

Transcriptome, Biochemical and Phenotypic Analysis of the Effects of a Precision Engineered Biostimulant for Inducing Salinity Stress Tolerance in Tomato

Elomofe Ikuyinminu ^{1,2}, Oscar Goñi ^{1,2,*}, Łukasz Łangowski ² and Shane O'Connell ^{1,2}

¹ Plant Biostimulant Group, Shannon Applied Biotechnology Centre,

Munster Technological University-Tralee (South Campus), Clash, V92 CX88 Tralee, Co. Kerry, Ireland

² Brandon Bioscience, V92 N6C8 Tralee, Co. Kerry, Ireland

* Correspondence: oscar.goni@mtu.ie

Supplementary Material

1 Supplementary Figures

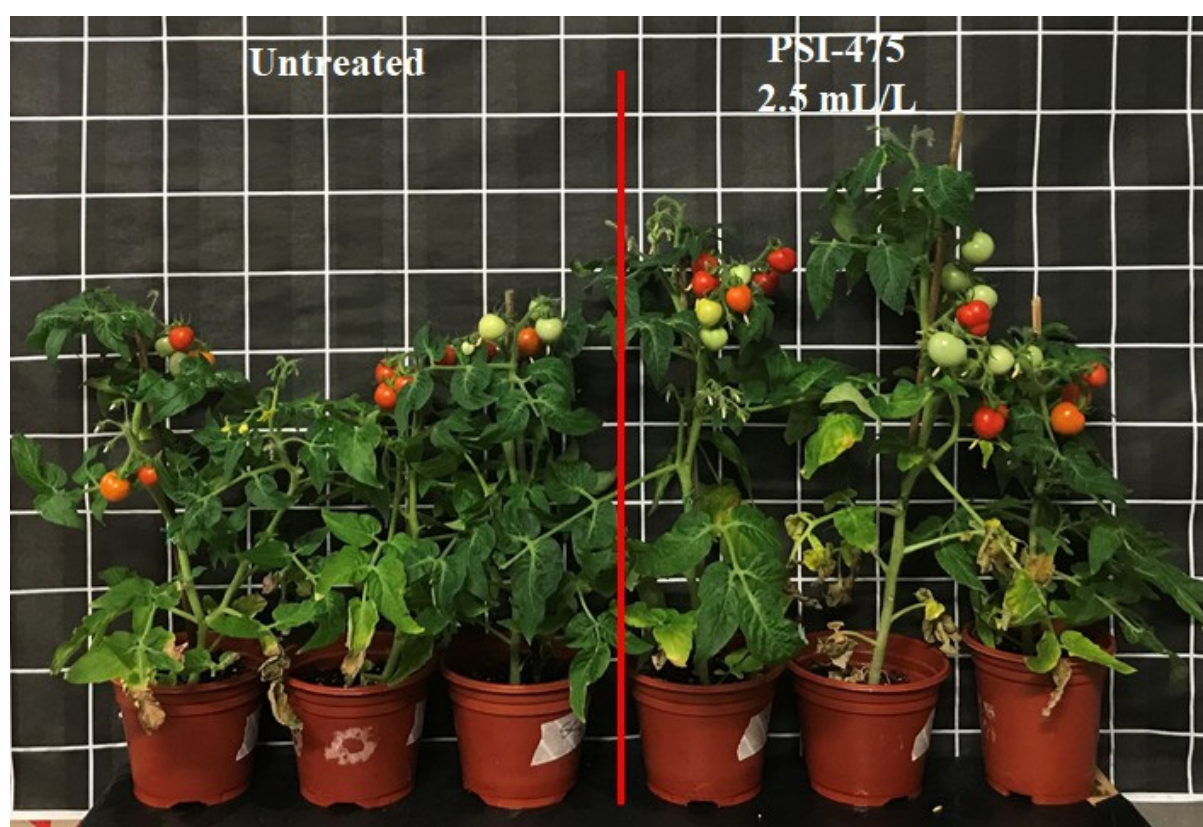


Figure S1. Pictures of the plants used to generate the transcriptomic data. Scale of background square equals 5 cm H X by 5 cm W.

2 Supplementary Tables

Table S1. Effect of salinity stress and PSI-475 on K⁺/Na⁺ ratio in different tomato plant tissues.

