Mechanism of Proline Biosynthesis and Role of Proline Metabolism Enzymes Under Environmental Stress in Plants

Szepesi, Ágnes [Szepesi, Ágnes (Növényélettan), szerző] Növénybiológiai Tanszék (SZTE / TTIK / BI); Szőllősi, Réka [Szőllősi, Réka (növényi oxidatív ...), szerző] Növénybiológiai Tanszék (SZTE / TTIK / BI)

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28. Mechanism of proline biosynthesis and role of proline metabolism enzymes under environmental stress in plants

Ágnes Szepesi* and Réka Szőllősi

Department of Plant Biology

University of Szeged, Hungary

H-Szeged, Közép fasor 52. 6726. Hungary

Telephone/fax: +36 62 544 307

Corresponding author: Ágnes Szepesi, PhD E-mail: szepesia@bio.u-szeged.hu

1 Introduction

Proline, as a multifunctional molecule in plant development and in response to a wide range of plant abiotic and biotics stresses can act as both compatible osmolyte and radical scavenger or by its degradation as a supply of energy for re-growth after stress situations. In this Chapter, we discuss the complexity of proline biosynthesis and catabolism deciphering components involved in the regulation of proper plant growth and development as well in the context of abiotic and biotic stress induced processes. Although numerous recent excellent reviews have been written, we provide a broad view of the most recent approaches of how proline can take part in other signaling pathways and interconnect with some phytohormones. Investigating the post-transcriptional modification of Pro-mediated enzymes provide a new uncovered area for

better understanding the efficiency of proline regulated stress responses and plant developmental processes.

2 Proline biosynthesis and degradation in plants

The place of proline biosynthesis in plants is the chloroplast and cytoplasm while degradation of proline occurs in the mitochondria. The pathways involved in proline synthesis and degradation are regulated by multiple enzymes. Proline dehydrogenase (ProDH) catalyzes the first and rate-limiting step in the transformation of proline (Pro) into glutamic acid (Glu) that takes place at mitochondria. Some studies indicated that as ProDH is bound to the inner mitochondrial membrane, may have a role in transferring electrons to ubiquinone in the mitochondrial electron chain in higher plants (Elthon and Stewart, 1981, Kiyosue et al., 1996, Liang et al., 2013). This enzyme oxidizes Pro into delta-1-pyrroline 5-carboxylate (P5C) using FAD as cofactor, which can transform non-enzymatically into glutamate semialdehyde (GSA). P5C dehydrogenase (P5CDH) can generate Glu and NADH from GSA.

Until now, our current knowledge about the transporters of proline inside the plant cell is limited, there may be glutamate/proline antiporters. In future more investigations also need to decipher the exporters of GSA/P5C into the cytoplasm or into the mitochondria from the cytoplasm.

The Pro-Glu pathway was confirmed an important role in supporting root growth as the proline oxidation can generate reducing equivalents for mitochondrial oxidative phosphorylation (Verslues and Sharma, 2010; Liang et al., 2013). Recently, Signorelli (2016) has proposed a fermentation analogy for proline accumulation in stressed plants.

The rate-limiting enzyme of the biosynthesis of proline in higher plants is D1- pyrroline-5-carboxylate synthetase (P5CS), which is responsible for converting glutamate into pyrroline -5-carboxylate (P5C). Next, the second enzyme D1- pyrroline-5-carbxylate reductase (P5CR) can reduce P5C to proline, encoding by only one gene in most plant species that have been investigated (Armengaud et al., 2004). P5CS is regulated by a lot of factors such as abscisic acid (ABA) nat-siRNAs and epigenetically or by alternative splicing (Abraham et al, 2003; Borsani et al., 2005; Kesari et al., 2012; Zhang et al., 2013).

Proline level varies depending from the species involved or severity and duration of stress situation (Delauney and Verma, 1993), however, the relation between the proline accumulation and stress adaptation is contradictory (Hare and Cress, 1997; Chen et al., 2007). Kishor et al (2014) suggest the importance of proline homeostasis over the proline accumulation with stress tolerance. Some studies provide evidence that in case of stress-induced proline accumulation could coexist with elevation of catabolic activities (Fabro et al., 2004; Kaplan et al., 2007). The overexpression of key proline synthesis pathway genes influences the modulation of the shoot/root biomass ratio, the inflorescence architecture and economic yield under stressful conditions (Kishor et al., 1995; Hare, Cress and van Staden, 1999) highlighted the importance of these by knockout transgenic Arabidopsis lines (Szekely et al., 2008; Funck et al., 2012).

P5CS1 and ProDH show different regulation profiles under stress, while the earlier is up-regulated under stress, the transcription of later enzyme in several plant species is down-regulated by moisture stress, dehydration and salinity (Verslues, Kim and Zhu, 2007; Sharma and Verslues, 2010) and up-regulated when stress is relieved (Yoshiba et al., 1997; Satoh et al., 2002).

The Pro synthetized in shoots can be transported to roots for subsequent degradation, sustaining root growth under unfavorable conditions (Sharma et al., 2011).

Proline responding1, gene encoding a P5CS catalyzing the biosynthesis of proline from glutamic acid, plays a critical role in regulating general protein synthesis and the cell cycle in maize (Wang et al., 2015). Loss of function of Pro1 showed inhibited proline biosynthesis and decreased its accumulation in the pro1 mutant. Proline deficiency triggered increasing of the level of uncharged tRNApro AGG accumulation and the phosphorylation of eukaryotic initiation factor 2α (eIF2α) in the pro1 mutant, which could lead to a general reduction in protein synthesis in this mutant. Downregulation of major cyclin genes at the transcriptional level, which caused cell cycle arrest and the suppression of cell proliferation due to proline deficiency were reversible when external proline is supplied to the mutant, suggesting a regulatory role of proline in the cell cycle transition. Based on these evidences, it can be demonstrated that proline plays an important role in the regulation of general protein synthesis and the cell cycle transition in plants.

Reproductive organs can show parallel induction of P5CS (Savouré et al., 1995), ProDH (Nakashima et al., 1998), and P5CDH (Deuschle et al., 2004), while expression of P5CS2, P5CR, and ProDH1 increase in meristematic tissues such as root tip, shoot apex, and inflorescences (Kavi Kishor and Sreenivasulu, 2014). In Brassica napus genome, six BnaProDH1 and two BnaProDH2 genes were described, the BnaProDH1 genes are mainly expressed in pollen and roots' organs while BnaProDH2 gene expression is associated with leaf vascular tissues at senescence (Faes et al., 2015).

The inner mitochondrial membrane is the place of the two-step oxidation of proline in all eukaryotes. The first step is the consecutive action of proline dehydrogenase (ProDH) that produces delta(1)-pyrroline-5-carboxylate (P5C) and the second is the P5C dehydrogenase

(P5CDH) that oxidizes P5C to glutamate. Once osmotic stress occurred, this catabolic route is down-regulated, allowing free Pro accumulation in plants. The cellular Pro to P5C ratio under ambient and osmotic stress conditions did not change in case of overexpression of MsProDH in tobacco and Arabidopsis or impairment of P5C oxidation in the Arabidopsis p5cdh mutant indicating that P5C excess was reduced to Pro in a mitochondrial-cytosolic cycle. This cycle, with ProDH and P5C reductase, exists in animal cells and Miller et al (2009) described also in plants. In this cycle, Pro oxidation by the ProDH-FAD complex delivers electrons to the mitochondrial electron transport chain and in the case of hyperactivity of the cycle, e.g. when an excess of exogenous Pro is provided, can generate reactive oxygen species (ROS) by delivering electrons to O_2. After exogenous Pro treatment, the lack of P5CDH activity induced higher ROS production under dark and light conditions, and rendered plants hypersensitive to heat stress. In order to avoid Pro mediated toxic effects it is important to balance mitochondrial ROS production during increased Pro oxidation by P5CDH which is the key enzyme to prevent P5C-Pro intensive cycling and avoid ROS production from electron run-off.

Fichman et al. (2015) reviewed proline biosynthesis pathways in different domains of life. The most prevalent pathway of proline synthesis is assisted by bi-functional Δ1-pyrroline-5-carboxylate synthase, in higher plants and animals, while there are alternative pathways of proline formation using the initial steps of the arginine biosynthetic pathway to ornithine, which can be converted to Δ1-pyrroline-5-carboxylate by ornithine aminotransferase and then reduced to proline or converted directly to proline by ornithine cyclodeaminase. In some organisms, the latter pathways contribute to or could be fully responsible for the synthesis of proline. Fichman et al (2015) provided novel data about proline biosynthesis in organisms contributing elucidating the conservation of proline biosynthetic enzymes, significance of specific residues for catalytic activity and allosteric regulation are analyzed on the basis of protein structural data, multiple sequence alignments, and mutant studies.

