

REVIEW

Role of amino acids in plant responses to stresses

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Plants subjected to stress show accumulation of proline and other amino acids. The role played by accumulated amino acids in plants varies from acting as osmolyte, regulation of ion transport, modulating stomatal opening, and detoxification of heavy metals. Amino acids also affect synthesis and activity of some enzymes, gene expression, and redox-homeostasis. These roles played by amino acids have been critically examined and reviewed.

Additional key words: drought resistance, enzymes, ion uptake, membranes, metal detoxification, osmolyte, proline, stomata.

Introduction

Amino acids have traditionally been considered as precursors and constituents of proteins. Many amino acids also act as precursors of other nitrogen containing compounds, *e.g.*, nucleic acids. In early fifties, with the advent of paper chromatography many scientists started studying the free amino acid spectra of plants under various stresses, specially pathogenic stress and observed that one of the earliest symptom was changes in free

amino acid spectra. This was due to disturbed amino acid and protein metabolism. In recent past, many experiments implicate that amino acids can play wide roles in plants including acting as regulatory and signalling molecules. This article tries an overview of different roles of amino acids in plants. The references quoted are representative only and not exhaustive.

Amino acids in water stress

Singh *et al.* (1972) were probably the first to assign a correlation between proline accumulation and drought resistance in barley cultivars. They showed that drought resistant cultivars accumulated many fold higher free proline than the susceptible cultivars. In the following decade similar correlation was found in many crop plants (see Aspinall and Paleg 1981). However, almost an equal number of reports showed a lack of correlation between proline accumulation and drought resistance (*e.g.*, Waldren and Teare 1974, Hanson *et al.* 1977, 1979, Singh *et al.* 1985). Some reports are available where accumulation of other free amino acids under drought has been shown, *e.g.*, asparagine, alanine and γ -amino butyric

acid in *Phaseolus mungo* (Rai and Bapat 1977), arginine in *Cryptomeria* (Mori *et al.* 1971), aspartic acid, glutamic acid and glutamine in cotton (Hanower and Brzozowska 1975), asparagine, aspartic acid, serine and glycine in maize (Slukhai and Shvedova 1972, Thakur and Rai 1982), aspartic acid and alanine in *Iris* (Paulin 1972), proline, ornithine, arginine, and glutamic acid in detached rice leaves (Yang *et al.* 2000), *etc.* Thakur and Rai (1982) examined the spectra of free amino acids under osmotic stress in maize cultivars and observed that in a resistant cultivar beside proline which showed *ca.* 4 fold increase, other amino acids, *e.g.*, asparagine (4.5 fold), serine and glycine (2.2 fold) and aspartic acid (1.5 fold) also

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Abbreviations: PEG - polyethylene glycol; P5CS - Δ -pyrroline-5-carboxylase synthetase.

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accumulated. In contrast in susceptible cultivar the accumulation pattern was proline (3.5 fold), asparagine (5.4 fold), serine and glycine (4.2 fold). Thus they argued why correlation with proline is of importance and why other amino acids are being ignored? The only way to answer this riddle was to forget about correlation and work out the roles of free amino acids in drought physiology, if any.

If proline has a role in drought resistance then we must first sort out whether the magnitude of stress induced increase in proline is important in drought resistance or the endogenous, absolute contents of proline are important. In rice cultivars, Mali and Mehta (1977) showed that under normal conditions, resistant cv. TKM-1 had $250 \mu\text{g g}^{-1}(\text{d.m.})$ of proline in leaves while under leaf water potential -1 MPa , proline content rose to *ca.* $1350 \mu\text{g g}^{-1}(\text{d.m.})$ (a 5.4 fold increase). However, in susceptible cv. Sabarmati the constitutive content of proline was $755 \mu\text{g g}^{-1}(\text{d.m.})$, which rose to *ca.* $900 \mu\text{g g}^{-1}(\text{d.m.})$ (1.2 fold increase) under the same stress. Thus it was found that increase in proline correlates with drought resistance but the constitutive content of proline was higher in susceptible cultivar.

