17. Kushwaha, H. L., Srivastava, A. P. and Singh, H., A study on physical properties of okra pod and seed. *J. Agric. Eng*., 2007, **44**, 88–91.
18. Joshi, D. C., Das, S. K. and Mukherjee, R. K., Physical properties of pumpkin seed. *J. Agric. Eng. Res*., 1993, **54**, 219–229.
19. Sahoo, P. K. and Srivastava, A. P., Physical properties of okra seed. *Biosyst. Eng*., 2002, **83**, 441–448.
20. Abdul-Baki, A. and Anderson, J. D., Vigor determination in soybean seed by multiple criteria. *Crop. Sci*., 1973, **13**, 630–633.
21. Priestley, J. T., Relations of protoplast permeability of cotton seed viability and pre-deposition of disease. *Plant Dis. Rep*., 1958, **42**, 582.
22. Cahsir, S., Marakoglu, T., Ogut, H. and Ozturk, O., Physical properties of rapeseed (*Brassica napus oleifera* L.). *J. Food Eng*., 2005, **69**, 61–66.
23. Kingsly, A. R. P., Singh, D. B., Manikantan, M. R. and Jain, R. K., Moisture dependent physical properties of dried pomegranate seeds (Anardana). *J. Food Eng*., 2006, **75**, 492–496.
24. Rasaq, A. A., Adebowale Sanni, L. O., Owo, O. H. and Karim, O. R., Effect of variety and moisture content on some engineering properties of paddy rice. *J. Food Sci. Technol*., 2011, **48**, 551–559.
25. Vishwakarma, R. K., Shivhare, U. S. and Nanda, S. K., Physical properties of guar seeds. *Food Bioprocess. Technol*., 2012, **5**, 1364–1371.
26. Izli, N., Effect of moisture on the physical properties of three varieties of kenaf seeds. *J. Food Sci. Technol*., 2015, **52**, 3254–3263.
27. Kashaninejad, M., Mortazavi, A., Safekordi, A. and Tabil, L. G., Some physical properties of pistachio (*Pistacia vera* L.). *J. Food Eng*., 2006, **72**, 30–38.
28. Isik, E. and Unal, H., Moisture-dependent physical properties of white speckled red kidney bean grains. *J. Food Eng*., 2007, **82**, 209–216.
29. Aviara, N. A., Power, P. P. and Abbas, T., Moisture-dependent physical properties of *Moringa oleifera* seed relevant in bulk handling and mechanical processing. *Ind. Crops Prod*., 2013, **42**, 96–104.
30. Mahawar, M. K., Sinha, J. P. ad Jalgaonkar, K., Design and development of seed priming prototype for hydropriming of okra (*Abelmoschus esculentus*) and pea (*Pisum sativum*) seeds. *Ind. J. Agric. Sci*., 2017, **87**(11), 1482–1486.
31. Pradhan, R. C., Naik, S. N., Bhatnagar, N. and Swain, S. K., Moisture-dependent physical properties of Karanja (*Pongamia pinnata*) kernel. *Ind. Crops Prod*., 2008, **28**, 155–161.
32. Mwithiga, G. and Sifuna, M. M., Effect of moisture content on the physical properties of three varieties of sorghum seeds. *J. Food Eng*., 2006, **75**, 480–486.
33. Yalcin, I., Ozarslan, C. and Akbas, T., Physical properties of pea (*Pisum sativum*) seed. *J. Food Eng*., 2007, **79**, 731–735.
34. Konak, M., Carman, K. and Aydin, C., Physical properties of chick pea seeds. *Biosyst. Eng*., 2002, **82**, 73–78.
35. Visvanathan, R., Palanisamy, P. T., Gothandapani, L. and Sreenarayanan, V. V., Physical properties of neem nut. *J. Agric. Eng. Res*., 1996, **63**, 19–26.

ACKNOWLEDGEMENTS. Financial support from the University Grants Commission (UGC) is duly acknowledged. We thank ICAR-IARI, New Delhi and ICAR-NBPGR, New Delhi for the research facilities.