3 Pro and abiotic stress factors

It is well-known that Pro has got a diversified role in plant metabolisms, especially in plant responses to several types of biotic and abiotic stresses, e.g. it does not only act as a non-enzymatic antioxidant but it may form complexes with heavy metals or it can be a signaling molecule (reviewed by Hare and Cress 1997; Hossain et al. 2014; Emamverdian et al. 2015). Numerous results have exhibited that Pro accumulation can be regarded as a general consequence of the stresses and its level depends on the degree and duration of the stress, as well as the ontogenetic stage of the plants (Kaur et al. 2011; Rejeb et al. 2013). Besides, other investigations executed on transgenic plants also confirmed that changes of Pro metabolism, mainly up- or down-regulation of enzymes improves the stress tolerance (review from Szabados and Savouré 2010; Ku et al. 2011; Lv et al. 2011; Rejeb et al. 2013). Moreover, exogenous application of Pro may also alleviate the negative effects of various abiotic stresses (Xu et al. 2009; Kaur et al. 2011; Zouari et al. 2016).

3.1 Salinity

Actually, due to climate change salinity has become one of the most serious abiotic stress factors, seldom in combination with drought stress, which may result in the loss of the yield and the quality of agricultural products (Mittler 2006; Shabala 2013). Salt-tolerant plants, namely halophytes (such as quinoa from Chenopodiaceae) have been acclimated to high salt concentration of the environment, while most of the plants (including the main crops) are non-tolerant and usually show e.g. disfunctions in photosynthetic activity, oxidative stress or other morpho-physiological changes of the organs, as it has been presented by several investigations (Meloni et al. 2003; Szepesi et al. 2008; Shabala 2013; Denlein et al. 2014; Singh et al. 2016). All the results pronounce that supra-optimal salt concentration provokes not only oxidative stress and usually the up-regulated activities of the enzymatic antioxidants, but it triggers Pro

accumulation accompanied by the changes of the enzymes of Pro metabolism (Table A). It was found that in the leaves of salt-stressed Indian mustard (Brassica juncea L. cv. Pusa Jai Kisan) or eggplant (Solanum melongena L. var. Neelam) the elevated level of Pro content was probably due to the enhanced activity of Δ1-pyrroline-5-carboxylate synthetase (P5CS) and disfunction of proline oxidase (PO) or proline dehydrogenase (PDH) (Iqbal et al., 2015; Singh et al., 2016; Table A). Moreover, transgenic potato plants with accrued level of P5CS and Δ1-pyrroline-5-carboxylate reductase (P5CR) activities expressed much higher Pro content compared to non-transgenic (control) lines (Hmida-Sayari et al., 2005; Table A).

3.2 Drought

Lack of water, beside salinization, may affect the fecundity of crops, especially in the arid fields. Therefore, the acclimation and the adaptation to water deprivation is a great defial for the intolerant plants. Several researchers displayed the detrimental impact of water deficit resulting in numerous morpho-physiological variations in plants e.g. a decline in photosynthetic efficiency, ROS-overproduction or reduced development of organs (Alexieva et al. 2001; Arora et al. 2002; Karataş et al. 2014). In both wild-type and transformed tobacco (Nicotiana tabacum L. cv. M51) plants exposed to water retention showed to have an increment of Pro level in leaves and roots, as well (Cvikrová et al. 2013). Dobrá et al. (2011) preformed that Pro accumulation was in correlation with the higher activity of P5CS and the lower level of PDH activity (Table A). Similarly, significant inhibition of Pro accumulation was observed in P5CS-silenced Nicotiana benthamiana plants (Ku et al. 2011).

3.3 Chilling and heat stress

Extremely high and low temperature values also might affect negatively the productivity of crops, though some plants have acclimated having specialized epidermal surface, e.g. waxy cuticle on the needles of pines or species covered with hairs can bear heat or cold stress. Most

of the commercial species (vegetables, cereals, etc.) can hardly bear weather extremities and several morphological alterations and/or physiological symptoms appear. Low temperature can cause disfunction of the chloroplasts or mitochondria in sensitive plants inducing oxidative stress. At the same time, chilling may also the change of Pro content (mainly accumulation) providing stabilization of the cellular structure, protection of enzymes and stress tolerance (Hare and Cress 1997; Prasad et al. 1994; Rivero et al. 2001; Szabados and Savouré 2010).

In chickpea (Cicer arietinum L. cv. GPF2) which is an important food legume in Asia, after one week- long chilling stress endogenous Pro level increased then decreased in the leaves at reproductive phase, while after foliar application of Pro it was significantly higher compared to non-treated chilling stressed plants (Kaur et al. 2011; Table A). Kumar and Yadav (2013) also confirmed the protective effects of exogenous usage of Pro. In the topmost leaves of cold stressed tea (Camellia sinensis (L.) O. Kuntze) lipid peroxidation diminished due to Pro exposure. In case of bamboo (Phyllostachys praecox f. prevernalis) induced activities of P5CS, PDH and ornithine-Δ-aminotransferase (OAT) were found which later attenuated (Liu et al. 2015; Table A).

Several studies have shown that heat stress can dramatically destroy the plants at physiological and/or morphological level, especially combined with water deficit or salt stress (Mittler 2006). Elevated temperature usually triggers the decline of photosynthetic rate, oxidative stress and Pro accumulation, as well, as it was found in the leaves if wheat, eggplant or chickpea (see Table A; Hare and Cress 1997; Kotak et al. 2007; Rejeb et al. 2013; Wu et al. 2014).

Type of abiotic stress factor	Plant name	Duration of pre-cultivation	Concentration or degree of the stress	Time of exposure	Plant organ investigated	Change in Pro content*	Activity of enzymes of Pro metabolism*	Reference
Salinity (NaCl)	*Brassica juncea* L. cv. Pusa Jai Kisan	no data	100 mM	30 days	leaves	↑	P5CS ↑, PO ↓	Iqbal et al., 2015
	Cucumis sativus L. var. Jumbo	30 days	150 mM	3 days	leaves	↑	no data	Fariduddin et al., 2013
	Cucumis sativus L. var. Rocket	30 days	150 mM	3 days	leaves	↑	no data	
	Mesembryanthemum crystallinum L.	4 weeks	100 mM	24 h	leaves	↑	no data	Shevyakova et al., 2009
		9 weeks	100 mM	24 h	leaves	↑	no data	
	Pisum sativum L. 'Sprinter'	20 days	100 mM	no data	leaves	↑	no data	Shahid et al. 2016
	Solanum melongena L. var. Neelam	15 days	0.3 g kg-1 (sand)	12 days	leaves	↑	P5CS ↑, PDH ↓	Singh et al., 2016
			0.5 g kg-1 (sand)			↑	P5CS ↑, PDH ↓	
	Solanum tuberosum L. cv. Nicola non-transgenic line	4-6 weeks	100 mM	10 days	leaves	↑ ns	non-detectable	Hmida-Sayari et al., 2005
			180 mM			↑	non-detectable	
	Solanum tuberosum L. cv. Nicola transgenic line 15B	4-6 weeks	100 mM	10 days	leaves	↑↑	P5CS and P5CR ↑	
			180 mM			↑↑	P5CS and P5CR ↑	
	Solanum tuberosum L. cv. Nicola transgenic line 17	4-6 weeks	100 mM	10 days	leaves	↑↑	P5CS and P5CR ↑	
			180 mM			↑↑	P5CS and P5CR ↑	
	Spirodela polyrhiza L., clone DR	24 ± 2 days	100 mM	4 days	frond (i.e. whole plant)	No change	P5CS ↑ ns, P5CR ↑ ns, GS ↓ ns	Cheng et al., 2013
			200 mM	4 days	frond (i.e. whole plant)	↓	P5CS ↑ ns, P5CR ↑, GS ↓	
Drought (water deficit)	*Nicotiana tabacum* L. cv. Wisconsin 38	6 weeks	No irrigation	1 day	leaves	↑ in upper and lower leaves but ↓ ns in middle leaves	PDH ↓, P5CS ↑	Dobrá et al., 2011
				6 days		↑	PDH ↓, P5CS ↑	
				1 day	roots	↑	PDH ↓, P5CS ↑	
				6 days		No change	PDH ↓, P5CS ↑	
	Nicotiana tabacum L. cv. M51 (wild type)	6 weeks	No irrigation	5 and 10 days	leaves	↑ and ↑↑	no data	Cvikrová et al., 2013
					roots	↑ ns	no data	
	Nicotiana tabacum L. M51-1 transformant			5 and 10 days	leaves	↑ and ↑↑	no data	
					roots	↑	no data	
Low temperature (chilling)	*Cicer arietinum* L. cv. GPF2	ca. 2 months	8.3–9.6/2.8–5.3 ◦C (max./ min.)	7 days	leaves	↑, later ↓	no data	Kaur et al., 2011
	Phyllostachys praecox f. prevernalis	14 days	1°C	42 days	shoots	↑	OAT ↑, later ↓; P5CS ↑; PDH ↑, later ↓	Liu et al., 2015
High temperature								
(heat)	*Cicer arietinum* L. cv. GPF2	no pre-cultivation	35-45 °C	10 days	leaves	↑ and ↑↑	no data	Kaushal et al., 2011
	Triticum aestivum L. cultivars (Unnat Halna, Halna, Raj3765, C306, NIAW34, WR544, WH730, HD2877 and PBW343)	4 weeks	38°C	4 h	leaves	↑	no data	Mishra et al., 2017
	Vigna aconitifolia L. varities (e.g. Jwala, RMO 40, RMO 225 and RMO 423)	7 days	42°C	1 h	leaves	↑ or ↓ ns	no data	Harsh et al., 2016
* ↑ indicates significant and ↑↑ significantly high increase, ↑ ns indicates non-significant increase, while ↓ refers to significant decrease and ↓ ns signs non-significant reduction in								

Table A: Summary of abiotic stress factors that affect proline metabolism in plants.

