Mexican Journal of Biotechnology 2018, 3(2):1-15

Journal homepage:www.mexjbiotechnol.com
ISSN:2448-6590

ORIGINAL RESEARCH

Differential expression of genes in response to salinity stress in tree tomato (*Solanum betaceum*)

Expresión diferencial de genes como respuesta a estrés salino en tomate de árbol (*Solanum betaceum*)

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Article history:
Received: 4 December 2017 / Received in revised form: 5 February 2018 / Accepted: 14 February 2018 / Published online: 1 April 2018.
https://doi.org/10.29267/mxjb.2018.3.2.1

ABSTRACT

The Andean “tree tomato” (*Solanum betaceum*) is an exotic fruit crop endemic to the high Andes, but principally cultivated in Colombia, Peru and Ecuador. The species displays broad agro-ecological adaptability and has proven resilient to different marginality factors, including high soil-salinity. This study presents a preliminary exploration of the genetic mechanisms underlying salinity tolerance in *S. betaceum*. To this end, we selected two *S. betaceum* genotypes contrasting in their ability to tolerate high salinity *in vitro*, and used differential display analysis to compare overall differences in gene expression between salinity-stressed and unstressed (control) plants in both genotypes. Overall, 171 differentially expressed transcripts (DETs) were identified; 30 of which showed homology with candidate genes associated with abiotic stress tolerance in different species. These were ascribed putative roles in stress-response, photosynthesis, cellular metabolism and cell wall metabolism. Several identified DETs (22 in total) also showed homology to proteins of unknown function. These sequences warrant further research for potentially novel abiotic stress tolerance mechanisms. Despite its inherent limitations, differential display analysis allowed us to identify and validate (via RT-qPCR) 3 salinity-stress induced DETs. Prospectively, expanding our analyses via the validation of additional DETs would likely contribute to the identification of genes which can be used as proxies for a better understanding of the regulatory, metabolic and physiological mechanisms used by *S. betaceum* to respond and adapt to salinity stress.

Keywords: Andean Highlands, Differential Display, Gene Expression Analysis, Orphan Crops, Salinity Tolerance, *Solanum betaceum*.

RESUMEN

El tomate de árbol (*Solanum betaceum*) es un cultivo frutal endémico de los Andes, producido principalmente en Colombia, Perú y Ecuador. Esta especie se ha adaptado a un rango amplio de condiciones agroecológicas y ha demostrado ser resistente a diferentes factores de marginalidad, incluyendo suelos salinos. Se presenta una exploración preliminar de los mecanismos genéticos que confieren tolerancia a la salinidad en *S. betaceum*. Se seleccionaron dos genotipos de *S. betaceum* que contrastan en su capacidad de tolerar alta salinidad in vitro. Se llevó a cabo un análisis de expresión diferencial para comparar los perfiles de expresión genética entre plantas expuestas a estrés salino y sin estrés (control) en ambos genotipos. Se identificaron 171 transcritos expresados diferencialmente (DETs); de estos, 30 mostraron homología con genes candidatos asociados a tolerancia a estrés salino en distintas especies. A éstos se les atribuyó roles putativos en respuesta a estrés, fotosíntesis, metabolismo celular y metabolismo de la pared celular. 22 DETs mostraron homología con proteínas sin función identificada. Estas secuencias identificadas abren posibilidades nuevas de investigación en busca de mecanismos que intervienen en la tolerancia al estrés salino en el tomate de árbol. A pesar de sus inherentes limitaciones, el análisis por despliegue diferencial permitió identificar y validar a través de RT-qPCR 3 DETs que se inducen en condiciones de estrés salino. El expandir nuestros análisis mediante la validación de DETs adicionales contribuiría a la identificación de genes que pueden usarse como base para un mejor entendimiento de los mecanismos regulatorios, metabólicos y fisiológicos usados por *S. betaceum* para responder a este tipo de estrés abiótico.

Palabras clave: Análisis de expresión genética, andino, cultivos huérfanos, despliegue diferencial, *Solanum betaceum*, tolerancia a la salinidad.

1. INTRODUCTION

The high Andes, with its broad biodiversity, is a strategic region for the search of genetic traits conferring resilience to abiotic stress. Agriculture in the region evolved under a varying range of agro-climatic niches, including areas where drought, frost and soil salinity exert extreme selective pressure (Jacobsen *et al.*, 2003; Luebert & Weigend, 2014). For decades, Andean agricultural biodiversity had been marginalized (Bermejo, 1994), but recent efforts are underway to preserve and promote the sustainable utilization of neglected species with superior agronomic resilience (Jacobsen *et al.*, 2003). A landmark example is quinoa (*Chenopodium quinoa*), which has gained commercial and scientific relevance owing to its nutritional quality and demonstrated ability to grow under drought and saline stress (Fuentes *et al*., 2009).

