

Characterization of β -amino- and γ -amino butyric acid-induced citrus seeds germination under salinity using nanoLC–MS/MS analysis

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Abstract

Key message BABA or GABA induces salinity acclimation during citrus seeds germination via alternation of specific proteins (e.g., citrin).

Abstract The impact of four elicitors, namely hydrogen peroxide (H₂O₂), β -amino butyric acid (BABA), γ -amino butyric acid (GABA) and hydrogen sulfide (H₂S) donor, sodium hydrosulfide (NaHS), in citrus seed germination under salinity (150 mM NaCl) was tested. The germination potential was adversely affected by NaCl-alone treatment. Pretreatment with H₂O₂ or the NaHS-H₂S donor prior to salinity had no significant effect in germination process, however, BABA and GABA substantially improved seed acclimation to salinity, as evidenced by increased germination percentage and radicle length. Total soluble proteins of radicle and cotyledons were separated by 1DE SDS-PAGE and proteins zones were analyzed by mass spectrometry. In total, 27 and 3 proteins were identified in radicle and cotyledons, respectively. The identified proteins mainly include redox-regulated enzymes (i.e., glutathione *S*-transferase, dehydroascorbate reductase, Mn-

superoxide dismutase, glutathione peroxidase), energy-related proteins (i.e., isocitrate lyase, malate synthase, pyruvate decarboxylase), stress proteins (i.e., stress-related protein, miraculin, thaumatin, disulfide isomerase), storage proteins (i.e., vicilin, Pis v 1 allergen 2S albumin) and transcriptional regulators (i.e., MarR family transcriptional regulator, MADS544 protein). Pretreatments with BABA or GABA altered the accumulation of protein zones exclusively corresponding to citrin, indicating that this protein may serve as a marker for salinity acclimation in citrus seeds.

Keywords BABA · Citrus · GABA · Proteomics · Salinity · Seed germination

During germination, the seed is highly sensitive to environmental stresses, notably salinity. Several signaling molecules, such as hydrogen peroxide (H₂O₂), hydrogen sulfide (H₂S) and the non-protein amino acids β -amino butyric acid (BABA) and γ -amino butyric acid (GABA) can confer protection against abiotic stresses. Evidence suggests that the salt responses ensure appropriate stress-related gene and protein expression that ultimately leads to acclimation (see Rajjou et al. 2012 for review). Citrus are among the most economically important fruit trees and characterized as relatively sensitive to salt stress (Tanou et al. 2012). In this study, we investigated whether seed pretreatment with four well-known inducers of plant resistance, H₂O₂, BABA, GABA or NaSH (a H₂S donor) can induce acclimation of citrus seeds to subsequent salinity. One-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (1DE SDS-PAGE) followed by nanoscale liquid chromatography coupled to tandem mass spectrometry (nano LC–MS/MS) was conducted in

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radicle and cotyledons to characterize, for the first time, citrus protein status during germination.

Citrus (*Citrus aurantium* L.) seeds were prepared for germination according to King and Roberts (1980). Seeds pretreated with 100 μ M of H_2O_2 , BABA, GABA, NaSH or distilled water (control) for 12 h in the dark at room temperature. Subsequently, seeds were allocated randomly in two Petri dishes (9 cm diameter) for each treatment (10 seeds in each Petri dish) containing three layers of filter paper, moistened with distilled water (control) or 150 mM NaCl solution. Germination was performed under standard conditions (King and Roberts, 1980).

Total proteins were extracted from radicle and cotyledon tissues in 50 mM potassium phosphate (pH 7.4) extraction buffer containing 1 mM EDTA, 0.5 % Triton X-100, 1 mM PMSF, 1 M NaCl, 1 % PVPP and the protease inhibitor cocktail 'Complete Mini' (Roche Diagnostics, Mannheim, Germany). Afterwards, proteins were separated by 1DE SDS-PAGE and gels were analyzed with Quantity One software (Bio-Rad) (Minas et al. 2012). Following digestion, peptides from protein zones of interest were analyzed by LTQ-Velos-Orbitrap online with a nanoLC Ultimate 3000 chromatography system (Thermo Fisher

Scientific, Bremen, Germany). Proteins were annotated based on the National Center for Biotechnology Information (NCBI) using BLASTp. All identified proteins had a MASCOT score greater than the significance level corresponding to $P < 0.05$. Moreover, among the positive matches, only protein identifications based on at least two different peptide sequences of more than six consecutive amino acids with an individual score above 20 were accepted. To validate protein identification with one peptide (e.g., as in protein zone 1, 4, 6 and 9; Supplementary Fig. 3), the threshold score was set above the identity threshold (value of 32) with at least a series of five consecutive y or b fragments in the spectrum.