3.4. Heavy metals

Up to now the emission of heavy metals (HMs) has been elevated due to anthropogenic activities such as mining, industrial, agricultural works and the output of municipal wastes which may have disadvantageous consequences in plants. Albeit, aluminum (Al) is not a HM because of its density (2.70 g cm−3), but due to its toxic effects it is often mentioned among the classical HMs such zinc (Zn), iron (Fe), copper (Cu), cadmium (Cd) or lead (Pb) (Matsumoto and Motoda 2012). The main visual symptoms of HM stress and /or toxicity in plants are chlorotic leaves, decreased or inhibited germination of seeds, disturbed development of vegetative parts (root and shoot) (Arora et al. 2002; Maksymiec 2007; Farooq et al. 2009; Emamverdian et al. 2015). Beside the toxic metals like Cd, Pb, Hg which can cause oxidative stress in the plant cells, the essential HMs (e.g. Fe, Zn, Ni or Cu) at supra-optimal concentration also might provoke the increment of ROS. Here we discuss the influence of excess HM on Pro metabolism in case of the main essential and non-essential HMs.

Copper (Cu) is an essential micronutrient for plants and its mean concentration in the soil is 2-40 mg kg-1 DM (dry matter), but it can reach 10-15-fold higher concentrations in agricultural soils due to the usage of sewage sludge, fertilizers and pesticides, and may induce dramatic changes in photosynthetic efficiency accompanied by reduced shoot growth and leaf chlorosis (Hirt and Shinozaki 2004; Janas et al. 2010; Yadav 2010; Emamverdain). Cu being a redox-active HM can cause lipid peroxidation, catalyze •OH radical formation trough Haber-Weiss and Fenton-reaction and change the activity of several enzymes (Arora et al. 2002; reviewed by Schützendübel and Polle 2002; Feigl et al. 2013; Sytar et al. 2013). In plants after Cu exposure increment of Pro content was observed which was in correlation with the elevated activity of P5CS, OAT and GDH but diminution of PDH activity (Table B).

Zinc (Zn) is also an essential trace element for plants, its mean concentration is 10-80 mg kg-1 DM (in soil) but in polluted areas the range can 150-300 mg kg-1 DM and becomes phytotoxic (Hirt and Shinozaki 2004; Emamverdian et al. 2015). Zn plays an important role in N-metabolism, chlorophyll biosynthesis, cell growth regulation, root development and acts as a constituent of several enzymes such as oxidoreductases, transferases, hydrolases, ligases (Rout and Das 2003; Broadley et al. 2007; Halušková et al. 2010; Emamverdian et al. 215). At supra-optimal concentrations, it may cause growth inhibition and chlorosis due to oxidative stress, lipid peroxidation or disorders in various metabolic processes (Wang et al. 2009; Halušková et al. 2010; Janas et al. 2010; Stoláriková-Vaculíková et al. 2015). Zinc is a non-redox active HM thus it evokes oxidative stress indirectly (Dat et al. 2000; Cuypers et al. 2002). Pro accumulation was reported in tomato shoots and the leaves and roots of wheat after Zn excess (Li et al.; Al Khateeb and Al-Qwasemeh 2014; Table B).

Nickel (Ni) is a micronutrient required by plants at very low concentration (Gajewska et al. 2006; Emamverdian et al. 2015). In natural soils its level ranges 10-1000 mg kg-1 DM but at 20-30-fold levels it becomes toxic (Yadav et al. 2010; Emamverdian et al. 2015; Pavlovkin et al. 2016). Several studies have exhibited that excess Ni may cause growth inhibition, chlorosis, tissue damage and functional disorders of plasma membrane or enhances ROS-generation in the plant cells (Gajewska et al. 2006; Kumar et al. 2007; Fiala et al. 2013; Pavlovkin et al. 2016). It was also proved that in crop plant such as Eleusine coracana (mainly in Africa and Asia) or pea high levels of Ni may provoke Pro accumulation in all vegetative plant organs (Gajewska and Skłodowska, 2005; Viswanath et al. 2016; Table B).

Cobalt (Co), similarly to Ni, is a transitional element and the co-factor of several enzymes. Excess Co may impact plant development, the integrity of plant cell wall and membrane, drought tolerance, photosynthetic activity or the uptake and translocation of other elements (Tewari et al. 2002; Jayakumar et al., 2007; Li et al. 2007; Arora et al. 2012). Though there are

not much data about the relationship between Co toxicity and Pro metabolism it seems that this HM also increases Pro content in plants (Table B).

Cadmium (Cd), known as a non-essential toxic heavy metal and can induce numerous morpho-physiological disorders in plants. Typical signs of Cd stress are reduced plant development and photosynthesis, chlorotic leaves or declined seed germination rate (Arora et al. 2002; Benavides et al. 2005; Halušková et al. 2010; Fiala et al. 2013). These symptoms are the probable consequences of oxidative stress and altered activity of several enzymes (Gallego et al. 1999; Szőllősi et al. 2009; Dietz et al. 2016). In natural soils Cd content is estimated to be about 0.06–0.50 mg kg-1 DM, but due to anthropogenic activities Cd output to agricultural environment has increased. Since Cd is not redox active it induces indirectly ROS-overproduction and has great affinity to bind to –SH groups of proteins which generally results in the dysfunction of the enzymes (Arora et al. 2002; Emamverdian et al. 2015). Several data support that plants at various age exposed to this toxic HM try to protect themselves by accumulating Pro (see Table B).

Up to now the level of toxic lead (Pb) has increased in soils as a result of several anthropogenic activities. Some plants are tolerant to Pb and can accumulate it, but most of the species exhibit toxicity symptoms like damage of root cells, declined root lenght or chlorotic leaves (Islam et al. 2008; Çavuşoğlu et al. 2009; reviewed by Nagajyoti et al. 2010). These visual symptoms are because of water imbalance, disturbed mineral nutrition or malfunction of enzymes (Seregin and Ivanov 2001; Yadav 2010). Crop plants such as soya or wheat showed to have elevated level of Pro in the leaves or expressed no change after Pb stress (Öncel et al., 2000; Imtiyaz et al., 2014; Table B).

Since aluminum (Al) is not a HM because of its density it is regarded to be toxic. At acidic pH (5-5.5) it produces a trivalent cation (Al^{3+}) and becomes toxic for plants (Emamverdian et al.

2015). Several studies have assessed that excess Al results in root growth inhibition and severe damage to root epidermal cells as a consequence of lipid peroxidation and ROS-overproduction (Dat et al. 2000; Matsumoto and Motoda 2012). Experiments executed on maize exhibited that the accrual of Pro level in both shoots and roots may be protective against Al stress, while in higher Al concentration can cause Pro degradation in both sensitive and resistant tomato plants (Khan et al., 2000; Surapu et al., 2014; Table B).

