We have evaluated in two *Solanum tuberosum* varieties, Soprano and Kondor, the adaptation to different abiotic stresses as heat, cold, and drought. For this purpose plants of both varieties were stressed, and when they showed symptoms, RNA extraction was carried out and a cDNA library for each sample was constructed. The objective of this study was to detect and analyse the genes involved in the responses to abiotic stresses in potato. The assay generated transcriptome sequences from both varieties, and a total of 5.579.655 reads and 8420 putative candidate genes were generated. 4.027 of the candidate genes were polymorphic and presented a different number of patterns defined by a varying number of SNPs. Many of the generated candidate genes showed differential expression, since the candidate gene was present in the stressed plant, but not in the control plant. The application of this methodology allows us to detect numerous candidate genes or specific alleles/allele combinations, which are differentially expressed in specific samples after the application of different abiotic stresses. This will be useful to identify superior alleles which can be used in Marker Assisted Selection for resistance and tolerance to abiotic stresses.

Additional Keywords: *Solanum tuberosum*, abiotic stresses, cDNA library, candidate gene, differential expression, allele.

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Identificación de genes candidatos implicados en la respuesta a diferentes estreses abióticos en papa (*Solanum tuberosum* L.)

Resumen

Las plantas que crecen en hábitats naturales están expuestas a múltiples estreses ambientales resultantes de factores abióticos como el calor, la sequía y el frío, que tienen un impacto significativo en la papa cultivada. Se ha evaluado en dos variedades de *Solanum tuberosum* L. Soprano y Kondor, la adaptación a diferentes estreses abióticos como calor, frío y sequía. Para ello, las plantas de ambas variedades fueron estresadas y cuando mostraron síntomas se llevó a cabo la extracción de ARN y se construyó una biblioteca de ADNc para cada muestra. El objetivo de este estudio fue detectar y analizar los genes involucrados en las respuestas al estrés abiótico en papa. El ensayo generó secuencias de transcriptomas de ambas variedades, con un total de 5579655 lecturas y 8420 genes candidatos posibles. 4027 de los genes candidatos fueron polimórficos y presentaron un número diferente de patrones definidos por un número variable de SNPs. Muchos de los genes candidatos generados mostraron una expresión diferencial, ya que el gen candidato estaba presente en la planta estresada, pero no en la control. La aplicación de esta metodología nos permite detectar numerosos genes candidatos o combinaciones específicas de alelos, que se expresan diferencialmente después de la aplicación de diferentes estreses abióticos. Esto será útil para identificar alelos superiores que se pueden usar en la Selección Asistida por Marcadores para la resistencia y tolerancia a estreses abióticos.

Palabras clave adicionales: *Solanum tuberosum*, estrés abiótico, librería cDNA, gen candidato, expresión diferencial, alelo.

Introduction

Plants growing in natural habitats are exposed to multiple environmental stresses resulting from abiotic and biotic factors. Adaptation and response to these stresses is highly complex and involve changes at the molecular, cellular, and physiological levels. Abiotic stress factors such as heat, drought, and cold have a significant impact on cultivated potato, affecting yield, tuber quality, and market value (Wang-Pruski and Schofield 2012). Water availability is by far the most important limiting factor in crop production. Potato plants use water relatively efficiently; however, due in part to its sparse and shallow root system, potato is considered a drought-sensitive

crop (Yuan *et al.* 2003). Climate change is expected to increase the frequency of drought events in many regions, affecting drought susceptible crops like potato (Simelton *et al.* 2012). High temperature drastically affects potato production too (Slater 1968). An important quantity of genes involved in drought and heat tolerance have been identified in the past few decades in potato (Monneveux *et al.* 2013).

The objective of this study was to detect and analyse the genes involved in the responses to abiotic stresses in two different varieties of *Solanum tuberosum* L.

Materials and methods

We have evaluated in two *Solanum tuberosum* varieties the adaptation to different abiotic stresses (heat, cold, drought). Plants of both varieties were stressed for different abiotic stresses, and when they showed symptoms of stress, RNA extraction was carried out and a cDNA library for each sample was constructed, including an unstressed control.

