BIHAREAN BIOLOGIST 8 (1): 16-20
Article No.: 141104

http://biozoojournals.ro/bihbiol/index.html

Identification of Transport Proteins in Arabidopsis leaf plasma membrane by 2DLC-MALDI-TOF

Hamid NAJAFI-ZARRINI[1] and Parviz HEIDARI[2,*]

1. Department of Plant Breeding, Sari Agricultural Sciences and Natural Resources University, Iran
2. PhD Student in Plant Breeding, Sari Agricultural Sciences and Natural Resources University, Sari, Iran
*Corresponding author, P. Heidari, Tel.: +98-9193803455, E-mail: parvizh63@gmail.com

Received: 4. December 2013 / Accepted: 29. January 2014 / Available online: 19. February 2014 / Printed: June 2014

Abstract. Plasma membrane forms the barrier between the cell and the environment but also forms the passageway for information from the outside to the inside of the cell. Molecular and ionic movement from one location to another is known as transport. Local transport of solutes into or within cells is regulated mainly by membranes. Identification of anion channel in plants represents a goal for a better understanding of their central role in cell signaling, osmoregulation, nutrition, and metabolism. By separating the proteins in the plasma membrane fraction with ion exchange and reverse phase chromatography and analyzing the resulting fractions on a MALDI-TOF mass spectrometer, over 900 proteins could be detected. Of these proteins, 304 were annotated as membrane proteins, 70 transporter proteins and 35 important proteins in biotic and abiotic stresses were, distributed among the different protein families in proportions reflecting the distribution in the genome. Strong cation exchange and reverse phase chromatography and analysis of the resulting fractions on a MALDITOF mass spectrometer, are a powerful tool for retrieving plasma membrane proteins.

Key word: plasma membrane, transporter protein, MALDI-TOF, Stress.

Introduction

Proteomics is the study of proteins, particularly their structures and functions (Kakaei et al. 2010). Proteomics has a central role in the systems biology workflow and complements the analysis of the transcriptome and the metabolome (Baginsky & Gruissem 2006). To date, most large scale proteome analyses with *Arabidopsis* were performed with isolated organelles, the plasma membrane (Alexandersson et al. 2004, Ephritikine et al. 2004, Marmagne et al. 2004, Borner et al. 2005). The plasma membrane (PM) forms the barrier between the cell and the environment but also forms the passageway for information from the outside to the inside of the cell (Najafi-zarrini et al. 2012, Cordwell & Thingholm 2010, Zaeifizadeh et al. 2013). In plant cells, as well as in animal cells, the PM is involved in many primary cellular functions, such as metabolite and ion transport, endocytosis, cell differentiation and proliferation (Alexandersson et al. 2004). In a study by Marmagne et al (2004), about 100 putative plasma membrane proteins were detected in plasma membrane fractions enriched in hydrophobic proteins and obtained from an *Arabidopsis* cell suspension culture. It has not been possible to use 2D electrophoresis for integral proteins of eukaryotic plasma membranes (Alexandersson et al. 2004). Also Alexandersson et al (2004), with aqueous two-phase partitioning method were isolated. Plasma membranes and in total, 238 putative plasma membrane proteins were identified. Ions, sugars, amino acids, and sometimes water must be transported by a group of integral membrane proteins including channels, transporters, and ATP-powered ion pumps. In the present study, we applied a gel-based proteomic approach followed by optimized 2DLC-MS for *Arabidopsis* leaf plasma membrane fractions. We optimized existing methods in order to obtain a comprehensive plasma membrane protein profile, specifically aimed at the detection of transporter proteins.

Materials and Methods

Plant material preparation

Arabidopsis plants were grown under controlled conditions in soil at a short day regime, (8 hours light/16h dark) and a daily temperature of 21 °C and night temperature of 19 °C. Leaf material was collected after 6 weeks.

Plasma membrane isolation

Plasma membranes were purified essentially by aqueous polymer two-phase partitioning as described by Larsson et al. (1987). The whole preparation procedure was performed at 4°C. The plasma membrane pellet, containing 1.5-2 mg of protein, was resuspended in 0.5 ml of resuspension medium, frozen in liquid nitrogen and stored at -80° C until further use. Protein concentration was determined according to Bradford (1976) with bovine serum albumin (BSA) as standard.