Phenotypical and physiological data showed that seed germination was depressed by NaCl (Fig. 1a–c). Although H_2O_2 or H_2S had no significant effect in germination, pretreatment with BABA or GABA improved germination, as documented by the increased germination percentage and radicle (Fig. 1b, c). Several distinct proteins zones (Fig. 1d; 1–11 arrows) showed significant variation in abundance (Supplementary Table 2). NanoLC–MS/MS analysis identified 27 and 3 proteins in radicle and cotyledons (Table S1), respectively, which mainly

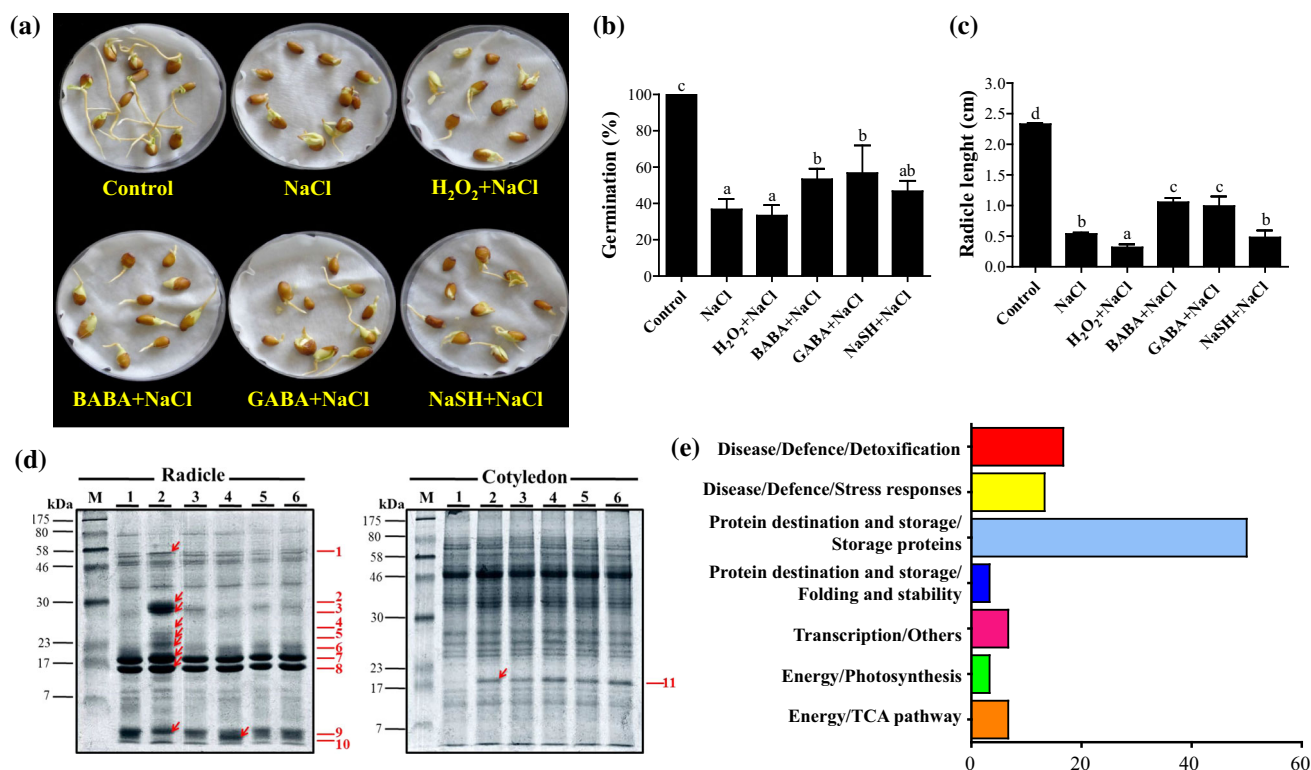


Fig. 1 Influence of chemical pretreatment on **a** citrus seed germination phenotype, **b** germination percentage (%) and **c** radicle length (cm). Bars marked by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$). Results are mean $\pm$ SD of three replications. **d** Representative total soluble protein 1DE SDS-PAGE analysis in radicle and cotyledon. Equal

amount (15 μ g) of total protein was loaded in each sample. After staining, gel was cut into 11 slices (1–11) each of which were then subjected to in-gel digestion with trypsin and nanoLC–MS/MS analysis. **e** Functional category of proteins identified in citrus tissues during germination. The x-axis represents the percentage of functional categories of proteins among the total proteins identified

localized in plastid and extracellular space (Figure S1). These proteins ($n = 30$) were primarily related to protein destination/storage (53.3 %) and plant defense (30.0 %) (Figure S2).

