ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus, 20(4) 2021, 17–28
https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 https://doi.org/10.24326/asphc.2021.4.2
ORIGINAL PAPER
Accepted: 13.01.2020

PROTEOMIC STUDIES IN THE SYMBIOTIC ASSOCIATIONS BETWEEN ARBUSCULAR MYCORRHIZAL FUNGI *Funneliformis mosseae* WITH MELON (*Cucumis melo* L.) UNDER SALT CONDITIONS

Yong-Hong Lin[1] ✉ Chung-Li Wang[1] Juei-Yu Chiu[2]

[1] Department of Plant Industry, National Pingtung University of Science and Technology, Pingtung, Taiwan

[2] Department of Environmental Science and Engineering, National Pingtung University of Science and Technology, Pingtung, Taiwan

ABSTRACT

Arbuscular mycorrhizal fungi (AMF) that can cause mutualism with higher plants. Some studies showed that the symbiosis of AMF will increase nutrients absorption, the capacity of anti-stress (e.g. drought, salt and disease) by melon (*Cucumis melo* L.). This study evaluated the roles of proteins on salt-tolerance mechanism after melon was symbiotic with AMF (*Funneliformis mosseae*). The melons were cultivated in the hydroponic solution containing 0 M, 0.042 M or 0.084 M NaCl for inoculated AMF and non-AMF inoculated seedlings. Root apice of AMF seedling after treating with different NaCl concentrations that were chosen for the estimation of proteins. The results showed that 12 proteins were significantly different after treating with different sodium chlorite (NaCl) concentrations, with proteins that four upregulated and eight downregulated. The tolerance of NaCl stress by root of melon that was inoculated by AMF were attributable to cellular activities involved in carbohydrate metabolism, energy metabolism, production of organic acid, relief of salt injury, which may be critical for promotion of nutrients absorption, anti-stress. This study can offer an important clue to advanced genomic exploration for the inoculation of AMF on different plants.

Key words: arbuscular mycorrhizal fungi (AMF), melon, salt-tolerance, protein

INTRODUCTION

Plants can diminish the damage of environmental stress through increasing root growth, absorption of nutrients and water, reducing the activities of antioxidant enzyme, and decreasing osmotic pressure in plant cells [Bray 1997, Huang et al. 2011]. For enhancing the capacity of anti-stress, an method of mycorrhizal inoculation can be applied [Auge 2001, 2004]. The symbiotic association will generated when arbuscular mycorrhizal fungi (AMF) are symbiotic with the roots of some higher plants [Gadkar et al. 2001]. Certain AMF strains can only be symbiotic with plants with specificity with certain was speculated [Roldan-Fajardo 1994]. In fact, different *Funneliformis mosseae* of AMF can induce various mycorrhizal dependencies in plants had been established [Fidelibus et al. 2000, 2001, Marulanda et al. 2003]. Some *Funneliformis mosseae* are more efficient for improving the water stress of citrus plants regard to the promotion of root growth, e.g. *G. geosporum*, *G. mosseae* [Wu et al. 2007].

Some proteins produced in plants can make the tolerance of NaCl injury were recognized [Dhingra and Varghese 1985, Singh and Haseguwa 1987]. Chen and

✉ yonghong@mail.npust.edu.tw

Lin [2010] showed that 5 proteins were significantly upregulated, however, 3 proteins were apparently inhibited in tomato root when treated with 0.5% NaCl concentration. They concluded that NaCl tolerance in tomato root could be characterized by some cell physiological activities, which are important for tomato enduring in the high salt environment.

Melon (*Cucumis melo* L.) is one of commonly cultivated crops in southern Taiwan. The symbiosis of plants with AMF can increase the absorption of nutrients (e.g. phosphate, potassium), the capacity of anti-stress (e.g. drought, salt and disease) for melon were proved [Huang et al. 2012]. However, some mechanism of symbiotic association between AMF and melon was still not discovered. Sarabi [2017] showed that melon is a kind of salt-tolerance crop. It can endure the environment that containing 0.4% (0.068 M) NaCl in the solution [Shih and Huang 1993]. When the NaCl concentration in Hoagland hydroponic solution was increased up to about 0.084 M, it accompanied by an significant inhibition in some physiological functions in melon leaf and stem [Sarabi et al. 2017]. Ye et al. [2019] showed that the symbiosis of AMF on watermelon will increase its growth, elemental uptake, antioxidant and stress-response gene expressions under salinity-alkalinity stresses. This study was conducted to evaluate the roles of proteins playing in arbuscular mycorrhizal fungi *Funneliformis mosseae* with melon associations under different NaCl concentrations.

MATERIALS AND METHOD

Plant culture and mycorrhizal inoculation. The cultivar of melon (*Cucumis melo* L.) was Chiu-Hwa No. 2 which was purchased from "Knownyou Seedling Cooperation", Taichung, Taiwan. All experimental seedlings were chosen uniformly. At first, the plants were cultivated in the cultivative medium (vermiculite) containing spores, roots and hyphae. The seedlings were plug cultivated, the volume of every plug was 50 cm^3, and 2 g (100 AMF spores/g) of the AMF spore material was added. Non-AMF plants were non-symbiosis by AMF, and they were plug cultivated, the volume of every plug was 50 cm^3.

The hydroponic cultivation of inoculated melon. 10 AMF seedlings and 10 non-AMF seedlings was planted in the hydroponic solutions containing 0.0, 0.42 and 0.84 M NaCl, respectively. And three replicates of every treatment were proceeded. After 2 weeks, 3 plants were used for the biometric analysis, and 3 plants were used for the proteomic analysis from every treatment. The colonization of AMF in roots and the characterstics of AMF and non-AMF seedlings were estimated, respectively. And the arbuscules and vesicles were observed by fluorescent microscopy after dyed by trypan blue. On the other hand, the roots of melon seedlings were cleaned with ultrasonic instruments to remove the attached soil, and then cultivated in the planting-pots (5 L) containing hydroponic solution 0%, 0.042 M (EC = 2.2)% or 0.084 M (EC = 4.1) NaCl in the solution for AMF and non-AMF seedlings. Triplicate samples were prepared for this study. All melon seedling were cultivated in growth chamber that the conditions were set at 27°C in the day (14 h, 65% relative humidity [RH]) and 23°C at night (10 h, 85% RH), and the roots were aerated continuously. The ingredients of hydroponic solution was allocated according to Konishi et al. [1985] and replaced every 3 days. After 14 days, ten root apices of every seedling and three replicates grown in 0.0 M, 0.042 M and 0.084 M NaCl were separately collected. Root apices of seedlings under different treatments were put in sonicater and shaked for 3 min to remove impurities which were adsorbed on the surface of roots. The root apices 1 cm were cut and preserved in eppendoff tube and waiting for analysis.