Prospectively, Andean agricultural biodiversity harbors novel abiotic stress tolerance mechanisms useful for plant breeding. The Andean "tree tomato" (*Solanum betaceum*) is a promising species matching these characteristics (Jaramillo *et al.*, 2014). *S. betaceum* is an exotic fruit crop endemic to Bolivia and northern Argentina, but principally cultivated in Colombia, Peru and Ecuador (Prohens & Nuez, 2000). The

crop has also been introduced in New Zealand, where its cultivation is directed primarily towards the supply of specialty markets in industrialized nations (Eagles *et al*., 1994). S. betaceum displays broad agro-ecological adaptability and is cultivated in humid and arid terrains (throughout the Andean corridor) at altitudes ranging from 1800 to 3000 m (Prohens & Nuez, 2000). Its fruits are commercially valued for their unique tropical flavor and superior nutritional quality. These have a low caloric content and are rich in micronutrients and bioactive compounds; including vitamins, minerals, anthocyanins, carotenoids and flavonoids (Mertz *et al*., 2009; Osorio *et al*., 2012). In addition to these qualities, the species appears resilient to high-salinity stress. S. betaceum is successfully cultivated on marginal soils with high pyroclastic contents (Feicán *et al*., 1999) and recent in-vitro studies demonstrate the plant's ability to sustain growth under high NaCl concentrations (Jaramillo *et al.*, 2014). As salinity stress gains relevance in global agriculture (Golldack, 2014), *S. betaceum* represents an attractive resource to study genetic mechanisms underlying fruit crop adaptability to marginal soils.

The aim of this research was to perform a preliminary screening of potential molecular traits underlying salt tolerance in *S. betaceum*. To this end, differential display analysis was used to identify genes with contrasting expression between salinity-stressed and unstressed plants of *S. betaceum*. Several salt-tolerance related genes were identified and their expression appeared to be genotype-dependent. *S. betaceum* demonstrated promising genetic characteristics regarding it's tolerance to salt stress; these need to be further explored to understand the adaptive mechanisms of Andean crops to marginal soils.

2. MATERIALS AND METHODS

2.1. Plant Material and Total RNA Extraction

S. betaceum plants from different localities of the Ecuadorian highlands were evaluated *in vitro* for their tolerance to salinity stress (Jaramillo *et al.*, 2014). From these assays, we found differences in salinity tolerance between plants originating from different locations of the country. The greatest salinity tolerance was found for individuals coming from the locality of Chaltura, in the province of Imbabura. The lowest tolerance was found in plants coming from the locality of Quero, in the province of Tungurahua (Jaramillo *et al.*, 2014). In these assays, salinity tolerance was measured as the percentage of plantlets that survived when exposed to different salt concentrations *in vitro*. For the differential display analysis, samples of seedlings treated with 100mM NaCl were collected after different times of exposure to salinity stress: 3, 9, 12, 24, and 48 hours. The salt concentration (100 mM NaCl) used corresponds to the maximum salt concentration at which *S. betaceum* could grow *in vitro* in the assays described by Jaramillo *et al.* (2014). Total RNA was isolated from leaf samples using Trizol Reagent (Invitrogen) following the instructions of the manufacturer. RNA concentration was measured by spectrophotometry using a Nanodrop 2000 (Thermo Scientific) and the quality and integrity of RNA samples were visually evaluated by electrophoresis in a 3% agarose gel.

