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REVIEW

Adaptive mechanisms of medicinal plants along altitude gradient: contribution of proteomics

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Abstract

Medicinal plants are a rich source of secondary metabolites extensively used in traditional health care systems. High altitude biodiversity encompasses the diversified and valuable medicinal plant species. The extreme environmental conditions of high altitude region *viz*. fluctuating temperatures, high UV radiation, salinity, low oxygen concentration, and high wind velocity limits the plant growth and distribution. Yet, how medicinal plants respond to these extreme conditions is not sufficiently understood. Therefore, addressing plant acclimation to different stresses presents an opportunity to unravel adaptive mechanism of medicinal plants along altitude gradient. This article reviews the recently published research that highlights the major role of proteins in plant adaptation to extreme environmental conditions. In the last few decades, climate change has made a profound impact on high altitude plants. Stress conditions alter cellular homeostasis of plants. With the advent of proteomics, it has become evident that stresses induce changes in proteome by synthesis/expression of novel stress responsive proteins. These proteins constitute a highly organized, complex network that leads to changes in the molecular, biochemical, physiological, and morphological responses of plants. Herein, we comprehensively discuss the proteomics of medicinal plants and its role in adaptation along altitude gradient. This review aims to provide impetus to current research in medicinal plants ranging from developmental to stress biology and to generate basis for genetic engineers and plant breeders to produce next-generation medicinal plants.

Additional key words: antifreeze proteins, climate change, heat shock proteins, photosynthesis, reactive oxygen species, secondary metabolites.

Introduction

In the post-genomic era, application of proteomics is important for understanding biological systems. Proteomic study offers a plethora of information from protein identification to quantitative profiling, sub-cellular localization, signalling pathways, post-translational modifications (PTMs), and protein-protein interactions in a tissue, cell, or organelle (Chattopadhyay *et al*. 2011, Agrawal *et al*. 2013, Jaiswal *et al*. 2013, Subba *et al*. 2013a,b, Kumar *et al*. 2014). In addition, proteomic study also highlighted plant adaptations to various stress conditions (Hu *et al*. 2015). Classical two-dimensional gel electrophoresis (2-DE) coupled with mass spectrometry (MS) has been widely used for proteomic studies of different abiotic stresses. The emergence of next generation proteomic tools, such as stable isotope labelling with amino acids in cell culture (SILAC), isobaric tags for relative and absolute quantitation (iTRAQ), multiple reaction monitoring

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Abbreviations: AFPs - antifreeze proteins; FACE - free air concentration enrichment; FATI - free air temperature increase; HSPs - heat shock proteins; iTRAQ - isobaric tags for relative and absolute quantitation; LEA - late embryogenesis abundant; LT - low temperature; MALDI - matrix-assisted laser desorption ionization; MRM - multiple reaction monitoring; MS - mass spectrometry; PTMs - post-translational modifications; ROS - reactive oxygen species; SILAC - stable isotope labelling with amino acids in cell culture; SM - secondary metabolite; SWATH - sequential window acquisition of all theoretical fragment ion spectra; 2-DE - two-dimensional gel electrophoresis.

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(MRM), single reaction monitoring, sequential window acquisition of all theoretical fragment ion spectra (SWATH), and matrix-assisted laser desorption ionization (MALDI) imaging, have paved the way for high throughput proteomic study (Langridge *et al*. 2011).

A variety of medicinal plants has been used directly or as precursors for the synthesis of herbal medicines. More than 70 % of the Indian population still depends on medicinal herbs (Gairola *et al*. 2010). The pharmaceutical industries have also expressed their interest in medicinal plants for bioactive phytochemicals, such as flavonoids, alkaloids, anthocyanins, terpenoids, lignans, quinones, and steroids that lead to the development of feasible drugs (Witzmann and Grant 2003). Most of the medicinal plant species are habitat-specific and flourish only within a narrow range of environmental conditions. It is expected that approximately 25 % of vascular plants would become extinct within the next 40 years from their natural habitats, due to unorganized cultivation and excessive harvesting (Kala *et al*. 2006). Since high altitude medicinal plants have well developed tolerance mechanism against harsh environmental conditions, the study of medicinal plant proteomes will be quite significant towards understanding, conservation, and sustainability. However, proteomic study of medicinal plants has certain limitations. First, limited information is available about the proteins involved in synthesis of secondary metabolites (SMs) and natural products. Second, protein expression profile of samples harvested from different developmental stage, tissue/organ, time-points, and locations vary significantly. Third, the stoichiometry of proteins particularly enzymes, transporters, and transcription factors involved in SM synthesis are generally low and these proteins are often obscured by polyphenols and pigments that impede protein extraction and subsequent proteome analysis. And fourth, the missing sequence data of both genes and proteins, represents a significant challenge.

Medicinal plants have a long association with human history. Various plant species served as a source of SMs or bioactive molecules. However, the characterization of biosynthetic pathways of bioactive molecules, needs to be fully explored. *Podophyllum hexandrum* produces lignans (podophyllotoxin) used for treating malignancies (Lau and Sattely 2015). *Picrorhiza kurroa,* a small perennial herb growing in Himalayan region (3 000 - 5 000 m) is one of the rich sources of iridoid glycosides (picroside I - V) with wide medicinal properties (Singh *et al*. 2013). *Panax ginseng* roots have been widely used as a traditional herbal medicine for anti-aging, anti-tumor, and immunity enhancement because of ginsenoids (Lee and Park 2016). Other medicinal plant species such as *Artemisia* produce artemisin, effective for the treatment of malaria, *Potentilla* species are used for gynecological disorders, *Dactylorhiza hatagirea* is used as nutritional supplement, neurostimulant, antibacterial agent and aphrodisiacum, and roots of *Valeriana jatamansi* have antioxidant activity (Graham *et al*. 2010, Warghat *et al*. 2012, Thusoo *et al*. 2014, Bryant *et al.* 2015, 2016). A gene encoding cysteine rich mini proteins called cyclotides was investigated using deep mining of transcriptome and proteome from *Viola tricolor* (Hellinger *et al*. 2015). This approach will be useful to generate a library of bioactive peptides. A substantial proteome analysis of developing rhizomes in *Curcuma comosa* and *Equisetum hyemale* revealed the possible role of proteins associated with rhizome trait in plant growth and development (Boonmee *et al*. 2011, Balbuena *et al*. 2012). These medicinal plants serve as primary source of income for high altitude habitats (Table 1 Suppl.).

There is an increasing demand of herbal drugs for the human well-being. Biotechnological approaches, namely cell suspension culture and metabolic engineering, *etc*., have been extensively used to increase the production of commercially important medicinal plants. However, these approaches are not sufficient due to being low productive, time consuming and troublesome (Verpoorte *et al*. 1999, Aghaei and Komastu 2013, Yue *et al*. 2016). Recently, proteomic approaches, which provide useful methods for comprehensive identification of SM related proteins including enzymes, transporters, and especially transcription factors have been employed. An overview of proteomic techniques has been reported for characterization of proteins involved in SM synthesis (Jacobs *et al*. 2000, Martinez-Esteso *et al*. 2015). The identified proteins have been transferred to heterologous hosts for current and future industrial production. Most of the proteomic studies have been carried out in *Catharanthus roseus* and *Papaver somniferum* for alkaloid and morphine biosyntheses (Decker *et al*. 2000, Jacobs *et al*. 2005), *Panax ginseng* for ginsenoside biosynthesis, hairy root analysis, and marker protein identification (Lum *et al.* 2002, Kim *et al.* 2003, Nam *et al*. 2005, Ma *et al.* 2013, 2016), *Mahonia bealei* for benzyl isoquinoline alkaloids production (Zhang *et al*. 2014), and *Euphorbia kansui* for laticifers development and disease responses (Zhao *et al*. 2014). Tea (*Camellia sinensis*) is an another important medicinal crop having different classes of flavonoids including flavonols, isoflavones, monomeric flavan-3-ols (catechins and epicatechins), anthocyanins, and oligomeric flavan-3-ols (proanthocyanidins). Most of the proteomic analysis of tea have been carried out at different storage conditions (Li *et al.* 2008), in leaves from different developmental stages (Li *et al*. 2011), and under drought and low temperature (LT) stress (Zhou *et al.* 2014, Lu *et al*. 2015). To check the effect of embryo development in plants propagation, various proteomic studies were carried out on different medicinal plants, *viz., Coffea arabica, Cyclamen persicum, Crocus sativa, Araucaria angustifolia*, and *Dianthus caryophyllus* (Sharifi *et al*. 2012, Mwangi *et al*. 2013, Campos *et al*. 2016, Dos-Santos *et al*. 2016, Muneer *et al*. 2016). These studies can be useful to find out the relation between protein expression and their involvement at different stages of somatic embryos development. Astonishingly, medicinal plant proteomics has gained attention in a few years ago as it is evident by increased number of