Estimation of arbuscular mycorrhizal colonization. After 14 days NaCl treatment, all experimental plants roots were carefully cut 1 cm fragments, the described method of Koske and Gemma [1989] was to estimate the colonization of AMF symbiotic association with the root of melon. AMF colonization (%) was calculated as the description by Graham and Syvertsen [1985].

The estimation of plant growth and dry weight. The height of experimental plant was estimated after 14 days NaCl treatment, and then roots were cleaned with distilled water and their lengths were estimated in each treatment. Parts of the seedlings were 65°C dried 24 h, and then the dry weights of plants were determined, respectively.

Protein extraction and labeling. The methods of protein extraction and labeling were refered to the method of Zhou et al. [2002]. Proteins from pineapple root apices were extracted using extraction buffer

(2% Triton, 50 mmol L)1 Tris, pH 7.4) and harvested by trichloroacetic acid precipitation. For each precipitated sample, proteins were dissolved in a standard 2-D electrophoresis (2-DE) rehydration buffer (8 mol L^{-1}) urea, 2% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate (CHAPS), 0.5% immobilized pH gradient (IPG) buffer and 7 mmol L)1 dithiothreitol (DTT)). Each prepared 2-DE sample was subject to isoelectric focusing (IEF) using a 13 cm (the medium format 2-DE) IPG strip (pH 3–10, linear) in the IPGphor II system (GE Healthcare, Piscataway, NJ, USA) with a total of 10,000 voltage hours applied.

Proceeding of two-dimensional electrophoresis (2-DE), gel staining and spot picking. Refered to the method of Gorg et al. [1998], the proteins were labeled and isoelectric focusing (IEF) was proceeded. And then SDS-PAGE was accomplished. At last, 2D Gel Image Analysis software was applied to interpreted the images of gels. As described by Lin et al. [2009], 2-D gels were indicated using a VisPRO 5 stain kit. And then selected protein were excised at 1 mm in diameter.

MS sample preparation, analysis and protein identification. The excised protein spots were made, analyzed and identified refering to the MS sample preparation protocol [Gharahdaghi et al. 1999].

RESULTS

Arbuscular mycorrhizal fungi colonization. The symbiotic situation of roots was well in this experiment after melon inoculated with *Funneliformis mosseae*, about 72% AMF colonization levels of tested root segments were found in different treatments of NaCl concentrations, and there are no significantly differences. However, the AMF colonization are all 0 M in that of non-AMF treatments (Tab. 1).

Plant growth and biomass production. In the non-AMF treatments, plant heights, fresh weights and dry weights were significantly reduced in the treatments of 0.084 M NaCl than that of 0 M and 0.042 M. However, the above investigated traits of AMF seedlings were not affected in different NaCl treatments (Tab. 2).

Proteins expression in roots. After two-dimensional electrophoresis analysis, three representative maps for the treatment of 0, 0.042 M and 0.084 M NaCl are respectively shown in Fig. 1. (pH 3.0–10.0 range). pH 4.0–8.5 strips were chosen because proteins from root of melon are distributed in the isoelectric point (pI) range. Over three hundred protein spots were presented in the pH range of the gel map (Fig. 1) and more than sixty protein spots were expressed significantly.

After comparing with the spots in the gel maps no NaCl , 0.042 M and 0.084 M NaCl treatments, twelve spots of significantly expressed were chosen and analyzed. By interpreting, four protein spots in 0.084 M NaCl treatment were significantly abundant in the samples, but not express apparently in the treatment of no NaCl, we speculating that these different proteins expressed which were induced in higher concentration of NaCl. On the other hand, eight proteins were significantly decreased in the treating concentration of 0.084 M NaCl (Fig. 1–3).

MASCOT searching engine was selected for the identities of twelve proteins. Principles of this searching engine refered to the proteins that similar sequences in the NCBInr database. After identifying these proteins, in addition to three proteins were hypothetical and four was not identified, five were thought as structural proteins (Tab. 3). By interpreting the function of these functional proteins, these proteins were involved in glycolysis pathway, production of energy and organic acids, decrease of protelytic activity and negative transcription factor, and then the root morphology were maintained.

DISCUSSION

Melon is a kind of medium salt-tolerance crop. However, it could be well grown in the hydroponic solution containing up to 0.068M (0.4%) NaCl [Shih and Huang 1993]. However, the plant growth was negatively affected in the 0.5% NaCl concertration. It may be the negative effect by salt stress on seedlings. In fact, high soil salinity is one of the damages for plant cultivation [Hashem et al. 2014, 2016a]. An increasing rate of salinity will result in some stresses in plants by hindering their physiological and biochemical procedures [Balliu et al. 2015, Hashem et al. 2016a], which obstucts the growth of many crops [Gamalero et al. 2010, Iqbal et al. 2015]. On the other hand, the reactive oxygen species (ROS) will be induced and injury the function of nucleic acids and the permeability of cell membrane was reduced [Alqarawi et al. 2014a, 2014b].