2.2. Differential mRNA Display

Differential display assays were performed using the RNAimage Kit (GenHunter Corporation, Nashville, TN). Reverse transcription (RT) reactions contained 5mM KCl, 10mM Tris-HCl (pH 8.3), 4mM $MgCl_2$, 0.5µM dNTP Mix, 0.2µM anchor primer (H-T11C or H-T11A; GenHunter Corporation, Nashville, TN), 200ng total RNA and 20U SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA). Reactions were carried out at 37°C for 60 min, followed by 15 min incubation at 70°C. Subsequently, first-strand cDNA synthesis was performed using 10 different primer combinations (Table 1). The PCR mixture contained 40ng cDNA, 0.5µM dNTP Mix, 1.5µM $MgCl_2$, 5mM KCl, 10mM Tris-HCl (pH 8.3), 5U Taq polymerase (Invitrogen, Carlsbad, CA) and 0.2µM anchor primer (*same as those used for reverse transcription*). Amplification was performed using a 40-cycle PCR reaction (30 s at 94°C, 2 min at 40°C, 30 s at 72°C, with a final extension of 5 min at 72°C). PCR products were then electrophoresed on 6% (w/v) denaturing polyacrylamide gels at 85 W for 3.5 h using a Bio-RAD GT (38 cm x 50 cm) Sequencing Cell (Bio-RAD, Hercules, CA). Gel band patterns were visualized after silver staining following the protocol described by Benbouza *et al.* (2006) The size of the bands was estimated using a 10bp DNA ladder (Life Technologies, Carlsbad, California). Differentially expressed transcripts (DETs) were selected by comparing band patterns between salinity stressed and unstressed samples.

Table 1. Primer combinations used for differential display analysis[a]

Combination Identity	Forward Sequence	Reverse Sequence
H-T11A/ H-AP1	5'-AAGCTTTTTTTTTTTA-3'	5'- AAGCTTGATTGCC- 3'
H-T11A/ H-AP2	5'-AAGCTTTTTTTTTTTA-3'	5'- AAGCTTCGACTGT- 3'
H-T11A/ H-AP6	5'-AAGCTTTTTTTTTTTA-3'	5'- AAGCTTGCACCAT- 3'
H-T11A/ H-AP7	5'-AAGCTTTTTTTTTTTA-3'	5'- AAGCTTAACGAGG- 3'
H-T11A/ H-AP11	5'-AAGCTTTTTTTTTTTA-3'	5'- AAGCTTCGGGTAA- 3'
H-T11C/ H-AP3	5'-AAGCTTTTTTTTTTTC-3'	5'- AAGCTTTGGTCAG- 3'
H-T11C/ H-AP4	5'-AAGCTTTTTTTTTTTC-3'	5'- AAGCTTCTCAACG- 3'
H-T11C/ H-AP7	5'-AAGCTTTTTTTTTTTC-3'	5'- AAGCTTAACGAGG- 3'
H-T11C/ H-AP11	5'-AAGCTTTTTTTTTTTC-3'	5'- AAGCTTCGGGTAA- 3'
H-T11C/ H-AP12	5'-AAGCTTTTTTTTTTTC-3'	5'- AAGCTTGAGTGCT- 3'

[a] *The sequences of the primers were derived from the RNAimage Kit (GenHunter Corporation, Nashville, Tennessee).*

2.3. Isolation, Sequencing and Functional Annotation of Differentially Expressed Transcripts

All bands showing a differential pattern between stressed and unstressed samples were excised from the gel, eluted in 50µL TE buffer and incubated at 37°C overnight. Purified bands were re-amplified using the respective primer combination utilized for selective amplification. PCR conditions for the re-amplification of DETs were as described earlier. Re-amplified products of great quality were sequenced at Functional Biosciences (Madison, WI). The nucleotide sequence and deduced amino acid sequence of DETs was compared with cDNA, CDS, and protein sequences from diverse

databases by means of the basic local alignment search tool (BLAST) (National Centre for Biotechnology Information, http://www.ncbi.nlm.nih.gov/).

2.4. RT-qPCR Analyses

Expression patterns for a subset of DETs were validated by EvaGreen®-based quantitative RT-PCR (RT-qPCR) using gene-specific primers. All primers were designed using the Primer3 software package based on the nucleotide sequence of selected DETS (Rozen & Skaletsky, 2000) (Table 2). Selected DETs were chosen based on their degree of homology with genes previously identified to be related with abiotic stress response in plants. RT-qPCR reactions were prepared in a final volume of 10 µl containing 1X SsoFast™ EvaGreen® SuperMix (Bio-Rad, Hercules, CA), 0.3µM of each primer and 20ng cDNA. Melting curve analyses were performed with the following temperature program: 2 min at 60 °C, followed by an initial denaturation at 95 °C for 10 min, 40 cycles of 15 s at 95 °C and 1 min at *Tm* for annealing and elongation. The relative expression level of mRNAs was normalized to that of internal control genes (*i.e., RPL8 and TIP41*) by using the 2-ΔCt cycle threshold method (Table 3). Primers for internal control genes were designed based on sequences from related species (*i.e.* tomato and potato).

Table 2. Primers designed for the amplification salinity-stress response candidate genes in *S. betaceum*.