Eight cDNA sub libraries were constructed, one for each sample. Four cv. Kondor plants and four cv. Soprano plants were used, both varieties of *Solanum tuberosum*. Soprano and Kondor varieties were chosen because these two varieties had been used in previous works and tested under different abiotic stresses. Soprano showed good behaviour after the application of abiotic stresses, not so Kondor, who showed a different behaviour and his yield was negatively affected under the application of stress. One plant of each variety was stressed to cold, another to heat and another to drought. At the same time, the fourth plant was used as a control. The plants were grown in the greenhouse at 20°C, in pots with 50% peat and 50% perlite. When the plants showed sufficient foliage, the stresses were applied. Plants that were stressed to drought were routinely left to irrigate while the control plant continued to be irrigated on a regular basis. When the plant showed signs of stress (withered leaves, yellowing) leaves were taken from both the stressed plant and the control plant, and were frozen at -80.

The plants stressed to drought were a total of 20 days without water. On the other hand, plants that were stressed at high temperatures were in a phytotron at 40°C for 10 days, with 16 hours of light and 8

hours of darkness. The plants were watered every day. When the plants showed wilt symptoms, leaves were picked and frozen at -80°C.

Finally, plants were stressed at low temperatures in a phytotron at 4°C for 10 days, with 16 hours of light and 8 hours of darkness. The plants were watered when necessary. When the plants showed wilt symptoms, leaves were picked and frozen at -80°C.

Following the protocol of Bachem *et al.* (1998), total RNA extraction was performed and the messenger RNAs were captured in each sample. The single-stranded and double-stranded cDNA was synthesized according to Sambrook *et al.* (1989). The double-stranded cDNA (ds-cDNA) was digested with TaqI and AseI (NEB Biolabs Inc., New Brunswick, NE, USA) and subsequently the ligation of the AseI and TaqI adapters with T4 DNA ligase (Invitrogen Inc, Barcelona, Spain) was performed. The primers that contained sequences complementary to the adapters and the indexes for each sample (Zhengjun *et al.* 2009) were used for PCR amplification as described by Bachem *et al.* (1998). After size selection and purification, the amplicons from all the samples were pooled in approximately equal concentrations and the library was sent for amplicon sequencing using the "Illumina MySeq" technology. The obtained sequences were processed using the RADLIB Software, developed in the Neiker institute.

Results and discussion

The assay generated transcriptome sequences from two potato varieties Soprano and Kondor. A total of 5.579.655 reads were obtained. The read number was different between libraries (samples). A total of 8420 putative candidate genes was generated from the eight cDNA

libraries (one per plant) constructed by pooling messenger ribonucleic acid (mRNA) from heat-, cold-, and drought-stressed potato leaves. Some of the putative candidate genes did not have sufficient reads and were discarded. We continued working with 6023 of them. A total of 4.865 of the putative candidate genes had annotations related with abiotic stresses such as cold, heat, drought and salt tolerance, amino acid metabolism degradation proteins, cell wall degradation proteins, cell division, cell

organization, DNA repair, DNA synthesis/chromatin structure, lipid metabolism, lipid degradation, protein degradation, Calvin cycle, etc. 4.027 of the candidate genes were polymorphic and presented a different number of patterns (from 1 to 5), defined by a varying number of SNPs (from 1 to 32). Many of the generated candidate genes showed differential expression, since the candidate gene was present in the stressed plant, but not in the control plant (Table 1).

Table 1. List of some of the candidate genes (CG) that showed differential expression in the sequenced library. These CGs are present in the stressed plants, but not in the control plants. Each candidate gene is defined by a different number of alleles and SNPs.