publications over the period 2000 - 2016 dealing with important medicinal plants (Fig. 1). Here, we discussed the effect of climate change on high altitude flora and also the recent developments in proteomics of plant adaptation.

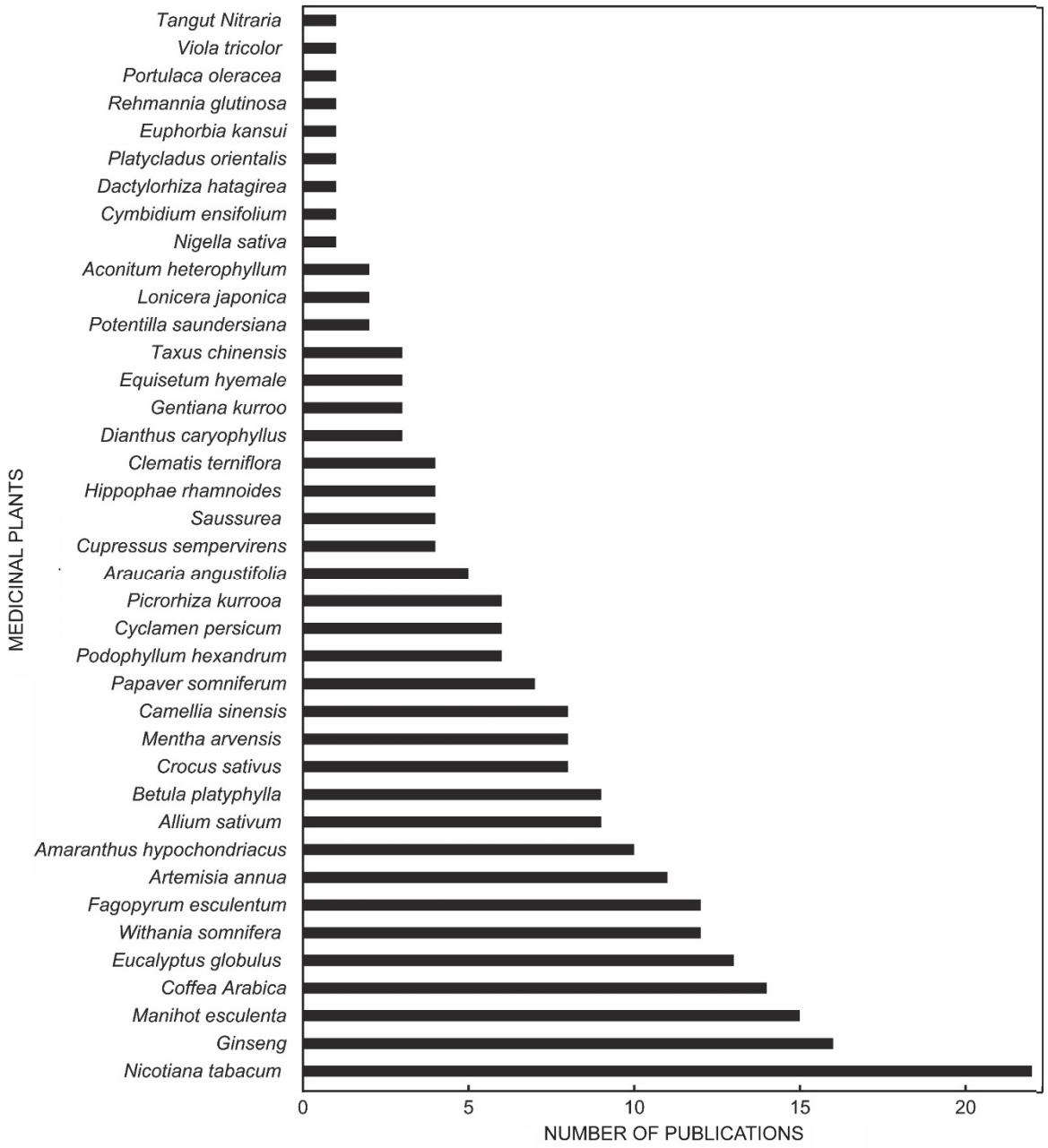

Fig. 1. An update of proteomics work on important medicinal plants of Himalayan region. Number of publications for 39 most often used medicinal plants over the period 2000 - 2016 (data from Web of Science).

Climate change affects medicinal plant distribution

Climate change is a long-term variation in the statistical distribution of weather patterns leading to shift in seasonal timings. They have begun to impact life cycles, distribution, as well as yield of various plant species. Ecologists have always been attracted to high altitude regions especially in light of the global climate change. Yet, how the medicinal plants respond to these changing environments is poorly understood. Therefore, addressing plant acclimation in the context of climate change is a matter of concern. The most evident effects of climate

change on high altitude plants over the few years involve phenology change, uneven distribution of plant species, and extinction of certain species (Anup and Ghimire 2015). Previous reports highlighted the shifting of various plant species from lower to higher altitudes in Himalayan region and anticipate uphill shifts in near future (Rumpf *et al*. 2018). Although, this trend of uphill shift is not applicable to all plant species, an unexpected response is generally observed. In a study conducted on distribution of plant species under climatic changes in California, 64 plant species had a significant downhill shift to optimum elevation. This downhill shift could be explained by regional changes in water balance (Crimmins *et al*. 2011). Furthermore, similar downhill shift of many other species is believed to be highly imminent. Elevated atmospheric CO_2 concentrations at high temperature have beneficial effects on plant growth by enhancing the rate of photosynthesis and reduction in stomatal conductance. In an attempt, to study the effect of different ozone (O_3) concentrations on redox proteome of soybean growth at free air concentration enrichment facility, Galant *et al*. (2012) identified 35 proteins, of which 22 proteins showed up to 5-fold higher abundance. Interestingly, reduced activity of phosphoenolpyruvate carboxylase/oxygenase (RuBisCO) decreases the photosynthetic rate, whereas increased activity of RuBisCO facilitates mobilization of leaf starch into sugar (Galant *et al*. 2012). In addition to soybean, several studies in diverse plant species like wheat, poplar, and tobacco tried to understand how environmental O_3 affects the redox-sensitive pathways which could facilitate crop adaptation to global climate changes (Baier *et al*. 2005, Bohler *et al*. 2007, Sarkar *et al*. 2010). At increased atmospheric temperature, high altitude plants increase their short-term growth, resulting in reduced time necessary for sufficient biomass accumulation. In addition, under such conditions, plants also start accumulating SMs because the fixed carbon is distributed for SM production instead of growth. An established facility of free air CO_2 enrichment (FACE) and free air temperature increase (FATI) in CSIR-IHBT was used to study climate change effects on proteome and metabolome of *Picrorhiza kurroa* in which increased accumulation of picrosides at elevated CO_2 was observed (Kumar 2016). Furthermore, climate change-induced drought stress has negative impact on plant growth and yield. At cellular level, climate change mainly affects the chlorophyll content, photosynthesis, and antioxidant system. Therefore, studies in these directions will be important for analysis of the impact of climate change on high altitude plants.