Received 24 March 2017; accepted 30 October 2017

doi: 10.18520/cs/v114/i04/909-915

Proline-rich proteins may regulate free cellular proline levels during drought stress in tomato

Ranjit Singh Gujjar, Suhas G. Karkute*, Ashutosh Rai, Major Singh and Bijendra Singh

Division of Crop Improvement, ICAR-Indian Institute of Vegetable Research, Varanasi 221 305, India

Proline (Pro)-rich proteins (PRPs), initially identified as structural proteins of cell wall, have emerged as multifunctional plant proteins in recent past. Their vibrant role in plant development and environmental stress promoted us to study a *SlPRP* gene of tomato, which was significantly downregulated under drought stress in a microarray experiment performed in our laboratory. Promoter analysis of *SlPRP* revealed a number of stress-responsive protein-binding sites, confirming its expression in response to stress. Expression of *SlPRP* gene in different tissues of tomato, viz. root, stem, leaf and flower was studied to analyse the gene expression pattern in response to drought stress. Further, we have correlated the expression of *SlPRP* gene with Pro levels of the respective plant tissues under drought stress. In anticipation, it has been observed that downregulation of *SlPRP* gene is coupled with simultaneous increase in cellular Pro concentration in all the tissues under drought stress, except the roots. This could help preserve the available cellular proline to function as osmoprotectant during stress. The present results propose a hypothesis where PRPs may regulate free cellular proline levels during drought stress by regulating their own gene expression. Thus, it may be concluded that transcription of PRPs in plants is synchronized with the cellular Pro concentration under environmental stress in order to provide drought tolerance to plants.

Keywords: Drought stress, gene expression, proline-rich proteins, tomato.

PROLINE (Pro)-rich proteins (PRPs) are structural cell-wall proteins that were initially identified as wound-induced gene products in carrot storage roots[1]. Environmental stress or physical damage to plants also causes PRPs to accumulate in cell walls, whereas their expression is temporally regulated during plant development[2]. PRPs have been categorized into three classes. One of these has PRPs with several copies of the POVEKPOV**X**K motif[3], whereas the other two classes (HyPRPs and NHyPRPs) have PRPs with a hybrid structure. HyPRPs contain a repetitive proline-rich region at the N-terminal domain and a conserved eight-cysteine motif (8 CM) at the C-terminal domain[4,5]. In contrast, NHyPRPs have a C-terminal region with a high percentage

*For correspondence. (e-mail: S.Karkute@icar.gov.in)

of proline residues organized in distinct repetitive sequence motifs, whereas its extended amino-terminus is essentially devoid of proline residues[6,7].

Though the role of PRPs in plant growth and development is predominant, a number of recent reports suggest their involvement in response to various environmental stresses like wounding, fungal infection, salt stress, drought stress, heat stress and cold stress. Abiotic stress affects the expression of PRPs in such a manner, whereby they are upregulated in one kind of stress while downregulated in another. In *Poncirus trifoliata*, *PtrPRP* gene gets induced under cold, salt and exogenous abscisic acid (ABA) treatment, but it is downregulated by dehydration treatment[8]. Upregulation of PRPs under abiotic stress is further supported by higher levels of CcHyPRP transcripts noticed in PEG, NaCl, heat (42°C), cold and ABA-treated *Cajanus cajan* plants. Overexpression of *CcHyPRP* gene in *C. cajan* under control of CaMV35S and rd29A promoters[9] and *HyPRP* gene in Arabidopsis[10] has been advantageous to plants under abiotic stress conditions. Inhibition of PRP expression is observed mainly under drought stress as established by downregulation of *StGCPRP* gene in potato[7], *SlPRP* gene in tomato[11] and *PtrPRP* gene in *Poncirus trifoliate*[8]. Extreme salt stress may also reduce the expression of *PRP* gene as evident by downregulation of soybean *SbPRP* gene when >0.4% NaCl solution is used[12]. Furthermore, their expression may be temporally and spatially regulated, i.e. increase or decrease in the transcription level depending on the time of exposure of a particular stress and the type of tissue[8,12].

On the other hand, Pro is an important osmoprotectant which accumulates specifically during abiotic stresses and its cellular concentration varies during environmental stresses. Under normal conditions, Pro levels in cells are primarily maintained by their *de novo* synthesis through P5CS (pyrroline-5-carboxylate synthase) and degradation by proline dehydrogenase (PDH)[13,14]. On exposure of plant to environmental stresses, rate of *de novo* synthesis gets accelerated to increase cellular Pro concentration to carry out osmolytic functions. Degradation of cell-wall-bound PRPs has also been suggested to regulate the cellular concentration of Pro[15–17]. In addition to these two ways of proline accumulation, another mechanism may also exist to equilibrate Pro concentration during abiotic stresses. Therefore, the present study aims at exploration of PRPs for balancing cellular Pro levels. We have correlated the transcription of *SlPRP* with cellular Pro level. We propose that downregulation or upregulation of PRPs may be an additional mechanism to maintain optimum concentration of Pro during stress.