The detection of several antioxidant enzymes in the radicle, such as glutathione *S*-transferase (GST), dehydroascorbate reductase (DHAR), Mn-superoxide dismutase (Mn-SOD) and glutathione peroxidase (GPX) together with the identification of several defense proteins (stress-related protein, miraculin, thaumatin) (Table S1) give the citrus tissues an advantage for survival in saline environment. The presence of energy-related proteins in radicle that participate into glyoxylate (TCA) cycle, such as malate synthase and isocitrate lyase (Table S1), suggests that lipids act as an energy source during radical growth under salinity. The identification of a MarR family transcriptional regulator in radical along with the detection of the transcription-related protein (MADS544 protein; Table S1) support that the post-germination development was mediated by transcription factors.

An important result revealed by this proteomic analysis was that the protein zones 2 and 3 (Fig. 1d), that exclusively corresponded to citrin (Table S1), were accumulated in NaCl-alone treated radicle compared with control (Table S2), possibly suggesting that mobilization of the two citrin subunits occurred during imbibition of seeds under salinity stress. Since citrin expression is tightly linked with polyembryony in citrus seeds (Nakano et al. 2013), we assume that the accumulation of citrin in the radicle and its presence in the cotyledons exposed to NaCl (Table S1) might be associated with the stimulation of polyembryony to improve the survival of at least one seedling per seed under salinity-originated unfavorable conditions, in a type of environmental-induced polyembryony mechanism. Interestingly, the accumulation of protein zones 2 and 3 of citrin in BABA- or GABA-treated tissues under salinity reversed to the levels of unstressed control (Table S2). Therefore, BABA- or GABA-depressed citrin accumulation could embody an acclimation mechanism that allows citrus seeds to depress polyembryony to avoid competition inside the seed or could be an indicator that the severity of salt stress is less sensed in tissues. An alternative explanation for the observed germination

behavior could be that salinity-induced solubilization/mobilization of citrin, as mentioned above, was detrimental for seedling establishment because it caused premature depletion of citrin-like storage compounds that are normally useful later during seedling establishment under non-stressed conditions. In this hypothesis, BABA and GABA under salinity would restore the normal seedling establishment behavior seen under optimal germination conditions. Further detailed researches are needed to characterize the potential roles of citrin in salt acclimation.

Author contribution statement AM, GT and GD conceived and designed the study, VZ—performed most of germination experiments, GT—performed seed germination and proteomic analysis, and MB—performed proteomic analysis. VZ, GT and AM wrote the paper.

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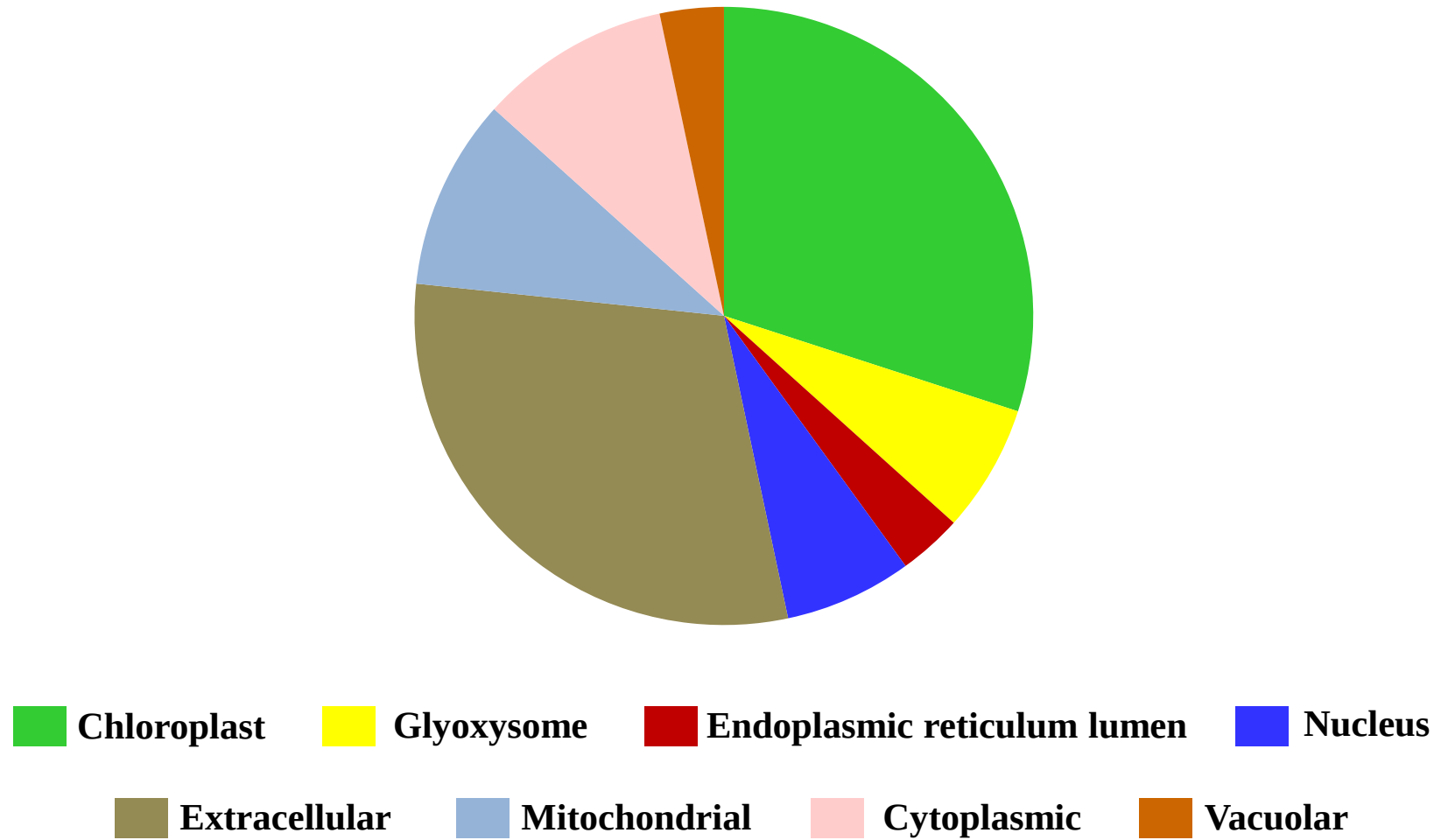
Compliance with ethical standards