Role of proteomics in plant adaptation

High altitude region is characterized by harsh environmental conditions that can influence both species richness and diversity. These environmental extremes alter cellular homeostasis, which leads to changes in the molecular, biochemical, physiological, and morphological responses of plants. These effects include production of reactive oxygen species (ROS), alteration of cellular structures, membrane functions, and eventually protein denaturation (Eldakak *et al*. 2013). Alpine plants have evolved many tolerance strategies to survive in extreme conditions prevailing at high altitudes; however, the mechanism underlying the evolution of these strategies remains less explored. Several proteomic studies of medicinal plants related to developmental biology and abiotic and biotic stresses have been reported in recent years which could be utilized for crop improvement (Sinha *et al.* 2011, Li *et al.*2014a, Correia *et al.* 2016, Zhang *et al.* 2015a, Zhu *et al.* 2015). In a proteomic study, Sud *et al.* (2014) revealed the role of putative cytochrome P_{450} superfamily protein, photosystem I reaction centre subunit V, 1-amino-cyclopropane-1-carboxylate oxidase, 2-oxo-glutarate ferrous-dependent oxygenase, and glyceraldehyde-3-phosphate dehydrogenase in biosynthesis of picrosides. Furthermore, leaf and root proteomic studies showed differential expression of proteins under the dark and under irradiance; they are probably involved in different biochemical pathways which could be used to develop new strategies for plant adaptation and increased biomass production in *Picrorhiza* (Parkash *et al.* 2014).

Along the altitude gradient, various environmental factors including soil temperature, UV radiation, oxygen concentration, rainfall, humidity, and photoperiod vary significantly, *e.g*., in the Himalayan region. Among these environmental factors soil temperature, rainfall, and UV radiation are three main factors that limit plant growth at high altitude. Plants show a decrease in height, a low leaf index, less biomass accumulation, change in stomatal control, root architecture, and increased accumulation of pigments at high altitude. Recently, it has been reported that alpine plant *Potentilla saundersiana* posseses independent main root system at low altitude and share joint form of root system among two or more plants at high altitudes (Ma *et al*. 2015). Interestingly, this shared root system is a tolerance strategy of plants caused by increased content of auxin and decreased content of strictosidine, which play a major role in lateral root formation. A decrease in stomatal aperture and stomata density, whereas increase in frequency of stomatal opening and closing is often observed at high altitudes. The rapid activation of MAPK signalling pathway is mainly responsible for stomatal movement. This adaptation is essential to avoid the damage from low temperature (LT), strong UV radiations, and low water content in soil at higher altitudes (Salvador *et al*. 1999, Sally *et al*. 2001). Photosynthetic pigments especially chlorophylls and carotenoids show an increased accumulation at high altitude. This increased content of

chlorophyll can absorb more sun radiation for photosynthesis, moreover, carotenoids might also be involved in prevention of damage from excess of visible radiation or from UV radiation. This is accompanied by increased expression of photosynthesis related proteins like chlorophyll *a*/*b* binding protein, light harvesting chlorophyll a/b binding protein, photosystem I reaction center subunit II, and photosystem I reaction center subunit IV in *Kobersia pygmaea* along elevational gradient (Li *et al*. 2014b). The structure and composition of cell wall is constantly modified to allow plant growth under changing environments (Lee *et al*. 2011). Increase in cell wall thickness is due to an accumulation of proteins involved in cellulose and lignin biosynthesis, including xylan-1,4-β-xylosidase, cellulose synthase, and caffeoyl-CoA-3-O-methyltransferase (Ma *et al.* 2015). These molecular adaptations help the plants to acclimatize in extreme conditions at high altitudes.

Seed germination and seedling growth are important physiological stages of plant development which are severely affected by altitude. It has been reported that lower mobilization of reserves, delayed cell division, and enlarged and injured hypocotyls are mainly due to fluctuations of environmental stimuli (Sosa *et al*. 2005). To decipher the molecular mechanism of developmental processes, particularly seed germination in *Podophyllum* at high altitude, 27 newly synthesized proteins were identified. The major group of proteins were involved in metabolism, signalling, and stress responses, suggesting their role in increased mobilization of reserves, cell division, as well as protection from stress conditions (Dogra *et al*. 2013). In an attempt to develop *Podophyllum* seed germination protein interaction network (PGN), Dogra *et al*. (2015) clustered 1 028 seed proteins. This study identified the key proteins involved in seed germination, particularly radicle protrusion, that are associated with diverse biological processes like metabolism, signalling, cell wall modification, protein modification, and cell cycle regulation. Furthermore, quantative proteomic study of seed germination was also carried out in *Magnolia sieboldii*, *Jatropha curcas, Ricinus communis,* and *Aconitum heterophyllum* (Nogueira *et al*. 2013, Pinheiro *et al*. 2013, Rana *et al.* 2013, Lu *et al*. 2016). Besides these studies, a web portal (SPWP http://www.seed-proteome.com/) offers qualitative and quantitative information regarding seed proteome (Galland *et al*. 2012). Recent proteomic studies in *Nigella sativa* indicated that majority of seed proteins were related to saccharide, lipid, and amino acid metabolism which is responsible for synthesis and storage of different oils and alkaloids that might be useful to treat many diseases (Alanazi *et al.* 2016).

Generation of ROS represents the most common and early plant response against abiotic and biotic stresses (Sewelam *et al*. 2016). The excess of ROS can damage the membrane lipids, saccharides, proteins, and DNA in the stressed cells. Thus, plant cells have evolved antioxidant systems. It has been reported that ROS lead to change in intracellular redox homeostasis that induces expression of many antioxidant enzymes (superoxide dismutase, peroxidase, ascorbate peroxidase, glutathione S-transferase, and catalase) in *Potentilla saundersiana, Kobersia pygmaea, Withania somnifera*, and *Phyllanthus amarus* at high altitude (Takshak and Agrawal 2014). It is predicted that higher activities of antioxidant enzymes may play an important role in plant adaptation to environmental stresses by orchestrating various key signalling events *via* their interaction with many other secondary messengers (protein kinases, phosphatases, phytohormones, and calcium ions). Proteins with thiols, such as glutathione and thioredoxin-1, are essential for many nuclear functions including DNA replication and repair, transcription, chromatin stability, and nucleo-cytoplasmic trafficking.

High altitude plant species respond to LT and freezing stress by spontaneous ice formation in their extracellular spaces or by deep super-cooling of their sap, thereby allowing plants to survive. LT induces expression of many genes encoding specific proteins including antifreeze proteins (AFPs), late embryogenesis abundant (LEA) proteins, heat shock proteins (HSPs), mRNA binding proteins, osmotin, enzymes of osmolyte biosynthesis, water channel proteins, and detoxification enzymes, which have a role in protecting cells from LT damage. AFPs are a class of polypeptides which binds to ice crystals in apoplast and prevent their growth and migration into other tissues (Griffith and Yaish 2004, Gupta and Deswal 2014, Sharma *et al.* 2016). Consistent with these observations, 61 LT stress responsive proteins including thaumatin-like and chitinase as putative AFPs were identified in *Hippophae rhamnoides* (Gupta and Deswal 2012). LEA proteins are important example of stress-induced hydrophilic proteins that functions as water binding molecule in ion sequestration and macromolecule and membrane stabilization. A study on *Caragana jubata,* revealed an up-regulation of 11 LEA proteins with chaperone activity exclusively present in this plant and provides tolerance to LT (Bhardwaj *et al*. 2013). This study predicted the involvement of LEA proteins in initial shock by LT, particularly in the period in which other chaperons are not induced. Another proteomic study of garlic showed that proteins associated with physiological and metabolic processes are highly accumulated under LT stress which suggested that plant acclimatization to stress condition is by changing their proteome composition (Dufoo-Hurtado *et al*. 2015). Similarly, in the leaves of birch, proteins involved in defense/stress response, C-N metabolism, hormone signalling, biosynthesis, and photosynthesis play a key role in cold hardiness (Wu *et al*. 2014, Zhang *et al*. 2015b). Therefore, it is proposed that protein-protein interaction based network might confer tolerance to LT stress. HSPs, especially HSP90, HSP70, HSP60, and sHSPs, and chaperones are the most important stress-induced proteins that enhance plant tolerance to various stresses including drought, salinity, and oxidative stress in addition to LT by preventing aggregation and promoting proper folding of proteins (Timperio *et al*.