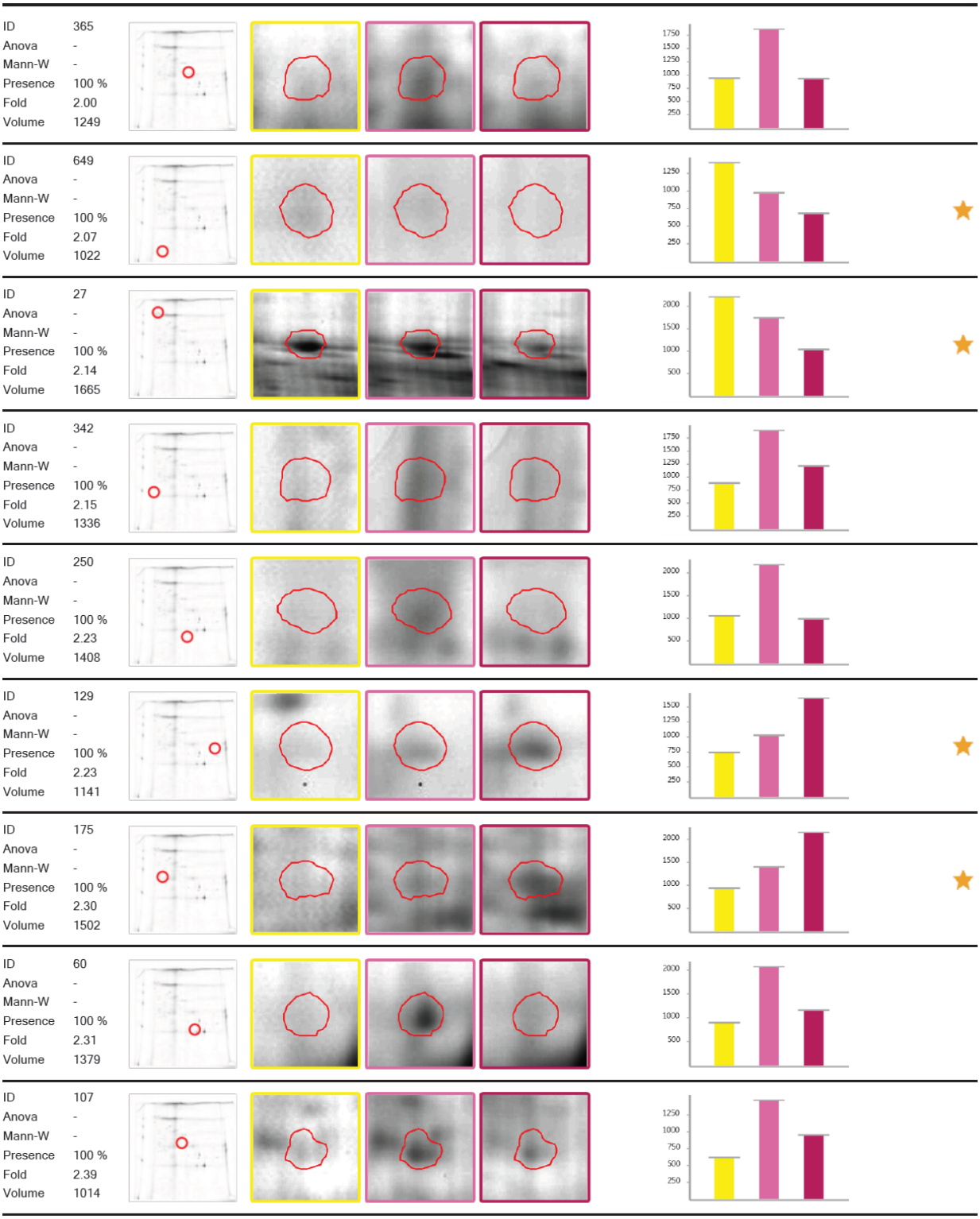

Fig. 1. Two upregulated and two downregulated significantly proteins on the 2-D proteins electrophoresis graphs of melon roots after the treatments of different NaCl concentration

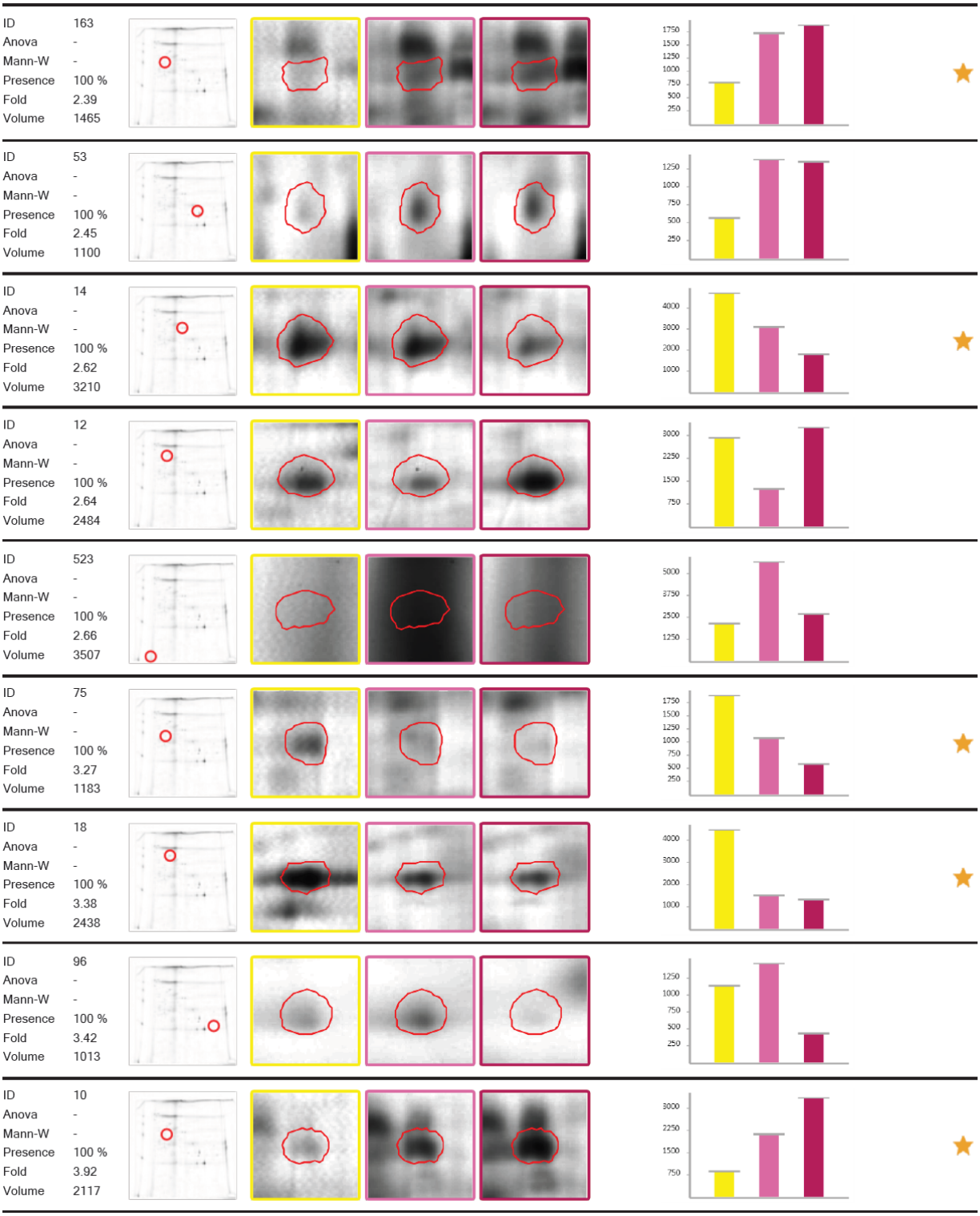

Fig. 2. Two upregulated and three downregulated significantly proteins on the 2-D proteins electrophoresis graphs of melon roots after the treatments of different NaCl concentration