CG	Reads per CG	Alleles	SNP s	Read							
				SCT	SD	SC	SH	KCT	KD	KC	KH
CG1032	217	2	1	0	0	17	0	0	147	23	30
CG1879	4736	3	2	1458	1700	891	1	0	3	342	341
CG1937	2757	4	3	232	1314	502	0	0	3	447	259
CG2073	784	3	3	131	256	221	1	0	0	140	35
CG3866	3421	3	4	0	301	3	1	37	173	2	2904
CG4867	271	2	3	0	0	86	0	36	0	54	95
CG4963	177	2	1	1	0	9	0	0	0	167	0
CG5422	178	2	1	0	24	36	0	73	0	40	5
CG5509	124	3	2	0	46	54	0	0	0	10	14
CG5680	257	2	1	0	15	1	0	0	0	24	217
CG5695	135	3	2	27	56	28	0	0	0	10	14
CG5725	152	4	3	77	16	14	0	0	0	15	30
CG5842	88	3	2	0	60	23	0	0	0	5	0
CG5921	130	2	2	0	21	105	1	0	0	3	0
CG6011	284	2	1	0	85	3	0	0	0	0	196
CG6015	291	2	4	0	18	0	0	0	0	0	273
CG6582	588	2	3	0	5	26	0	20	523	8	6
CG7044	162	2	1	0	9	0	0	0	122	31	0
CG7326	94	3	13	0	3	75	0	1	14	1	0
CG7402	728	2	8	5	32	2	0	0	2	2	685
CG7553	136	2	1	0	0	92	0	0	0	44	0
CG7670	140	3	2	64	21	2	0	0	0	32	21
CG7731	170	3	2	0	41	75	0	0	0	25	29
CG8163	173	2	1	0	14	1	0	152	0	6	0
CG8277	105	3	2	47	15	12	0	0	0	5	26
CG5296	348	3	2	0	28	202	0	0	0	45	73
CG6331	85	2	1	0	27	58	0	0	0	0	0

SCT: Soprano Control; SD: Soprano Drought; SC: Soprano Cold; SH: Soprano Heat. KCT: Kondor Control; KD: Kondor Drought; KC: Kondor Cold; KH: Kondor Heat

Using as reference the most interesting genes with marked differential expression, we will design primers for an allele specific primer assay in order to identify specific alleles for different abiotic stresses. Here, we explored in depth the transcriptome of potato in response to abiotic factors using data from our cDNA library and comparing the results with other cDNA library data.

The application of this methodology and methods of analysis allows us to detect numerous candidate genes or specific alleles/allele combinations, which are differentially expressed in specific

samples after the application of different abiotic stresses.

Based on the annotations we observed that many CG have a relevant biological meaning that explains the observed expression patterns. On the other hand, allelic variability has been determined in the CG. In the future we will perform association mapping of CGs in a set of germplasm with varying degree of resistance/tolerance to abiotic stresses. This will be useful to identify superior alleles which can be used in Marker Assisted Selection for resistance and tolerance to abiotic stresses.

Patterns of the CG5509 candidate gene defined by two SNPs in the position 21 (A/G) and 152 (C/T), respectively, defining three different alleles (Figure 1).

Candidate gene: CG5509

PATTERN 1:

TAATCCCTTCCATACCATATATGTCATATATTTGTCCTTTTCGTCTCATCCGATAAAATTTTCATAAGCTTCACATA
TTCGTTGGAAATTCTCTGTTGCAATTCCTTCATCTCAAGAGCTTGATATTTATCAGGGTGATAGATTTGAGCC
CACTGACGATAAGCTTTTCG

PATTERN 2:

TAATCCCTTCCATACCATATATGTCATATATTTGTCCTTTTCGTCTCATCCGATAAAATTTTCATAAGCTTCACATA
TTCGTTGGAAATTCTCTGTTGCAATTCCTTCATCTCAAGAGCTTGATATTTATCAGGGTGATAGATTTGAGCC
CATTGACGATAAGCTTTTCG

PATTERN 3:

TAATCCCTTCCATACCATATGTCATATATTTGTCCTTTTCGTCTCATCCGATAAAATTTTCATAAGCTTCACATA
TTCGTTGGAAATTCTCTGTTGCAATTCCTTCATCTCAAGAGCTTGATATTTATCAGGGTGATAGATTTGAGCC
CATTGACGATAAGCTTTTCG

Figure 1. Means for stem diameter at senescence, survival rate and plant height in ten varieties of native potato planted under aeroponic system conditions. Letters denote differences (LSD $\alpha = 0.05$).

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Conflict of interest

The authors declare no conflict of interest.

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