Conflict of interest The authors declare no competing financial interests.

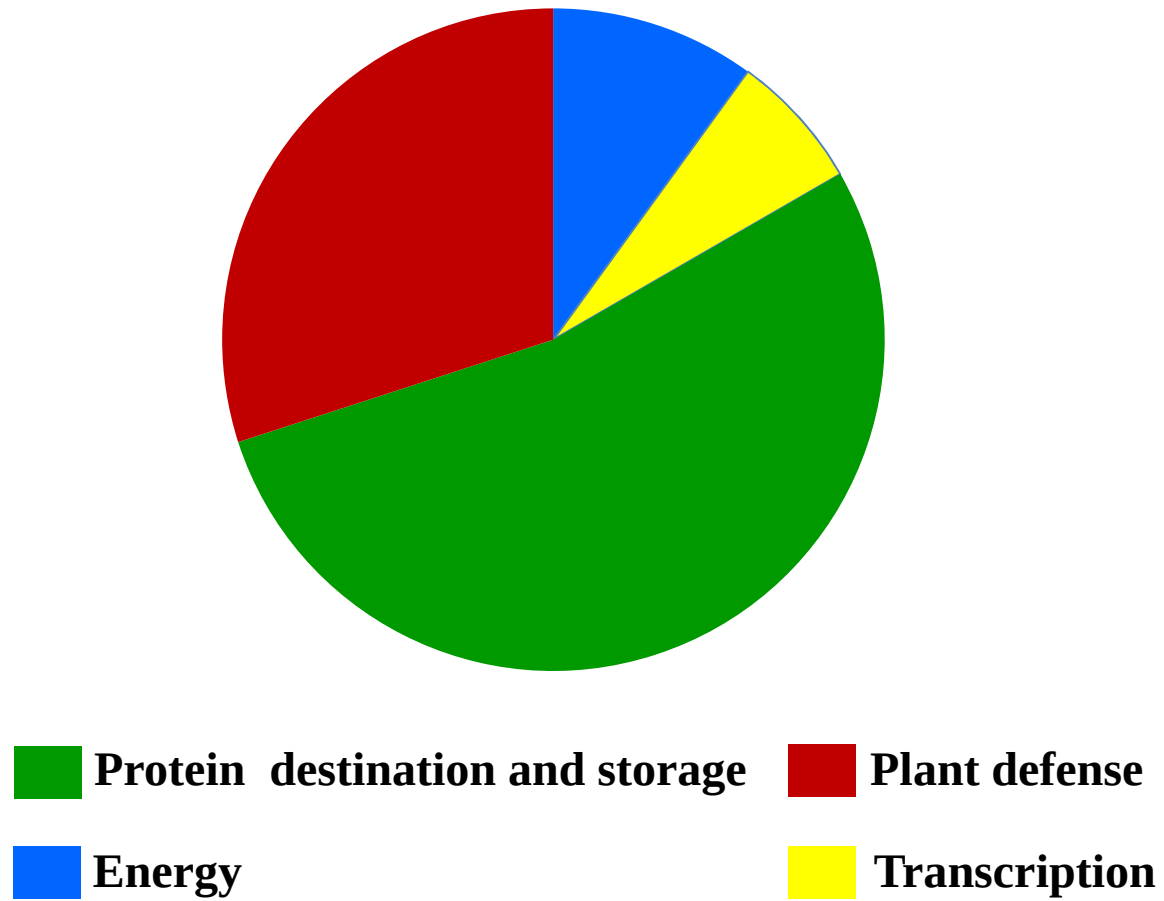
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Supplementary Figure 1, Ziogas et al.