Type of abiotic stress factor	Plant name	Duration of pre-cultivation	Concentration or degree of the stress	Time of exposure	Plant organ investigated	Change in Pro content*	Activity of enzymes of Pro metabolism*	Reference
Heavy metals								
Cu	*Cucumis sativus* L. var. Jumbo	30 days	100 mg kg−1 (sand)	3 days	leaves	↑	no data	Fariduddin et al., 2013
	Cucumis sativus L. var. Rocket	30 days	100 mg kg−1 (sand)	3 days	leaves	↑	no data	
	Solanum nigrum L.	no data	50, 100, 150 and 200 μM	5 weeks	shoot	↑	no data	Al Khateeb and Al-Qwasemeh 2014
	Solanum lycopersicum L.	no data	50, 100, 150 and 200 μM	5 weeks	shoot	↑	no data	Al Khateeb and Al-Qwasemeh 2014
	Zea mays var. rugosa Bona	54 h	50 μM	4-24 h	shoot	↑ns and ↑ at 12-24h	P5CS ↑ at 12-24h, GDH ↑ at 12-24h, OAT ↑, PDH ↓ at 8-24h	Wen et al., 2013
Zn								
	Solanum nigrum L.	no data	0.25, 0.5, 0.75 and 1.0 mM	5 weeks	shoot	↑	no data	Al Khateeb and Al-Qwasemeh 2014
	Solanum lycopersicum L.	no data	0.25, 0.5, 0.75 and 1.0 mM	5 weeks	shoot	↑ns and ↑ at 1.0 mM	no data	Al Khateeb and Al-Qwasemeh 2014
	Triticum aestivum L. cv. Xihan 3	no data	0.5, 1 and 3 mM	6 days	leaves	↑	OAT↑, PDH ↑	Li et al., 2013
					roots	↑	OAT ↑ns and ↑ at 1 and 3 mM; PDH no change and ↓ at 3 mM	
Ni								
	Eleusine coracana L. (Sri Chaitanya VR-847)	7 days	0.5 mM	7 days	shoot	↑	no data	Viswanath et al. 2016
					root	↑	no data	
	Pisum sativum L. cv. 'Cud Kelvedonu'	14 days	10, 100 and 200 μM	1-9 days	leaves	↑	no data	Gajewska and Skłodowska, 2005
					roots	↑ and ↑ ns	no data	
	Pisum sativum L. 'Sprinter'	20 days	100 μM	no data	leaves	↑	no data	Shahid et al. 2016
Co	*Glycine max* L. varieties (SL-688, PS-1347 and DS-9712)	no data	50, 100 and 150 μM	no data	leaves	↑↑	no data	Imtiyaz et al., 2014
Cd	*Avicennia marina* (Forsk.)	2 weeks	0.5 mg kg−1	30 days	leaves	↑	no data	Dai et al., 2017
			5 mg kg−1			↑	no data	
	Kandelia obovata (S., L.)	2 weeks	0.5 mg kg−1	30 days	leaves	↑ ns	no data	Dai et al., 2017
			5 mg kg−1			↑	no data	
	Olea europaea L. cv Chemlali	2 years	10 and 30 mg kg-1 soil	5 months	leaves	↑	no data	Zouari et al., 2016
					roots	↑	no data	
	Solanum nigrum L.	no data	100, 200 and 400 μM	5 weeks	shoot	↑	no data	Al Khateeb and Al-Qwasemeh 2014
	Solanum lycopersicum L.	no data	100, 200 and 400 μM	5 weeks	shoot	↓ns and ↑ at 400 mM	no data	Al Khateeb and Al-Qwasemeh 2014
	Triticum aestivum L. cv. Gerek-79	7 days	50, 100, 250, 500 mg l−1	6 days	leaves	↑	no data	Öncel et al., 2000
	Triticum aestivum L. cv. Bolal-2973	7 days	50, 100, 250, 500 mg l−1	6 days	leaves	↑ ns	no data	
Pb								
	Glycine max L. varieties (SL-688, PS-1347 and DS-9712)	no data	50, 100 and 150 μM	no data	leaves	↑	no data	Imtiyaz et al., 2014
	Sesamum indicum	30 days	2 mM	48h	root	↑	no data	Amooaghaie et al., 2017
	Triticum aestivum L. cv. Gerek-79	7 days	50, 100, 250, 500 mg l−1	6 days	leaves	No change	no data	Öncel et al., 2000
	Triticum aestivum L. cv. Bolal-2973					No change	no data	
Al								
	Lycopersicum esculentum Mill. cv. Gowri (sensitive)	no data	500 μM	2 weeks	leaves	↓	no data	Surapu et al., 2014
	Lycopersicum esculentum Mill. cv. Siri (resistant)					↓ ns	no data	
	Zea mays L. cv. Zea 769, (tolerant)	3 days	0.22 mM	7 days	shoot	↑	no data	Khan et al., 2000
					roots	↑↑	no data	
	Zea mays L. cv. Bozm 1335 (tolerant)				shoot	↑	no data	
					roots	↑↑	no data	
	Zea mays L. cv. Reward (sensitive)				shoot	↑ ns	no data	
					roots	↑ ns	no data	
	Zea mays L. cv. Golden (sensitive)				shoot	↑ ns	no data	
					roots	↑ ns	no data	

* ↑ indicates significant and ↑↑ significantly high increase, ↑ ns indicates non-significant increase, while ↓ refers to significant decrease and ↓ ns signs non-significant reduction in Pro content and enzyme activities

Table B: Changes of proline metabolism during heavy metal stress in plants

Oh et al. (2017) investigated Pro accumulation and related gene expression during spring regrowth in three Rosaceae species, suggesting that Pro accumulation in response to higher temperatures during the spring regrowth is a more general phenomenon across Rosaceae fruit trees, and although peach shoots may accumulate Pro via the ornithine pathway during spring regrowth, there was no evidence for this in plum and apple.

Lipid signaling can be involved in the regulation of proline biosynthesis as a negative regulators (Thiery et al., 2004), however, during high salt stress (200 mM NaCl) calcium signaling and phospholipase C could trigger P5CS1 transcription and proline accumulation (Parre et al., 2007). Halophyte plants, such as Thelungiella salsuginea, function in an opposite manner in the case of lipid signaling (Ghars et al., 2012).

PLD signaling for proline biosynthesis shows similar process to RD29A gene expression and different from the abscisic acid-dependent RAB18 gene expression. Some studies revealed that PLDs play positive and negative roles in hyperosmotic stress signal transduction in plants, contributing to a precise regulation of ion homeostasis and plant salt tolerance. In cashew plants treated with NaCl, parallel increases of Orn and Pro have also been observed which activated OAT and curiously repressed P5CDH (Da Rocha et al., 2012).

In addition, NADPH oxidases trigger proline accumulation in response to salt stress independently of the SOS pathway (Ben Rejeb et al., 2015).

P5CS1 expression has also been confirmed to be stimulated by light (Hayashi et al., 2000) and nitric oxide (NO) (Zhao et al., 2009) in Arabidopsis. Under osmotic and salt stresses, ABA can induce AtP5CS1 expression through the cis-acting ABA responsive (ABRE) element (Strizhov et al., 1997). However, Savouré et al. (1997) showed that stress induction of P5CS1 occurs independently of ABA upon cold and osmotic stresses. Sharma and Verslues (2010) provided

evidences supporting these by indicating significant ABA-independent expression of P5CS1 at low water potential demonstrated by comparing ABA-deficient (aba2-1), proline accumulation deficient (p5cs1-4) and double (p5cs1-4/aba2-1) mutants.

4 Proline metabolism during plant biotic stress

Pro metabolism appears to be tightly regulated by environmental and endogenous signals. During plant-pathogen interactions, it has been described increases in glutamate, proline and γ-aminobutyric acid (GABA) that can, directly or indirectly, exhibit ROS scavenging activity (Takahashi et al., 2008; Verslues and Sharma, 2010). Recently, ProDH has been implicated in defences against pathogens. In Arabidopsis plants, Cecchini et al (2011a) reported enhanced gene expression and enzyme activity on elicitation of the hypersensitive response (HR) in the case of treatment with Pseudomonas syringae pv. tomato (Pst) AvrRpm1 (Pst-AvrRpm1). Senthil-Kumar and Mysore (2012) stated that ProDH1/2 genes are also induced in Nicotiana benthamiana plants establishing a non-host interaction with Pst T1. In both cases, silencing of ProDH genes compromises the accumulation of reactive oxygen species (ROS) (Cecchini et al. 2011a), the generation of cell death and disease resistance (Senthil-Kumar and Mysore, 2012). Moreover, ProDH activation is believed to sustain the oxidative burst and to reduce cell viability in other kingdoms (Cecchini et al., 2011b). ProDH1 and ProDH2 genes could be differentially regulated in infected tissues (Cecchini et al., 2011a). More recent, Rizzi et al. (2016) found that ProDH1 and ProDH2 are both necessary for achieving maximum resistance against Pst-AvrRpm1 and B. cinerea, but ProDH2 has more significant effect on early restriction of B. cinerea growth. It is important to note that while ProDH1 is upregulated by SA and JA, ProDH2 is only activated by JA, and both genes show transcriptional inter-regulation at basal and infection conditions. Rizzi et al. (2016) provided the first evidence of the contribution of ProDH2 to disease resistance showing the differential regulation and

complementary function of both enzyme isoforms in infected tissues, deciphering the fundamental role of ProDH in the control of biotrophic and necrotrophic pathogens. Transient exposure to exogenous Pro or infection with Pseudomonas syringae pv. tomato could trigger increase of ProDH activity in Arabidopsis tissues (Rizzi et al., 2015). Wild type (Col-0) and p5cdh Arabidopsis plants showed consecutively activated ProDH and Pro biosynthetic genes under both conditions by different coordination between the biosynthetic pathways, whereas in Col-0 plants induced both Pro biosynthetic routes, p5cdh mutant plants may preferentially activate the Orn route.

5 Proline and redox signalling in plants

Szabados and Savouré summarized in an excellent review (2010) that proline has multifunctional roles. There are some data about how proline can impact on ROS generation processes affecting relationship between ROS and proline metabolism (Ben Rejeb et al., 2014; 2015). Some recent studies, such as Zhang and Becker (2015) proved evidence that proline metabolic ROS production appears to be a general phenomenon in diverse organisms potentially impacting cellular processes such as aging and plant senescence. Recent studies have highlighted the roles of proline as an important player in the redox signaling in plants. Giberti et al. (2014) studied the different regulation of P5CR activity by proline and chloride ions depending on the co-factor was used NADH or NADPH. Sharma et al (2011) also confirmed that proline metabolism has a special effect on the ratio of NADP and NADPH (Sharma et al., 2011). Some studies also indicated the influence of redox sensitive enzymes on proline accumulation eg. thioredoxins (Verslues et al., 2014) and mitochondrial NAD dehydrogenases (Lovell et al., 2015).