Source of variance	Root	Lower stem	Old leaf	Upper stem	Young leaf
Salinity (S)					
Unstressed	1.66 b	30.60 b	36.24 b	24.01 b	102.73 b
Salinity stressed	0.51 a	2.86 a	1.76a	2.21 a	1.32 a
PSI-475 (P)					
Untreated	0.62 a	16.25	19.60 b	12.52	32.87 a
PSI-475	1.54 b	17.21	18.40 a	13.70	71.19 b
S × P					
Unstressed × Untreated	0.81 c	29.46	37.46 c	22.82	65.52 b
Unstressed × PSI-475	2.51 d	31.75	35.03 b	25.19	140.94 c
Salinity stressed × Untreated	0.43 a	3.05	1.74 a	2.21	1.22 a
Salinity stressed × PSI-475	0.58 b	2.67	1.78 a	2.21	1.43 a
Statistical significance					
Salinity (S)	***	***	***	***	***
PSI-475 (P)	***	ns	***	ns	*
S × P	***	ns	***	ns	*

All data are expressed as average per sample collected at harvest sample points. ns, *, and *** means non-significant or significant at $p \leq 0.05$, and $p \leq 0.001$, respectively. Different letters indicate statistical differences with $p \leq 0.05$ based on t-test (S, P) or Tukey's HSD test (S × P). Number of biological replicates ($n \geq 3$).

Table S2. Effect of salinity stress and PSI-475 on glucose content in different tomato plant tissues.

Source of variance	Root (mg/g DW)	Lower stem (mg/g DW)	Old leaf (mg/g DW)	Upper stem (mg/g DW)	Young leaf (mg/g DW)
Salinity (S)					
Unstressed	0.24	0.79 b	0.68 a	1.43 a	0.80 a
Salinity stressed	0.30	0.56 a	2.06 b	0.73 b	1.78 b
PSI-475 (P)					
Untreated	0.21 a	0.32 a	1.20 a	0.71 a	0.98 a
PSI-475	0.33 b	1.04 b	1.55 b	1.45 b	1.60 b
S × P					
Unstressed × Untreated	0.16	0.24 a	0.54	0.48 a	0.70
Unstressed × PSI-475	0.32	1.35 c	0.83	2.37 b	0.90
Salinity stressed × Untreated	0.25	0.40 a	1.86	0.93 a	1.26
Salinity stressed × PSI-475	0.34	0.73 b	2.26	0.53 a	2.31
Statistical significance					
Salinity (S)	ns	*	***	**	***
PSI-475 (P)	*	***	***	**	*
S × P	ns	***	ns	***	ns

All data are expressed as average per sample collected at harvest sample points. ns, *, **, and *** means non-significant or significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. Different letters indicate statistical differences with $p \leq 0.05$ based on t-test (S, P) or Tukey's HSD test (S × P). Number of biological replicates ($n \geq 3$).

Table S3. Effect of salinity stress and PSI-475 on fructose content in different tomato plant tissues.

Source of variance	Root (mg/g DW)	Lower stem (mg/g DW)	Old leaf (mg/g DW)	Upper stem (mg/g DW)	Young leaf (mg/g DW)
Salinity (S)					
Unstressed	0.50	0.75	1.01 a	1.36 b	1.25 a
Salinity stressed	0.47	0.89	4.76 b	0.87 a	2.45 b
PSI-475 (P)					
Untreated	0.35 a	0.50 a	2.63	0.69 a	1.17 a
PSI-475	0.62 b	1.14 b	3.15	1.54 b	2.53 b
S × P					
Unstressed × Untreated	0.27 a	0.26 a	0.73	0.46 a	0.82 a
Unstressed × PSI-475	0.72 b	1.24 c	1.29	2.26 c	1.68 b
Salinity stressed × Untreated	0.42 a	0.74 b	4.52	0.91 b	1.53 ab
Salinity stressed × PSI-475	0.51 ab	1.04 bc	5.00	0.82 b	3.38 c
Statistical significance					
Salinity (S)	ns	ns	***	***	***
PSI-475 (P)	**	***	ns	***	***
S × P	*	**	ns	***	*

All data are expressed as average per sample collected at harvest sample points. ns, *, **, and *** means non-significant or significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. Different letters indicate statistical differences with $p \leq 0.05$ based on t- test (S, P) or Tukey's HSD test (S × P). Number of biological replicates ($n \geq 3$).

Table S4. Effect of salinity stress and PSI-475 on soluble protein content in different tomato plant tissues.