Supplementary Figure 2, Ziogas et al.



Supplementary Table 1. List of the 30 soluble proteins identified by mass spectrometry in the radicle and cotyledons of citrus seeds.

Legend: Zone number, Zone label on the reference gel maps presented in [Figure 1d](#); Protein name, identified peptide names; Acces. number, accession number in NCBI or in Uniprot database; Matching criteria, adopted criteria for the valuation of similarity for proteins identified after manual blast against current databases. Blank cells correspond to proteins readily identified without further blast; Organism, organism in which the protein has been identified; Subcellular localization, Sub-cellular localization was assigned based on database searches; Functional category, proteins ontologically classified into functional categories proposed by [Bevan et al. \(1998\)](#); Theor.pI/Mw, theoretical isoelectric point/molecular weight; % Cov, Mascot coverage %, percentage of sequence coverage obtained with identified peptides with Mascot software for the orthologous protein; Score, a minimum of two different peptides was used (score>65 for search in NCBI nr, score > 32 when using UNIPROT database and score>76 when using the EST database). To validate protein identification with one single peptide Supporting Information are provided in [Supplementary Figure 3](#); m/z, mass to charge ratio; Mass(exp), experimental mass of peptide; Sequences, list of peptides identified with Mascot software

Zone number	Protein name	Acces.number	Matching criteria	Organism	Subcellular localization	Functional category	Theor. pI/Mw:	% Cov	Score	MW	m/z	Mass (exp)	Sequences
Zone 1	Pyruvate decarboxylase	Q4JIY7 Q4JIY7_CITSI		Citrus sinensis	Plastidiac	11.06-Disease/Defense /Detoxification	5.24/22091	6	64	22091	619.3735	12.367.324	R.VSAANSRPPNPQ.-
Zone 1	Isocitratelase	gi 56533701	87 % to gi 113029 Gossypiumhirsutum (upland cotton), E value:1e-118	Citrus sinensis	Glyoxysome	02.10-Energy/ TCA pathway	8.84/31924	18	221	31924	451.2983	9.005.820	K.GSVDAAIIR.G
											462.2689	9.225.232	K.GVTEDQFK.E
											477.7838	9.535.530	R.GMLAYVER.I + Oxidation (M)
											555.8413	11.096.680	R.RGMLAYVER.I + Oxidation (M)
											598.8577	11.957.008	R.NNGVDTLAHQK.W
											662.3936	13.227.726	K.TVQGGISSTAAMGK.G + Oxidation (M)
Zone 1	Malate synthase	gi 56533075	84 % to gi 255540321 Ricinuscommunis (castor bean), E value:8e-157	Citrus sinensis	Glyoxysome	02.10-Energy/ TCA pathway	9.04/30401	16	187	30401	437.2440	8.724.734	R.YSEEFK.I
											500.3197	9.986.248	R.VEITGPVER.K

Zone 1	Disulfide-isomerase	gi 38033087	80 % to gi 225459587 Vitisvinifera, E value:4e-132	Citrus sinensis	Endoplasmic reticulum lumen	06.01 Folding and stability	5.01/26697	12	121	26697	564.3748	11.267.350	R.VEITGPVERK.M											
											574.3643	11.467.140	K.MIINALNSGAK.V + Oxidation (M)											
											574.3684	11.467.222	K.MIINALNSGAK.V + Oxidation (M)											
											578.3773	11.547.400	R.RVEITGPVER.K											
											741.4286	14.808.426	R.YNEGALPGFDAATR.Y											
											462.7751	9.235.356	K.FFNSPNAK.A											
											494.3023	9.865.900	K.QSGPASAEIK.S											
											544.3403	10.866.660	R.GESSVTGPVVR.L											
											Zone 1	Rubisco subunit binding-protein alpha subunit,	gi 110848135	80 % to gi 255587664 Ricinuscommunis (castor bean), E value:4e-170	Citrus clementina	Plastidiac	02.30-Energy/Photosynthesis	9.57/46532	4	85	46532	401.7724	8.015.302	R.VLVTDQK.F
																						429.1564	8.562.982	K.ASAEGNCFG.-
522.3408	10.426.670	K.VVNDGVTIAR.A																						
Zone 1	Vicilin	gi 188256916	61 % to gi 133711974 Pistaciavera, E value:4e-104	Citrus trifoliata	Nuclear	06.20-Protein destination and storage/Storage proteins	7.90/32034	6	80	32034												425.7635	8.495.124	K.ELAFNTR.A
																						600.8313	11.996.480	K.GAMAAPFYNSR.S + Oxidation (M)
																						600.8324	11.996.502	K.GAMAAPFYNSR.S + Oxidation (M)