DET	Gene	Length	Forward Sequence (5'-3')	Reverse Sequence (5'-3')
DET 1	Arabinogalactan-Like Protein	100	CCACATTGGCTCCCTTTTTA	TAGAGGAAAGCGCAAGTCCA
DET 2	Cinnamic Acid 4-Hidroxylase	120	CCCATTGTGAGGAAACCAAG	GAAGGCCAGCAGTTTAGCAC
DET 3	Methyl Pectin Esterase	121	TTGGGGTTGGAATTGTGTTT	TCCGGCTTTTCACTTGAGAT
DET 21	Oxygen Evolving Complex	152	CCCTGATATGGGAGCAACGG	AAAGGGGTCACAGCAAGGTT

Table 3. Primers designed for the amplification of endogenous controls.

ID	Length	Forward Sequence (5'-3')	Reverse Sequence (5'-3')
ACT	107	AGGCAGGATTTGCTGGTGATGATGCT	ATACGCATCCTTCTGTCCCATTCCGA
αTUB	163	AGCTCATTAGCGGCAAAGAA	AGTACCCCCACCAACAGCA
TIP41	235	ATGGAGTTTTTGAGTCTTCTGC	GCTGCGTTTCTGGCTTAGG
RPL8	184	CCGAAGGAGCTGTTGTTTGTA	ACCTGACCAATCATAGCACGA
UBI	119	TCGTAAGGAGTGCCCTAATGCTGA	CAATCGCCTCCAGCCTTGTTGTAA

3. RESULTS

3.1. Identification of Differentially Expressed Transcripts

Band pattern comparison between salinity stressed and unstressed samples yielded a total of 171 differentially expressed transcripts (DETs) with a size range of 100 to 600bp. From these, 99 were re-amplified and sequenced; 36 DETs presented no homology with previously described sequences, and the remaining 63 DETs could be categorized into 3 groups: ribosomal related genes (11 DETs), cDNAs with undefined function (22 DETs) and coding sequences (30 DETs, 21 unique protein sequences). The latter could be further classified into five groups with respect to their biological activity (*i.e.* cell wall related, metabolism, stress response, photosynthesis and regulation of gene expression). A detail survey of these 30 genes is presented in Table 4.

From the set of DETs with homology to coding sequences, eight genes were selected for further validation via RT-qPCR; these genes are readily identified by an asterisk on Table 4. Selected genes were chosen on the basis of previous reports associating them with salinity stress tolerance in other species (Bell-Lellong *et al.*, 1997; Kosova *et al.*, 2013; Machuka *et al.*, 1999; Micheli *et al.*, 2001; Thompson *et al.*, 2000; Zhang *et al.*, 1999). The selected DETs showed homology with secondary cell wall (DET #1, DET #2, DET #3), photosynthesis (DET #6, DET #12, DET #19) and stress response-related (DET #5) genes.

Table 4. Specific information for the 30 TDFs related with coding proteins.

DET	GenBank Accession	Homologous Sequence	E-value	% Homology
DET #1*	NM001247514.1	Arabinogalactan protein (AGP-1), *Solanum lycopersicum*	4e-73	95%
DET #2*	AF212318.1	Cinnamic acid 4-hydroxylase (C4H), *Capsicum annum*	1e-22	78%
DET #3*	NM001288040.1	Pectin Methyl Esterase (Pest2), *Solanum tuberosum*	4e-24	76%
DET #4	NM001247259.2	Tyrosine- and lysine-rich protein (TLRP), *Solanum lycpersicum*	7e-33	80%
DET #5*	XM006340829.1	Pathogenesis-related protein STH-2-like, *Solanum tuberosum*	6e-24	89%
DET #6*	HM013964.1	Zeaxanthin epoxidase (ZEP)), *Solanum tuberosum*	1e-38	79%
DET #7	XM006352075.1	Translation factor GUF1 homolog, *Solanum tuberosum*	8e-69	94%
DET #8	00075597.1	trnH-psbA (Fotosistema II), *Oenothera magellanica*	3e-8	85%