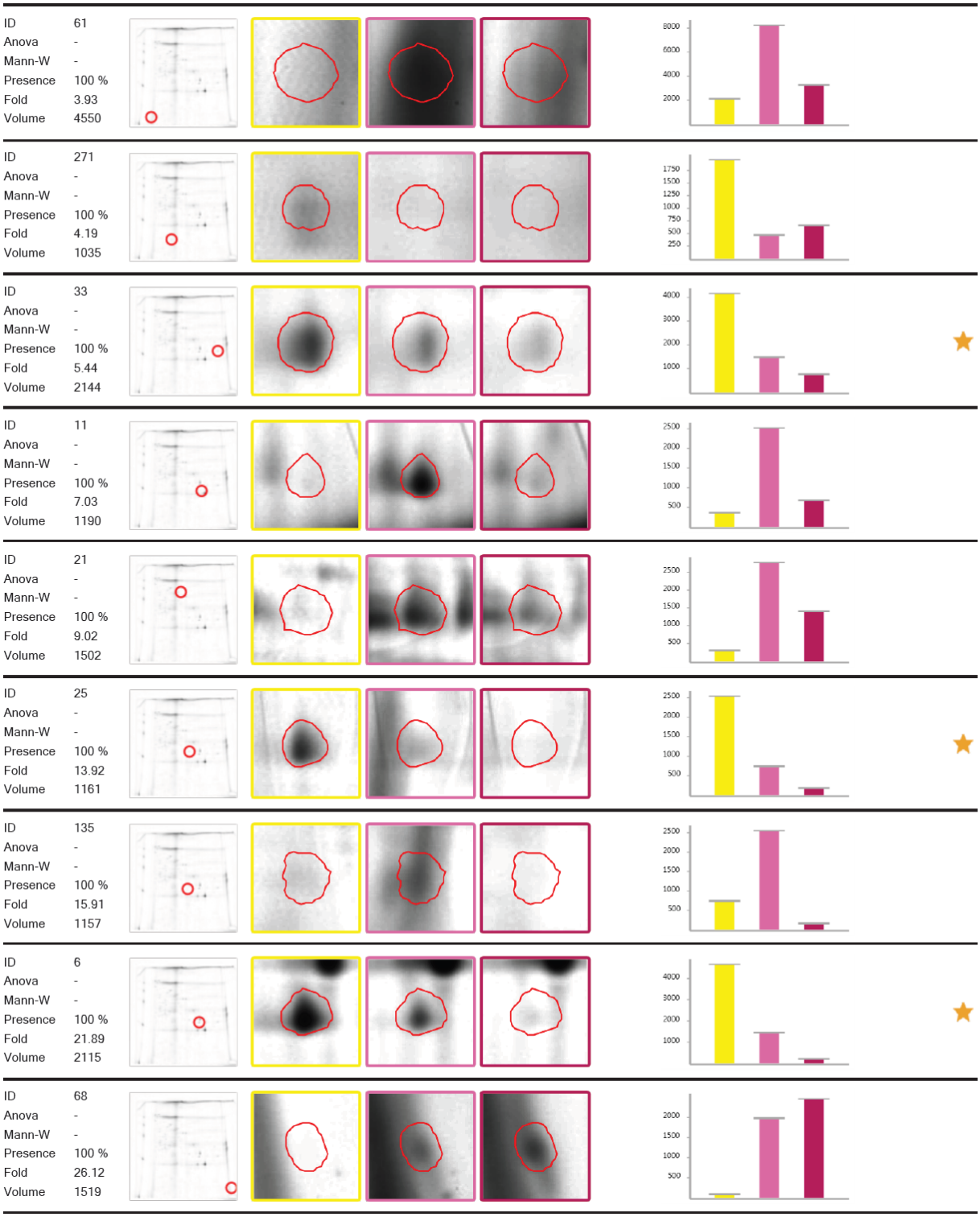

Fig. 3. Three downregulated significantly proteins on the 2-D proteins electrophoresis graphs of melon roots after the treatments of different NaCl concentrations

Table 1. Root arbuscular mycorrhizal colonization, vesicules and arbuscules of melon seedlings symbiosis with *Funneliformis mosseae* (AMF) or non-symbiotic plants (non-AMF) under saline stress

Treatment	AMF colonization (%)	Vesicles ($no \cdot cm^{-1}$)	Arbuscular ($no \cdot cm^{-1}$)
AMF + 0 M NaCl	72.5 ±1.9a	9.6 ±1.2a	13.3 ±1.7a
AMF + 0.042 M NaCl	71.8 ±1.1a	7.9 ±1.3a	10.6 ±1.3a
AMF + 0.084 M NaCl	72.1 ±1.3a	8.2 ±1.0a	11.0 ±1.2a
Non-AMF + 0% NaCl	0 ±0b	0 ±0b	0 ±0b
Non-AMF + 0.042 M NaCl	0 ±0b	0 ±0b	0 ±0b
Non-AMF + 0.084 M NaCl	0 ±0b	0 ±0b	0 ±0b

Values are means ±SD

Table 2. Aboveground plant height, root length, dry weight of melon seedlings symbiosis with *Funneliformis mosseae* (AMF) or non-symbiotic (non-AMF) plants under saline stress

Treatment	Plant height (cm)	Root length (cm)	Dry weight (g)
AMF + 0% NaCl	9.0 ±0.8a	22.5 ±0.9a	1.23 ±0.11a
AMF + 0.042 M NaCl	8.6 ±0.9a	20.7 ±0.7b	1.19 ±0.14a
AMF + 0.084 M NaCl	8.4 ±0.8a	21.2 ±0.8ab	1.18 ±0.15a
Non-AMF + 0% NaCl	8.1 ±0.4a	19.1 ±0.6bc	1.01 ±0.05a
Non-AMF + 0.042 M NaCl	7.4 ±0.6ab	16.8 ±0.5d	0.84 ±0.06b
Non-AMF + 0.084 M NaCl	6.7 ±0.3b	14.9 ±0.5e	0.66 ±0.03c

Values are means ±SD

Plant growth was promoted when arbuscular mycorrhiza fungi (AMF) was symbiotic with many land plants [Tang et al. 2009, Mo et al. 2016]. Additionally, it will improve tolerance of some abiotic stresses [Alqarawi et al. 2014a, Hashem et al. 2015].