Nowadays, some evidences appeared to prove that Pro has a role in controlling redox status in plants. In a recent study, Shinde et al. (2016) observed that lipid metabolism mutants have

higher proline accumulation under stress, and the greater proline accumulation is caused mainly by the altered redox status of these mutants as ProDH can load electrons into the mitochondrial electron transport chain regulating the cellular redox state. Based on studies by using ProDH inhibitors and exogenous Pro treatment as a ProDH inducer process, Fabro et al. (2016) demonstrated that Arabidopsis ProDH could modulate by affecting RBOHD generating ROS and contributing to flagellin-mediated PAMP-triggered immunity. Ben Rejeb et al. (2015) reported that hydrogen peroxide produced by NADPH oxidases increases proline accumulation during salt or mannitol stress in Arabidopsis thaliana. Some potential mechanisms by which proline metabolism influences plant senescence was studied in the case of petal and leaf, suggesting that proline content is influenced by the energy demands of senescing cells. Proline metabolism may influence ROS signal pathways in the flower and leaf in order to delay senescence progression. In the future, it should be elucidating those mechanisms by which proline could regulate senescence offering new methods to improve and preserve post-harvest agricultural products (for review see Zhang et al.2015). Very recent, Filippou et al (2016) reported enhanced proline accumulation after priming of Medicago truncatula plants by kresoxim-methyl, a type of strobilurins widely used as important agricultural fungicide. Data highlighted the necessity of monitoring the proline level during priming processes and if controlling the effects of different biotic compounds are needed. The above mentioned data can help decipher the complex regulation of proline mediated redox status and further investigations are needed to uncover novel steps in proline metabolism.

6 Post-transcriptional modification processes of proline metabolism enzymes

Despite some recent interesting reports, our knowledge about post-translational modification of Pro metabolism enzymes is limited. Redox sensitive modification is one possible type of these modifications, such as in the case of ProDH1 involved in electron transport (Servet et al., 2012;

Schertl et al., 2014). Recent studies revealed that enzymes involved in Pro metabolism can be affected at different levels (Fichman et al., 2014; Kavi Kishor and Sreenivasulu, 2014), eg. OAT, P5CS, and ProDH at transcriptional and post-transcriptional levels while P5CS can be inhibited by allosterically by its endproduct. P5CDH has post-transcriptional regulation by cis antisense transcripts, while P5CR shows sensitivity to redox regulation (Giberti et al., 2014). Despite of Bhaskara et al. (2015) proved by immunoblot data for post-translational modifications of both P5CS1 and ProDH1, our knowledge about these modifications are limited. Further studies to decipher these post-translational modifications of Pro metabolism enzymes will advance our understanding of the Pro-mediated biotic and abiotic stress tolerance of plants important for agriculture and food safety.

7 Proline and other signal pathways in plants

An interaction between proline metabolism and the phytohormone abscisic acid under stress was reported by some studies (Savoure et al., 1997; Strizhov et al., 1997, Abraham et al., 2003); despite of the fact that ABA alone cannot duplicate drought-induced proline accumulation (Sharma and Verslues, 2010). Our current knowledge about how proline can coordinate and affect cell redox status by other metabolic pathways is very limited. Very recently, Shinde et al. (2016) proved evidence that both proline and VLCFA (very-long-chain fatty acid) synthesis can help buffer cellular redox state and also produce some products, eg. compatible osmolytes and cuticle lipids for improving stress resistance.

8 Future perspectives

A recent review by Mansour and Ali (2017) provided an excellent evaluation of proline functions in saline conditions and concluded that comprehensive future research is needed to establish the proline exact mechanism by which it enhances plant salt tolerance. Further investigations are needed to determine effective proline concentration range for external proline

application or the best mode of application eg. seed soaking and focusing the fruit quality of this plants controlling food safety. Recent examples of exogenous proline application is in the case of olive fruits and date palm (Zouari et al., 2016a and b) also provided evidence that exogenous treatment with proline improved mineral nutrition, enzymatic and non-enzymatic antioxidant systems counteracting cadmium inhibitory effects in young date palm plants. Aksakal et al. (2017) described that proline applied by exogenously could enhance the levels of GA, IAA, the concentrations of soluble sugars and organic acids and expressions of PAL, γ-TMT and ProDH genes as compared to the control. The results obtained in this study suggest that pre-treatment with exogenous Pro provides important contributions to the increase in the UV-B tolerance of lettuce by regulating the biochemical mechanisms of UV-B response. There are some contradictionary data about effectiveness of exogenous Pro treatment, eg. Wani et al (2016) reported that proline was not highly effective in alleviating the undesirable effects of higher degrees of salt stress in either Brassica juncea cultivars investigated. As in the study of Aliferis et al. (2014) that provide metabolic data reporting in plant-pathogen pathosystems, metabolomics of proline homeostasis in plants may uncover novel possible strategies for improve crop management and food safety.

Abbreviations used in this Chapter

ABA, abscisic acid

bZIP, basic leucine zipper transcription factor

Ca^{2+}, calcium

GABA, gamma aminobutyric acid

GSA, glutamic γ-semialdehyde

H_2O_2, hydrogen peroxide

OAT, ornithine D-aminotransferase

P5C, pyrroline-5-carboxylate

P5CR, pyrroline-5-carbxylate reductase

P5CS, pyrroline-5-carboxylate synthetase

P5CDH, P5C dehydrogenase

ProDH, proline dehydrogenase

PLC, phospholipase C

PLD, phospholipase D

SiRNA, small interfering RNA

SRO5, similar to radical induced cell death one which shares a 3' overlapping region with P5CDH

SNAC, stress responsive NAC transcription factor

References

Abraham, E., Rigo, G., Szekely, G., Nagy, R., Koncz, C., and Szabados, L.2003. Light-dependent induction of proline biosynthesis by abscisic acid and salt stress is inhibited by brassinosteroid in Arabidopsis Plant Mol Biol 51: 363-372. doi: 10.1023/A:1022043000516.

Aksakal, O., Tabay, D., Esringu, A., Aksakal, F.I., Esim, N. 2017. Effect of proline on biochemical and molecular mechanisms in lettuce (Lactuca sativa L.) exposed to UV-B radiation. Photochem Photobiol Sci 16: 246-254. doi: 10.1039/C6PP00412A.

Alexieva, V., Sergiev, I., Mapelli, S., Karanov, E. 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant, Cell and Environment 24, 1337-1344.

Aliferis, K.A., Faubert, D., Jabaji, S. 2014. A metabolic profiling strategy for the dissection of plant defense against fungal pathogens. Plos One doi: 10.1371/journal.pone.0111930.

Al Khateeb, W., Al-Qwasemeh, H. 2014. Cadmium, copper and zinc toxicity effects on growth, proline content and genetic stability of Solanum nigrum L., a crop wild relative for tomato; comparative study. Physiology and Molecular Biology of Plants 20(1), 31-39.

Amooaghaie, R., Enteshari, S. 2017. Role of two-sided crosstalk between NO and H2S on improvement of mineral homeostasis and antioxidative defense in Sesamum indicum under lead stress. Ecotoxicology and Environmental Safety 139, 210-218.

Armengaud, P., Thiery, L., Buhot, N., Grenier-de March, G., and Savoure, A. 2004. Transcriptional regulation of proline biosynthesis in Medicago truncatula reveals developmental and environmental specific features. Physiol Plant 120: 442-450.

Arora, P., Bhardwaj, R., Kanwar, M.K. 2012. Effect of 24-epibrassinolide on growth, protein content and antioxidative defense system of Brassica juncea L. subjected to cobalt ion toxicity. Acta Physiologiae Plantarum 34, 2007-2017.

Arora, A., Sairam, R.K., Srivastava, G.C. 2002. Oxidative stress and antioxidative system in plants. Current Science 82, 1227-1238.

Bashkara, G.B., Yang, T.H., and Verslues, P.E. 2015. Dynamic proline metabolism: importance and regulation in water limited environments. Front Plant Sci 6:1-7.

Benavides, M.P., Gallego, S.M., Tomaro, M.L. 2005. Cadmium toxicity in plants. Brazilian Journal of Plant Physiology 17, 21-34.

Broadley, M.R., White, P.J., Hammond, J.P., Zelko, I., Lux, A. 2007. Zinc in plants. New Phytologist 173, 677-702.

Çavuşoğlu, K., Ergene, A., Yalçin, E., Tan, S., Yapar, K. 2009. Cytotoxic effects of lead (Pb) and mercury (Hg) ions on root tip cells of Cicer arietinum L. Fresenius Environmental Bulletin 18(9), 1654-1661.

Cecchini, N.M., Monteoliva, M.I., Alvarez, M.E. 2011a. Proline dehydrogenase contributes to pathogen defense in Arabidopsis. Plant Physiol 155: 1947-1959.

Cecchini, N.M., Monteoliva, M.I., Alvarez, M.E. 2011b. Proline dehydrogenase is a positive regulator of cell death in different kingdoms. Plant Signal Behav 6: 1195-1197.

Cheng, T.S., Hung, M.J., Cheng, Y.I., Cheng, L.J. 2013. Calcium-induced proline accumulation contributes to amelioration of NaCl injury and expression of glutamine synthetase in greater duckweed (Spirodela polyrrhiza L.). Aquatic Toxicology 144, 265-274.

Cvikrová, M., Gemperlová, L., Martincová, O., Vanková, R. 2013. Effect of drought and combined drought and heat stress on polyamine metabolism in proline-over-producing tobacco plants. Plant Physiology and Biochemistry 73, 7-15.