Seeds of *Solanum lycopersicum* were sown in pots filled with a mixture of soil and compost. Germinated seedlings were maintained at 25°C under optimal conditions in a glass house with regular watering. To induce expression of the target genes, drought stress was imposed on 45-day-old plants by withholding water till the appearance of drought symptoms (17 days). After treatment, leaves were taken in three biological replicates from drought-treated and control plants, and immediately frozen in liquid nitrogen. Samples were taken in three biological replicates from root, stem, leaf and flower for simultaneous investigation of both proline quantification and Q-RTPCR expression of *SlPRP* gene.

SlPRP, a PRP gene, corresponding to probe set ID Les.228.1.S1_a_at, showing drastic downregulation in tomato under drought stress in an earlier experiment in our laboratory was selected for the present study. Protein sequence of SlPRP was analysed using Interpro tool for understanding its composition, structure and probable function. Chemical and physical properties were computed using ProtParam tool. Further, it was manually analysed for presence of any particular repetitive pattern of amino acid sequence. To scrutinize the presence of specific cis-acting regulatory elements, promoter analysis was performed using 'PlantCare' tool[18]. For this purpose, the promoter region of 1000 bp upstream to the *PRP* gene was retrieved from NCBI.

Total RNA samples were extracted from all the experimental tissues using TRI Reagent (Ambion). Isolated RNA samples were treated with RNase-free DNase to remove genomic DNA contamination and stored at –20°C until further analysis. The first-strand cDNA was synthesized by 1.0 µg of total RNA in 20 µl reaction volume using cDNA synthesis kit, according to the manufacturer's instructions (Bio-Rad). Further, Q-RTPCR was done in all tissues using iQ SYBR Green Supermix (Bio-Rad) according to the manufacturer's instructions. Relative expression levels of *SlPRP* were normalized to the expression level of internal control gene (α-tubulin). Primers of *SlPRP* gene (forward: CAACAACAAAGGCAACATGC and reverse: GGATCACCAAGGCCAATATG) and α-tubulin gene (forward: CACTAGTGTCGCTGAGGTTTTCT and reverse: TGACCCGTCAAACTCTTACTCAT) were used for the experiment. The reverse transcription efficiency of *SlPRP* and tubulin gene was almost equal as analysed by comparing the C_T values at different dilutions of cDNA. Three technical replications were taken and the mean value was considered.

Proline was estimated in different drought-stressed tissues of tomato plants[19]. Fresh samples (500 mg) were homogenized in 10 ml of 3% aqueous sulphosalicylic acid and centrifuged at 22,000 *g* for 5 min. To 2 ml of the supernatant, 2 ml of acid ninhydrin was added. Further, 2 ml of glacialacetic acid was added and the content was boiled in water bath for 1 h at 100°C. The mixture was then extracted with 10 ml of toluene by mixing it thoroughly in a test tube with vigorous stirring. Absorption of chromophore was read at 515 nm in an UV-Vis spectrophotometer (Perkin Elmer, USA). L-Proline (Sigma) was used for preparation of standard curve. The amount of proline in different samples was calculated and expressed in terms of mg (proline) g^{-1} fresh weight.