2008, Huang *et al*. 2016). *Kobresia pygmaea* accumulates more HSP and especially HSP20 along altitude gradient, which strongly suggests that HSPs are involved in plant tolerance to this harsh environment (Li *et al*. 2014b). Similarly, an up regulation of HSP101, HSP70, HSP90, and the molecular chaperones (DnaK and DnaJ) in *Portulaca oleracea* has been observed under high temperature and humidity (Yang *et al.* 2012). Selected stress-responsive proteins from medicinal plants are presented in Table 2 Suppl.

The gradual increase in atmospheric temperature coupled with limited rainfall induce drought, which leads to retardation of growth, development, and eventually plant mortality. Therefore, plants under drought stress usually activate their defense mechanisms to re-establish the cellular homeostasis (Zurbriggen *et al.* 2008, Gharechahi *et al.* 2014). Comparative proteomic study in *Eucalyptus globulus* revealed that drought tolerant genotype has more developed root system, smaller seeds with low water content in the mature state, enhanced accumulation of endogenous ABA, and up-regulation of responsive to abscisic acid 17 **(**RAB17) and 28 (RAB28) proteins (Valdes *et al.* 2013). Kashyap *et al*. (2014) investigated the molecular response of *Picrorhiza kurroa* to drought and identified a total of 13 and 18 proteins in leaves and roots, respectively. They observed that proteins involved in transcription, stress, and defence response are up-regulated suggesting that plants undergo reprogramming of their gene expression in drought conditions. In addition, abundance of proteins related to signalling, metabolism, and transport was also increased, which probably involved synthesis of SMs and their transport to different organelles. Zhou *et al*. (2014) examined the effect of exogenous ABA on the tea leaf proteome under drought stress. The study revealed that proteins particularly related to photosynthesis, energy metabolism, and S-adenosylmethionine biosynthesis such as cytochrome b6-f complex, iron-sulfur subunit 2, aldehyde dehydrogenase family 2 member B4, and S-adenosyl-methionine synthase 3 are down regulated which supports the hypothesis that plants undergo metabolic adjustment for energy consumption in response to drought stress. Identification of these novel proteins, their expression, and understanding of their functions would provide the basis for effective engineering strategies to improve stress tolerance of medicinal plants with enhanced SM content. Indeed, by deliberately applying moderate drought stress during their cultivation, the quality of medicinal plants can be enhanced significantly, however, this aim requires much more research.

High altitude plants are able to modulate the content of SMs like isoprenoids, alkaloids, and phenols during different abiotic stresses and the scientific knowledge in this field is accumulating. An increased content of alkaloids, flavonols, anthocyanidines, hydroxybenzoic acids, taxanes, phytosterol, and carotenoids has been reported in *Clematis terniflora*, *Ribes nigrum, Malus domestica*, and *Taxus chinensis* after UV-B exposure (Alothman *et al*. 2009, Gao *et al*. 2016, Yang *et al.* 2016, Zheng *et al.* 2016). Recently, Ma *et al*. (2015) reported a consistent increase in flavonoid metabolism related proteins (flavone 3′-O-methyltransferase 1, isoflavone reductase, and chalcone synthase D) leads to an increase in the accumulation of flavonoids and anthocyanins in *Potentilla saundersiana,* suggesting their role in plant tolerance to environmental stresses. Elicitation of plant cells in cell-culture represents a useful biotechnological tool to improve the production of valuable metabolites. The cell suspension culture of *Podophyllum hexandrum* produces more podophyllotoxin (PTOX) in response to methyl jasmonate elicitation (Bhattacharyya *et al*. 2012). An enhanced production of PTOX is attributed to up-regulation of many phenylpropanoid and monolignol pathway enzymes like chalcone synthase, caffeoyl CoA 3-O-methyltransferase, polyphenol oxidase, caffeic acid-O-methyl transferase, S-adenosyl-L-methionine dependent methyltransferases, *etc*. Similarly, poppy cell culture produces more sanguinarine in response to fungal elicitor (Desgagne-Penix *et al*. 2010). A list of proteins involved in SM synthesis is given in Table 3 Suppl. It is supposed that an increased content of SMs is involved in plant protection by modulating antioxidant system and chaperone proteins.

Conclusions and questions

With an increasing shift to environmental extremes, it is essential to explore the molecular mechanism of plant adaptation along an elevation gradient. Extreme environmental conditions may impact several key biological processes including photosynthesis, transpiration, antioxidant defence systems, and hormone signalling. Therefore, plants use multiple strategies to adapt to high altitude environmental conditions. There is a great variability in expression of different stress induced proteins, namely HSPs, AFPs, RBP, and LEA, and detoxification enzymes which likely contribute to plant adaptation. Furthermore, recent molecular, physiological, and morphological studies have taken a big leap forward to reveal molecular mechanism of plant adaptation to extreme environmental conditions at high altitude. However, complex studies of ”omics” in different developmental stages and under variable stress conditions will be required for better understanding signalling pathways. Successful acclimation of medicinal plants to the alpine environment has raised many key questions, which continue to remain unanswered. For example, what are morphological, physiological, and biochemical characteristics acquired by medicinal plants for stress tolerance at high altitude? How many and which type of proteins are involved in signalling pathways for stress tolerance? Is there any post-

translational modification of proteins, particularly phosphorylation involved in plant adaptations? How differently do the cell organelles integrate their signals to regulate nuclear gene expression and other cellular activities? To what extent does the SMs content contribute to plant adaptations along altitude gradient? What type of antifreeze proteins are involved in survival at low temperature? Does epigenetic reprogramming play any role in plant survival at high altitude environment? These questions need to be addressed for establishment of a mechanistic model of plant survival under extreme environmental conditions. To answer these questions, proteomics has evolved as an essential tool along with different "omics" approaches for better understanding plant tolerance mechanisms. This study could help researchers in identification, cultivation, and production of economically important medicinal plants. In near future, the effect of climate change will become more prominent so it is important to develop new plant genotypes that are more tolerant to changing climate conditions.

References

Aghaei, K., Komatsu, S.: Crop and medicinal plants proteomics in response to salt stress. - Front. Plant Sci. **4**: 8, 2013.

Agrawal, G.K., Sarkar, A., Righetti, P.G., Pedreschi, R., Carpentier, S., Wang, T., Barkla, B., Kohli, A., Ndimba, B.K., Bykova, N.V., Rampitsch, C., Zolla, L., Rafudeen, M.S., Cramer, R., Bindschedler, L.V., Tsakirpalglou, N., Ndimba, R.J., Farrant, J.M., Renaut, J., Job, D., Kikuchi, S., Rakwal, R.: A decade of plant proteomics and mass spectrometry: translation of technical advancements to food security and safety issues. - Mass Spectrom. Rev. **32**: 335-365, 2013.

Alanazi, I.O., Benabdelkamel, H., Alfadda, A.A., AlYahya, S.A., Alghamdi, W.M., Aljohi, H.A., Almalik, A., Masood, A.: Proteomic analysis of the protein expression profile in the mature *Nigella sativa* (Black seed). - App. Biochem. Biotechnol. **179**: 1184-1201, 2016.