Cuypers, A., Vangronsveld, J., Clijsters, H. 2002. Peroxidases on roots and primary leaves of Phaseolus vulgaris copper and zinc phytotoxicity: a comparison. Journal of Plant Physiology 159, 869-876.

Dai, M., Lu, H., Liu, W., Jia, H., Hong, H., Liu, J., Yan, C. 2017. Phosphorus mediation of cadmium stress in two mangrove seedlings Avicennia marina and Kandelia obovata differing in cadmium accumulation. Ecotoxicology and Environmental Safety 139, 272-279.

Da Rocha, I.M., Vitorello, V.A., Silva, J.S., Ferreira-Silva, S.L., Viegas, R.A., Silva, E.N., et al. 2012. Exogenous ornithine is an effective precursor and the D-ornithine amino transferase pathway contributes to proline accumulation under high N recycling in salt-stressed cashew leaves. J. Plant Physiol 169, 41-49.

Dat, J., Vandenabeele, S., Vranova, E., Van Montagu, M., Inzé, D., Van Breusegem, F. 2000. Dual action of the active oxygen species during plant stress responses. Cellular and Molecular Life Sciences 57, 779-795.

Deinlein, U., Stephan, A.B., Horie, T., Luo, W., Xu, G., Schroeder, J.I. 2014. Plant salt-tolerance mechanisms. Trends in Plant Science 19(6), 371-379.

Deuschle, K., Funck, D., Forlani, G., Stransky, H., Biehl, A., Leister, D. et al. 2004. The role of delta1-pyrroline-5-carboxylate dehydrogenase in proline degradation. Plant Cell 16, 3413-3425.

Dietz, K.J., Mittler, R., Noctor, G., 2016. Recent progress in understanding the role of reactive oxygen species in plant cell signaling. Plant Physiology 171(3), 1535-1539.

Dobrá, J., Vanková, R., Havlová, M., Burman, A.J., Libus, J., Štorchová, H. 2011. Tobacco leaves and roots differ in the expression of proline metabolism-related genes in the course of drought stress and subsequent recovery. Journal of Plant Physiology 168(13), 1588-1597.

Elthon, T.E. and Stewart, C.R. 1981. Submitochondrial location and electron transport characteristics of enzymes involved in proline oxidation. Plant Physiol 67: 780-784.

Emamverdian, A., Ding, Y., Mokhberdoran, F., Xie, Y. 2015. Heavy metal stress and some mechanisms of plant defense response. The Scientific World Journal 2015 Article ID 756120. http://dx.doi.org/10.1155/2015/756120

Fabro, G., Kovacs, I., Pavet, V., Szabados, L., and Alvarez, M.E., 2004. Proline accumulation and *AtP5CS2* gene activation are induced by plant-pathogen incompatible interactions in Arabidopsis. Mol Plant-Microbe Interact. 17, 343–350.

Fabro, G., Rizzi, Y.S., and Alvarez, M.E. 2016. Arabidopsis proline dehydrogenase contributes to flagellin-mediated PAMP-triggered immunity by affecting RBOHD. Mol Plant Microbe Interactions 29(8): 620-628.

Faes, P., Deleu, C., Ainouche, A., Le Cahérec, F., Montes, E., Clouet, V., Gouraud, A.M., Albert, B., Orsel, M., Lassalle, G., Leport, L., Bouchereau, A., Niogret, M.F. 2015. Molecular evolution and transcriptional regulation of the oilseed rape proline dehydrogenase genes suggest distinct roles of proline catabolism during development. Planta 241 (2): 403-409.

Fariduddin, Q., Khalil, R.R., Mir, B.A., Yusuf, M., Ahmad, A. 2013. 24-Epibrassinolide regulates photosynthesis, antioxidant enzyme activities and proline content of Cucumis sativus under salt and/or copper stress. Environmental Monitoring and Assessment 185(9), 7845-7856.

Farooq, Z.R., Iqbal, M.Z., Kabir, M., Shafiq, M. 2009. Toxic effects of lead and cadmium on germination and seedling growth of Albizia lebbeck (L.) Benth. Pak. J. Bot. 41(1), 27-33.

Feigl, G., Kumar, D., Lehotai, N., Tugyi, N., Molnár, Á., Ördög, A., Szepesi, Á., Gémes, K., Laskay, G., Erdei, L., Kolbert, Z. 2013. Physiological and morphological responses of the root system of Indian mustard (Brassica juncea L. Czern.) and rapeseed (Brassica napus L.) to copper stress. Ecotoxicology and Environmental Safety 94, 179-189.

Fiala, R., Kenderešová, L., Syshchykov, D.V., Martinka, M., Repka, V., Pavlovkin, J., Čiamporová, M. 2013. Comparison of root growth and morphological responses to cadmium and nickel in two maize cultivars. Modern Phytomorphology 3, 131-137.

Fichman, Y., Gerdes, S.Y., Kovács, H., Szabados, L., Zilberstein, A., Csonka, L., N. 2015 Evolution of proline biosynthesis: enzymology, bioinformatics, genetics, and transcriptional regulation. Biol Reviews. 90 (4), 1065-1099.

Filippou, P., Antoniou, C., Obata, T., Van Der Kelen, K., Harokopos, V., Kanetis, L., Aidinis, V., Van Breusegem, F., Fernie, A.R., Fotopoulos, V. 2016. Kresoxim-methyl primes Medicago truncatula plants against abiotic stress factors via altered reactive oxygen and nitrogen species signalling leading to downstream transcriptional and metabolic readjustment. J Exp Bot 67(5): 1259-1274.

Funck, D., Winter, G., Baumgarten, L., and Forlani, G. 2012. Requirement of proline synthesis during Arabidopsis reproductive development. BMC Plant Biol 12:191.

Gajewska, E., Skłodowska, M. 2005. Antioxidative responses and proline level in leaves and roots of pea plants subjected to nickel stress. Acta Physiologiae Plantarum 27, 329-340.

Gallego, S.M., Benavides, M.P., Tomaro, M.L. 1999. Effect of cadmium ions on antioxidant defense system in sunflower cotyledons. Biologia Plantarum 42, 49-55.

Ghars, M.A., Richard, L., Lefebvre-De Vos, D., Leprince, A.S., Parre, E., Bordenave, M., Abdelly, C., Savouré, A. 2012. Phospholipases C and D modulate proline accumulation in Thelungiella halophile/salsuginea differently according to the severity of salt or hyperosmotic stress. Plant Cell Physiol 53 (1): 183-192.

Giberti, S., Funck, D., and Forlani, G. 2014. Delta1-Pyrroline-5-carboxylate reductase from Arabidopsis thaliana: stimulation or inhibition by chloride ions and feedback regulation by proline depend on whether NADPH or NADH acts as co-substrate. New Phytol 202, 911-919.

Halušková, L.U., Valentovičová, K., Huttová, J., Mistrík, I., Tamás, L. 2010. Effect of heavy metals on root growth and peroxidase activity in barley root tip. Acta Physiologiae Plantarum 32(1), 59-65.

Hare, P.D., Cress, W.A., 1997. Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regul. 21, 79-102.

Harsh, A., Sharma, Y.K., Joshi, U., Rampuria, S., Singh, G., Kumar, S., Sharma, R. 2016. Effect of short-term heat stress on total sugars, proline and some antioxidant enzymes in moth bean (Vigna aconitifolia). Annals of Agricultural Sciences 61(1), 57-64.

Hirt, H., Shinozaki, K. 2003. Plant responses to abiotic stress. Topics in Current Genetics Vol. 4. Springer-Verlag, Berlin-Heidelberg, pp. 188-189.

Hmida-Sayari, A., Gargouri-Bouzid, R., Bidani, A., Jaoua, L., Savouré, A., Jaoua, S. 2005. Overexpression of Δ1-pyrroline-5-carboxylate synthetase increases proline production and confers salt tolerance in transgenic potato plants. Plant Science 169(4), 746-752.

Hossain, M.A., Hoque, M.A., Burritt, D.J., Fujita, M. 2014. Proline protects plants against abiotic oxidative stress: biochemical and molecular mechanisms. Oxidative damage to plants. Elsevier, San Diego, USA, pp.477-522.

Imtiyaz, S., Agnihotri, R.K., Ganie, S.A., Sharma, R. 2014. Biochemical response of Glycine max (L.) Merr. to cobalt and lead stress. Journal of Stress Physiology and Biochemistry 10(3), 259-272.

Iqbal, N., Umar, S., Khan, N.A. 2015. Nitrogen availability regulates proline and ethylene production and alleviates salinity stress in mustard (Brassica juncea). Journal of Plant Physiology 178, 84-91.

Islam, E., Yang, X., Li, T., Liu, D., Jin, X., Meng, F. 2007. Effect of Pb toxicity on root morphology, physiology and ultrastructure in the two ecotypes of Elsholtzia argyi. Journal of Hazardous Materials 147(3), 806-816.

Janas, K.M., Zielińska-Tomaszewska, J., Rybaczek, D., Maszewski, J., Posmyk, M.M., Amarowicz, R., Kosińska, A. 2010. The impact of copper ions on growth, lipid peroxidation, and phenolic compound accumulation and localization in lentil (Lens culinaris Medic.) seedlings. Journal of Plant Physiology 167, 270-276.