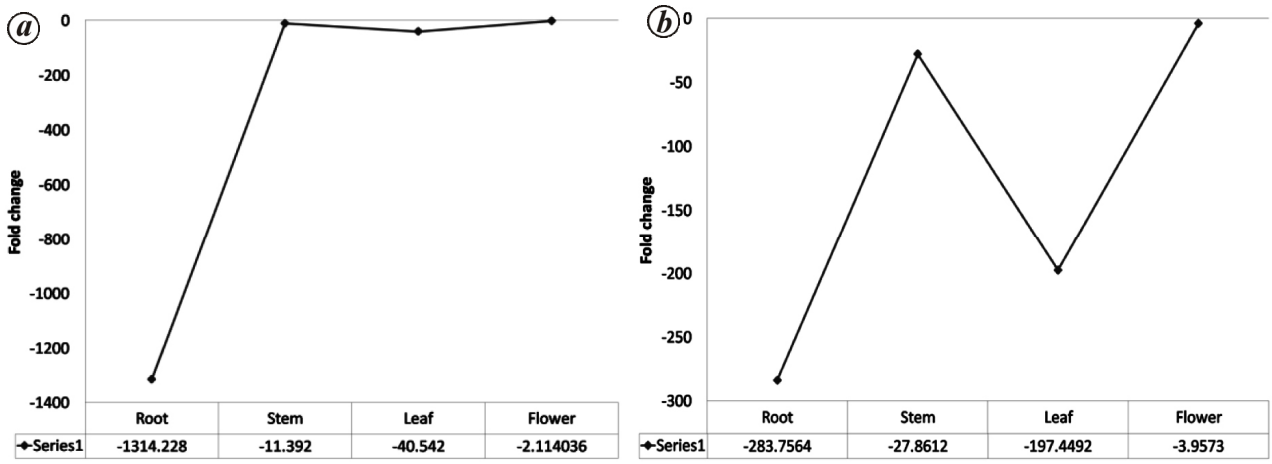

Figure 1. Drought-induced expression of *SlPRP* gene in different tissues of (***a***) drought-tolerant and (***b***) susceptible lines of tomato.

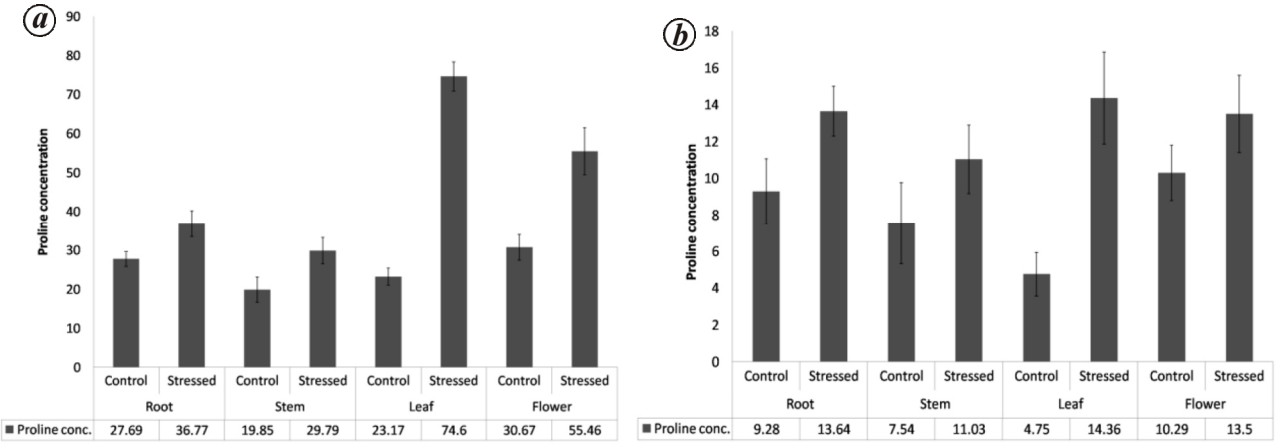

Figure 2. Drought-induced accumulation of proline in different tissues of (***a***) drought-tolerant and (***b***) susceptible lines of tomato.

Expression analysis of *SlPRP* gene was performed in different tissues taken for the experiment, viz. root, stem, leaf and flower under drought stress, whereby downregulation was observed in all these tissues (Figure 1). Surprisingly, highest downregulation was observed in roots of both susceptible (–283-fold) and tolerant (–1314-fold) lines. Nevertheless, this drastic reduction of *SlPRP* expression in roots could pertain to their physiological feature of being the first-level tissues to experience water deficit. *SlPRP* expression is also significantly reduced in leaves, followed by a rather low downregulation in the stems. Owing to its sensitive nature, slightest upshot of drought stress is perceived by the flower, where least downregulation of *SlPRP* gene has been observed. Pattern of *SlPRP* expression is fairly similar in both tolerant and susceptible lines of tomato. However, *SlPRP* expression is significantly reduced in the roots of tolerant line (–1314-fold) and leaves of susceptible line (–197-fold), under drought stress.