Rajagopal and Sinha (1980) were probably the first

who show that exogenous proline given to wheat in nutrient solution, delayed wilting in such plants when exposed to osmotic stress. Subsequently, Rajagopal (1981) also showed that in *Vicia faba* exogenous proline decreased stomatal conductance. He also noted sufficient uptake of exogenous proline into leaves. Thakur and Rai (1985) provided exogenous amino acids to maize seedlings and observed that beside proline, alanine, serine, and asparagine also delayed wilting under stress conditions. Further, Rai and Sharma (1991) showed that in *Vicia faba* leaf epidermal peels, proline, glycine, alanine, leucine, threonine, lysine, arginine, tryptophan and phenylalanine inhibited stomatal opening while histidine, methionine, aspartic acid, glutamic acid, asparagine and glutamine promoted stomatal opening. They also observed that amino acids promoting stomatal opening could also revert the ABA (Rai and Sharma 1991) or mannitol induced stomatal closure (Sharma and Rai 1989). They suggested that this could be a balancing mechanism for stomatal regulation under drought, since ABA induced stomatal closure under drought may conserve water but would lead to severe constraints on gaseous exchange which could be deleterious for the plant.

Effects of amino acids on membrane permeability and ion transport

In the experiments of Rai and Sharma (1991), it was also observed that amino acids promoting stomatal opening also promoted K^+ influx into the guard cells while amino acids inhibiting stomatal opening inhibited K^+ flux into the guard cells. That opened up the question about the amino acids affecting ion transport across the membrane. Rai and Kumari (1983) monitored the passage of H^+ or OH^- ions across the *Vinca* petal membrane as influenced by exogenous amino acids. They found that proline, arginine, asparagine, glutamine, alanine and leucine reduced membrane permeability by 100 %; phenylalanine, threonine, serine and γ -aminobutyric acid reduced it by 40 %, while glycine, glutamic acid and aspartic acid did not show any effect. Rana and Rai (1996) showed that exogenous histidine, proline, glutamine, methionine and glycine promoted calcium uptake in *Phaseolus* seedlings while alanine, aspartic acid, glutamic acid and tryptophan inhibited the same.

Proline relieved salt toxicity in barley plantlets by changing salt transport from root to shoot (Lone *et al.* 1987). Increasing proline content increased K^+ content and alleviated salt stress effects on growth of *Vigna radiata* cultures (Kumar and Sharma 1989). Thioproline

also increased K^+ uptake in *V. radiata* but not the uptake of Na^+ or Cl^- (Kumar *et al.* 1990); Tipirdamaz and Karakullucku (1993) confirmed this in cultured tomato embryo. Khanna (1998) showed exogenous proline to promote K^+ uptake by *ca.* 15 %, in *Raphanus* seedlings. Similar was the case with histidine, γ -amino butyric acid, hydroxy proline, leucine, glutamine and phenylalanine. On the other hand threonine, aspartic acid and alanine did not show any effect on K^+ uptake. The promotory effects of amino acids were different in control and osmotically stressed seedlings.

Flowering in *Lemna paucicostata* is affected by presence of amino acids in the growing media. Tanaka *et al.* (1987) found that asparagine, aspartic acid, glutamic acid, alanine, glycine or serine when added to the media promoted flowering and cystine, cysteine, glutamine, arginine, threonine and phenylalanine inhibited flowering. This effect was related to their promotion or inhibition of iron uptake by the plants. Thus exogenous amino acids can modulate membrane permeability and ion uptake and probably this is the major component by which amino acids help in mitigating drought or salt stress effects.

Amino acids as osmolyte

The work of Singh *et al.* (1972) showing correlation between proline accumulation and drought resistance also led to suggestions that proline can act as a non-toxic osmolyte. Palfi *et al.* (1974) using *Sinapis alba* seed germination as a test showed that proline was least inhibitory (at 3.5×10^{-2} M) while at same concentration alanine, glutamic acid and aspartic acid were highly inhibitory. They also pointed out that proline was about 300 times more soluble in water than other amino acids, thus it can act as a non-toxic osmolyte. In an extensive study Handa *et al.* (1983) measured contents of various solutes under polyethylene glycol (PEG) stress in tomato cell cultures and calculated the contribution of these (%) to total osmotic potential (*e.g.* under -2.2 MPa): sucrose (2.1 %), reducing sugars (1.4 %), free amino acids (8.1 %), proline (2.1 %), quarternary ammonium salts (12.5 %), malate (1.3 %), citrate (1.5 %), Na^+ (2.4 %), K^+ (12.4 %), NO_3^- (4.5 %) and Cl^- (2.7 %). Thus free proline plays only a minor role, while reducing sugars and K^+ are major contributors to osmoregulation. These calculations are based on cell volumes, but if these components are differentially partitioned in the cell, *e.g.*, if proline is sequestered into the cytoplasm and cytoplasm represents 10 % of the cell volume, then proline would contribute 0.7 MPa equivalent of osmoticum in the cytoplasm of PEG (20 %) treated cells. Such propositions need careful investigations on the exact distribution of different solutes in the cell in order to assign any specific role to them in contributing towards osmoticum.