Alothman, M., Bhat, R., Karim, A.A.: Effects of radiation processing on phytochemicals and antioxidants in plant produce. - Trends Food Sci. Tech. **20**: 201-212, 2009.

Alves, M., Moes, S., Jenö, P., Pinheiro, C., Passarinho, J., Ricardo, C.P.: The analysis of *Lupinus albus* root proteome revealed cytoskeleton altered features due to long-term boron deficiency. - J. Proteomics **74**: 1351-1363, 2011.

An, F., Fan, J., Li, J., Li, Q.X., Li, K., Zhu, W., Wen, F., Carvalho, L.J., Chen, S.: Comparison of leaf proteomes of cassava (*Manihot esculenta Crantz*) cultivar NZ199 diploid and autotetraploid genotypes. - PLoS ONE **9**: 4 e85991, 2014.

Anup, K.C., Ghimire, A.: High-altitude plants in era of climate change: a case of Nepal Himalayas. - In: Munir, O., Khalid, R.H., Faridah-Hanum, I., Recep, E. (ed.): Climate Change Impacts on High-Altitude Ecosystems. Pp. 177-187. Springer, Dordrecht 2015.

Baier, M., Kandlbinder, A., Golldack, D., Dietz, K.J.: Oxidative stress and ozone: perception, signalling and response. - Plant Cell Environ. **28**: 1012-1020, 2005.

Balbuena, T.S., He, R., Salvato, F., Gang, D.R., Thelen, J.J.: Large-scale proteome comparative analysis of developing rhizomes of the ancient vascular plant *Equisetum hyemale*. - Front. Plant Sci. **3**: 131, 2012.

Bhardwaj, P.K., Kapoor, R., Mala, D., Bhagwat, G., Acharya, V., Singh, A.K., Vats, S.K., Ahuja, P.S., Kumar, S.: Braving the attitude of altitude: *Caragana jubata* at work in cold desert of Himalaya. - Sci. Rep. **3**: 1022, 2013.

Bhattacharyya, D., Sinha, R., Ghanta, S., Chakraborty, A., Hazra, S., Chattopadhyay, S.: Proteins differentially expressed in elicited cell suspension culture of *Podophyllum hexandrum* with enhanced podophyllotoxin content. - Proteome Sci. **10**: 34, 2012.

Bohler, S., Bagard, M., Oufir, M., Planchon, S., Hoffmann, L., Jolivet, Y., Hausman, J.F., Dizengremel, P., Renaut, J.:A DIGE analysis of developing poplar leaves subjected toozone reveals major changes in carbon metabolism. - Proteomics **7**: 1584-1599, 2007.

Boonmee, A., Srisomsap, C., Chokchaichamnankit, D., Karnchanatat A., Sangvanich, P.: A proteomic analysis of *Curcuma comosa* Roxb. rhizomes. - Proteome Sci. **9**: 43, 2011.

Bryant, L., Flatley, B., Patole, C., Brown, G.D.,Cramer, R.: Proteomic analysis of *Artemisia annua* - towards elucidating the biosynthetic pathways of the antimalarial pro-drug artemisinin. - BMC Plant Biol. **15**: 1, 2015.

Bryant, L., Patole, C., Cramer, R.: Proteomic analysis of the medicinal plant *Artemisia annua*: data from leaf and trichome extracts. - Data Brief **7**: 325-331, 2016.

Campos, N.A., Paiva, L.V., Panis, B., Carpentier, S.C.: The proteome profile of embryogenic cell suspensions of *Coffea arabica* L. - Proteomics **16**: 1001-1005, 2016.

Chattopadhyay, A., Subba, P., Pandey, A., Bhushan, D., Kumar, R., Datta, A., Chakraborty, S., Chakraborty, N.: Analysis of grass pea proteome and identification of stress-responsive proteins upon exposure to high salinity, low temperature and abscisic acid treatment. - Phytochemistry **72**: 1293-1307, 2011.

Cheng, T., Chen, J., Zhang, J., Shi, S., Zhou, Y., Lu, L., Wang, P., Jiang, Z., Yang, J., Zhang, S., Shi, J.: Physiological and proteomic analyses of leaves from the halophyte tangut *Nitraria* reveals diverse response pathways critical for high salinity tolerance. - Front. Plant Sci. **6**: 30, 2015.

Correia, B., Valledor, L., Hancock, R.D., Renaut, J., Pascual, J., Soares, A.M.V.M., Pinto, G.: Integrated proteomics and metabolomics to unlock global and clonal responses of *Eucalyptus globulus* recovery from water deficit. - Metabolomics **12**: 141, 2016.

Crimmins, S.M., Dobrowski, S.Z., Greenberg, J.A., Abatzoglou, J.T., Mynsberge, A.R.: Changes in climatic water balance drive downhill shifts in plant species optimum elevations. - Science **331**: 324-327, 2011.

Decker, G., Wanner, G., Zenk, M.H., Lottspeich, F.: Characterization of proteins in latex of the opium poppy (*Papaver somniferum*) using two-dimensional gel electrophoresis and microsequencing. - Electrophoresis **21**: 3500-3516, 2000.

Desgagne-Penix, I., Khan, M.F., Schriemer, D.C., Cram, D., Nowak, J., Facchini, P.J.: Integration of deep transcriptome and proteome analyses reveals the components of alkaloid metabolism in opium poppy cell cultures. - BMC Plant Biol. **10**: 252, 2010.

Dogra, V., Ahuja, P.S., Sreenivasulu, Y.: Change in protein content during seed germination of a high altitude plant *Podophyllum hexandrum* Royle. – J. Proteomics **78**: 26-38, 2013.

Dogra, V., Bagler, G., Sreenivasulu, Y.: Re-analysis of protein data reveals the germination pathway and up accumulation mechanism of cell wall hydrolases during the radicle protrusion step of seed germination in *Podophyllum hexandrum* – a high altitude plant. - Front. Plant Sci. **6**: 874, 2015.

Dos-Santos, A.L., Elbl, P., Navarro, B.V., De Oliveira, L.F., Salvato. F., Balbuena, T.S., Floh, E.I.: Quantitative proteomic analysis of *Araucaria angustifolia* (Bertol.) Kuntze cell lines with contrasting embryogenic potential. - J. Proteomics **130**: 180-189, 2016.

Duby, G., Degand, H., Faber, A.M., Boutry, M.: The proteome complement of *Nicotiana tabacum* Bright-Yellow-2 culture cells. - Proteomics **10**: 2545-2550, 2010.

Dufoo-Hurtado, M.D., Huerta-Ocampo, J.Á., Barrera-Pacheco, A., Barba de la Rosa A.P., Mercado-Silva, E.M.: Low temperature conditioning of garlic (*Allium sativum* L.) "seed" cloves induces alterations in sprouts proteome. - Front. Plant Sci. **6**: 332, 2015.

Eldakak, M., Milad, S.I.M., Nawar, A.I., Rohila, J.S.: Proteomics: a biotechnology tool for crop improvement. - Front. Plant Sci. **4**: 35, 2013.

Gairola, S., Shariff, N.M., Bhatt, A., Kala, C.P.: Influence of climate change on production of secondary chemicals in high altitude medicinal plants: issues needs immediate attention. - J. med. Plants Res. **4**: 1825-1829, 2010.

Galant, A., Koester, R.P., Ainsworth, E.A., Hicks, L.M., Jez, J.M.: From climate change to molecular response: redox proteomics of ozone-induced responses in soybean. - New Phytol. **194**: 220-229, 2012.

Galland, M., Job, D., Rajjou, L.: The seed proteome web portal. - Front. Plant Sci. **3**: 98, 2012.

Gao, C., Yang, B., Zhang, D., Chen, M., Tian, J.: Enhanced metabolic process to indole alkaloids in *Clematis terniflora* DC. after exposure to high level of UV-B irradiation followed by the dark. - BMC Plant Biol. **16**: 231, 2016.