It is well known that cellular Pro level increases rapidly under all types of environmental stress. In the present study also, Pro concentration increased in all the tissues, viz. root, stem, leaf and flower under drought stress (Figure 2). Drought-induced accumulation of Pro was relatively high in leaves of both tolerant and susceptible lines. In leaves of susceptible line, Pro level amplified by approximately threefold and the increase was even more in leaves of tolerant line (> three fold). In other tissues like root, stem and flower, effect of drought on free cellular Pro concentration was moderately less (< two fold). Though drought-induced accumulation of Pro was similar in both the cultivars, tolerant line had reasonably high cellular Pro under normal as well as stress conditions.

Over the years, build-up of cellular Pro concentration in plants during environmental stress has been solely attributed to its synthesis through P5CS enzyme[13,14]. Some studies proposed that proline accrual during stress is not

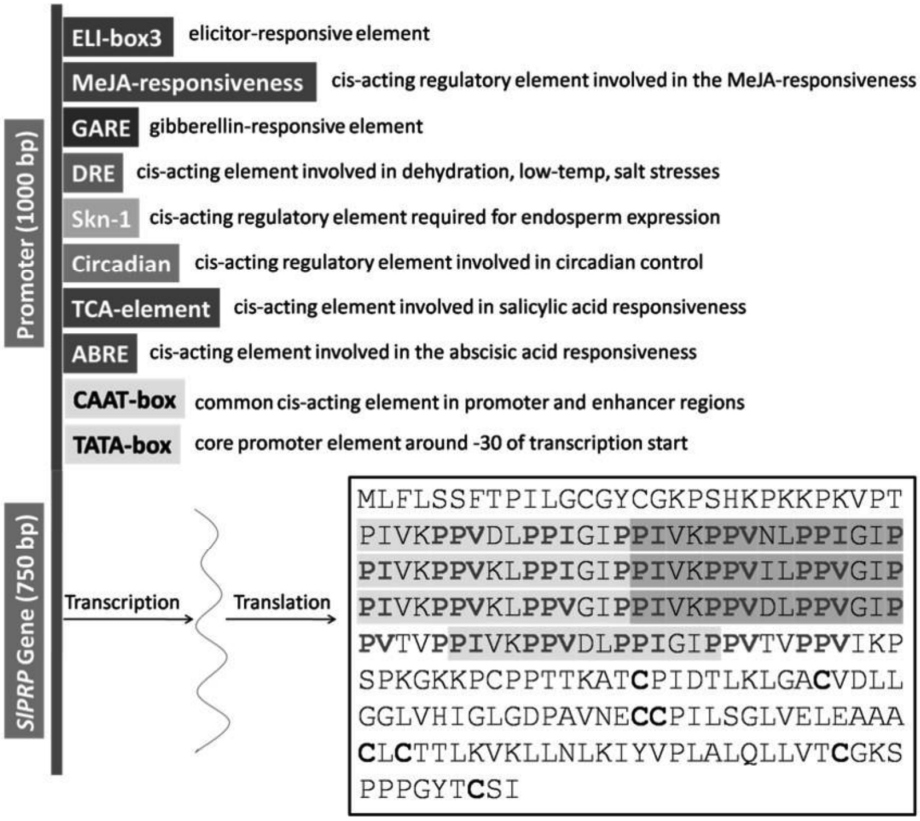

Figure 3. Regulatory elements present in the promoter region of *SlPRP* gene. Highlighted region at N-terminal of SlPRP protein represents praline-rich simple tandem repeats, while eight-cysteine motif at the C-terminal is indicated by bold letters.

exclusively dependent on P5CS[17,20]. Alternatively, degradation of cell-wall-bound PRPs may also contribute in the accumulation of free Pro during environmental stress[15,16]. In the present study, on exposure to drought stress, Pro concentration increased in all the plant tissues (root, stem, leaf and flower), with simultaneous downregulation of *SlPRP* gene. Besides roots which exhibited maximum downregulation of *SlPRP* gene, decline in *SlPRP* expression clearly corresponded to the increase in Pro concentration in stem, leaf and flower. However, major effect of drought was detected on the leaves where Pro concentration enhanced drastically and a concurrent reduction was also observed in the expression of *SlPRP*. Contrasting levels of Pro and PRP transcript in roots observed in the study could be the consequence of upward transportation of Pro from roots to leaves through xylem[17].

