Glutathione and proline can coordinately make plants withstand the joint attack of metal(loid) and salinity stresses

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INTRODUCTION
Agricultural soils in the vicinity of extensive anthropogenic activities may exhibit salinity together with high levels of metals/metalloids (hereafter termed as “metal(loid)”) as co-stressors. Elevated concentrations of metals (such as As, Cd, Cr, Hg, Ni, and Pb) may affect photosynthetic apparatus, electron transport chain and chlorophyll biosynthesis, induce cellular damage, impair cellular redox homeostasis, and finally cause cellular metabolic arrest (Anjum et al., 2010, 2012a; Gill and Tuteja, 2010; Talukdar, 2012; Talukdar and Talukdar, 2014). Saline soil conditions, on the other hand, can cause osmotic stress that in turn can inhibit cell expansion and cell division, impact stomatal closure, induce cell turgor via lowering water potential, and alter the normal homeostasis of cells (Miller et al., 2010). However, the generation of osmotic stress through impaired plant water relations, and oxidative stress caused by uncontrolled generation of varied reactive oxygen species (ROS; such as such as -OH, H2O2, O2−) are common in plants exposed to high levels of salinity and/or metals (Benavides et al., 2005; Anjum et al., 2010, 2012a).

Diverse plant taxa have been reported to adapt metabolically to salinity and exposure to metals by enhancing synthesis of sulfur (S)-rich peptides (such as glutathione, GSH) and low-molecular-weight nitrogenous and proteogenic amino acids/osmolytes (such as proline, Pro) (Khan et al., 2009; Anjum et al., 2010, 2012a; Talukdar, 2012; Kishor and Sreenivasulu, 2014; Talukdar and Talukdar, 2014). Nevertheless, both GSH and Pro share L-glutamate as a common biosynthesis precursor (Moat et al., 2003) (Figure 1). However, very little or no effort has been made so far to dissect the intricacies of potential metabolic inter-relationships between the GSH and Pro induction either under salinity/osmotic or metal stress conditions.

Therefore, we discuss and interpret through this note the facts related with the mainstays (chemistry, biosynthesis, compartmentalization, significance) commonly and potentially shared by these two enigmatic compounds (GSH and Pro) in plants. The outcome of the present endeavor can be useful in designing future research aimed at sustainably alleviating isolated and/or joint impact of metal and salinity stresses in crop plants through exploiting the GSH and Pro metabolism.

CROSS-TALKS AND PERSPECTIVES

Both GSH and Pro, with molecular formula C10H17N3O6S and C5H9NO2, respectively, belong to the “glutamate or α-ketoglutarate” family and originate from a common precursor L-glutamate (Moat et al., 2003). Although cellular compartments and changing growth conditions may influence their levels, biosynthesis of both GSH (Preuss et al., 2014) and Pro (Lehmann et al., 2010) is predominantly plastidic. Of the two major GSH-biosynthesis enzymes, glutamate cysteine ligase (GCL; γ-glutamylcysteine synthetase; E.C. 6.3.2.2) is localized to plastid stroma; whereas GSH synthetase (GS; E.C. 6.3.2.3) is targeted to plastid stroma and cytosol (Raviliou and Jez, 2012). On the other hand, the Pro-biosynthesis enzymes, namely Δ1-pyrroline-5-carboxylate synthetase (P5CS) and Δ1-pyrroline-5-carboxylate reductase (P5CR), occur in cytosol and plastids (reviewed by Szabados and Savouré, 2010). Since plastids are among the major organelles with: (a) a highly oxidizing metabolic activity; (b) an intense rate of electron flow; and (c) plastid signal-mediated regulation of different cellular processes (Barajas-López et al., 2013), localization of both GSH and Pro is apt to their role as the major ROS-scavenger and singlet-oxygen quencher during photosynthesis (Szekely et al., 2008).