Plant height, some root characteristics (length, dry weight) of AMF melon seedlings were not affected in different NaCl treatments (Tab. 2). Shekoofeh et al. [2012] showed the inoculation of arbuscular mycorrhiza fungi (AMF) *Ocimum basilicum* L. will improve some functions of plant including absorption of mineral elements and utilization efficiency of water in the environment of high salinity stress. Comparing to controls, the leaf area, some nutrients absorption of tomato seedlings will be increasing when inoculating AMF [Balliu et al. 2015]. The growth of cucumber was enhanced by the symbiosis of AMF due to the promoted adsorption of some essential elements [Hashem et al. 2018].

The root plays an important mechanism in soil environmental stress that were identified [Delhaize and Ryan 1995, Kochian 1995, Rengel 1996]. Formerly, genomics and transcripts were used as technology for studying the characteristics of various plants, but they were not sufficient for studying the defensive capacity

of some stress or providing favorable evidence to the effect of protein transformation and protein modification [Brumbarova et al. 2008]. In this experiment, non-AMF melon seedlings were apparently damaged in the increasing NaCl concentration, hence, the further study on the proteins variation of salt tolerance is meaningless. Differences among proteins in melon root apices symbiosis with arbuscular mycorrhizal fungi under varied NaCl concentration treatments are discussed below.

Enhancement of pentose phosphate pathway. In the melon roots inoculated with arbuscular mycorrhizal fungi and 0.5% NaCl treatment, the endochitinase MCHT-2 and orf774 were downregulated, their functions were more likely the process of sucrose synthesis, and causing the pathway for glycolysis was promoted [Selig et al. 1997] (Tab. 3). They supported that the fructose metabolism was downregulated in melon root apices under 0.5% NaCl concentration, and then causing glycolysis process were upregulat-

Table 3. LC/MS/MS identification of the differentially expressed proteins in melon roots

Spot No.		Accession No.	Protein description	MOWSE score	Experimental Mr / pI	Theoretical Mr / pI	Protein expression in
6	1	gi|224057715 gi|255547734*	predicted protein (*Populus trichocarpa*) PLE, putative (*Ricinus communis*)	46	31.01/7.36	66.66/5.91 66.32/6.38	0%
10	1	gi|53791748	hypothetical protein (*Oryza sativa* Japonica Group)	56	44.97/4.39	42.64/9.72	0.084 M
14	1 3	gi|218198433 gi|6911142	hypothetical protein OsI_23479 (*Oryza sativa* Indica Group) putative glycine-rich RNA binding protein 1 (*Catharanthus roseus*)	205 52	48.47/5.81	52.23/4.64 14.27/8.71	0%
18	1	gi|159472671	heat shock protein 70C (*Chlamydomonas reinhardtii*)	138	67.05/4.71	65.44/5.60	0%
25	1	gi|384250526	malate dehydrogenase (*Coccomyxa subellipsoidea* C-169)	90	32.45/6.45	34.06/5.54	0%
27	1	gi|195627118	rhomboid family protein (*Zea mays*)	48	110.86/3.58	43.58/6.05	0%
33	1	gi|23496435	endochitinase MCHT-2 (*Cucumis melo*)	83	31.35/9.13	34.80/8.57	0%
75	1 4	gi|302794672 gi|384253773	hypothetical protein SELMODRAFT_153081 (*Selaginella moellendorffii*) ARM repeat-containing protein (*Coccomyxa subellipsoidea* C-169)	69 49	42.09/4.28	43.45/6.19 100.35/5.17	0%
129	1 3	gi|205830697 gi|13486803	RecName: Full = Unknown protein 18 Epstein-Barr virus EBNA-1-like protein (*Oryza sativa* Japonica Group)	76 58	36.62/8.89	1.39/5.80 40.27/11.08	0.084 M
163	1 2	gi|205830697 gi|54606795	RecName: Full = Unknown protein 18 orf774 (*Beta vulgaris* subsp. *vulgaris*)	65 52	44.72/4.20	1.39/5.80 90.37/7.61	0.084 M
175	1 4	gi|303285200 gi|255070611	predicted protein (*Micromonas pusilla* CCMP1545) glutathione s-transferase (*Micromonas* sp. RCC299)	96 41	39.15/3.99	31.71/7.64 58.87/6.08	0.084 M
649	1	gi|2832470	R2R3-MYB transcription factor (*Arabidopsis thaliana*)	53	17.34/4.00	5.26/10.88	0%

*: Sameset protein

ed. Therefore the pentose phosphate pathway was enhanced. Step by step, the converting of glyceraldehyde-3-phosphate to pyruvate and to Acetyl CoA is promoted. Afterward, tricarboxylic acid (TCA) cycle is proceeded from the converting of acetyl CoA. There are some spots were identified as similar to MCHT-2 and orf774. It may be the expression of post translational modifications by the same gene.

Resistance of oxidative injury and secretion of organic acids. The inhibition of root growth is the typical injury with environmental stress (e.g. soil with strongly acidity, high salt concentration). In the results, glutathione s-transferase in root apices of melon was upregulated in response to high NaCl concentration. The studies of Hayes and Mclellan [1999] identified that the upregulation of glutathione s-transferase was for the increasing resistance of oxidative injury, and then maintaining the cellular structure [Sheehan et al. 2001].

The secretion of organic acid is the first mechanism for the stress-resistance of root apices, however, the important roles of proteins are now still unknown [Ling et al. 2002]. TCA cycle can produce energy in crops for NaCl-tolerance [Bansal et al. 2002], or provides organic acids for cations chelating cations for absorption in plant roots [Tiffin 1966, Lopez-Millan et al. 2000], and then causing the root protection. During TCA cycle process, the amount of organic acids will be accumulated abundantly. In our study, the malate dehydrogenase was downregulated in the treatment of 0.5% NaCl. The same phenomenon was described in the experimental results of sugar beet [Lopez-Millan et al. 2000] and Arabidopsis [Thimm et al. 2001].