Handa *et al.* (1986) in cultured tomato cells showed that endogenous proline content could be correlated to adaptability of cell lines to PEG induced osmotic stress. They also observed that besides exogenous proline, other amino acids *viz.*, arginine, asparagine, aspartic acid, valine, glutamine and glutamic acid enhanced tolerance in non-adapted cells. It was also observed that these amino acids (1 mM) increased proline content under osmotic stress. Increase in proline contents on supplementation of other amino acids has also been shown in *Daucus carota* cells (Carbonera *et al.* 1989) and *Raphanus* seedlings (Khanna and Rai 1998).

To what extent the accumulated proline plays a role as an osmolyte remains a riddle. Perez-Aflocea *et al.* (1993) showed that in tomato leaves sugars, organic acids, and amino acids contributed to *ca.* 50 % in osmoregulation while the other 50 % was contributed to by Na^+ , K^+ , Cl^- ,

NO_3^- and Ca^{2+} , *etc.* Handa *et al.* (1983) worked out contribution of solutes in osmoregulation (optimal under different stress levels), as sucrose 4.9 %, reducing sugars 26.3 %, total amino acids 8.1%, proline 3.0 %, organic acids 2.8 %, potassium 13.8 % and other inorganic ions 9.6 %. Thus the role of proline as an osmolyte remains at a very low level.

However, exogenous amino acids have been shown to promote K^+ uptake, *e.g.*, in *Raphanus* seedlings by *ca.* 15 % (Khanna 1998) and calcium uptake in *Phaseolus* seedlings by 20 - 60 % (Rana and Rai 1996). Therefore, proline and other amino acids may contribute to osmoregulation not only *per se*, but also by regulating the contents of inorganic solutes, which in turn may contribute to osmotolerance.

Kavikishore *et al.* (1995) produced a transgenic tobacco line, over-producing proline, by transferring mothbean Δ -pyrroline-5-carboxylase synthetase (P5CS). P5CS catalyses conversion of glutamate to pyrroline-5-carboxylate. Such transgenic plants showed better growth and osmotolerance under osmotic stress and their growth rate was correlated to proline content in plants. In transgenic plants proline content rose to *ca.* 6000 $\mu\text{g g}^{-1}$ (f.m.) in comparison to *ca.* 200 $\mu\text{g g}^{-1}$ (f.m.) in wild plants under stress created by withholding watering. They concluded that proline accumulation in transgenic plants helped in maintaining osmotic potential and thereby to tolerate water stress. However, Maggio *et al.* (1997) selected proline overproducing carrot cell lines and observed that tolerance to water stress induced by PEG was not different in normal and proline over-producing lines at early phase of growth but proline content at this phase was hardly different in two lines. They also calculated that in experiment of Kavikishore *et al.* (1995) with P5CS tobacco, the accumulated proline was *ca.* 8.68 $\mu\text{mol g}^{-1}$ (f.m.) (*i.e.*, *ca.* 9 mM) and this would contribute only *ca.* 0.2 % of total osmotic adjustment. Maggio *et al.* (1997) also suggested that proline may not be important as an osmolyte but function through some indirect way, *i.e.*, by protecting against active oxygen or hydroxyl radicals. Mansour (2000) also concluded that accumulation of nitrogen containing compounds (*i.e.*, amino acids, amides, quarternary ammonium compounds and polyamines) under saline stress correlated to salt tolerance of plants but is based on untested hypothesis.