GSH and Pro may occur in the concentrations of few mM (2–3 mM) in various plant tissues (Noctor et al., 2002; Kishor et al., 2005). The GSH and Pro levels of plant tissues are indicators of the S (reduced) (Hubberten et al., 2012) and nitrogen (N) (Sánchez et al., 2001) nutritional status of the plant respectively. GSH and Pro have also been reported to act as sources of (reduced)-S (Anjum et al., 2010) and N (reviewed by Kishor and Sreenivasulu, 2014), respectively, under
FIGURE 1 | Schematic representation of the points of interrelationships in the major metabolic pathway of sulfur-rich peptide—glutathione (GSH) and nitrogenous and proteogenic amino acid—proline (Pro).

stress conditions. Additionally, their status may presumably be improved through enhancing L-glutamate level via N and S nutrition, respectively (Anjum et al., 2012b). Moreover, modulation of biosynthesis of GSH (Bartoli et al., 2009) and Pro (Abraham et al., 2003) is reportedly light dependent. In particular, GSH levels may depend on growth and photosynthetically active photon flux density at low light intensities (up to ca. 100 μmol m$^{-2}$ s$^{-1}$) (Ogawa et al., 2004). GSH (Son et al., 2014) and Pro (Sivakumar et al., 2001) can negatively/positively modulate the photosynthesis functions by influencing the activity of ribulose-1,5-bisphosphate oxygenase, an enzyme involved in the first major step of carbon fixation. Moreover, an increased intracellular ROS-availability can shift the reduced GSH toward a more oxidized GSH (i.e., GSSG) status (Anjum et al., 2010, 2012a; Noctor et al., 2012). In contrast, increased status of cellular H$_2$O$_2$ (or exogenous H$_2$O$_2$) can increase Pro level by modulating the ex-novo synthesis of Pro (Matsysik et al., 2002). Oxidation of Pro generates NADP/NADPH cycling or redox balance (Kishor et al., 2005) that in turn may regulate the reduction of GSSG to GSH via GSH reductase (Anjum et al., 2010, 2012a; Noctor et al., 2012). Interaction of Pro (Iqbal et al., 2014) and GSH (Mhamdi et al., 2010; Ghanta et al., 2014) with a number of defense-related phytohormones (such as ethylene, jasmonic acid and salicylic acid) and/or their analogs has also been reported to modulate plant stress tolerance.

Both GSH (Ogawa, 2005) and Pro (Lehmann et al., 2010) perform multiple functions in plants including the modulation of plant growth and developmental processes. In particular, under metal stress, apart from the induction of GSH-based defense system (Anjum et al., 2010, 2012a; Noctor et al., 2012; Talukdar, 2012; Talukdar and Talukdar, 2014), elevated accumulation of osmolytes such as Pro has been extensively noticed (reviewed by Gill et al., 2014). Under salinity stress also, in addition to the accumulation of Pro that maintains both cell turgor and cellular redox homeostasis (Lehmann et al., 2010; Szabados and Savouré, 2010; Kishor and Sreenivasulu, 2014), GSH-based defense system is activated to maintain reduced cellular redox environment via metabolizing the varied ROS and their reaction products (Ruiz and Blumwald, 2002; Kocsy et al., 2004). Nevertheless, reports are available on the efficient Pro-metal, GSH-metal or Pro-GSH-metal sequestration, scavenging of
been extensively evidenced to protect cellular metabolism against a range of abiotic stresses.

The causal relationships of Pro accumulation and significance of GSH metabolism with enhanced tolerance to single stress factor (either metal or salinity) have been reported extensively in separate studies using natural variants, mutants or transgenic plants (Matysik et al., 2002; Anjum et al., 2010, 2012a; Noctor et al., 2012; Kishor and Sreenivasulu, 2014). However, significance of the potential “metabolic interrelationships” between GSH and Pro with reference to the plant’s adaptive responses to prevailing multiple stressors has not been fully appreciated and the molecular insights of these relationships have yet to be developed.

Nevertheless, owing to the facts that: (a) deficiency of S and N has become extensive in agricultural soils on the globe (reviewed by Anjum et al., 2012b); (b) plant’s S requirement and S metabolism are closely related to N nutrition, and the N metabolism is strongly affected by the plant’s S status (Fazili et al., 2008; Anjum et al., 2012b); and (c) both GSH (Kopriva and Rennenberg, 2004; Anjum et al., 2012b) and Pro (Sánchez et al., 2001; Rais et al., 2013) are closely related to these nutrients, integrated efforts should be made to work-out the coordinated role of S and N in the GSH and Pro metabolic pathways, develop more insights into their biochemistry/physiology and molecular biology and understand potential interrelationships among different components of these pathways.

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