SlPRP promoter exposed several stress-inducible elements (Figure 3), which is in contrast to its expression under drought stress. In conjunction with core promoter elements and common cis-acting elements, the promoter region of *SlPRP* gene contains a number of abiotic and biotic stress responsive elements, viz. DRE (dehydration responsive element), ABRE (ABA-responsive element), TCA-element (salicylic acid responsive element), ELI-box3 (elicitor responsive element) and MeJA-responsiveness (methyl jasmonic acid responsive element). Besides, few other regulatory elements involved in gibberellin responsiveness, endosperm expression and circadian control are also present. *SlPRP* gene translates into a 25.67 kDa protein consisting of almost 26% Pro residues with unique repetitive pattern (PIVKPPV × LPPI/VGIP) of six simple tandem repeats at N-terminal (Figure 3). C-terminal of the protein is predominant in hydrophobic amino acids with conserved 8 CM, which is a characteristics signature of lipid transfer proteins.

Osmolytic function of Pro is largely appreciated for counteracting water-deficit stress in plants[21,22]. On sensing drought stress, Pro gets accumulated in cells either by its *de novo* synthesis[13,14] or by degradation of cell-wall-bound PRPs[15,16]. Conversely, when plants recover from stress, excess quantity of free cellular Pro is moderated by proline dehydrogenase[14]. Also, high Pro concentration in plant cells may inhibit growth, cell division[23,24], seed germination[25] and root growth[26], therefore Pro level needs to be maintained below toxicity. The present study discusses one more mechanism to fine-tune the cellular Pro concentration during stress, where PRPs are the main regulators (Figure 4). Upon exposure to drought stress, the plant instantly requires Pro to prevent cellular damage that may be caused by osmotic imbalance. On the other