Decrease of protelytic activity. Kanaoka et al. [2005] proved that molecular properties of Rhomboid family proteins in plants, and they characterized the Rhomboid family proteins stored in the Golgi apparatus of plant cells and play an important role in the specificity of substrate in the root of plants. Our results showed that the Rhomboid family proteins are downregulated when the concentration of NaCl was rise to 0.5%, it may cause the decrease of protein proteolytic activity and then maintain the integrity of root cells. Except that, endochitinase MCHT-2 is downregulated by increasing salt concentration up to 0.5% NaCl. Although the endochitinase influence generally the composition of hemicellulose and cellulose [Sánchez-Rodríguez et al. 2012], and it is advancely proved to has the resistant function against fungus disease [Cletus et al. 2013]. However, this protein may be not involved with salt treatment, or be negatively correlated with the increase of salt concentration.

Decrease of negative transcription factor. In common, GAs including ga1-3 (RGA) and GA-insensitive (GAI) can identify several signal transduction pathway [Bethke and Jones 1998; Sun 2000]. However, some proteins of negative regulator were encoded by RGA and GAI (including ARM repeat-containg protein) which was shown in some articles [Silverstone et al. 1998, Pysh et al. 1999]. About these proteins, some functional activities appears to be influenced by the ARM repeat-containg protein. The downregulation of ARM repeat-containg protein in this study was beneficial for the expression of some advantaging proteins, and made the salt tolerance of melon.

Unknown function of proteins. In addition to six main identified proteins which were involved in cultured in high NaCl concentration, there are some unknown function of proteins, for example, the orf774 in soybean [Gao 2014] and Espein-Barr virus EBNA-1--like protein in virus [Ceccarelli et al. 2000], were both upregulated. R2R3-MYB transcription factor which may be involved with the functions of some biological activities in plants [Du et al. 2012], is downregulated. On the other hand, one hypothetical protein that was upregulated and two dowregulated proteins that were unknown which may be furtherly estimated for the physiological functions in the tolerance of NaCl.

CONCLUSION

This result provides an information about the melon roots inoculated AMF response to high NaCl concentration. Melon roots require more energy to prevent injury and organic acids secreted for chelating Na in the environment containing high Na concentration. Thus, the enhancement of pentose phosphate pathway and TCA cycle are critical for melon roots providing more organic acids and ATP to adapt high salt concentration. On the other hand, the promotion of alleviating redox damage play an important role for root morphological maintenance that is critical, too. These results can make us to understand the adaptation reasons of melon root inoculated AMF to NaCl-stress.

ACKNOWLEDGEMENTS

The Innovation and Incubation Center in Fu-Jen Catholic University, Taipei, Taiwan, was appreciated for the analysis and identification of proteins. The English was critically corrected by Wallace Academic Editing for this article.

REFERENCES

Alqarawi, A.A., Hashem, A., Abd Allah, E.F., Alshahrani, T.S., Huqail, A.A. (2014b). Effect of salinity on moisture content, pigment system, and lipid composition in Ephedra alata Decne. Acta Biol. Hung., 65, 61–71. https://doi.org/10.1556/ABiol.65.2014.1.6

Alqarawi, A.A., Abd Allah, E.F., Hashem, A. (2014a). Alleviation of salt-induced adverse impact via mycorrhizal fungi in Ephedra aphylla Forssk. J. Plant Interact., 9, 802–810. https://doi.org/10.1080/17429145.2014.949886

Auge, R.M. (2001). Water relations, drought and vesiculararbuscular mycorrhizal symbiosis. Mycorrhiza, 11, 3–42. https://doi.org/10.1007/s005720100097

Balliu, A., Sallaku, G., Rewald, B. (2015). AMF inoculation enhances growth and improves the nutrient uptake rates of transplanted, salt-stressed tomato seedlings. Sustainability, 7(12), 15967–15981. https://doi.org/10.3390/su71215799

Bansal, P., Sharma, P., Goyal, V. (2002). Impact of lead and cadmium on enzyme of citric acid cycle in germinating pea seeds. Biol. Plant., 45, 125–127. https://doi.org/10.1023/A:1015173112842

Bray, E.A. (1997). Plant responses to water deficit. Trends Plant Sci., 2, 48–54. https://doi.org/10.1016/S1360-1385(97)82562-9

Bethke, P.C., Jones, R.L. (1998). Gibberellin signaling. Curr. Opin. Plant Biol., 1, 440–446. https://doi.org/10.1016/S1369-5266(98)80270-7

Brumbarova, T., Matros, A., Mock, H.P., Bauer, P. (2008). A proteomic study showing differential regulation of stress, redox regulation and peroxidase proteins by iron supply and the transcription factor FER. Plant J., 54, 321–334. https://doi.org/10.1111/j.1365-313X.2008.03421.x

Ceccarelli, D.F.J., Frappier, L. (2000). Functional analyses of the EBNA1 origin DNA binding protein of Epstein-Barr virus. J. Virol., 74, 4939–4948. https://doi.org/10.1128/.74.11.4939-4948.2000

Chen, J.H., Lin, Y.H. (2010). Sodium chloride causes variation in organic acids and proteins in tomato root. Afr. J. Biotechnol., 9, 8161–8167. https://doi.org/10.5897/AJB09.1994

Cletus, J., Balasubramanian, V., Vashisht, D. (2013). Transgenic expression of plant chitinases to enhance disease resistance. Biotechnol. Lett., 35, 1719–1732. https://doi.org/10.1007/s10529-013-1269-4

Delhaize, E., Ryan, P.R. (1995). Aluminum toxicity and tolerance in plants. Plant Physiol., 107, 315–321. https://doi.org/10.1104/pp.107.2.315

Du, H., Feng, B.R., Yang, S.S., Huang, Y.B., Tang, Y.X. (2012). The R2R3-MYB transcription factor gene family in maize. PloS ONE, 7(6): https://doi.org/10.1371/journal.pone.0037463

Dhingra, H.R., Varghese, T.M. (1985). Effect of growth regulators on the in vitro germination and tube growth of maize (*Zea mays* L.) pollen from plants raised under sodium chloride salinity. New Physiol., 100, 563–569. https://doi.org/10.1111/j.1469-8137.1985.tb02802.x

Fidelibus, M.W., Martin, C.A., Wright, G.C., Stutz, J.C. (2000). Effect of arbuscular mycorrhizal (AM) fungal communities on growth of 'Volkamer' lemon in continually moist or periodically dry soil. Sci. Hortic., 84, 127–140. https://doi.org/10.1016/S0304-4238(99)00112-0