Gharechahi, J., Alizadeh, H., Naghavi, M.R., Sharifi, G.: A proteomic analysis to identify cold acclimation associated proteins in wild wheat (*Triticum urartu* L.). - Mol. Biol. Rep. **41**: 3897-3905, 2014.

Gill, T., Dogra, V., Kumar, S., Ahuja, P. S., Sreenivasulu, Y.: Protein dynamics during seed germination under copper stress in *Arabidopsis* over-expressing *Potentilla* superoxide dismutase. - J. Plant Res. **125**: 165-172, 2012.

Graham, I.A., Besser, K., Blumer, S., Branigan, C.A., Czechowski, T.,Elias, L., Guterman, I., Harvey, D., Isaac, P.G., Khan, A.M., Larson, T.R., Li, Y. Pawson, T., Penfield, T., Rae, A.M., Rathbone, D.A., Reid, S., Ross, J., Smallwood, M.F., Segura, V., Townsend, T., Vyas, D., Winzer, T., Bowles, D.: The genetic map of *Artemisia annua* L. identifies loci affecting yield of the antimalarial drug artemisinin. - Science **327**: 328-331, 2010.

Griffith, M., Yaish, M.W.F.: Antifreeze proteins in over wintering plants: a tale of two activities. - Trends Plant Sci. **9**: 399-405, 2004.

Guerra-Guimaraes, L., Tenente, R., Pinheiro, C., Chaves, I., Silva Mdo, C., Cardoso, F.M., Planchon, S., Barros, D.R., Renaur, J., Ricardo, C.P.: Proteomic analysis of apoplastic fluid of *Coffea arabica* leaves highlights novel biomarkers for resistance against *Hemileia vastatrix*. - Front. Plant Sci. **6**: 478, 2015.

Gupta, R., Deswal, R.: Antifreeze proteins enable plants to survive in freezing conditions. - J. Biosci. **39**: 931-944, 2014.

Gupta, R., Deswal, R.: Low temperature stress modulated secretome analysis and purification of antifreeze protein from *Hippophae rhamnoides*, a Himalayan wonder plant. - J. Proteome Res. **11**: 2684-2696, 2012.

Hafidh, S., Potesil, D., Fila, J., Capkova, V., Zdrahal, Z., Honys, D.: Quantitative proteomics of the tobacco pollen tube secretome identifies novel pollen tube guidance proteins important for fertilization. - Genome Biol. **17**: 81, 2016.

Hellinger, R., Koehbach, J., Soltis, D.E., Carpenter, E.J., Wong, G.K.S., Gruber, C.W.: Peptidomics of circular cysteine-rich plant peptides: Analysis of the diversity of cyclotides from *Viola tricolor* by transcriptome and proteome mining. - J. Proteome Res. **14**: 4851-4862, 2015.

Hu, J., Rampitsch, C., Bykova,N.V.: Advances in plant proteomics toward improvement of crop productivity and stress resistance. - Front. Plant Sci. **6**: 209, 2015.

Huang, Y., Jin, D., Lu, C., Lan, X., Qiao, P., Li, H., Chen, Y.: Proteomic responses associated with freezing tolerance in the callus of the Tibetan alpine plant *Saussurea laniceps* during cold acclimation. - Plant Cell Tissue Organ Cult. **124**: 81-95, 2016.

Huerta-Ocampo, J.A., Briones-Cerecero, E.P., Mendoza-Hernández, G., De Leon-Rodriguez, A., Barba de la Rosa, A.P.: Proteomic analysis of amaranth (*Amaranthus hypochondriacus* L.) leaves under drought stress. - Int. J. Plant Sci. **170**: 990-998, 2009.

Jacobs, D.I., Gaspari, M., Van der Greef, J., Van der Heijden, R., Verpoorte, R.: Proteome analysis of the medicinal plant *Catharanthus roseus*. - Planta **221**: 690-704, 2005.

Jacobs, D.I., Van der Heijden, R., Verpoorte, R.: Proteomics in plant biotechnology and secondary metabolism research. - Phytochem. Anal. **11**: 277-287, 2000.

Jaiswal, D.K., Ray, D., Choudhary, M.K., Subba, P., Kumar, A., Verma, J., Kumar, R., Datta,A., Chakraborty, S., Chakraborty, N.: Comparative proteomics of dehydration response in the rice nucleus: new insights into the molecular basis of genotype-specific adaptation. - Proteomics **13**: 3478-3497, 2013.

Kala, C.P., Dhyani, P.P., Sajwan, B.S.: Developing the medicinal plants sector in northern India: challenges and opportunities. - J. Ethnobiol. Ethnomed. **2**: 1, 2006.

Kim, S.I., Kim, J.Y., Kim, E.A., Kwon, K.H., Kim, K.W., Cho, K., Lee, J.H., Nam, M.H., Yang,D.C., Yoo, J.S., Park, Y.M.: Proteome analysis of hairy root from *Panax ginseng* C.A. Meyer using peptide fingerprinting, internal sequencing and expressed sequence tag data. - Proteomics **3**: 2379-2392, 2003.

Kashyap, S., Parkash, J., Kalita, P.J., Devi, M., Pathania, J., Joshi, R., Dutt, S.: Comparative proteome analysis of *Picrorhiza kurroa* Royle *ex* Benth. in response to drought. - J. Proteome Sci. Comput. Biol. **3**: 2, 2014.

Kumar, R., Kumar, A., Subba, P., Gayali, S., Barua, P., Chakraborty, S., Chakraborty, N.: Nuclear phosphoproteome of developing chickpea seedlings (*Cicer arietinum* L.) and protein-kinase interaction network. - J. Proteomics **105**: 58-73, 2014.

Kumar, S.: Understanding Altered Molecular Dynamics in the Targeted Plant Species in Western Himalaya in Relation to Environmental Cues: Implications Under Climate Change Scenario. - Wiley, New York 2016.

Langridge, P., Fleury, D.: Making the most of 'omics' for crop

breeding. - Trends Biotechnol. **29**: 33-40, 2011.

Lau, W., Sattely, E.S.: Six enzymes from mayapple that complete the biosynthetic pathway to the etoposide aglycone. - Science **349**: 1224-1228, 2015.

Lee, K., Park, K.: Proteomic variation in Korean ginseng (*Panax ginseng* CA Meyer) isolates from different geographic regions. - Afr. J. Biotechnol. **15**: 1738-1745, 2016.

Lee, K.J., Marcus, S.E., Knox, J.P.: Cell wall biology: perspectives from cell wall imaging. - Mol. Plants **4**: 212-219, 2011.

Li, J., Chen, J., Zhang, Z., Pan, Y.: Proteome analysis of tea pollen (*Camellia sinensis*) under different storage conditions. - J. Agr. Food Chem. **56**: 7535-7544, 2008.

Li, Q., Huang, J., Liu, S., Li, J., Yang, X., Liu, Y., Liu, Z.: Proteomic analysis of young leaves at three developmental stages in an albino tea cultivar. - Proteome Sci. **9**: 44, 2011.

Li, X., Xu, W., Chowdhury, M.R., Jin, F.: Comparative proteomic analysis of labellum and inner lateral petals in *Cymbidium ensifolium* flowers. - Int. J. mol. Sci. **15**: 19877-19897, 2014a.

Li, X., Yang, Y., Ma, L., Sun, X., Yang, S., Kong, X., Hu,X., Yang, Y.: Comparative proteomics analyses of *Kobresia pygmaea* adaptation to environment along an elevational gradient on the Central Tibetan Plateau. - PLoS ONE **9**: e98410, 2014b.

Lu, X.J., Zhang, X.L., Mei, M., Liu, G.L., Ma, B.B.: Proteomic analysis of *Magnolia sieboldii* K. Koch seed germination. - J. Proteomics **133**: 76-85, 2016.