SDS-PAGE and western blot analyses

SDS-polyacrylamide gel electrophoresis was carried out according to the method of Laemmli (1970) on a 10% acrylamide separation gel and a 4% stacking gel. For western blot analysis, proteins were transferred from gel to Polyvinylidene Fluoride (PVDF) sheet by semidry approach. Detection was accomplished by the alkaline phosphatase-based chemiluminescent detection assay; Western light produced using a goat anti-rabbit antibody linked to alkaline phosphatase (Sigma, A-3687) with a CDP-Star as substrate (Roche). The chemiluminescence was detected by using the CCD camera system (Lumi-Image F1, Roch). To test the enrichment and purity of the plasma membrane fraction the presence of proteins that are considered to be characteristic for specific membrane types were used (Table 1). The enrichment of PM was assessed by immunological tests using antibodies raised against proteins specifically associated with the different cellular membranes.

In-gel tryptic digestion and MALDI-TOF

For mass spectrometry based protein identifications, 50 µg of the purified membrane fraction was separated on SDS-PAGE. Following staining with coomassie blue, the gel lane was divided in 24 slices. Excised gel fractions were washed with 50% acetonitrile in 50 mM NH_4HCO_3, at room temperature, until complete destain. For off-line peptide pre-fractionation, a silica-based Polysulfoethyl Aspartamide strong cation exchange (SCX) column was used (PolyLC Inc., Columbia USA). The separation was run on an Ettan-MDLC system

Table 1. Characteristics of the antibodies rose against specific membranes (MW=molecular weight).

Antibody name	Target protein	MW	Target membrane	Dilution
758	pm-H+-ATPase	104	Plasma membrane	1:10000
Anti-VVP	pyrophosphatase	80.8	Tonoplast	1:1000
Anti-Toc75	atToc75	75	Chloroplast	1:1000
PM022	β-subunit ATPase	55	Mitochondria	1:100
PM035	Porin	29-35	Mitochondria	1:100

(Amersham Biosciences AB, Uppsala, Sweden) at a flow rate of 200 µL/min. Depending on the complexity, either separate fractions or pools of two fractions were analyzed by RP-LC MALDI-TOF as described above, with the only exception that no internal standard were used for MS spectra calibration. Collected mass spectrograms were quantified using Protein Pilot (Applied Biosystems).Protein identifications were confirmed using Mascot (version2.1) and the TAIR7 sequence database as described above. All matches with a confidence of identification higher than 95% were considered.

Protein identification and data mining

Protein identification was carried out using Mascot (version 2.1, Matrix Science, London, UK) and ProteinPilot (Applied Biosystems, Foster City, CA, USA) searching against a the TAIR7 release of the *Arabidopsis* proteome (www.arabidopsis.org), combined with reversed entries for all protein sequences. Peptide tolerance was set to 0.2 and 0.4 Da for MS and MS/MS respectively, allowing for 1 missed trypsin cleavage. Oxidation of methionine residues, deamidation of asparagine and glutamine were specified as variable modifications. Protein identifications based on at least 2 peptides, identified independently with probability higher than 95%, were accepted.

Result and Discussion

Plasma membrane isolation and assessment of PM enrichment

As one of the objectives of this study was the identification of transporter proteins. We did not use any procedure aimed at 'stripping' the vesicles like washing with organic solvents, alkaline treatments, salt treatments or sonication (Blackler et al. 2008, Marmagne et al. 2008). Western blot analysis indicated that antigens from the mitochondrial membranes (PM022 and PM035) and chloroplast membranes (Toc37) were presented in microsomal fractions, but cannot be detected in the PM fraction. Only a weak anti-VVP signal, a marker for the tonoplast, was presented in the PM fraction. The mitochondrial, chloroplastic and vacuolar marker proteins were enriched in the endomembrane fraction. The H+-ATPase, a marker for the plasma membrane, was enriched in the PM fraction. Using the Mascot search algorithm, more than 1000 proteins could be matched with *Arabidopsis* proteins in the TAIR7 protein database. From this total number of proteins, 818 were identified with a confidence level > 99% by a minimum of two unique peptides. The two phase partition plasma membrane purification followed by 2DLC results in the most comprehensive profile so far, even though some of the proteins identified by other methods were not presented in our profile. Using on Targetp (www.cbs.dtu.dk/services/TargetP), 304 of the identified proteins are predicted to be membrane proteins. Among them 177 are indicated to be secreted proteins. 66 are mitochondrial and 61 are chloroplastic proteins. According to SUBA (http://www.plantenergy.uwa.edu.au/suba2/) 143 proteins of all identified proteins in this study have been used previously in GFP/YFP-based localization experiments. More than 40% of these proteins were localized in the plasma membrane. Proteomics studies have associated some of the proteins in our profile with other cell compartments. Proteins that were identified in the profile, but have no transmembrane domain, might be anchored to the PM through myristoylation or prenylation (GTP-binding proteins) or by other uncharacterized means like for instance P24 (Marmagne et al. 2004). The fairly high number (126) of unknown proteins is an illustration of our still sketchy knowledge of the plasma membrane composition, mainly due to the technical difficulties in extraction, separation and identification of hydrophobic proteins by mass spectrometry.