Fidelibus, M.W., Martin, C.A., Stutz, J.C. (2001). Geographic isolates of *Glomus* increase root growth and whole-plant transpiration of citrus seedlings grown with high phosphorus. Mycorrhiza, 10, 231–236. https://doi.org/10.1007/s005720000084

Gadkar, V., David-Schwartz, R., Kunik, T., Kapulnik, Y. (2001). Arbuscular mycorrhizal fungal colonization. Factors involved in host recognition. Plant Physiol., 127, 1493–1499. https://doi.org/10.1104/pp.010783

Gamalero, E., Berta, G., Massa, N., Glick, B.R., Lingua, G. (2010). Interactions between *Pseudomonas putida* UW4 and *Gigaspora rosea* BEG9 and their consequences for the growth of cucumber under salt-stress conditions. J. Appl. Microbiol., 108, 236–245. https://doi.org/10.1111/j.1365-2672.2009.04414.x

Gao, D., Abernathy, B., Rohksar, D., Schmutz, J., Jackson, S.A. (2014). Annotation and sequence diversity of transposable elements in common bean (*Phaseolus vulgaris*). Front Plant Sci., 5, 339. https://doi.org/10.3389/fpls.2014.00339

Gharahdaghi, F., Weinberg, C.R., Meagher, D.A., Imai, B.S., Mische, S.M. (1999). Mass spectrometric identification of proteins from silver-stained polyacrylamide gel: a method for the removal of silver ions to enhance sensitivity. Electrophoresis, 20, 601–605. https://doi.org/10.1002/(SICI)1522-2683(19990301)20:3<601::AID-ELPS601>3.0.CO;2-6

Gorg, A., Postel, W., Gunther, S. (1998). The current state of two-dimensional electrophoresis with immobilized pH gradients. Electrophoresis, 9, 531–546. https://doi.org/10.1002/elps.1150090913

Graham, J.H., Syvertsen, J.P. (1985). Host determinants of mycorrhizal dependency of citrus rootstock seedlings. New Phytol., 101, 667–676. https://doi.org/10.1111/j.1469-8137.1985.tb02872.x

Hashem, A., Abd Allah, E., Alqarawi, A.A., El-Didamony, G., Alwhibi, M.S., Egamberdieva, D., Ahmad, P. (2014). Alleviation of adverse impact of salinity on faba bean (*Vicia faba* L.) by arbuscular mycorrhizal fungi. Pak. J. Bot., 46, 2003–2013. https://doi.org/10.1007/BF00041276

Hashem, A., Abd Allah, E.F., Alqarawi, A.A., Aldubise, A., Egamberdieva, D. (2015). Arbuscular mycorrhizal fungi enhances salinity tolerance of *Panicum turgidum* Forssk by altering photosynthetic and antioxidant pathways. J. Plant Interact., 10, 230–242. https://doi.org/10.1080/17429145.2015.1052025

Hashem, A., Abd Allah, E.F., Alqarawi, A.A., Al-Huqail, A.A., Wirth, S., Egamberdieva, D. (2016a). The interaction between arbuscular mycorrhizal fungi and endophytic bacteria enhances plant growth of *Acacia gerrardii* under salt stress. Front Microbiol., 7, 1089. https://doi.org/10.3389/fmicb.2016.01089

Hashem, A., Alqarawi, A.A., Radhakrishnan, R., Al-Arjani, A.B.F., Aldehaish, H.A., Egamberdieva, D., Abd Allah, E.F. (2018). Arbuscular mycorrhizal fungi regulate the oxidative system, hormones and ionic equilibrium to trigger salt stress tolerance in *Cucumis sativus* L. Saudi J. Biol. Sci., 25, 1102–1114. https://doi.org/10.1016/j.sjbs.2018.03.009

Hayes, J.D., McLellan, L.I. (1999). Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. Free Rad. Biol. Med. Res., 31, 273–300. https://doi.org/10.1080/10715769900300851

Huang, J.C., Jiang, W.J., Lin, G.C. (2012). Research on application of a growth medium containing mycorrhizal for nursery in melons (*Cucumis melo* L.) crops. Bull. Tainan District Agric. Res. Ext. Station, 60, 38–47. https://doi.org/ 10.29558/XLZY.201212.0005 (in Chinese)

Huang, Z., Zou, Z., He, C. et al. (2011). Physiological and photosynthetic responses of melon (*Cucumis melo* L.) seedlings to three Glomus species under water deficit. Plant Soil, 339, 391–399. https://doi.org/10.1007/s11104-010-0591-z

Iqbal, N., Umar, S., Khan, N.A. (2015). Nitrogen availability regulates proline and ethylene production and alleviates salinity stress in mustard (*Brassica juncea*). J. Plant Physiol., 178, 84–91. https://doi.org/10.1016/j.jplph.2015.02.006

Kanaoka, M., Urbanb, S., Freemanc, M., Okada, K. (2005). An Arabidopsis Rhomboid homolog is an intramembrane protease in plants. FEBS Letters, 579, 5723–5728. https://doi.org/10.1016/j.febslet.2005.09.049

Kochian, L.V. (1995): Cellular mechanisms of aluminum toxicity and resistance in plants. Plant Mol. Biol., 46, 237–260. https://doi.org/10.1146/annurev.pp.46.060195.001321

Konish, S., Miyamoto, S., Taki, T. (1985). Stimulatory effect of aluminum on tea plants grown under low and high phosphorus supply. Soil Sci. Plant Nutr., 31, 361–368. https://doi.org/10.1080/00380768.1985.10557443

Koonin, E.V., Makarova, K.S., Rogozin, I.B., Davidovic, L., Letellier, M.C., Pellegrini, L. (2003). The rhomboids: a nearly ubiquitous family of intramembrane serine proteases that probably evolved by multiple ancient horizontal gene transfers. Genom Biol., 4, 19. https://doi.org/10.1007/978-1-4020-6311-4_4

Koske, R.E., Gemma, J.N. (1989). A modified procedure for staining roots to detect VA mycorrhizas. Mycol. Res., 92, 486–505. https://doi.org/10.1016/S0953-7562(89)80195-9

Lin, C.Y., Wang, V.C., Shui, H.A., Juang, R.H., Hour, A.L., Chen, P.S., Huang, H.M., Wu, S.Y., Lee, J.C., Tsai, T.Z., Chen, H.M. (2009). A comprehensive evaluation of imidazole-zinc reverse stain for current proteomic researches. Proteomic, 9, 696–709. https://doi.org/10.1002/pmic.200700470