Amino acids and enzyme activity

Proline has also been proposed as a protector of enzymes against heat, salinity, chilling and dilution under *in vitro* conditions (Paleg *et al.* 1981, Krall *et al.* 1989). The

possibility is that in 3-D structure of protein the stability of structure is governed by hydrophobic/hydrophilic, ionic interactions and interaction between side chains of

constituent amino acids. Thus free amino acids (or exogenous amino acids) could interfere with these side chain bondings and introduce conformational changes in the enzyme protein and thus affect their activity. Amino acid-protein interaction in solution have not been studied, however, proline-protein interaction has been suggested when proline protected enzymes against denaturation by salinity, heat or dilution (Paleg *et al.* 1981, Schobert 1977, Schobert and Tschesche 1978). Therefore, amino

Heavy metal tolerance and amino acids

Many plants have been shown to accumulate proline when exposed to heavy metals (Alia and Saradhi 1991, Bassi and Sharma 1993a,b, Costa and Morel 1994, Talanova *et al.* 2000). Metal tolerant populations of *Deschampsia* and *Silene* have been shown to have higher constitutive content of proline as compared to non-tolerant counterparts (Smirnoff and Stewart 1987, Schat *et al.* 1997). Smirnoff and Stewart (1987) further noted that metal non-tolerant ecotypes of *Deschampsia* accumulated large amounts of proline under metal stress while tolerant ecotypes lacked this response. It was further shown that Cu, Zn, and Ni caused massive proline accumulation in leaves of non-tolerant ecotype of *Silene vulgaris*. The constitutive content of proline was also 5 - 6 times higher in tolerant ecotype (Schat *et al.* 1997). That such proline accumulation was related to metal induced water deficit, became clear when plants were kept under high relative humidity and then treated with heavy metals. Under such conditions plants failed to develop water deficit or accumulate proline.

Other suggested roles

Modulation of ion uptake is often related to trans-membrane potentials and amino acids have been shown to significantly alter these. Franz and Tattar (1981) observed that in sugarcane protoplast which showed a trans-membrane potential of -135 mV, it changed to 27, 19, 18 mV on addition of glycine, serine and alanine, respectively. Grout and Coutt (1974) found that in *Nicotiana* and *Petunia* membranes negative charge changed to positive on addition of polyornithine or polylysine. Proline, γ -amino butyric acid, hydroxy proline, and aspartate have been shown to act as an excellent cryoprotectant in case of cultured cells of *Zea mays* (Withers and King 1979) and proline in *Solanum tuberosum* (Hellerger and Li 1981).

Hare *et al.* (1998) while discussing the possible roles of proline pointed out that if the regeneration of NADP⁺ is limited under continued photon absorption, redox imbalance is likely to result in photoinhibition and

acid protein interaction must be looked in detail to understand the *in vitro* and *in vivo* effects of amino acids on enzyme activities.

Holowach *et al.* (1986) showed exogenous methionine to inhibit synthesis of β -subunits of 75 storage proteins in methionine treated soybean cotyledons. They suggested that methionine acts as an "effector" controlling the levels of β -m-RNA at the level of transcription, processing or turn over.

The possibility that proline may protect plants against heavy metals by forming chelates has also been examined. Sharma *et al.* (1998) showed that proline protected the activity of glucose-6-phosphate dehydrogenase and nitrate reductase, *in vitro*, against inhibition by Cd and Zn. They further showed that this was due to formation of a proline-metal complex. Cu in case of metal tolerant *Armeria* has been shown to exist as Cu-proline complex (Frago and Mullen 1979).

Krammer *et al.* (1996) reported that nickel hyper accumulator, *Alyssum*, when exposed to nickel produced histidine, proportionally to applied Ni. Histidine accumulation was not noted in nickel non-accumulator. They showed Ni-histidine complex in the xylem sap. Non tolerant clones of *Deschampsia*, are shown to accumulate asparagine in the roots and Zn-asparagine complex has been suggested to reduce metal toxicity (Smirnoff and Stewart 1987). Thus amino acids can have an important role in regulating metal toxicity in plants and needs extensive study.

enhanced use of O₂ instead of NADP⁺ as electron acceptor in photosynthesis. Thus stress induced increase in transfer of reducing equivalents into proline by Δ -pyrroline-5-carboxylate (P5C) and P5C reductase may be a protective mechanism whereby many species may ameliorate shifts in redox potentials which accompany stress (Hare and Cress 1997). The observation that in *Arabidopsis*, dehydration is associated with induction of both proline dehydrogenase and P5C synthase with no net accumulation of free proline (Kiyosue *et al.* 1996); corroborates the notion that cycling between proline and its precursors may be an important homeostasis to forestall redox imbalance associated with small water deficits.