Transport proteins

As expected, transporters, pumps, and channels were identified in the plasma membrane (Table 2). Phylogenetic and gene structure analysis of plant H+-ATPases divided them into five subfamilies (Arango et al. 2003).The AHA3 isoform, present in the current profile, was earlier demonstrated in leaf tissue (Arango et al., 2003), where it is located in the plasma membrane of phloem companion cells (DeWitt & Sussman 1995, Chen et al. 2011). However, AHA4 that was identified in the plasma membrane proteome by Alexandersson et al. (2004) could not be detected in the present study. Transmembrane ATPases import many of the metabolites necessary for cell metabolism and export toxins, wastes, and solutes that can hinder cellular processes. Of the 13 plasma membrane aquaporins (PIPs) present in the *Arabidopsis* genome, 9 were identified. The localization of PIP1-2, PIP2-1 and PIP2-7 to the plasma membrane has been confirmed by GFP fusion proteins (Cutler et al. 2000). Some transport proteins that were not endogenous to the plasma membrane such as the H+-PPase (AVP1), two ABC-transporters (PDR8 and MDR11), V-ATPase subunits and one TIP isoform (TIP2) , usually regarded as vacuolar membrane proteins (Heazlewood et al., 2004), were also identified in our plasma membrane profile. Auxin transport is mediated by several membrane transport proteins such as PIN-FORMED (PIN), AUXIN RESISTANT1 (AUX1)/LIKE-AUXIN RESISTANT1, and ABCB/PGP/MDR (Cho et al. 2012). All plants require nitrogen (N) as an essential nutrient and are able to acquire N from nitrate (NO3) and ammonium (NH4) in the soil. Nitrate acquisition begins with its transport into root cells, accomplished by NO3 transporters (Bagchi et al. 2012). In addition detected ammonium transport, nitrate transporter and Major facilitator superfamily protein (type of nitrate transporter). The integral membrane proteins of the ammonium transporter (AMT/Rh) family provide the major route for shuttling ammonium (NH4/NH3) across bacterial, archaeal, fungal and plant

Table 2. List of identified transporter and stress proteins with confidence level ≥ 99%.

AGI acc no	Protein	MW
Transporter		
AT1G17840	white-brown complex homolog protein 11	78
AT1G59870	ABC-2 and Plant PDR ABC-type transporter	165
AT2G36910	ATP binding cassette subfamily B1	141
AT2G39480	P-glycoprotein 6	156
AT3G28860	ATP binding cassette subfamily B19	137
AT3G62150	P-glycoprotein 21	140
AT3G62700	multidrug resistance-associated protein 10	172
AT4G25960	P-glycoprotein 2	140
AT5G06530	ABC-2 type transporter family protein	83
AT1G58360	amino acid permease 1	53
AT5G01240	like AUXIN RESISTANT 1	55
AT5G40780	lysine histidine transporter 1	50
AT5G40780.2	lysine histidine transporter 1	50
AT5G49630	amino acid permease 6	53
AT2G38290	ammonium transporter 2	51
AT4G03560	two-pore channel 1	85
AT5G57110	autoinhibited Ca2+ -ATPase, isoform 8	116
AT1G01620	plasma membrane intrinsic protein 1C	31
AT2G37170	plasma membrane intrinsic protein 2	30
AT2G37180	Aquaporin-like superfamily protein	30
AT2G39010	plasma membrane intrinsic protein 2E	31
AT2G45960	plasma membrane intrinsic protein 1B	31
AT3G53420	plasma membrane intrinsic protein 2A	30
AT3G61430	plasma membrane intrinsic protein 1A	31
AT4G00430	plasma membrane intrinsic protein 1;4	31
AT4G23400	plasma membrane intrinsic protein 1;5	31
AT4G35100	plasma membrane intrinsic protein 3	30
AT1G22530	PATELLIN 2	76
AT1G29310	SecY protein transport family protein	52
AT1G30690	Sec14p-like phosphatidylinositol transfer family protein	61
AT1G32050	SCAMP family protein	30
AT1G61250	secretory carrier 3	33
AT1G72150	PATELLIN 1	64
AT1G72160	Sec14p-like phosphatidylinositol transfer family protein	56
AT2G27810	nucleobase-ascorbate transporter 12	77
AT3G51670	phosphoglyceride transfer family protein	47
AT1G12110	nitrate transporter 1.1	65
AT2G26690	Major facilitator superfamily protein	64
AT3G21670	Major facilitator superfamily protein	65
AT1G57990	purine permease 18	44
AT1G12840	vacuolar ATP synthase subunit C (VATC)	43
AT1G78900	vacuolar ATP synthase subunit A	69
AT2G21410	vacuolar proton ATPase A2	93
AT3G01390	vacuolar membrane ATPase 10	12
AT4G23710	vacuolar ATP synthase subunit G2	12
AT4G39080	vacuolar proton ATPase A3	93
AT5G62670	H(+)-ATPase 11	105
AT2G18960	H(+)-ATPase 1	104
AT4G30190	H(+)-ATPase 2	104
AT5G57350	H(+)-ATPase 3	104
AT1G76030	ATPase, V1 complex, subunit B protein	54
AT3G28710	ATPase, V0/A0 complex, subunit C/D	41
AT3G42050	vacuolar ATP synthase subunit H family protein	50
AT3G58730	vacuolar ATP synthase subunit D (VATD)	29
AT4G02620	vacuolar ATPase subunit F family protein	14
AT4G11150	vacuolar ATP synthase subunit E1	26
AT4G38510	ATPase, V1 complex, subunit B protein	54
AT1G52190	Major facilitator superfamily protein	67