DET #9	XM006362445.1	Photosystem I reaction center subunit psaK, *Solaum tuberosum*	1e-100	95%
DET #10	JN559757.1	ndhK inside photosystem II, *Nicotiana tometonsiformis*	7e-93	93%
DET #11	XM006350225.1	Photosystem I reaction center subunit IV B, Solanum tuberosum	3e-69	93%
DET #12	XM004233367.1	33kDa precursor protein of oxygen-evolving complex (PSBO), *Solanum lycopersicum*	3e-62	85%
DET #13	XM010317242.1	ATP synthase subunit, *Solanum lycopersicum*	2e-6	78%
DET #14	NM001308943.1	Ribulose-1,5-bisphosphate carboxylase, small subunit precursor, transcript variant 2, *Solanum lycopersicum*	1e-7	70%
DET #15	AB762697.1	ATP synthase subunit 1, *Solanum aethiopicum*	0	99%
DET #17	EF438866.1	Maturase K, *Nolana paradoxa*	2e-54	84%
DET #18	XM006342391.1	Zinc finger protein CONSTANS, *Solanum lycopersicum*	1e-81	92%
DET #19*	DO228333.1	ADP-ribosylation factor, *Solanum tuberosum*	3e-12	90%
DET #20	DQ226996.1	26S proteasome subunit alpha 4, *Nicotiana benthamiana*	9e-14	76%
DET #21*	NM001309365.1	Oxygen-evolving enhancer protein 1 (OEE1), *Solanum lycopersicum*	1e-68	88%
DET #22	XM006354802.1	Serine/threonine-protein kinase, *Solanum tuberosum*	5e-11	98%
DET #23	XM006363625.1	Aluminum Sensitive Protein, *Solanum tuberosum*	4e-23	79%
DET #24	XM006356172.1	Proline--tRNA ligase, *Solanum tuberosum*	2e-11	74%
DET #25	XM004242296.1	Nuclease HARBI1-like, *Solanum lycopersicum*	1e-14	84%
DET #26	XM010320534.1	Heparanase-like protein, *Solanum lycopersicum*	2e-4	92%

DET #27	XM004233150.2	Photosystem I reaction center subunit III, *Solanum lycopersicum*	1e-9	89%
DET #28	XM006339758.1	Casein kinase II, *Solanum tuberosum*	2e-5	81%
DET #29	XM006357847.1	Putative glycerol-3-phosphate, *Solanum tuberosum*	4e-17	87%
DET #30	KP962981.1	NADH dehydrogenase subunit 4, *Monsonia emarginata*	1e-29	94%

* Indicates DET selected for RT-qPCR validation

3.2. RT-qPCR Validation

The expression patterns of eight selected genes (Table 4; marked with asterisks) were validated against endogenous controls displaying constant expression under stressed and unstressed conditions. The expression stability of five candidate endogenous controls was evaluated for this purpose. These genes were actin (ACT), α-tubulin (TUB), ubiquitin (UBI), RPL8 and TIP41. The Ct values obtained for these genes ranged from 23.4 (ACT) to 30.1 (TUB); their amplification efficiency and specificity was confirmed by melting curve analysis. In order to establish which gene showed the most constant expression across differential treatments, the entire Ct dataset was analyzed using the geNorm v.0 software. The results of this analysis (Figure 1) revealed RPL8 as the most stable gene, whereas the least stable was α-tubulin. Based on these results, RPL8 was selected as a reference gene to quantify the expression of selected candidate genes.