Zone 2	Citrin	gi 1061408	Citrus sinensis	Extracellular	06.20-Protein destination and storage/Storage proteins	8.70/54913	10	252	54913	436.8013	8.715.880	R.FNLPILR.D
										452.2782	9.025.418	R.DLQLSAEK.G
										519.8483	10.376.820	R.LQRPQIQR.G
										519.8493	10.376.840	R.LQRPQIQR.G
										519.8514	10.376.882	R.LQRPQIQR.G
										519.8517	10.376.888	R.LQRPQIQR.G
										519.8521	10.376.896	R.LQRPQIQR.G
										793.0010	1583.9874	R.LLAEEAFNVNPDLIR.R
										793.0045	15.839.944	R.LLAEEAFNVNPDLIR.R
										793.5226	15.850.306	R.LLAEEAFNVNPDLIR.R
										649.3675	19.450.807	R.FQTQCNIQNLNALEPR.Q + Carbamidomethyl (C)
										973.5613	19.451.080	R.FQTQCNIQNLNALEPR.Q + Carbamidomethyl (C)
Zone 3	Citrin	gi 1061408	Citrus sinensis	Extracellular	06.20-Protein destination and storage/Storage proteins	8.70/54913	9	245	54913	436.8022	8.715.898	R.FNLPILR.D
										519.8481	10.376.816	R.LQRPQIQR.G
										792.9982	15.839.818	R.LLAEEAFNVNPDLIR.R
										792.9997	15.839.848	R.LLAEEAFNVNPDLIR.R
										793.5330	15.850.514	R.LLAEEAFNVNPDLIR.R
										649.3648	19.450.726	R.FQTQCNIQNLNALEPR.Q + Carbamidomethyl (C)
										973.5536	19.450.926	R.FQTQCNIQNLNALEPR.Q + Carbamidomethyl (C)

Zone 6	Glutathione peroxidase	A1ECK3 A1ECK3_9ROSI	Citrus hybrid cultivar	Nuclear	11.06-Disease/Defense /Detoxification	5.72/18584	7	51	18584	653.8947	13.057.748	R.YAPTTSPLSIEK.D
Zone 6	Citrin	gi 1061408	Citrus sinensis	Extracellular	06.20-Protein destination and storage/Storage proteins	8.70/54913	14	322	54913	436.7946	8.715.746	R.FNLPILR.D
										436.7986	8.715.826	R.FNLPILR.D
										452.2691	9.025.236	R.DLQLSAEK.G
										452.2723	9.025.300	R.DLQLSAEK.G
										622.8758	12.437.370	R.QELTVFTPGPR.S
										622.8759	12.437.372	R.QELTVFTPGPR.S
										682.3802	13.627.458	K.TNDVAMTSQLAGR.A
										690.3768	13.787.390	K.TNDVAMTSQLAGR.A + Oxidation (M)
										792.5148	15.830.150	R.EGQLIVVPQGFAVVK.R
										649.3599	19.450.579	R.FQTQCNIQNLNALEPR.Q + Carbamidomethyl (C)
										973.5438	19.450.730	R.FQTQCNIQNLNALEPR.Q + Carbamidomethyl (C)
Zone 6	Vicilin	gi 55400879	54 % to gi 356547194 Glycine max (soybean), E value:1e-41 Citrus sinensis	Plastidiac	06.20-Protein destination and storage/Storage proteins	5.53/22656	5	69	22656	568.3085	11.346.024	R.VVQTEAGEMR.V + Oxidation (M)
										568.3121	11.346.096	R.VVQTEAGEMR.V + Oxidation (M)
										431.2444	12.907.114	R.RVVQTEAGEMR.V + Oxidation (M)
Zone 7	Citrin	gi 1061408	Citrus sinensis	Extracellular	06.20-Protein destination and storage/Storage proteins	8.70/54913	25	451	54913	436.7949	8.715.752	R.FNLPILR.D