Continued on next page

Table 2. (contiued).

AGI acc no	Protein	MW
AT3G17650	YELLOW STRIPE like 5	79
AT3G47960	Major facilitator superfamily protein	71
AT3G54140	peptide transporter 1	64
AT4G16370	oligopeptide transporter	82
AT3G18830	polyol/monosaccharide transporter 5	58
AT4G02050	sugar transporter protein 7	56
AT1G71880	sucrose-proton symporter 1	55
AT3G51895	sulfate transporter 3;1	73
AT1G11260	sugar transporter 1	58
AT3G19930	sugar transporter 4	57
AT5G26340	Major facilitator superfamily protein	57
AT1G22710	sucrose-proton symporter 2	55
Abiotic stress		
AT3G17020	Adenine nucleotide alpha hydrolases-like superfamily protein	18
AT3G53990	Adenine nucleotide alpha hydrolases-like superfamily protein	18
AT5G15970	stress-responsive protein (KIN2) / cold-responsive protein	7
AT5G52300	CAP160 protein	66
AT5G52310	low-temperature-responsive protein 78 (LTI78)	78
AT1G13930	Involved in response to salt stress	16
AT1G30360	Early-responsive to dehydration stress protein (ERD4)	82
AT4G04340	ERD (early-responsive to dehydration stress) family protein	88
AT5G25610	BURP domain-containing protein	42
AT1G79930	heat shock protein 91	92
AT3G09440	Heat shock protein 70 (Hsp 70) family protein	71
AT4G16660	heat shock protein 70 (Hsp 70) family protein	97
AT5G56010	heat shock protein 81-3	80
AT5G56030	heat shock protein 81-2	80
AT1G20440	cold-regulated 47	30
AT1G20450	Dehydrin family protein	30
AT1G54410	Dehydrin family protein	11
AT1G76180	Dehydrin family protein	21
AT2G21620	Adenine nucleotide alpha hydrolases-like superfamily protein	21
AT3G26450	dehydrase and lipid transport superfamily protein	18
AT4G23670	dehydrase and lipid transport superfamily protein	18
Biotic stress		
AT1G20780	senescence-associated E3 ubiquitin ligase 1	88
AT3G28940	AIG2-like (avirulence induced gene) family protein	19
AT5G43470	Disease resistance protein (CC-NBS-LRR class) family	105
AT1G12280	LRR and NB-ARC domains-containing disease resistance protein	103
AT1G62630	Disease resistance protein (CC-NBS-LRR class) family	102
AT2G32680	receptor like protein 23 (PR)	98
AT3G50950	HOPZ-ACTIVATED RESISTANCE 1 (PR)	97
AT5G40170	receptor like protein 54 (PR)	89
AT5G45490	P-loop containing nucleoside triphosphate hydrolases superfamily protein	40
AT5G48620	Disease resistance protein (CC-NBS-LRR class) family	104
AT1G19110	inter-alpha-trypsin inhibitor heavy chain-related	83
AT1G72500	EXPRESSED DURING: inter-alpha-trypsin inhibitor	85
AT5G47910	respiratory burst oxidase homologue D	104
AT4G02600	Seven transmembrane MLO family protein	59
AT3G17020	Adenine nucleotide alpha hydrolases-like superfamily protein	88