Ling, H. Q., Bauer, P., Bereczky, Z., Keller, B. (2002). The tomato fer gene encoding a bHLH protein controls iron-uptake responses in roots. Proc. Natl. Acad Sci. USA, 99, 13938–13943. https://doi.org/10.1073/pnas.212448699

Lopez-Millan, A.F., Morales, F., Abadia, A., Abadia, J. (2000). Effects of iron deficiency on the composition of the leaf apoplastic fluid and xylem sap in sugar beet. Implications for iron and carbon transport. Plant Physiol., 124, 873–884. https://doi.org/10.1104/pp.124.2.873

Mo, Y., Wang, Y., Yang, R., Zheng, J., Liu, C., Li, H., Ma, J., Zhang, Y., Wei, C., Zhang, X. (2016). Regulation of plant prowth, photosynthesis, antioxidation and osmosis by an arbuscular mycorrhizal fungus in watermelon seedlings under well-watered and drought conditions. Front. Plant Sci., 7, 644. https://doi.org/10.3389/fpls.2016.00644

Pysh, L.D., Wysocka-Diller, J.W., Camilleri, C., Bouchez, D., Benfey, P.N. (1999). The GRAS gene family in Arabidopsis: sequence characterization and basic expression analysis of the SCARECROW-LIKE gene. Plant J., 18, 111–119. https://doi.org/10.1046/j.1365-313X.1999.00431.x

Rengel, Z. (1996). Uptake of aluminium by plant cells. New Phytol., 134, 389–406. https://doi.org/10.1111/j.1469-8137.1996.tb04356.x

Roldan-Fajardo, B.E. (1994). Effect of indigenous arbuscular mycorrhizal endophytes on the development of six wild plants colonizing a semi-arid area in south-east Spain. New Phytol., 127, 115–121. https://doi.org/10.1111/j.1469-8137. 1994.tb04265.x

Sarabi, B., Bolandnazar, S., Ghaderi, N., Ghashghaie, J. (2017). Genotypic differences in physiological and bio-

chemical responses to salinity stress in melon (*Cucumis melo* L.) plants: Prospects for selection of salt tolerant landraces. Plant Physiol. Biochem., 119, 294–311. https://doi.org/10.1016/j.plaphy.2017.09.006

Sánchez-Rodríguez, C. (2012). Chitinase-like1/pom-pom1 and its homolog CTL2 are glucan-interacting proteins important for cellulose biosynthesis in *Arabidopsis*. Plant Cell, 24, 589–607. https://doi.org/10.1105/tpc.111.094672

Selig, M., Xavier, K.B., Santos, H., Schönheit, P. (1997). Comparative analysis of Embden-Meyerhof and Entner-Doudoroff glycolytic pathways in hyperthermophilic archaea and the bacterium Thermotoga. Arch. Microbiol., 167, 217–232. https://doi.org/10.1007/BF03356097

Sheehan, D., Meade, G., Foley, V.M., Dowd, C.A. (2001). Structure, function, and evolution of glutathione tranferrases: Implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem. J., 360, 1–16. https://doi.org/10.1042/0264-6021:3600001

Shekoofeh, E., Sepideh, H., Roya, R. (2012). Role of mycorrhizal fungi and salicylic acid in salinity tolerance of *Ocimum basilicum* resistance to salinity. J. Biotech., 11, 2223–2235. https://doi.org/10.5897/AJB11.1672

Shevchenko, A., Wilm, M., Vorm, O., Mann, M. (1996). Mass spectrometric sequencing of proteins from silver stained polyacrylamide gels. Anal. Chem., 68, 850–858. https://doi.org/10.1021/ac950914h

Shih, S.C., Huang, H. (1993). Effects of salt content on the germination and seedling growth of muskmelon. Bull. Kaoh. Agric. Ext. Station., 5, 62–70. (In Chinese) https://doi.org/10.19045/bspab.2018.700218

Silverstone, A.L., Ciampaglio, C.N., Sun, T.P. (1998). The Arabidopsis RGA gene encodes a trascriptional regulator repressing the gibberellin signal transduction pathway. Plant Cell., 10, 155–169. https://doi.org/10.2307/3870695

Singh, N.K., Haseguwa, P.M. (1987). Protein associate with adaptation of cultured to tobacco cells to NaCl. Plant Physiol., 7, 126–137, https://doi.org/10.1104/pp.79.1.126

Sun, T.P. (2000). Gibberellin signal transduction. Curr. Opin. Plant Biol., 3, 374–380, https://doi.org/10.1016/S1369-5266(00) 00099-6Tang, M., Chen, H., Huang, J.C., Tian, Z.Q. (2009). AM fungi effects on the growth and physiology of *Zea mays* seedlings under diesel stress. Soil Biol. Biochem., 41, 936–940, https://doi.org/10.1016/j.soilbio.2008.11.007

Thimm, O., Essigmann, B., Kloska, S., Altmann, T. (2001). Response of Arabidopsis to iron deficiency stress as revealed by microarray analysis. Plant Physiol., 127, 1030–1043. https://doi.org/10.1104/pp.010191

Tiffin, L.O. (1966). Iron translocation I. plant culture, exudate sampling, iron-citrate analysis. Plant Physiol., 41, 510–514. https://doi.org/10.1104/pp.41.3.510

Wu, Q.S., Zou, Y.N., Xia, R.X., Wang, M.Y. (2007). Five Glomus species affect water relations of Citrus tangerine during drought stress. Bot. Studies, 48, 147–154. https://doi.org/10.1093/aob/mcm113

Yamaguchi, T. (1983). World vegetables. AVI Press, Westport. https://doi.org/10.1007/978-94-011-7907-2_1

Ye, L., Zhao, X., Bao, E., Cao, K., Zou, Z. (2019). Effects of arbuscular mycorrhizal fungi on watermelon growth, elemental uptake, antioxidant, and photosystem II activities and stress-response gene expressions under salinity-alkalinity stresses. Front Plant Sci., 10, 863. https://doi.org/10.3389/fpls.2019.00863

Zhou, G., Li, H., DeCamp, D., Chen, S., Shu, H., Gong, Y., Flaig, M., Gillespie, J.W., Hu, N., Taylor, P.R., Emmert-Buck, M.R., Liotta, L.A., Petricoin, E.F., Zhao, Y. (2002). 2D differential in-gel electrophoresis for the identification of esophageal scans cell cancer-specific protein markers. Mol. Cell. Proteomics, 1, 117–124. https://doi.org/10.1074/mcp.M100015-MCP200