Jayakumar, K., Jaleel, C.A., Vijayarengan, P. 2007. Changes in growth, biochemical constituents, and antioxidant potentials in radish (Raphanus sativus L.) under cobalt stress. Turkish Journal of Biology 31(3), 127-136.

Karataş, İ., Öztürk, L., Demir, Y., Ünlükara, A., Kurunç, A., Düzdemir, O. 2014. Alterations in antioxidant enzyme activities and proline content in pea leaves under long-term drought stress. Toxicology and Industrial Health 30(8), 693-700.

Kaur, G., Kumar, S., Thakur, P., Malik, J.A., Bhandhari, K., Sharma, K.D., Nayyar, H., 2011. Involvement of proline in response of chickpea (Cicer arietinum L.) to chilling stress at reproductive stage. Scientia Horticulturae 128(3), 174-181.

Kaushal, N., Gupta, K., Bhandhari, K., Kumar, S., Thakur, P., Nayyar, H. 2011. Proline induces heat tolerance in chickpea (Cicer arietinum L.) plants by protecting vital enzymes of carbon and antioxidative metabolism. Physiology and Molecular Biology of Plants 17(3), 203-213.

Kavi Kishor, P. B., and Sreenivasulu, N. 2014. Is proline accumulation per se correlated with stress tolerance or is proline homeostasis a more critical issue? Plant Cell Environ 37, 300-311.

Khan, A.A., McNeilly, T., Collins, J.C. 2000. Accumulation of amino acids, proline, and carbohydrates in response to aluminum and manganese stress in maize. Journal of Plant Nutrition 23(9), 1303-1314.

Kiyosue, T., Yoshiba, Y., Yamaguchi-Shinozaki, K., and Shinozaki, K. 1996. A nuclear gene encoding mitochondrial proline dehydrogenase, an enzyme involved in proline metabolism, is upregulated by proline but downregulated by dehydration in Arabidopsis. Plant Cell 8: 1323-1335.

Kotak, S., Larkindale, J., Lee, U., von Koskull-Döring, P., Vierling, E., Scharf, K.D. 2007. Complexity of the heat stress response in plants. Current Opinion in Plant Biology 10, 310-316.

Ku, H.M., Hu, C.C., Chang, H.J., Lin, Y.T., Jan, F.J., Chen, C.T. 2011. Analysis by virus induced gene silencing of the expression of two proline biosynthetic pathway genes in Nicotiana benthamiana under stress conditions. Plant Physiology and Biochemistry 49(10), 1147-1154.

Kumar, P., Tewari, R., Sharma, P. 2007. Excess nickel–induced changes in antioxidative processes in maize leaves. Journal of Plant Nutrition and Soil Science 170, 796-802.

Kumar, V., Yadav, S.K. 2009. Proline and betaine provide protection to antioxidant and methylglyoxal detoxification systems during cold stress in Camellia sinensis (L.) O. Kuntze. Acta Physiologiae Plantarum 31(2), 261-269.

Li, M., Zhu, Q., Hu, C.W., Chen, L., Liu, Z.L., Kong, Z.M. 2007. Cobalt and manganese stress in the microalga Pavlova viridis (Prymnesiophyceae): Effects on lipid peroxidation and antioxidant enzymes. Journal of Environmental Sciences 19, 1330-1335.

Li, Z.G., Ding, X.J., Du, P.F. 2013. Hydrogen sulfide donor sodium hydrosulfide-improved heat tolerance in maize and involvement of proline. Journal of Plant Physiology 170(8), 741-747.

Li, X., Yang, Y., Jia, L., Chen, H., Wei, X. 2013. Zinc-induced oxidative damage, antioxidant enzyme response and proline metabolism in roots and leaves of wheat plants. Ecotoxicology and Environmental Safety 89, 150-157.

Liang, X., Zhang, L., Natarajan, S.K., and Becker, D.F. 2013. Proline mechanism of stress survival. Antiox Redox Signal 19, 998-1011.

Liu, Z., Li, L., Luo, Z., Zeng, F., Jiang, L., Tang, K. 2016. Effect of brassinolide on energy status and proline metabolism in postharvest bamboo shoot during chilling stress. Postharvest Biology and Technology 111, 240-246.

Lovell, J.T., Mullen, J.L., Lowry, D.B., Awole, K., Richards, J.H., Sen, S et al. (2015) Exploiting differential gene expression and epistasis to discover candidate genes for drought-associated QTLs in Arabidopsis thaliana. Plant Cell 27: 969-983. doi: 10.1105/tpc.15.00122.

Lv, W.T., Lin, B., Zhang, M., Hua, X.J. 2011. Proline accumulation is inhibitory to Arabidopsis seedlings during heat stress. Plant Physiology 156(4), 1921-1933.

Maksymiec, W. 2007. Signaling responses in plants to heavy metal stress. Acta Physiologiae Plantarum 29, 177-187.

Mansour, M.M.F., Ali, E.F. 2017. Evaluation of proline functions in saline conditions. Phytochemistry 140: 52-68.

Matsumoto, H., Motoda, H. 2012. Aluminum toxicity recovery processes in root apices. Possible association with oxidative stress. Plant Science 185, 1-8

Meloni, D. A., Oliva, M. A., Martinez, C. A., Cambraia, J. 2003. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environmental and Experimental Botany 49, 69-76.

Miller, G., Honig, A., Stein, H., Suzuki, N., Mittler, R., Zilberstein, A. 2009. Unraveling delta1-pírrolyne-5-carboxylate-proline cycle in plants by uncoupled expression of proline oxidation enzymes. J Biol Chem 284(39): 26482-26492.

Mishra, D., Shekhar, S., Agrawal, L., Chakraborty, S., Chakraborty, N. 2017. Cultivar-specific high temperature stress responses in bread wheat (Triticum aestivum L.) associated with physicochemical traits and defense pathways. Food Chemistry 221, 1077-1087.

Mittler, R. 2006. Abiotic stress, the field environment and stress combination. Trends in Plant Science 11, 15-19.

Nagajyoti, P.C., Lee, K.D., Sreekanth, T.V.M. 2010. Heavy metals, occurrence and toxicity for plants: a review. Environmental Chemistry Letters 8(3), 199-216.

Nakashima, K., Satoh, R., Kiyosue, T., Yamaguchi-Shinozaki, K., and Shinozaki, K. 1998. A gene encoding proline dehydrogenase is not only induced by proline and hypoosmolarity, but is also developmentally regulated in the reproductive organs of Arabidopsis. Plant Physiol 118, 1233-1241.

Oh, S., Shin, H., Arora, R., Kim, K., and Kim, D. 2017. Proline accumulation and related gene expression during spring regrowth in three Rosaceae species. Hortic Environ Biotechnol 58, 1: 21-26.

Öncel, I., Keleş, Y., Üstün, A.S. 2000. Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. Environmental Pollution, 107(3), 315-320.

Parre, E., Ghars, M.A., Leprince, A.S., Thiery, L., Lefebvre, D., Bordenave, M., Luc, R., Mazars, C., Abdelly, C., Savouré, A. 2007. Calcium signaling via phospholipase C is essential for proline accumulation upon ionic but not nonionic hyperosmotic stresses in Arabidopsis. Plant Physiology 144: 503-512.

Pavlovkin, J., Fiala, R., Čiamporová, M., Martinka, M., Repka, V. 2016. Impact of nickel on grapevine (Vitis vinifera L.) root plasma membrane, ROS generation, and cell viability. Acta Botanica Croatica 75(1), 25-30.

Prasad, T.K., Anderson, M.D., Martin, B.A., Stewart, C.R. 1994. Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. The Plant Cell 6, 65-74.

Rejeb, I.B., Pastor, V., Mauch-Mani, B., 2014. Plant responses to simultaneous biotic and abiotic stress: molecular mechanisms. Plants (Basel) 3(4): 458-475.

Rejeb, I.B., Lefebvre-De Vos, D., Le Disquet, I., Leprince, A.S., Bordenave, M., Maldiney, R., Jdey, A., Abdelly, C., Savouré, A. 2015. Hydrogen peroxide produced by NADPH oxidases increases proline accumulation during salt or mannitol stress in Arabidopsis thaliana. New Phytol 208: 1138-1148.

Rivero, R.M., Ruiz, J.M., García, P.C., López-Lefebre, L.R., Sánchez, E., Romero, L. 2001. Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants. Plant Science 160, 315-321.

Rizzi, Y., S., Monteoliva, M., I., Fabro, G., Grosso, C., L., Laróvere, L., E., Alvarez, M., E., 2015. P5CDH affects the pathways contributing to Pro synthesis after ProDH activation by biotic and abiotic stress conditions. Front Plant Sci. https://doi.org/10.3389/fpls.2015.00572.

Rizzi, Y.S., Cecchini, N.M., Fabro, G., Alvarez, M.E. 2016. Differential control and function of Arabidopsis ProDH1 and ProDH2 genes on infection with biotrophic and necrotrophic pathogens. Mol Plant Pathol. DOI: 10.1111/mpp.12470.

Rout, G.R., Das, P. 2003. Effect of metal toxicity on plant growth and metabolism: I. Zinc. Agronomie 23, 3-11.