membranes (Ludewing et al. 2007) Identification and characterization of anion channel genes in plants represent a goal for a better understanding of their central role in cell signaling, osmoregulation, nutrition, and metabolism (Marmagne et al. 2004). Monosaccharide transporter, sucrose-proton symporter and sugar transporter have detected in plasma membrane. Sucrose transporters (SUTs) are important for both phloems loading in source tissue and sucrose uptake into some sink cells. SUTs utilize the transmembrane proton gradient to drive sucrose uptake into the cytoplasm (Sun et al. 2010).Oxidized glutathione and L-cystine, strongly enhanced St SUT1–mediated sucrose uptake, and the increased rates of transport were reached immediately after the addition of the oxidants (Krugel et al. 2008). Sucrose transporters (SUTs) are important for both phloem loading in source tissue and sucrose uptake into some sink cells. SUTs utilize the

transmembrane proton gradient to drive sucrose uptake into the cytoplasm (Sun et al. 2010). Molecular and ionic movement from one location to another is known as transport. Local transport of solutes into or within cells is regulated mainly by membranes.

Proteins involved in stress

35 proteins, which are involved in biotic (14 proteins) and abiotic (21 proteins) stresses, were identified (Table 2). Four identified proteins are involved in heat stress. Heat shock proteins (Hsp) are involved in the folding of denatured proteins. Most heat shock proteins are molecular chaperones. Hsp70, 91 and 81 are identified, however Heat-shock proteins are named according to their molecular weight. The HSP70 family is necessary for protein synthesis, translocation, and folding (Tkacova & Angelovicova 2012). Dehydrin family protein and adenine nucleotide alpha hydrolases, also identified in the protein profile, are involved in cold, drought and salt stresses. Nine proteins, which are effective in disease resistance, were identified.

With more than 900 proteins that were identified, this study has yielded the largest dataset on the plant plasma membrane proteome so far. The analysis resulted in the identification of 70 transporter proteins and 35 important proteins in biotic and abiotic stresses with a high confidence level, most of which were detected at the protein level for the first time and for most there is no functional annotation present. The data exemplify that the combination of plasma membrane enrichment, peptide purification with strong cation exchange and reverse phase chromatographyand analysis of the resulting fractions on a MALDITOF mass spectrometer, is a powerful tool for retrieving plasma membrane proteins.

References

Alexandersson, E., Saalbach, G., Larsson, C., Kjellbom, P. (2004): *Arabidopsis* plasma membrane proteomics identifies components of transport, signal transduction and membrane trafficking. Plant Cell Physiology 45: 1543–1556.

Arango, M., Gevaudant, F., Oufattole, M., Boutry, M. (2003): The plasma membrane proton pump ATPase: the significance of gene subfamilies. Planta 216: 355-365.

Bagchi, R., Salehin, M. O., Adeyemo, S., Salazar, C., Shulaev, V., Sherrier, D.J., Dickstein, R. (2012): Functional Assessment of the *Medicago truncatula* NIP/LATD Protein emonstrates That It Is a High-Affinity Nitrate Transporter. Plant Physiology 160: 906-916.

Baginsky, S., Gruissem, W. (2006): *Arabidopsis thaliana* proteomics: from proteome to genome. Journal of Experimental Botany 57: 1485–1491.

Blackler, A.R., Speers, A.E., Ladinsky, M.S., Christine, C.W. (2008): A shotgun proteomic method for the identification of membrane-embedded proteins and peptides. Journal of Proteome Research 7: 3028-3034.

Borner, G.H., Sherrier, D.J., Weimar, T., Michaelson, L.V., Hawkins, N.D., Macaskill, A., Napier, J.A., Beale, M.H., Lilley, K.S., Dupree P. (2005): Analysis of detergent-resistant membranes in *Arabidopsis*: evidence for plasma membrane lipid rafts. Plant Physiology 137: 104–116.