Most of the stresses are also associated with generation of free radicals. Proline has been shown to scavenge hydroxyl radicals *in vitro*, the product of the reaction was not determined but hydroxy proline could be

formed (Smirnoff and Cumbes 1989). Proline does not interact with superoxide radicals. Alia *et al.* (1995) showed that in *Brassica* and *Cajanus*, Zn treatment resulted in formation of free radicals as measured by thiobarbituric acid (TBA) reactive material which was also associated with increased proline levels. They made a suggestion that this proline could act as scavenger for free radicals.

Kiyosue *et al.* (1996) showed that a nuclear gene encoding proline dehydrogenase is upregulated by proline. Iyer and Caplan (1998) demonstrated that P5C, an intermediate in proline metabolism, can selectively increase the expression of at least three osmotically regulated genes in rice. It is in full accordance with that a signal derived from proline metabolic pathway, possibly the redox potential of the pyridine metabolic pool may control gene expression in response to osmotic stress (Hare and Cress 1997). Proline has also been shown to

destabilise DNA helix, it reduced DNA melting point. Increased susceptibility to S1 nuclease and insensitivity to DNase I, in presence of increasing proline concentration was noted. It partially counteracted effects of NaCl and spermidine on DNA stability (Rajendrakumar *et al.* 1997).

Altman *et al.* (1977) in their experiment on *Zea mays* protoplast observed that addition of polyamines delayed their senescence. Polyamine precursor, *e.g.*, arginine or lysine also delayed senescence. Results were interpreted that amino acids were converted to polyamines that delayed senescence. However, direct effects of arginine or lysine on senescence could not be ruled out. Earlier, Shibaoka and Thimann (1970) had noted that in *Avena* coleoptile, senescence was differentially affected by amino acids, L-serine promoted senescence and even acted as antikinetin. Effect of serine was countered by arginine.

Conclusions

The overview presented here clearly shows that large array of physiological processes in plants are affected/regulated by amino acids. Role of proline has been examined in some details whereas role of other amino acids needs extensive study. Experiments with exogenous amino acids suffer with some basic limitations, *e.g.*, what is the extent of uptake, what is their sub-cellular compartmentation and most important what is their stability in the tissue? Amino acid inter-conversions are very rapid. Whole amino acid spectra changes on addition of proline (Carbonera *et al.* 1989) and addition of other amino acids also result in elevated proline content (Handa *et al.* 1986, Khanna and Rai 1998). Is this readjustment in amino acid spectra homeostasis? All these questions must be examined carefully. Amino acids and membrane interactions need extensive examination since membranes

play central role in a large number of physiological responses. Another important observation is that in all these amino acid effects, no relationship with structure and function of the amino acids could be established and most effects seem to be "general" in nature and change with kind of response or genetic material. This often puts under doubt the *in vivo* role of amino acids in observed responses towards any given amino acid. However, now with tools like specific amino acid deficient mutants or specific transgenics overproducing specific amino acids, some of these questions can be attempted (Kavikishore *et al.* 1995, Nanjo *et al.* 1999a,b). The common assumption that increase in free amino acids under biotic or abiotic stress is a symptom of damage needs review. Many of these responses could be adaptive in nature.

References

- Alia, Saradhi, P.: Proline accumulation under heavy metal stress. - *J. Plant Physiol.* **138**: 504-508, 1991.
- Alia, Prasad, K.V.S.K., Saradhi, P.: Effect of zinc on free radicals and proline in *Brassica* and *Cajanus*. - *Phytochemistry* **39**: 45-47, 1995.
- Altman, A., Kaur-Sawhney, R., Galston, A.W.: Stabilisation of leaf protoplast through polyamine mediated inhibition of senescence. - *Plant Physiol.* **60**: 570-574, 1977.
- Aspinall, D., Paleg, L.G.: Proline accumulation physiological aspects. - In: Paleg, L.G., Aspinall, D. (ed.): *Physiology and Biochemistry of Drought Resistance in Plants*. Pp. 205-240. Academic Press, Sydney 1981.
- Bassi, R., Sharma, S.S.: Changes in proline content accompanying the uptake of zinc and copper by *Lemna minor*. - *Ann. Bot.* **72**: 151-154, 1993a.
- Bassi, R., Sharma, S.S.: Proline accumulation in wheat seedlings exposed to zinc and copper. - *Phytochemistry* **33**: 1339-1342, 1993b.
- Carbonera, D., Iadarola, P., Cella, R.: Effect of exogenous amino acids on the intracellular content of proline and other amino acids in *Daucus carota* cells. - *Plant Cell Rep.* **8**: 422-424, 1989.
- Costa, G., Morel, J.L.: Water relations, gas exchange and amino acid content in Cd-treated lettuce. - *Plant Physiol. Biochem.* **32**: 561-565, 1994.
- Farago, M.E., Mullen W.A.: Plants which accumulate metals. IV. A possible copper-proline complex from roots of *Armeria maritima*. - *Inorg. chim. Acta* **32**: L93-94, 1979.
- Franz, S.L., Tattar, T.A.: Effects of sugars and amino acids on membrane potentials in two clones of sugarcane. - *Plant*