												452.2700	902.5254	R.DLQLSAEK.G
												490.2917	9.785.688	R.GLEWISFK.T
												490.2960	978.5774	R.GLEWISFK.T
												622.8716	12.437.286	R.QELTVFTPGPR.S
												690.3318	13.786.490	K.TNDVAMTSQLAGR.A + Oxidation (M)
												515.0007	15.419.803	R.VTTVNRFNLPILR.D
												515.0014	15.419.824	R.VTTVNRFNLPILR.D
												772.0085	15.420.024	R.VTTVNRFNLPILR.D
												515.0085	15.420.037	R.VTTVNRFNLPILR.D
												792.5052	15.829.958	R.EGQLIVVPQGFAVVK.R
												793.5330	15.850.514	R.EGQLIVVPQGFAVVK.R
												837.0089	16.720.032	R.GLPLDVIQNSFQVSR.D
												559.9879	1676.9419	K.YNRQELTVFTPGPR.S
												559.9901	16.769.485	K.YNRQELTVFTPGPR.S
												560.3316	16.779.730	K.YNRQELTVFTPGPR.S
												580.7029	17.390.869	R.EGQLIVVPQGFAVVKR.A
												870.5701	17.391.256	R.EGQLIVVPQGFAVVKR.A
												441.4911	1761.9353	R.HNIDKPSHADVYNPR.A
												441.4911	17.619.353	R.HNIDKPSHADVYNPR.A
												969.0222	19.360.298	R.MQIVAENGENVFDGQIR.E + Oxidation (M)
												758.1033	22.712.881	R.GLPLDVIQNSFQVSRDEAQ R.L
Zone 8	Citrin	gi 56534330	84 % to gi 1061408 Citrus sinensis, E value:2e-170	Citrus sinensis	Cytoplasmic	06.20-Protein destination and storage/Storage proteins	9.30/34222	40	526	34222	436.7940	8.715.734	R.FNLPILR.Y	
											436.7975	8.715.804	R.FNLPILR.Y	

436.7986	8.715.826	R.FNLPILR.Y
476.2916	950.5686	R.YIQLSAEK.G
490.8167	9.796.188	R.GLEWISFK.T
615.8677	12.297.208	R.QELTVFSPGPR.S
690.3717	13.787.288	K.TNDVAMTSQLAGR.A + Oxidation (M)
514.9980	15.419.722	R.VTTVNRFNLPILR.Y
514.9990	15.419.752	R.VTTVNRFNLPILR.Y
515.0015	15.419.827	R.VTTVNRFNLPILR.Y
515.0037	15.419.893	R.VTTVNRFNLPILR.Y
772.0133	15.420.120	R.VTTVNRFNLPILR.Y
792.5173	15.830.200	R.EGQLIVVPQGFAVVK.R
547.6127	16.398.163	K.HNINDPSGADAYNPR.G
547.6139	16.398.199	K.HNINDPSGADAYNPR.G
547.6145	16.398.217	K.HNINDPSGADAYNPR.G
555.3181	16.629.325	K.YNRQELTVFSPGPR.S
555.3201	16.629.385	K.YNRQELTVFSPGPR.S
555.3209	16.629.409	K.YNRQELTVFSPGPR.S
837.0146	16.720.146	R.GLPLDVIQNSFQVSR.D
471.2578	18.810.021	K.LKHNINDPSGADAYNPR.G
471.2601	18.810.113	K.LKHNINDPSGADAYNPR.G
646.0172	1935.0298	R.MQIVAENGENVFDGQIR.E + Oxidation (M)
968.5347	19.350.548	R.MQIVAENGENVFDGQIR.E + Oxidation (M)
758.1061	22.712.965	R.GLPLDVIQNSFQVSRDEAQ R.L

Zone 9	Miraculin	A1ECJ7 A1ECJ7_9ROSI		Citrus hybrid cultivar	Extracellular	11.05-Disease/Defense /Stress responses	8.18/23211	5	52	23211	619.4205	12.368.264	R.ALAVVLVPGNER.S
Zone 9	MarR family transcriptional regulator	G2LZT9 G2LZT9_9XANT		Xanthomona saxonopodis pv. Citrumelo	Plastidiac	04.99-Transcription/Others	6.59/16892	5	39	16892	508.3100	10.146.054	R.QLKQELEK.L
											508.3143	10.146.140	R.QLKQELEK.L
											508.3177	10.146.208	R.QLKQELEK.L
											508.3188	10.146.230	R.QLKQELEK.L
											508.3230	10.146.314	R.QLKQELEK.L
Zone 9	MADS544 protein	gi 38229879		Asarumcaudigerum	Plastidiac	04.99-Transcription/Others	9.32/24346	3	65	24346	5.083.100	10.146.054	K.QLKQLETR.L
											5.083.143	10.146.140	K.QLKQLETR.L
											5.083.177	10.146.208	K.QLKQLETR.L
											5.083.188	10.146.230	K.QLKQLETR.L
											5.083.230	10.146.314	K.QLKQLETR.L
Zone 9	Pis v 1 allergen 2S albumin	gi 55392779	51% to gi 110349081 Pistaciavera, E value:1e-37	Citrus sinensis	Vacuolar	06.20-Protein destination and storage/Storage proteins	8.94/28386	21	284	28386	7.959.252	15.898.358	R.QCCQQLQQLDNR.C
											5.222.352	10.424.558	R.CPGLEQALR.S
											5.223.121	10.426.096	R.CPGLEQALR.S
											5.228.157	10.436.168	R.CPGLEQALR.S
											7.804.659	15.589.172	R.SQLQQGQIQGMLR.Q
											6.253.573	12.487.000	R.QAYESASQIPR.K
											6.253.63	12.487.116	R.QAYESASQIPR.K