Bradford, M.M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248–254

Chen, J., Liu, Y., Ni, J., Wang, Y., Bai, Y., Shi, J., Gan, J., Wu, Z., Wu P. (2011): OsPHF1 Regulates the Plasma Membrane Localization of Low- and High-Affinity Inorganic Phosphate Transporters and Determines Inorganic Phosphate Uptake and Translocation in Rice. Plant Physiology 157: 269–278.

Cho, M., Lee, Z.W., Cho, H.T. (2012): ATP-Binding Cassette B4, an Auxin-Efflux Transporter, Stably Associates with the Plasma Membrane and Shows Distinctive Intracellular Trafficking from That of PIN-FORMED Proteins. Plant Physiology 159: 642–654.

Cordwell, S.J., Thingholm, T.E. (2010): Technologies for plasma membrane proteomics. Proteomics 10: 611–627.

Cutler, S.R., Ehrhardt, D.W., Griffitts, J.S., Somerville, C.R. (2000): Random GFP::cDNA fusions enable visualization of subcellular structures in cells of *Arabidopsis* at a high frequency. PNAS 97: 3718–3723.

DeWitt, N.D., Sussman, M.R. (1995): Immunocytological localization of an epitope-tagged plasma membrane proton pump (H+-ATPase) in phloem companion cells. Plant Cell 7: 2053–2067.

Ephritikhine, G., Ferro, M., Rolland,N. (2004): Plant membrane proteomics. Plant Physiology and Biochemistry 42: 943–962.

Heazlewood, J.L., Tonti-Filippini, J.S., Gout, A.M., Day, D.A., Whelan, J., Millar, A.H. (2004): Experimental analysis of the *Arabidopsis* mitochondrial proteome highlights signaling and regulatory components, provides assessment of targeting prediction programs, and indicates plant-specific mitochondrial proteins. Plant Cell 16: 241–256.

Kakaei, M., Kahrizi, D., Mostafaie, A. (2010): Study on powdery mildew disease related proteins expression in winter wheat cultivars via SDS-PAGE. Biharean Biologist 2: 169-171.

Krugel, U., Veenhoff, L.M., Langbein, J., Wiederhold, E., Liesche, J., Friedrich, T., Grimm, B., Martinoia, E., Poolman, B., Kuhna, C. (2008): transport and Sorting of the *Solanum tuberosum* Sucrose Transporter SUT1 Is Affected by Posttranslational Modification. The Plant Cell 20: 2497–2513.

Laemmli, U.K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685.

Larsson, C., Widell, S., Kjellbom, P. (1987): Preparation of high-purity plasma membranes. Methods in Enzymology 148: 558–568.

Ludewing, U., Neuhauser, B., Dynowski, M. (2007): Molecular mechanisms of ammonium transport and accumulation in plant. FEBS Letters 581: 2301-2308.

Marmagne, A., Ferro, M., Meinnel, T., Bruley, T., Kuhn, L., Garin, J., Barbier-Brygoo, H., Ephritikhine, G. (2008): A high content in lipid-modified peripheral proteins and integral receptor kinases features in the *Arabidopsis* plasma membrane proteome. Molecular and Cellular Proteomics 6: 1980-1996.

Marmagne, A., Rouet, M.A., Ferro, M., Rolland, N., Alcon, C., Joyard, J., Garin, J., Barbier, B.H., Ephritikhine, G. (2004): Identification of new intrinsic proteins in *Arabidopsis* plasma membrane proteome. Molecular and Cellular Proteomics 3: 675–691.

Najafi-Zarrini, H., Fusetti, F., Elzenga, J.T.M., Lanfermeijer, F.C. (2012): *Arabidopsis* leaf plasma membrane proteome using a gel free method: Focus on receptor–like kinases. Journal of Plant Molecular Breeding 1: 25-33.

Sun, Y., Reinders, A., LaFleur, K.R., Mori, T., Ward, J.M. (2010): Transport Activity of Rice Sucrose Transporters OsSUT1 and OsSUT5. Plant Cell Physiol 51: 114–122.

Tkacova, J., Angelovicova, M. (2012): Heat Shock Proteins (HSPs): a Review. Animal Science and Biotechnologies 45: 349-353.

Zaeifizadeh, M., Tahmasebi Enferadi, S., Mousavi, A., Heidari, P., Ahmadizadeh, M. (2013): Quick method for screening of tolerant sunflower (*Helianthus annuus* L.) genotypes to *Sclerotinia sclerotiorum* at seedling stage. Biharean Biologist 7: 29-32.