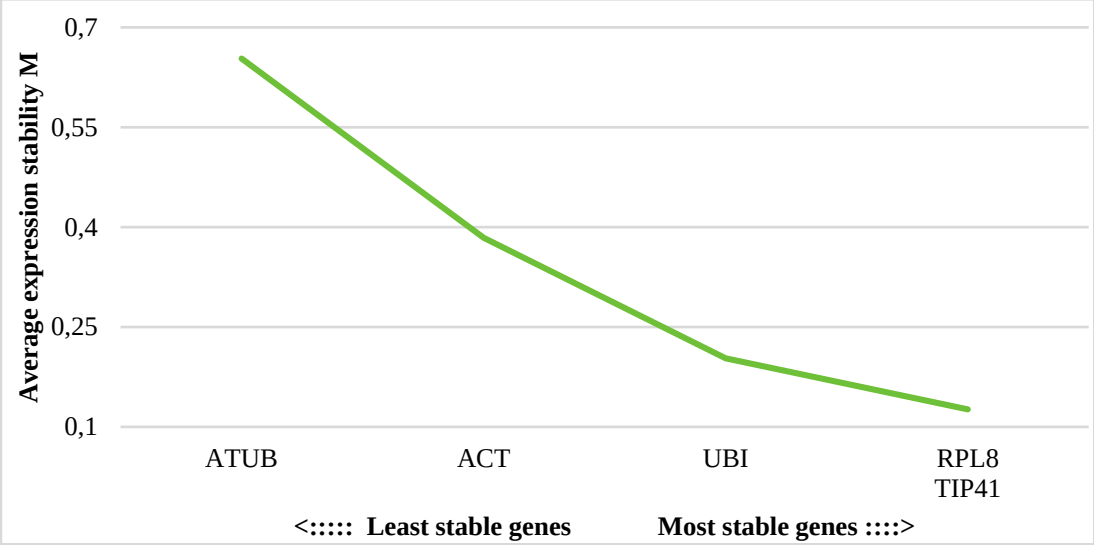

Fig. 1. Analysis of the most stable genes by geNorm. M value of the five candidate reference genes shown for leaf tissue sample of plants exposed to salinity stress after 3 hours.

Overall, it was only possible to validate the expression of 4 of the 8 selected DETs. For DET #5, DET #6, DET #12 and DET #19 it was not possible to synthetize primers that amplified the desired sequences. This was due to the lack of a species-dedicated genome for *S. betaceum* and the broad degree of genetic dissimilitude between this species and the selected reference species (tomato and potato). For the other 4 candidate genes, expression patterns were as follows:

DET #1 showed homology to an Arabinogalactan (AGP-1) protein from *Solanum lycopersicum*. In the differential display analysis, this fragment was identified as "up-regulated" in salt-stressed plantlets from the potentially salt-tolerant genotype (originating from Chaltura) after 3 hours of stress treatment. However, RT-qPCR results did not show significant differences in expression for this gene between salt-stressed and unstressed plants for either genotype (Figure 2).

DET #2, which showed homology to a Cinnamic Acid 4-Hydroxylase (C4H) gene from *Capsicum anumm,* was also "up-regulated" in salt-stressed plantlets from the salt-tolerant genotype after 3 hours of stress treatment, as determined by differential display analysis. While differential expression of this gene was validated via RT-qPCR, the directionality of the expression pattern was discordant. RT-qPCR results showed that this transcript was down-regulated in salt-stressed and unstressed plantlets of the salt-tolerant genotype, but the latter showed a markedly higher down-regulation of DET #2. For the salt-sensitive genotype (originating from Quero), DET #2 was also down-regulated in stressed and unstressed plantlets, but there was no significant difference between treatments (Figure 2).

DET #3 showed homology to a Pectin Methyl Esterase (Pest2) gene of *Solanum tuberosum.* In the differential display analysis, this fragment appeared to be "down-regulated" in the salt-tolerant genotype after 3 hours of stress treatment. This result was confirmed by RT-qPCR analysis (Figure 2).

Finally, DET #21 was homologous to an Oxygen-Evolving Enhancer Protein 1 gene from *S. lycopersicum*. The fragment was identified as being "down-regulated" in salt-stressed plants from both genotypes analyzed after 24 hours of stress treatment. RT-qPCR results confirmed this trend for the salt-tolerant genotype, but DET #21 did not show significant differences in expression between treatments in the salt-sensitive genotype.