- Physiol. **67**: 150-155, 1981.
- Grout, D.W.W., Coutt, R.H.A.: Additives for the enhancement of fusion and endocytosis in higher plant protoplasts, an electrophoretic study. - *Plant Sci. Lett.* **2**: 397-403, 1974.
- Handa, S., Bressan, R.A., Handa, A.K., Caprita, N.C., Hasegawa, P.M.: Solutes contributing to osmotic adjustments in cultured plant cells adapted to water stress. - *Plant Physiol.* **73**: 834-843, 1983.
- Handa, S., Handa, A.K., Hasegawa, P., Bressan, R.A.: Proline accumulation and the adaptation of cultured plant cells to water stress. - *Plant Physiol.* **80**: 938-945, 1986.
- Hanower, P., Brzozowska, J.: Effects of osmotic stress on composition of free amino acids in cotton leaves. - *Phytochemistry* **14**: 1691-1694, 1975.
- Hanson, A.D., Nelson, C.E., Everson, E.H.: Evaluation of free proline accumulation as an index of drought resistance using two contrasting barley cultivars. - *Crop Sci.* **17**: 720-726, 1977.
- Hanson, A.D., Nelson, C.E., Pedarson, A.R., Everson, E.H.: Capacity for free proline accumulation during water stress in barley and its implication for drought resistance. - *Crop Sci.* **19**: 489-493, 1979.
- Hare, P.D., Cress, W.A.: Metabolic implications of stress induced proline accumulation in plants. - *Plant Growth Regul.* **21**: 79-102, 1997.
- Hare, P.D., Cress, W.A., Van Staden, J.: Dissecting the role of osmolyte accumulation during stress. - *Plant Cell Environ.* **21**: 535-553, 1998.
- Hellergren, J., Li, P.H.: Survival of *Solanum tuberosum* suspension culture to -14 °C: mode of action of proline. - *Plant Physiol.* **52**: 444-449, 1981.
- Holowach, L.P., Madison, J.T., Thompson, J.F.: Studies on mechanism of regulation of the m-RNA level for a soybean storage protein subunit by exogenous L-methionine. - *Plant Physiol.* **80**: 561-567, 1986.
- Iyer, S., Caplan, A.: Products of proline catabolism can induce osmotically regulated genes. - *Plant Physiol.* **116**: 203-211, 1998.
- Kavikishore, P.B., Hong, Z., Miao, G.H., Hu, C.H., Verma, D.P.S.: Over expression of Δ -pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. - *Plant Physiol.* **108**: 1887-1894, 1995.
- Khanna, S.: Regulation of K⁺ uptake by exogenous amino acids, glycine betaine and abscisic acid in turgid and stressed *Raphanus sativus* L. seedlings. - Ph. D. Thesis. H.P. University, Shimla 1998.
- Khanna, S., Rai, V.K.: Changes in proline levels in response to osmotic stress and exogenous amino acids in *Raphanus sativus* L. seedlings. - *Acta Physiol. Plant.* **20**: 393-397, 1998.
- Kiyosue, T., Yoshida Y., Yamaguchi-Shinozaki, K., Shinizaki, K.: A nuclear gene encoding mitochondrial proline dehydrogenase, an enzyme involved in proline metabolism, is upregulated by proline but down regulated by dehydration in *Arabidopsis*. - *Plant Cell* **8**: 1323-1335, 1996.
- Krall, J.P., Edwards, G.E., Andreo, C.S.: Protection of pyruvate, Pi dikinase from maize against cold lability by compatible solutes. - *Plant Physiol.* **89**: 280-284, 1989.
- Krammer, U., Cotter-Howells, J.D., Charnock, J.M., Baker, A.J.M., Smith, J.A.C.: Free histidine as a metal chelator in plant that accumulate nickel. - *Nature* **379**: 635-638, 1996.