Source of variance	Root (mg/g DW)	Lower stem (mg/g DW)	Old leaf (mg/g DW)	Upper stem (mg/g DW)	Young leaf (mg/g DW)
Salinity (S)					
Unstressed	17.11 b	8.24	20.52 b	10.60	43.91
Salinity stressed	10.76 a	7.79	14.01 a	9.02	42.44
PSI-475 (P)					
Untreated	13.33	7.44	18.93 b	9.60	39.80 a
PSI-475	14.55	8.60	15.61 a	10.03	46.54 b
S × P					
Unstressed × Untreated	16.87	7.31	20.04 c	10.99	41.61
Unstressed × PSI-475	17.37	9.18	21.01 c	10.22	46.20
Salinity stressed × Untreated	9.79	7.58	17.82 b	8.21	37.99
Salinity stressed × PSI-475	11.73	8.01	10.21 a	9.36	46.88
Statistical significance					
Salinity (S)	***	ns	***	ns	ns
PSI-475 (P)	ns	ns	***	ns	***
S × P	ns	ns	***	ns	ns

All data are expressed as average per sample collected at harvest sample points. ns and *** means non-significant or significant $p \leq 0.001$. Different letters indicate statistical differences with $p \leq 0.05$ based on t- test (S, P) or Tukey's HSD test (S × P). Number of biological replicates ($n \geq 3$).

Table S5. RNA-seq QC data summary in young leaf and root tissue samples of salinity stressed tomato plants.

Sample name ¹	Raw reads ²	Clean reads ³	Error rate (%) ⁴	Q20 (%) ⁵	Q30 (%) ⁶	GC content (%) ⁷
LeafTrt2	28,233,239	27,758,977	0.02	98.56	95.47	42.79
LeafTrt3	25,557,968	25,230,307	0.02	98.66	95.70	42.74
LeafTrt1	27,191,658	26,799,960	0.02	98.68	95.75	43.06
RootTrt2	21,395,116	20,865,057	0.02	98.65	95.64	42.34
RootTrt3	25,815,340	25,134,755	0.02	98.52	95.32	42.48
RootTrt1	22,280,918	21,782,527	0.02	98.61	95.50	42.34
LeafCtr3	23,909,857	23,588,287	0.02	98.64	95.67	42.86
LeafCtr2	22,323,457	21,922,394	0.02	98.54	95.42	42.65
LeafCtr1	24,066,624	23,750,535	0.02	98.56	95.49	42.58
RootCtr3	23,636,644	23,136,802	0.02	98.62	95.58	42.20
RootCtr2	23,023,155	22,730,435	0.02	98.51	95.32	42.17
RootCtr1	21,560,363	21,210,996	0.02	98.50	95.27	42.14

¹ “Trt” and “Ctr” correspond to treated with PSI-475 and control samples.

² Reads count from the raw data, four rows as a unit, with statistics of reads count for every sequencing.

³ Reads count filtered from raw data. Statistics method is similar with raw reads. All the following analysis is based on clean data.

⁴ Base error rate of whole sequencing.

⁵ The percentage of the bases whose Q Phred values is greater than 20 (99% correct rate).

⁶ The percentage of the bases whose Q Phred values is greater than 30 (99.9% correct rate).

⁷ The percentage of G&C base numbers of total bases.

Table S6. Primers sequences used for qRT-PCR analysis in tomato

Gene	Primer Forward	Primer Reverse
<i>TIP41 (Soly07g025390.4)</i>	CTCGACCAAGTCCCTACGAT	TGAAAAGCTTGCGACACTCG
<i>NHX4 (Soly01g098190.3)</i>	TGGGAGCAATTTACGACGA	TTCCAGCGCCTTTACCAAT
<i>SPS (Soly09g092130.3.1)</i>	AAAACGCCGTCAAGAACGTG	GCAATCGGCCCTCTGGTACTT
<i>PDH (Soly02g089630.3.1)</i>	GGTTTTTCGTGACCTCCGAC	AGAACCATAGTTGCCGGTGA
<i>CHL (Soly03g115980.1)</i>	TGGCCAACATACAAGGTGCT	AATGGGGTTTCTGGTGCAA
<i>TAS14 (Soly02g084850.3)</i>	TGCCTGGACAACATGAAGGT	GCGTCAGCACACTTTACACG
<i>HSP101 (Soly03g115230.3.1)</i>	ACCCGATCAGATTGCGGAAG	GAACCAGTTGGTTGCTGTGG
<i>GS1 (Soly04g014510.3.1)</i>	ATCGCGGAGACTACCATCCT	AGGTCATGTGAGGAAAGGGC
<i>AKT2/3 (Soly01g104030.3.1)</i>	CGGAGACGCTCAAGGAAGAA	TGCATCCATGTCTAAGAAGCAC
<i>PIP2.1 (Soly10g084120.2.1)</i>	CAGCTGGCATTCTGGTGGA	CAGCACCTAATCCAGTGGCTA
<i>POR2 (Soly07g054210.3.1)</i>	GCTGCATTGCTGAACTGGT	TCTCTGCATCACTGGCTTCC
<i>HKT1;2 (Soly07g014680.3)</i>	CCTACCGTCTTTTCGTCTCA	AGGTAAAAGCTTCCCCACCA