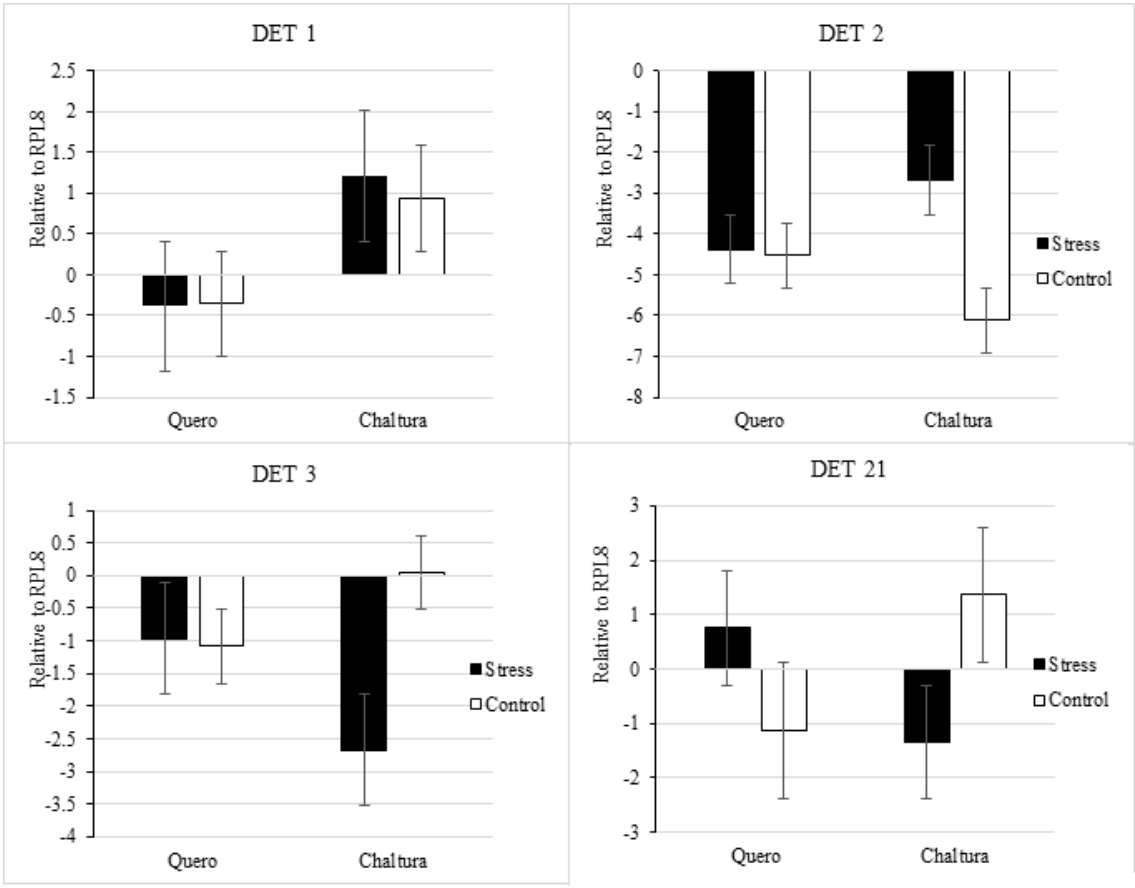

Fig. 2. Expression analysis of DETs validated by RT-qPCR in response to salt stress after 3 hours for DETs 1, 2 and 3, and after 24 for DET 21 in salt tolerant cv. On the x-axis, Quero represents the salt-sensitive genotype and Chaltura represents the salt-tolerant genotype, as defined by Jaramillo *et al.* (2014).

4. DISCUSSION

The Andean "tree tomato" (*S. betaceum*) is an exotic fruit species with great potential to develop into a novel high-value export product, but its productivity is challenged by biotic and abiotic factors (National Research Council, 1989). Efforts to improve *S. betaceum* have concentrated on fruit quality and disease resistance (Acosta-Quezada, 2015), but the development of varieties tolerant to environmental stresses should also be prioritized. *S. betaceum* shows broad adaptation to a diverse range of agro-climatic environments, and has proven resilient to several marginality factors (Feicán *et al.*, 1999; Jaramillo *et al.*, 2014). By understanding the mechanisms underlying its adaptability to environmental stresses, breeders can develop novel tools and strategies to increase *S. betaceum* productivity and to expand its cultivated area.

This study presents a preliminary exploration of the genetic mechanisms underlying salinity tolerance in *S. betaceum*. To this end, we selected two *S. betaceum* genotypes contrasting in their ability to tolerate high salinity *in vitro* (Jaramillo *et al.*, 2014), and used differential display analysis to compare overall differences in gene expression between salinity-stressed and unstressed (control) plants in both genotypes. Previously, differential display analysis was successfully used in commercial (*e.g. camellia sinensis*) and neglected crop species (*e.g. Setaria italica*) towards the elucidation of

genes underlying crop adaptiveness to environmental stress (Liu & Baird, 2003; Jayaraman *et al.*, 2008; Gupta *et al.*, 2013).