- Kumar, V., Sharma, D.R.: Effects of exogenous proline on growth and ion content in sodium chloride stressed and non-stressed cells of mung bean, *Vigna radiata* L. var. *radiata*. - *Indian J. exp. Biol.* **27**: 813-815, 1989.
- Kumar, V., Sharma, D.R., Sheoran, I.S.: Effects of proline on growth, ionic content and osmotic potential of thioproline stressed and nonstressed wild type callus cultures of mung bean (*Vigna radiata* var. *radiata*). - *Indian J. exp. Biol.* **29**: 661-664, 1990.
- Lone, M.I., Kueh, J.S.H., Wyn-Jones, R.G., Bright, S.W.J.: Influence of proline and glycine betaine on salt tolerance of cultured barley embryo. - *J. exp. Bot.* **38**: 479-490, 1987.
- Maggio, A., Bressan, R.A., Hasegawa, P.M., Locy, R.D.: Moderately increased constitutive proline does not alter osmotic stress tolerance. - *Physiol. Plant.* **101**: 240-246, 1997.
- Mali, P.C., Mehta, S.L.: Effect of drought on enzyme and free proline in rice varieties. - *Phytochemistry* **16**: 1355-1358, 1977.
- Mansour, M.M.F.: Nitrogen containing compounds and adaptation of plants to salinity stress. - *Biol. Plant.* **43**: 491-500, 2000.
- Mori, T., Sakagami, Y., Doi, K.: Changes in free amino acid contents in *Cryptomeria japonica* transplants under various soil moisture conditions. - *Jap. J. Forest Sci.* **52**: 350-354, 1971.
- Nanjo, T., Kobayashi, M., Yoshida, Y., Kakubari, Y., Yamaguchi-Shinozaki, K., Shinozaki, K.: Antisense suppression of proline degradation improves tolerance to freezing and salinity in *Arabidopsis thaliana*. - *FEBS Lett.* **46**: 205-210, 1999a.
- Nanjo, T., Kobayashi, M., Yoshida, Y., Sunada, Y., Wada, K., Tsukaya, H., Kakubari, Y., Yamaguchi-Shinozaki, K., Shinozaki, K.: Biological functions of proline in morphogenesis and osmotolerance revealed in antisense transgenic *Arabidopsis thaliana*. - *Plant J.* **18**: 185-193, 1999b.
- Paleg, L.G., Douglas, T.J., Van Daal, A., Keech, D.B.: Proline and betaine protect enzymes against heat inactivation. - *Aust. J. Plant Physiol.* **8**: 107-114, 1981.
- Palfi, G., Kovacs, F., Bito, M., Sebestyen, R.: The role of amino acids during water stress in species accumulating proline. - *Phyton Rev. Inst. Bot. Exp.* **32**: 121-127, 1974.
- Paulin, A.: The influence of a temporary water deficit on nitrogen metabolism in cut flowers of *Iris germinica*. - *Compt. rend. Acad. Sci. Paris, Sér. D* **275**: 209-212, 1972.
- Perez-Aflocea, F., Estan, M.T., Caro, M., Guerrier, G.: Osmotic adjustment in *Lycopersicon esculentum* and *Lycopersicon pennellii* under sodium chloride and polyethylene glycol 6000 iso-osmotic stress. - *Physiol. Plant.* **87**: 493-498, 1993.
- Rai, V.K., Bapat, C.M.: Water stress effects on amino acid metabolism during early vegetative growth in *Phaseolus mungo* L. - *Geobios* **4**: 231-234, 1977.
- Rai, V.K., Kumari, A.: Modulation of membrane permeability by amino acids in *Vinca* petals. - *Experientia* **39**: 301-303, 1983.
- Rai, V.K., Sharma, U.D.: Amino acids can modulate ABA induced stomatal closure, stomatal resistance and K⁺ fluxes