Lu, Y., Hu, Y., Zhang, X., Li, P.: Responses of electrical properties of tea leaves to low-temperature stress. - Int. J. Agr. Biol. Eng. **8**:170-176, 2015.

Lum, J.H., Fung, K.L., Cheung, P.Y., Wong, M.S., Lee, C.H., Kwok, F.S., Leung, M.C., Hui,P.K., Lo, S.C.: Proteome of oriental ginseng *Panax ginseng* C.A. Meyer and the potential to use it as an identification tool. - Proteomics **2**: 1123-1130, 2002.

Ma, L., Sun, X., Kong, X., Galvan, J.V., Li, X., Yang, S., Yang, Y., Yang, Y., Hu, X.: Physiological, biochemical and proteomics analysis reveals the adaptation strategies of the alpine plant *Potentilla saundersiana* at altitude gradient of the Northwestern Tibetan Plateau. - J. Proteomics **112**: 63-82, 2015.

Ma, R., Sun, L., Chen, X., Jiang, R., Sun, H., Zhao, D.: Proteomic changes in different growth periods of ginseng roots. - Plant Physiol. Biochem. **67**: 20-32, 2013.

Ma, R., Sun, L., Chen, X., Mei, B., Chang, G., Wang, M., Zhao, D.: Proteomic analyses provide novel insights into plant growth and ginsenoside biosynthesis in forest cultivated *Panax ginseng* (F. Ginseng). - Front. Plant Sci. **7**: 1, 2016.

Martinez-Esteso, M.J., Martinez-Marquez, A., Selles-Marchart, S., Morante-Carriel, J.A., Bru-Martinez, R.: The role of proteomics in progressing insights into plant secondary metabolism. - Front. Plant Sci. **6**: 504, 2015.

Muneer, S., Soundararajan, P., Jeong, B.R.: Proteomic and antioxidant analysis elucidates the underlying mechanism of tolerance to hyperhydricity stress in *in vitro* shoot cultures of *Dianthus caryophyllus*. - J. Plant Growth Regul. **35**: 667-679, 2016.

Mwangi, J.W., Rode, C., Colditz, F., Haase, C., Braun, H.P., Winkelmann, T.: Proteomic and histological analyses of endosperm development in *Cyclamen persicum* as a basis for optimization of somatic embryogenesis. - Plant Sci. **201**: 52-65, 2013.

Nam, M.H., Kim, S.I., Liu, J.R., Yang, D.C., Lim, Y.P., Kwon, K.H., Yoo, J.S., Park, Y.M.: Proteomic analysis of Korean ginseng (*Panax ginseng* C.A. Meyer). - J. Chromatogr. B Anal. Technol. Biomed. Life Sci. **815**: 147-155, 2005.

Nogueira, F.C., Palmisano, G., Schwammle, V., Soares, E.L., Soares, A.A., Roepstorff, P., Domomt, G.B., Campos, F.A.: Isotope labeling-based quantitative proteomics of developing seeds of castor oil seed (*Ricinus communis* L.). - J. Proteome Res. **12**: 5012-5024, 2013.

Parkash, J., Kashyap, S., Kalita, P.J., Devi, M., Ahuja, P.S., Dutt, S.: Differential proteomics of *Picrorhiza kurroa* Royle *ex* Benth. in response to dark stress. - Mol. Biol. Rep. **41**: 6051-6062, 2014.

Pinheiro, C.B., Shah, M., Soares, E.L., Nogueira, F.C., Carvalho, P.C., Junqueira, M., Araujo, G.D., Soares, A.A., Domont, G.B., Campos, F.A.: Proteome analysis of plastids from developing seeds of *Jatropha curcas* L. - J. Proteome Res. **12**: 5137-5145, 2013.

Rana, B., Sreenivasulu, Y.: Protein changes during ethanol induced seed germination in *Aconitum heterophyllum*. - Plant Sci. **198**: 27-38, 2013.

Rumpf, S.B., Hulber, K., Klonner, G., Moser, D., Schutz, M., Wessely, J., Willner, W., Zimmermann, N.E., Dullinger S.: Range dynamics of mountain plants decrease with elevation. - Proc. Nat. Acad. Sci. USA **20**:1848-1853, 2018.

Sallets, A., Beyaert, M., Boutry, M., Champagne, A.: Comparative proteomics of short and tall glandular trichomes of *Nicotiana tabacum* reveals differential metabolic activities. - J. Proteome Res. **13**: 3386-3396, 2014.

Sally, W., Alison L.C., William, J.D.: Rapid low temprature-induced stomatal closure occurs in cold-tolerant *Commelina communis* leaves but not in cold-sensitive tobacco leaves, *via* a mechanism that involves apoplastic calcium but not abscisic acid. - Plant Physiol. **126**:1566-1578, 2001.

Salvador, N., Damian, J.A., James, I.L.M., Neil, R.B.: Characterization of stomatal closure caused by ultraviolet-B radiation.- Plant Physiol. **121**: 489-496, 1999.

Sarkar, A., Rakwal, R., Agrawal, S.B., Shibato, J., Ogawa, Y., Yoshida, Y., Agrawal, G.K., Agrawal, M.: Investigating the impact of elevated levels of ozone on tropical wheat using integrated phonotypical, physiological, biochemical, and proteomics approaches. - J. Proteome Res. **9**: 4565-4584, 2010.

Scarafoni, A., Ronchi, A., Prinsi, B., Espen, L., Assante, G., Venturini, G., Duranti, M.: The proteome of exudates from germinating *Lupinus albus* seeds is secreted through a selective dual-step process and contains proteins involved in plant defence. - FEBS J. **280**: 1443-1459, 2013.

Schmitz, G.J.H., De Magalhaes Andrade, J., Valle, T.L., Labate, C.A., Do Nascimento, J.R.O.: Comparative proteome analysis of the tuberous roots of six cassava (*Manihot esculenta*) varieties reveals proteins related to phenotypic traits. - J. Agr. Food Chem. **64**: 3293-3301, 2016.

Sewelam, N., Kazan, K., Schenk, P.M.: Global plant stress signaling: reactive oxygen species at the cross-road. - Front. Plant Sci. **7**: 187, 2016.

Shahali, Y., Sutra, J.P., Haddad, I., Vinh, J., Guilloux, L., Peltre, G., Senechal, H., Poncet, P.: Proteomics of cypress pollen allergens using double and triple one-dimensional electrophoresis. - Electrophoresis **33**: 462-469, 2012.

Sharifi, G., Ebrahimzadeh, H., Ghareyazie, B., Gharechahi, J., Vatankhah, E.: Identification of differentially accumulated proteins associated with embryogenic and non-embryogenic calli in saffron (*Crocus sativus* L.). - Proteome Sci. **10**: 3, 2012.

Sharma, B., Gupta, R., Deswal, R.: Mining the protein repertoire of a Himalayan shrub, *Hippophae rhamnoides* for antifreeze proteins. - J. Proteins Proteomics **7**: 199-211, 2016.

Sheffield, J., Taylor, N., Fauquet, C., Chen, S.: The cassava (*Manihot esculenta* Crantz) root proteome: protein identification and differential expression. - Proteomics **6**: 1588-1598, 2006.

Shemesh-Mayer, E., Ben-Michael, T., Rotem, N., Rabinowitch, H.D., Doron-Faigenboim, A., Kosmala, A., Perlikowski, D., Sherman, A., Kamenetsky, R.: Garlic (*Allium sativum* L.) fertility: transcriptome and proteome analyses provide insight into flower and pollen development. - Front. Plant Sci. **6**: 271, 2015.