												1		
												4.377.644	8.735.142	R.KCNISPR.Q
												4.377.662	8.735.178	R.KCNISPR.Q
												4.377.677	8.735.208	R.KCNISPR.Q
Zone 10	Citrin	gi 56534768	86% to gi 1061408 Citrus sinensis, E value:1e-140	Citrus sinensis	Cytoplasmic	06.20-Protein destination and storage/Storage proteins	9.39/32434	13	161	32434	436.7902	8.715.658	R.FNLPILR.Y	
											476.2903	9.505.660	R.YIQLSAEK.G	
											522.8173	10.436.200	K.LQRPQM <u>Q</u> K.G + Oxidation (M)	
											822.5076	1643.0006	R.LLA ~~E~~ AFNVNPDLISK.L	
Zone 10	Pis v 1 allergen 2S albumin	gi 55392023	52% to gi 24473800 Anacardiummoccidentale, E value:7e-37	Citrus sinensis	Extracellular	06.20-Protein destination and storage/Storage proteins	8.46/23859	8	93	23859	508.3108	10.146.070	R.QLQ ~~Q~~ LETR.C	
											515.2954	10.285.762	R. <u>C</u> PGLEQAVR.R + Carbamidomethyl (C)	
Zone 11	Miraculin	gi 38032794	98 % to gi 119367468 Citrus hybrid cultivar, E value:4e-147	Citrus reticulata	Plastidiac	11.05-Disease/Defense /Stress responses	9.27/27659	23	326	27659	451.3063	9.005.980	R.RLALTNGR.A	
											451.3092	9.006.038	R.RLALTNGR.A	
											451.3095	9.006.044	R.RLALTNGR.A	

Zone 11	Stress related protein	gi 21650315	97 % to gi 116643152 Citrus sinensis (navel orange), E value:5e-106	Citrus sinensis	Plastidiac	11.05-Disease/Defense /Stress responses	7.08/22657	12	100	22657	469.7285	9.374.424	R.VDSYDPSR.G
											469.7617	9.375.088	R.VDSYDPSR.G
											503.3227	10.046.308	R.GAGGGGLTLFR.G
											503.3249	10.046.352	R.GAGGGGLTLFR.G
											531.2936	10.605.726	R.CNEPTVWR.V + Carbamidomethyl (C)
											531.2982	10.605.818	R.CNEPTVWR.V + Carbamidomethyl (C)
											550.3453	10.986.760	R.DYYLVSAIR.G
											562.3229	11.226.312	R.VDSYDPSRGK.W
											562.3242	11.226.338	R.VDSYDPSRGK.W
											619.4237	12.368.328	R.ALAVVLVPGNER.S
											619.4258	12.368.370	R.ALAVVLVPGNER.S
											619.4262	12.368.378	R.ALAVVLVPGNER.S
											619.4265	12.368.384	R.ALAVVLVPGNER.S
											619.4292	12.368.438	R.ALAVVLVPGNER.S
											619.4301	12.368.456	R.ALAVVLVPGNER.S
											619.9299	12.378.452	R.ALAVVLVPGNER.S
											619.9390	12.378.634	R.ALAVVLVPGNER.S
											619.9481	12.378.816	R.ALAVVLVPGNER.S
Zone 11	Stress related protein	gi 21650315	97 % to gi 116643152 Citrus sinensis (navel orange), E value:5e-106	Citrus sinensis	Plastidiac	11.05-Disease/Defense /Stress responses	7.08/22657	12	100	22657	458.7849	9.155.552	K.VVGTPDGGSK.S
											721.9543	14.418.940	K.NVELISGDGGPGSIK.K
Zone 11	Citrin	gi 56531223	85 % to gi 1061408 Citrus sinensis, E value:6e-128	Citrus sinensis	Cytoplasmic	06.20-Protein destination and storage/Storage proteins	9.53/30997	7	99	30997	476.3036	9.505.926	R.YIQLSAEK.G