- in *Vicia faba* leaves. - Beitr. Biol. Pflanz. **66**: 393-405, 1991.
- Rajendrakumar, C.S.V., Suryanarayan, T., Reddy, A.R.: DNA helix destabilisation by proline and betaine: Possible role in salinity tolerance process. - FEBS Lett. **410**: 201-205, 1997.
- Rajagopal, V.: The influence of exogenous proline on stomatal resistance in *Vicia faba*. - Physiol. Plant. **52**: 292-296, 1981.
- Rajagopal, V., Sinha, S.K.: Influence of exogenously supplied proline on relative water content in wheat and barley. - Indian J. exp. Biol. **18**: 1523-1524, 1980.
- Rana, U., Rai, V.K.: Modulation of calcium uptake by exogenous amino acids in *Phaseolus vulgaris* seedlings. - Acta Physiol. Plant. **18**: 117-120, 1996.
- Schat, H., Sharma, S.S., Vooijs, R.: Heavy metal induced free proline in a metal tolerant and a non-tolerant ecotype of *Silene vulgaris*. - Physiol. Plant. **101**: 477-482, 1997.
- Schobert, B., Tschesche, H.: Unusual solution properties of proline and its interaction with proteins. - Biochem. biophys. Acta. **541**: 270-277, 1978.
- Schobert, B.: Is there an osmotic regulatory mechanism in algae and higher plants? - J. theor. Biol. **541**: 17-26, 1977.
- Sharma, S.S., Schat, H., Vooijs, R.: *In vitro* alleviation of heavy metal-induced enzyme inhibition by proline. - Phytochemistry **49**: 1531-1535, 1998.
- Sharma, U.D., Rai, V.K.: Modulation of osmotic closure of stomata, stomatal resistance and K⁺ fluxes by exogenous amino acids in *Vicia faba* L. leaves. - Biochem. Physiol. Pflanz. **185**: 369-376, 1989.
- Shibaoka, H., Thimann, K.V.: Antagonism between kinetin and amino acids. Experiments on mode of action of cytokinins. - Plant Physiol. **46**: 212-220, 1970.
- Singh, T.N., Aspinall, D., Paleg, L.G.: Proline accumulation and varietal adaptation to drought in barley, a potential metabolic measure of drought resistance. - Nature new Biol. **236**: 188-190, 1972.
- Singh, G., Thakur, P.S., Rai, V.K.: Free amino acid pattern in stressed leaves of two contrasting resistant and susceptible cultivars of chick pea. - Experientia **41**: 40-41, 1985.
- Slukhai, S.I., Shvedova, O.E.: [Dynamics of free amino acid contents in maize plants in connection with soil water regimes.] - Fiziol. Biochim. kul't. Rast. **4**: 151-156, 1972. [In Russ.]
- Smirnoff, N., Cumbes, Q.J.: Hydroxyl radical scavenging activity of compatible solutes. - Phytochemistry **28**: 1057-1059, 1989.
- Smirnoff, N., Stewart, G.R.: Nitrogen assimilation and zinc toxicity to zinc-tolerant and non-tolerant clones of *Deschampsia caespitosa* (L) Beau. - New Phytol. **107**: 671-680, 1987.
- Talanova, V.V., Titov, A.F., Boeva, N.P.: Effect of increasing concentration of lead and cadmium on cucumber seedlings. - Biol. Plant. **43**: 441-444, 2000.
- Tanaka, O., Nasu, Y., Sonoyama, A., Machara, Y., Kobayashi, T., Nawafune, H., Kugimoto, M.: Effects of exogenous amino acids on iron uptake in relation to their effects on photoperiodic flowering in *Lemna pausicostata* 6746. - Plant Cell Physiol. **28**: 697-702, 1987.
- Thakur, P.S., Rai, V.K.: Dynamics of amino acid accumulation in two differentially drought resistant *Zea mays* cultivars in response to osmotic stress. - Environ. exp. Bot. **22**: 221-226, 1982.
- Thakur, P.S., Rai, V.K.: Exogenously supplied amino acids and water deficits in *Zea mays* cultivars. - Biol. Plant. **27**: 458-461, 1985.
- Tipirdamaz, R., Karakullukcu, S.: Effects of proline and glycine betaine on growth and some internal solute changes of cultured tomato embryos under saline conditions. - Turk. J. Biol. **17**: 57-64, 1993.
- Waldren, R.D., Teare, I.D.: Free proline accumulation in drought stressed plants under laboratory conditions. - Plant Soil **40**: 689-692, 1974.
- Withers, L.A., King, P.J.: Proline: a novel cryoprotectant for the freeze preservation of cultured cells in *Zea mays*. - Plant Physiol. **64**: 675-678, 1979.
- Yang, C.-W., Lin, C.C., Kao, C.H.: Proline, ornithine, arginine and glutamic acid contents in detached rice leaves. - Biol. Plant. **43**: 305-307, 2000.