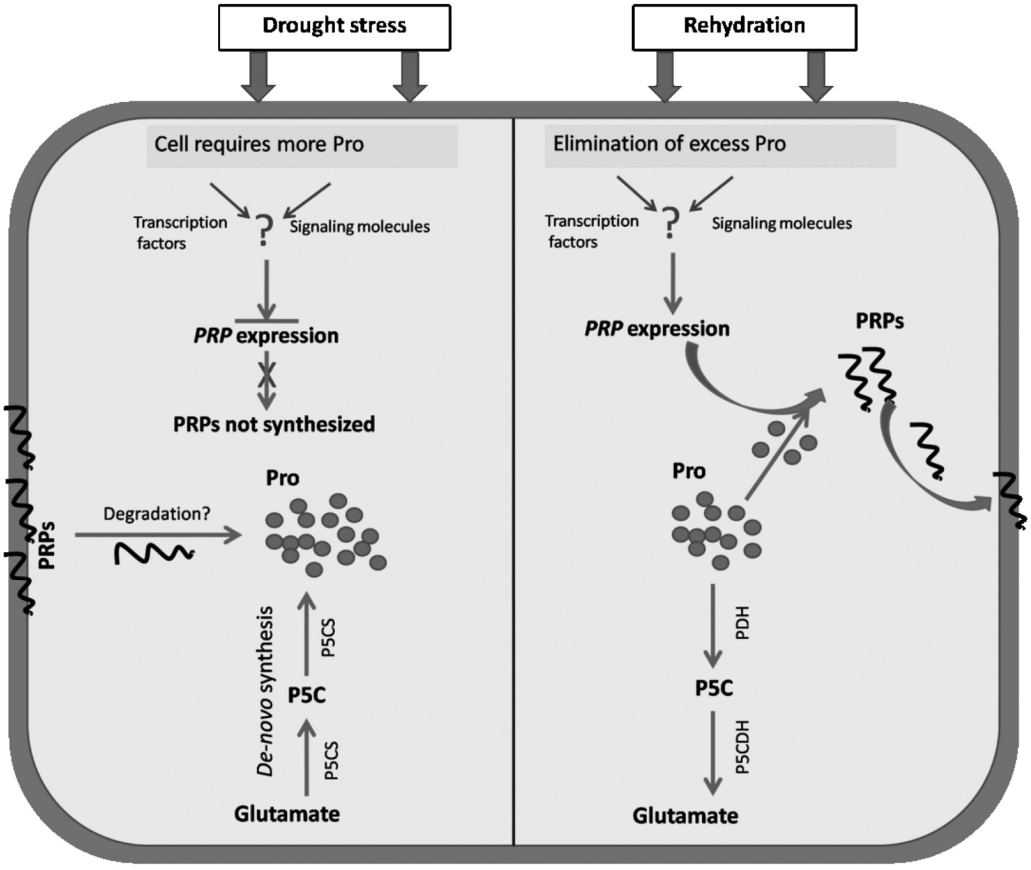

Figure 4. Regulation of cellular Pro through modulation of *PRP* expression. Drought perception induces accumulation of Pro in cells by three ways – (1) *de novo* synthesis is mediated through P5CS, whereby glutamate is converted into Pro. (2) Cell-wall-bound proline-rich proteins are degraded to release free Pro. (3) Transcription of *PRP* gene is blocked via signalling cascade (?) to avoid consumption of free Pro for synthesis of proline-rich proteins. Rehydration of plants resumes the expression of *PRP* gene and thereby synthesis of proline-rich proteins that leads to consumption of excess Pro. Alternatively, excess Pro is also eliminated by the process of glutamate synthesis guided by PDH and P5CDH.

hand, drought perception causes drastic reduction in *PRP* gene expression in order to avoid full use of available free Pro molecules in cells[8,11]. This assures the availability of adequate Pro molecules to function as osmolyte till their *de novo* synthesis begins. On withdrawal of stress, surplus cellular Pro is consumed by enhanced transcription of PRPs[8]. Furthermore, temporal expression of PRP genes during various stages of environmental stresses may be attributed to equilibrate the Pro level according to the requirements of the cell[8,12].

Though PRPs have several well-defined developmental functions right from the germination of seed to flower development and cell death, their precise role during stress is still unknown. In the present study, we observed that accumulation of Pro during drought stress is synchronized with simultaneous reduction in *SlPRP* expression. Besides *de novo* synthesis by P5CS and degradation by PDH, the study suggests a novel mechanism that may contribute in regulating cellular Pro concentration during stress through modulation of *PRP* expression. Though the occurrence of stress responsive elements on promoter region is supposed to enhance the transcription of *SlPRP* under stress environment, reduced expression of the aforementioned gene could be the consequence of some unknown mechanism involved in its regulation. Thus, future studies should focus to unravel the signalling cascades involved in the proposed regulatory mechanism.

1. Chen, J. and Varner, J. E., Isolation and characterization of cDNA clones for carrot extension and a proline-rich 33-kDa protein. *Proc. Natl. Acad. Sci. USA*, 1985, **82**, 4399–4403.
2. Fowler, T. J., Bernhardt, C. and Tierney, M. L., Characterization and expression of four proline-rich cell wall protein genes in *Arabidopsis* encoding two distinct subsets of multiple domain proteins. *Plant Physiol.*, 1999, **121**, 1081–1091.
3. Hong, J. C., Nagao, R. T. and Key, J. L., Characterization of a proline- rich cell wall protein gene family of soybean: a comparative analysis. *J. Biol. Chem.*, 1990, **265**, 2470–2475.
4. Subramaniam, K., Ranie, J., Srinivasa, B. R., Sinha, A. M. and Mahadevan, S., Cloning and sequence of a cDNA encoding a novel hybrid proline-rich protein associated with cytokinin-induced