Differential display analysis allowed us to identify 171 differentially expressed transcripts (DETs). The number of detected transcripts resembled values reported in similar studies (Jayaraman *et al.*, 2008; Gupta *et al.*, 2013) and manifests the drastic and extensive changes in gene expression patterns and metabolic fluxes which underlie plant responses to abiotic stress (Tsukagoshi *et al.*, 2015). Sequence analyses revealed that several of the identified DETs (30 in total) showed homology with candidate genes putatively involved with abiotic stress tolerance in different species; a factor which reiterates the universal nature of abiotic stress responses across the plant kingdom (Vydia *et al.*, 1999; Yan *et al.*, 2006; Hee-Yeo *et al.*, 2008; Tsukagoshi *et al.*, 2015). In addition to their association with ontology terms related to stress response, these DETs were ascribed putative roles in photosynthesis, cellular metabolism and cell wall metabolism. Photosynthesis is the most fundamental physiological process in plants and is among the primary processes to be affected by abiotic stress. Physiological studies show that saline environments can directly or indirectly affect the different components of the photosynthesis apparatus (*i.e.*, electron transport system, gas exchange system, oxidative reduction pathways, photosynthetic pigments, etc.), often leading to inadequate photosynthesis and growth-rate reduction (Chaves *et al.*, 2008; Flood *et al.*, 2011; Ashraf & Harris, 2013). However, quantitative and transcript-profiling studies also demonstrate that plants could perceive and respond to salinity stress by rapidly modifying genes related to photosynthetic metabolism in an attempt to sustain or restore normal growth (Chaves *et al.*, 2008; Flood *et al.*, 2011). In a similar manner, several studies show that plants can remodel their cell walls as an adaptive response to stressful environments. The plant cell wall is a complex and dynamic structure that plays a prominent role in the shaping of cells, and in providing mechanical strength against turgor pressure (Tenhaken, 2015). The complexity of cell wall composition and its role in cell biomechanics has been well characterized (Braidword *et al.*, 2014). Changes in gene expression, as well concomitant modifications in the chemical composition of the cell wall under saline stress have also been reported (Byrt *et al.*, 2017). Cell expansion is generally controlled by a coordinated increase in cellular turgor pressure and an increase in cell wall extensibility (Braidword *et al.*, 2014). By modifying the genes and components responsible for this coordinated process, turgor and cell size can be optimized and modulated under osmotic stress conditions, such as those created by high salinity (Braidword *et al.*, 2014). Identified DETs also showed homology to proteins of unknown function. These sequences should be explored further for potentially novel abiotic stress tolerance mechanisms. Historically, Andean agriculture has favored breeding for agronomic resilience over high yields, ease of harvest and low-toxicity (Jacobsen 2003; Palmgren *et al.*, 2015). Therefore, Andean neglected crops, like *S. betaceum*, constitute an untapped source for survival traits presumably lost in commodity crops where plant breeding has homogenized gene pools (Palmgren *et al.*, 2015). Certainly, the allelic basis of these traits could be exploited in plant breeding programs to expedite the advance of cultivars resilient to the detrimental effects of climate change.

This research constitutes the first attempt to inquire the transcriptome of *Solanum betaceum* as a means to elucidate the molecular basis of genotype-specific differences in response to salinity stress. Overall, we have shown that differential display analysis constitutes an effective, easy-to-implement and economically-accessible tool for the genetic exploration of neglected crop species (Liang *et al.*, 2002). However, relevant

limitations observed in this study and reported in literature should be taken into consideration when using this technology. These limitations include the inconsistent reproducibility of band patterns, a detection bias favoring high-copy number mRNAs (Ledakis *et al*., 1998) and inherent artefacts of the technique (*e.g.* multiple DETs migrate through the gel at the same rate, two or more DETs are in close proximity) which lead to the equivocal identification of false positives (Stein & Liang, 2002). With regards to the latter, RT-qPCR results in this study showed that DET #1 did not differ significantly between salinity-stressed and unstressed plants in both genotypes. Another key factor influencing the effectiveness of differential display analysis is the availability of species-dedicated genomic tools. In this study, the lack of a reference genome for *S. betaceum* was a limiting factor of our research. It restricted the characterization (*e.g*. assignment of gene identity) of numerous DETs and made it difficult to design functional primers for DETs showing putative associations with salinity stress tolerance. Despite these limitations, however, our analytical pipeline did allow us to functionally characterize at least 4 salinity-stress induced DETs (i.e. DET#1, DET #2, DET #3 and DET #21), and to successfully validate the expression of 3 of them (*i.e.* DET#1, DET #2, DET #3 and DET #21) via RT-qPCR in the salt-tolerant genotype. As previously mentioned, these DETs belong to different metabolic pathways, supporting the complexity and diversity of physiological mechanisms for salt tolerance. Expanding our analyses via validation of additional DETs would likely contribute to the identification of genes which can be used as proxies for a better understanding of the regulatory, metabolic and physiological mechanisms used by *S. betaceum* to respond and adapt to abiotic stress. This research provides a framework for the exploration of resilience mechanisms in neglected Andean crops. We strongly believe that *S. betaceum* underlies interesting adaptations to detrimental conditions, and its tolerance to salinity could be potentially enhanced by the development of genetic and molecular resources for further characterization of the species.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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