Saradhi, P.P. 1991. Proline accumulation under heavy metal stress. Journal of Plant Physiology 138(5), 554-558.

Savouré, A., Jaoua, S., Hua, X.J., Ardiles, W., Van Montagu, M., and Verbruggen, N. 1995. Isolation, characterization, and chromosomal location of a gene encoding the delta1-pyrroline-5-carboxylate synthetase in Arabidopsis thaliana. FEBS Lett 372, 13-19.

Schertl, P., Cabassa, C., Saadallah, K., Bordenave, M., Savoure, A., Braun, H.P. 2014. Biochemical characterization of proline dehydrogenase in Arabidopsis mitochondria. FEBS J. 281: 2794-2804.

Schützendübel, A., Polle, A. 2002. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. Journal of Experimental Botany 53, 1351-1365.

Senthil-Kumar, M. and Mysore, K.S. 2012. Ornithine-delta-aminotransferase and proline dehydrogenase genes play a role in non-host disease resistance by regulating pyrroline-5-carboxylate metabolism-induced hypersensitive response. Plant Cell Environ 35: 1329-1343.

Seregin, I.V., Ivanov, V.B. 2001. Physiological aspects of cadmium and lead toxic effects on higher plants. Russian Journal of Plant Physiology 48, 523–544.

Servet, C., Ghelis, T., Richard, L., Zilberstein, A., and Savoure, A. 2012. Proline dehydrogenase: a key enzyme in controlling cellular homeostasis. Front Biosci 17:3947. doi:10.2741/3947.

Shabala, S. 2013. Learning from halophytes: physiological basis and strategies to improve abiotic stress tolerance in crops. Annals of Botany 112, 1209-1221.

Sharma, S., and Verslues, P.E. 2010. Mechanisms independent of abscisic acid (ABA) or proline feedback have a predominant role in transcriptional regulation of proline metabolism during low water potential and stress recovery. Plant Cell Environ 33, 1838-1851.

Sharma, S., Villamor, J.G., and Verslues, P.E. 2011. Essential role of tissue-specific proline synthesis and catabolism in growth and redox balance at low water potential. Plant Physiol 157, 292-304.

Sheokand, S., Kumari, A., Sawhney, V. 2008. Effect of nitric oxide and putrescine on antioxidative responses under NaCl stress in chickpea plants. Physiology and Molecular Biology of Plants 14, 355-362.

Shevyakova, N.I., Bakulina, E.A., Kuznetsov, V.V. 2009. Proline antioxidant role in the common ice plant subjected to salinity and paraquat treatment inducing oxidative stress. Russian Journal of Plant Physiology 56(5), 663-669.

Shinde S., Villamor, J.G., Lin, W., Sharma, S., Verslues, P.E. 2016. Proline coordination with fatty acid synthesis and redox metabolism of chloroplast and mitochondria. Plant Physiol 172, 1074-1088.

Signorelli, S., 2016. Thermentation analogy: a point of view for understanding the intriguing role of proline accumulation in stressed plants. Front Plant Sci 7:1339. doi: 10.3389/fpls.2016.01339.

Singh, M., Singh, V.P., Prasad, S.M. 2016. Nitrogen modifies NaCl toxicity in eggplant seedlings: Assessment of chlorophyll a fluorescence, antioxidative response and proline metabolism. Biocatalysis and Agricultural Biotechnology 7, 76-86.

Stoláriková-Vaculíková, M., Romeo, S., Minnocci, A., Luxová, M., Vaculík, M., Lux, A., Sebastiani, L. 2015. Anatomical, biochemical and morphological responses of poplar Populus deltoides clone Lux to Zn excess. Environmental and Experimental Botany 109, 235-243.

Strizhov, N., Abraham, E., Okresz, L., Blickling, S., Zilberstein, A., Schell, J., et al. 1997. Differential expression of two P5CS genes controlling proline accumulation during salt-stress requires ABA and is regulated by ABA1, ABI1 and AXR2 in Arabidopsis. Plant J 12: 557-569. doi: 10.1111/j.09607412.1997.00557.x

Surapu, V., Ediga, A., Meriga, B. 2014. Salicylic acid alleviates aluminum toxicity in tomato seedlings (Lycopersicum esculentum Mill.) through activation of antioxidant defense system and proline biosynthesis. Advances in Bioscience and Biotechnology

Sytar, O., Kumar, A., Latowski, D., Kuczynska, P., Strzałka, K., Prasad, M.N.V. 2013. Heavy metal-induced oxidative damage, defense reactions, and detoxification mechanisms in plants. Acta Physiologiae Plantarum 35(4), 985-999.

Szabados, L., Savouré, A., 2010. Proline: a multifunctional amino acid. Trends Plant Sci. 15 (2), 89-97.

Szekely, G., Abraham, E., Cseplo, A., Rigo, G., Zsigmond, L., Csiszar, J., et al. 2008. Duplicated P5CS gene of Arabidopsis play distinct roles in stress regulation and developmental control of proline biosynthesis. Plant J 53: 11-28.

Szepesi, Á., Poór, P., Gémes, K., Horváth, E., Tari, I. 2008. Influence of exogenous salicylic acid on antioxidant enzyme activities in the roots of salt stressed tomato plants. Acta Biologica Szegediensis 52, 199-200.

Szőllősi, R., Varga, I.Sz., Erdei, L., Mihalik, E. 2009. Cadmium-induced oxidative stress and antioxidative mechanisms in germinating Indian mustard (Brassica juncea L.) seeds. Ecotoxicology and Environmental Safety 72, 1337-1342.

Takahashi, H., Matsumura, H., Kawai-Yamada, M., Uchimiya, H. 2008. The cell death factor, cell wall elicitor of rice blast fungus (Magnaporthe grisea) causes metabolic alterations including GABA shunt in rice cultured cells. Plant Signal Behav 3: 945–953.

Tewari, R.K., Kumar, P., Sharma, P.N., Bisht, S.S. 2002. Modulation of oxidative stress responsive enzymes by excess cobalt. Plant Science 162(3), 381-388.

Thiery, L., Leprince, AS., Lefebvre, D., Ghars, M.A., Debarbieux and Savouré, A. 2004. Phospholipase D is a negative regulator of proline biosynthesis in Arabidopsis thaliana. J Biol Chem 279, 14812-14818.

Verslues, P.E. and Sharma, S. 2010. Proline metabolism and its implications for plant-environment interaction. Arabidopsis Book 8:e0140.

Viswanath, K.K., Palaka, B.K. and Ampasala, D.R. 2016. Alleviation of nickel toxicity in finger millet (Eleusine coracana L.) germinating seedlings by exogenous application of salicylic acid and nitric oxide. doi:10.1016/j.cj.2016.09.002

Zhang, L., and Becker, D., F., 2015. Connecting proline metabolism and signalling pathways in plant senescence. Front Plant Sci. https://doi.org/10.3389/fpls.2015.00552.

Zouari, M., Ben Ahmed, C., Elloumi, N., Bellassoued, K., Delmail, D., Labrouuse, P., Ben Abdallah, F., Ben Rouina, B. 2016a. Impact of proline application on cadmium accumulation, mineral nutrition and enzymatic antioxidant defense system of Olea europea L. cv Chemlali exposed to cadmium stress. Ecotoxicol Environmental Safety 128: 195-205.

Zouari, M., Elloumi, N., Ben Ahmed, C., Delmail,D., Ben Rouina, B., Ben Abdallah, F., Labrousse, P. 2016b. Exogenous proline enhances growth, mineral uptake, antioxidant defense, and reduces cadmium-induced oxidative damage in young date palm (Phoenix dactylifera L.). Ecological Engineering 86: 202-209.

Wang, G., Zhang, J., Wang, G., Fan, X., Sun, X., Qin, H., Xu, N., Zhong, M., Qiao, Z., Tang, Y., Song, R. 2014. Proline responding1 plays a critical role in regulating general protein synthesis and the cell cycle in maize. Plant Cell 26, 2582-2600.

Wang, S.H., Yang, Z.M., Yang, H., Lu, B., Li, S.Q., Lu, Y.P. 2004. Copper-induced stress and antioxidative responses in roots of Brassica juncea L. Botanical Bulletin of Academia Sinica 45, 203-212.

Wani, A.S., Ahmad, A., Hayat, S., Tahir, I. 2016. Is foliar spray of proline sufficient for mitigation of salt stress in Brassica juncea cultivars? Environ Sci Pollution Research 23 (13): 13413-13423.

Wen, J.F., Gong, M., Liu, Y., Hu, J.L., Deng, M.H. 2013. Effect of hydrogen peroxide on growth and activity of some enzymes involved in proline metabolism of sweet corn seedlings under copper stress. Scientia Horticulturae 164, 366-371.

Wu, X.X., He, J., Zhu, Z.W., Yang, S.J., Zha, D.S. 2014. Protection of photosynthesis and antioxidative system by 24-epibrassinolide in Solanum melongena under cold stress. Biologia Plantarum 58(1), 185-188.

Xu, J., Yin, H., Li, X. 2009. Protective effects of proline against cadmium toxicity in micropropagated hyperaccumulator, Solanum nigrum L. Plant Cell Reports 28(2), 325-333.

Yadav, S.K. 2010. Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. South African Journal of Botany 76, 167-179.