Shin, D.H., Kamal, A.H.M., Suzuki, T., Yun, Y.H., Lee, M.S., Chung, K.Y., Jeong, H.S., Park, C.H., Choi, J.S., Woo, S.H.: Reference proteome map of buckwheat (*Fagopyrum esculentum* and *Fagopyrum tataricum*) leaf and stem cultured under light or dark. - Aust. J. Crop. Sci. **4**: 633-641, 2010.

Singh, H., Gahlan, P., Kumar, S.: Cloning and expression analysis of ten genes associated with picrosides biosynthesis in *Picrorhiza kurroa*. - Gene **515**: 320-328, 2013.

Sinha, R., Chattopadhyay, S.: Changes in the leaf proteome profile of *Mentha arvensis* in response to *Alternaria alternata* infection. - J. Proteomics **74**: 327-336, 2011.

Sosa, L., Llanes, A., Reinoso, H., Reginato, M., Luna, V.: Osmotic and specific ion effect on the germination of *Prosopis strombulifera*. - Ann. Bot. **96**: 261-267, 2005.

Subba, P., Barua, P., Kumar, R., Dutta, A., Soni, K.K., Chakraborty, S., Chakraborty, N.: Phosphoproteomic dynamics of chickpea (*Cicer arietinum L.*) reveals shared and distinct components of dehydration response. - J. Proteome Res. **12**: 5025-5047, 2013a.

Subba, P., Kumar, R., Gayali, S., Shekhar, S., Parveen, S., Pandey, A., Dutta, A., Chakraborty, S., Chakraborty, N.: Characterization of the nuclear proteome of a dehydration-sensitive cultivar of chickpea and comparative proteomic analysis with a tolerant cultivar. - Proteomics **13**: 1973-1992, 2013b.

Sud, A., Chauhan, R.S., Tandon, C.: Mass spectrometric analysis of differentially expressed proteins in an endangered medicinal herb, *Picrorhiza kurroa*. - Biomed. Res. Int. **326405**: 1-12, 2014.

Suzuki, H., Takashima, Y., Ishiguri, F., Yoshizawa, N., Yokota, S.: Proteomic analysis of responsive proteins induced in Japanese birch plantlet treated with salicylic acid. - Proteomes **2**: 323-340, 2014.

Takshak, S., Agrawal, S.B.: Effect of ultraviolet-B radiation on biomass production, lipid peroxidation, reactive oxygen species, and antioxidants in *Withania somnifera*. - Biol. Plant. **58**: 328-334, 2014.

Thusoo, S., Gupta, S., Sudan, R., Kour, J., Bhagat, S., Hussain, R., Bhagat, M.: Antioxidant activity of essential oil and extracts of *Valeriana jatamansi* roots. - Biomed. Res. Int. **2014**: 614187, 2014.

Timperio, A.M., Egidi, M.G., Zolla, L.: Proteomics applied on plant abiotic stresses: role of heat shock proteins (HSP). - J. Proteomics **71**: 391-411, 2008.

Valdes, AE., Irar, S., Majada, J.P., Rodriguez, A., Fernandez, B., Pages, M.: Drought tolerance acquisition in *Eucalyptus globulus* (Labill.): A research on plant morphology, physiology and proteomics. - J. Proteomics **79**: 263-276, 2013.

Verpoorte, R., vander Heijden, R., Hoopen Ten, H.J.G., Memelink, J.: Metabolic engineering of plant secondary metabolite pathways for the production of fine chemicals. - Biotechnol. Lett. **21**: 467-479, 1999.

Wang, X., Chang, L., Tong, Z., Wang, D., Yin, Q., Wang, D., Jin, X., Yang, Q., Wang, L., Sun, Y., Huang, Q., Guo, A., Peng, M.: Proteomics profiling reveals carbohydrate metabolic enzymes and 14-3-3 proteins play important roles for starch accumulation during cassava root tuberization. - Sci. Rep. **6**: 19643, 2016.

Warghat, A.R., Bajpai, P.K., Murkute, A.A., Sood, H., Chaurasia, O.P., Srivastava, R.B.: Genetic diversity and population structure of *Dactylorhiza hatagirea* (Orchidaceae) in cold desert Ladakh region of India. - J. med. Plants Res. **6**: 2388-2395, 2012.

Witzmann, F.A., Grant, R.A.: Pharmacoproteomics in drug development. - Pharmacogenomics J. **3**: 69-76, 2003.

Wu, F.-Z., Wang, B.-C., Yang, C.-P.: Proteomic analysis of the cold stress response in the leaves of birch (*Betula platyphylla* Suk). - Plant Omics J. **7**: 195-204, 2014.

Wu, L., Wang, H., Zhang, Z., Lin, R., Zhang, Z., Lin, W.: Comparative metaproteomic analysis on consecutively *Rehmannia glutinosa*-monocultured rhizosphere soil. - PLoS ONE **6**: e20611, 2011.

Yang, B., Wang, X., Gao, C., Chen, M., Guan, Q., Tian, J., Komatsu, S.: Proteomic and metabolomic analyses of leaf from *Clematis terniflora* DC. exposed to high-level ultraviolet-B irradiation with dark treatment. - J. Proteome Res. **15**: 2643-2657, 2016.

Yang, Y., Chen, J., Liu, Q., Ben, C., Todd, C.D., Shi, J., Yang, Y., Hu, X.: Comparative proteomic analysis of the thermotolerant plant *Portulaca oleracea* acclimation to combined high temperature and humidity stress. - J. Proteome Res. **11**: 3605-3623, 2012.

Yue, W., Ming, Q.L., Lin, B., Rahman, K., Zheng, C.J., Han, T., Qin, L.P.: Medicinal plant cell suspension cultures: pharmaceutical applications and high-yielding strategies for the desired secondary metabolites. - Crit. Rev. Biotechnol. **36**: 215-232, 2016.

Zhang, C.X., Tian, Y., Cong, P.H.: Proteome analysis of pathogen-responsive proteins from apple leaves induced by the alternaria blotch *Alternaria alternata*. - PloS ONE **10**: e0122233, 2015a.

Zhang, L., Zhu, W., Zhang, Y., Yang, B., Fu, Z., Li, X., Tian, J.: Proteomics analysis of *Mahonia bealei* leaves with induction of alkaloids via combinatorial peptide ligand libraries. - J. Proteomics **110**: 59-71, 2014.

Zhang, S., Zhang, L., Chai, Y., Wang, F., Li, Y., Su, L., Zhao, Z.: Physiology and proteomics research on the leaves of ancient *Platycladus orientalis* (L.) during winter. - J. Proteomics **126**: 263-278, 2015b.

Zhao, X., Si, J., Miao, Y., Peng, Y., Wang, L., Cai, X.: Comparative proteomics of *Euphorbia kansui Liou* milky sap at two different developmental stages. - Plant Physiol. Biochem. **79**: 60-65, 2014.

Zheng, W., Komatsu, S., Zhu, W., Zhang, L., Li, X., Cui, L., Tian, J.: Response and defense mechanisms of *Taxus chinensis* leaves under UV-A radiation are revealed using comparative proteomics and metabolomics analyses. - Plant Cell Physiol. **57**: 1839-1853, 2016.

Zhou, L., Xu, H., Mischke, S., Meinhardt, L.W., Zhang, D., Zhu, X., Li, X., Fang, W.: Exogenous abscisic acid significantly affects proteome in tea plant (*Camellia sinensis*) exposed to drought stress. - Hort. Res. **1**: 14029, 2014.

Zhu, W., Xu, X., Tian, J., Zhang, L., Komatsu S.: Proteomic

analysis of *Lonicera japonica* Thunb. immature flower buds using combinatorial peptide ligand libraries and polyethylene glycol fractionation. - J Proteome Res. **15**: 166-181, 2015.

Zurbriggen, M.D., Tognetti, V.B., Maria, F.F., Hajirezaei, M.R.,Valle, E.M., Carrillo, N.: Combating stress with flavodoxin: a promising route for crop improvement. - Trends Biotechnol. **26**: 531-537, 2008.