haustoria formation in *Cuscuta reflexa*. *Gene*, 1994, **14**, 207–210.
5. Dvorakova, L., Srba, M., Opatrny, Z. and Fischer, L., Hybrid proline-rich proteins: novel players in plant cell elongation. *Ann. Bot.*, 2012, **109**, 453–462.
6. Castonguay, Y., Laberge, S., Nadeau, P. and Vezina, L. P., A cold induced gene from *Medicago sativa* encodes a bimodular protein similar to developmentally regulated proteins. *Plant Mol. Biol*., 1994, **24**, 799–804.
7. Menke, U., Renault, N. and Mueller-Roeber, B., *StGCPRP*, a potato gene strongly expressed in stomatal guard cells, defines a novel type of repetitive proline-rich proteins. *Plant Physiol*., 2000, **122**, 677–686.
8. Peng, T., Jia, M. M. and Liu, J. H., RNAi-based functional elucidation of *PtrPRP*, a gene encoding a hybrid proline rich protein, in cold tolerance of *Poncirus trifoliate*. *Front. Plant Sci*., 2015, **6**, 808.
9. Priyanka, B., Sekhar, K., Reddy, V. D. and Rao, K. V., Expression of pigeonpea hybrid-proline-rich protein encoding gene (*CcHyPRP*) in yeast and Arabidopsis affords multiple abiotic stress tolerance. *Plant Biotechnol. J.*, 2010, **8**, 76–87.
10. Li, W. *et al.*, Identification of early salt stress responsive proteins in seedling roots of upland cotton (*Gossypium hirsutum* L.) employing iTRAQ-based proteomic technique. *Front. Plant Sci.*, 2015, **6**, 732.
11. Gujjar, R. S., Akhtar, M., Rai, A. and Singh, M., Expression analysis of drought induced genes in wild tomato line (*Solanum habrochaites*). *Curr. Sci.*, 2014, **107**, 496–502.
12. He, C. Y., Zhang, J. S. and Chen, S. Y., A soybean gene encoding a proline-rich protein is regulated by salicylic acid, an endogenous circadian rhythm and by various stresses. *Theor. Appl. Genet.*, 2002, **104**, 1125–1131.
13. Kishor, P. K., Hong, Z., Miao, G. H., Hu, C. A. and Verma, D. P., Overexpression of [delta]-pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. *Plant Physiol*., 1995, **108**, 1387–1394.
14. Perez-Arellano, I., Carmona-Alvarez, F., Martinez, A. I., Rodriguez-Diaz, J. and Cervera, J., Pyrroline-5-carboxylate synthase and proline biosynthesis: from osmotolerance to rare metabolic disease. *Protein Sci.*, 2010, **19**, 372–382.
15. Chen, D., Kessler, B. and Monselise, S. P., Studies on water regime and nitrogen metabolism of citrus seedlings grown under water stress. *Plant Physiol.*, 1964, **39**, 379–386.
16. Barthakur, S., Babu, V. and Bansal, K. C., Over-expression of osmotin induces proline accumulation and confers tolerance to osmotic stress in transgenic tobacco. *J. Plant Biochem. Biotechnol*., 2001, **10**, 31–37.
17. Verbruggenm, N. and Hermans, C., Proline accumulation in plants: a review. *Amino Acids*, 2008, **35**, 753–759.
18. Lescot, M. *et al.*, PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for *in silico* analysis of promoter sequences. *Nucleic Acids Res*., 2002, **30**, 325–327.
19. Bates, L. S., Waldren, R. P. and Teare, I. D., Rapid determination of free proline for water stress studies. *Plant Soil*, 1973, **39**, 205–207.
20. Stines, A. P., Naylor, D. J., Hoj, P. B. and Heeswijack, R., Proline accumulation in developing grapevine fruit occurs independently of changes in the levels of delta1-pyrroline-5-carboxylate synthetase mRNA or protein. *Plant Physiol*., 1999, **120**, 923–923.
21. Szabados, L. and Savoure, A., Proline: a multifunctional amino acid. *Trends Plant Sci*., 2010, **15**, 89–97.
22. Meringer, M. V. *et al.*, Saline and osmotic stresses stimulate PLD/diacylglycerol kinase activities and increase the level of phosphatidic acid and proline in barley roots. *Environ. Exp. Bot.*, 2016, **128**, 69–78.
23. Maggio, A. *et al.*, Does proline accumulation play an active role in stress-induced growth reduction. *Plant J*., 2002, **31**, 699–712.
24. Yamada, M., Morishita, H., Urano, K., Shiozaki, N., Yamaguchi-Shinozaki, K., Shinozaki, K. and Yoshiba, Y., Effects of free proline accumulation in petunias under drought stress. *J. Exp. Bot.*, 2005, **56**, 1975–1981.
25. Hare, P. D., Cress, W. A. and Van-Staden, J., A regulatory role for proline metabolism in stimulating *Arabidopsis thaliana* seed germination. *Plant Growth Regul*., 2003, **39**, 41–50.
26. Kant, S., Kant, P., Raveh, E. and Barak, S., Evidence that differential gene expression between the halophyte *Thellungiella halophila* and *Arabidopsis thaliana* is responsible for higher levels of the compatible osmolyte proline and tight control of Na^+ uptake in *T. halophila*. *Plant Cell Environ*., 2006, **29**, 1220–1234.

ACKNOWLEDGEMENT. This study was financially supported by ICAR-Indian Institute of Vegetable Research, Varanasi.

Received 19 November 2016; revised accepted 21 September 2017

doi: 10.18520/cs/v114/i04/915-920