

Proline accumulation in plants

Not only stress

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In addition to its role in protein synthesis and the plant cells' response to environmental stresses, circumstantial evidence suggest that proline may also play a role in flowering and development both as a metabolite and as a signal molecule. Although there is a growing consensus that proline is of special importance throughout the reproductive phase (from flower transition to seed development) a general agreement on the molecular and genetic mechanisms proline is involved in, is yet to be established. In this paper we shall review and critically discuss most of the evidence supporting a role for proline in plant development, paying special attention to the recently reported role of proline in flower transition.

Proline Accumulates, Under Non-Stressed Conditions, in Reproductive Organs and Tissues

It is well described¹ that under stress conditions many plant species accumulate proline as an adaptive response to adverse conditions. Although a clear-cut relationship between proline accumulation and stress adaptation has been questioned by some authors,² it is generally believed that the increase in proline content following stress injury is beneficial for the plant cell.

However, ever since the early 80s different research groups found a significant amount of proline in the reproductive organs of different plant species, raising the possibility that the accumulation of this amino acid may also occur in physiological non-stressed conditions for developmental purposes. Chiang and Dandekar,³ for example, reported that in *Arabidopsis* reproductive tissues, such as florets, pollen, siliques and seeds, proline represents up to 26% of the total amino acid pool, while in vegetative tissues it only accounts for 1–3%. Even more striking, Schwacke et al.⁴ observed that the content of free proline in tomato flowers was 60-fold higher than in any other organ analyzed. Similar physiological accumulations of proline have been reported, at different concentrations, in reproductive organs of other plant species,³ and in most cases the overall levels of this amino acid seem too high to be accounted for only by an increased demand of protein synthesis.

At the molecular level, the differential accumulation of proline in reproductive tissues is thought to be primarily determined by

upregulation of proline synthesis and transport genes, as upregulation of Δ^1 -pyrroline-5-carboxylate synthetase (*P5CS*), a gene encoding the rate-limiting enzyme of proline synthesis from glutamate, and *Proline transporter T (ProT)*, a gene encoding a specific proline transporter, has been found in flower organs.⁴⁻⁶ The role exerted by the proline catabolic genes in the process of developmental proline accumulation, in contrast, appear different from the role played by these genes during stress-induced proline accumulation, as *proline dehydrogenase (PDH)* and Δ^1 -pyrroline-5-carboxylate dehydrogenase (*P5CDH*) catabolic genes are upregulated in the former case,⁷⁻⁹ and downregulated in the latter case.^{8,10}

Although the developmental accumulation of proline in reproductive organs has been repeatedly reported, and seems to be a widespread phenomenon among plant species, its functional meaning is still matter of debate. An obvious function of proline in development may be the protection of developing cells from osmotic damages, especially in those developmental processes, such as pollen development and embryogenesis, in which tissues undergo spontaneous dehydration. Similarly to the osmotic stress caused by environmental factors, the desiccation process that spontaneously occurs in reproductive tissues may seriously damage the plant cell, and it is likely to be counteracted by proline accumulation. Accordingly, higher levels of proline have been measured³ in tissues with low water content as compared as to tissues with high water content. The correlation between proline accumulation and water content, however, is not very tight. Florets, for example, have been described by Chiang and colleagues as the organs with the highest proline concentration, in spite of their relatively high water content.

As an alternative possibility, proline has been proposed to provide energy to sustain metabolically demanding programs of plant reproduction. In a similar way, proline is used in animal systems to fuel the initial phase—the most energy-dependent—of the flight of many insects, such as bees and butterflies.¹¹ Since the oxidation of one molecule of proline yields 30 ATP equivalents,¹² this amino acid seems well suited to sustain high energy-requiring processes. The upregulation of the proline catabolic genes typically observed in flowers, siliques and seeds is consistent with the need to provide the plant with energy throughout the whole reproductive phase.

Involvement of Proline in Stem Elongation

A further argument supporting a role of proline in plant reproductive development, comes from the analysis of loss of function *Arabidopsis* mutants and transgenics impaired in proline synthesis

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which confirms a specific role of proline in the rapid elongation of the inflorescence stem. Because in higher plants the proline synthesis pathway proceeding from glutamate is regarded as the main, if not the only, biochemical route to synthesize proline,¹³ and P5CS is the rate limiting enzyme of this pathway, mutants of either *P5CS1* or *P5CS2*, two paralog genes present in the Arabidopsis genome that encode P5CS, were mainly used in these works.

The antisense expression of *P5CS1* in Arabidopsis was shown by Nanjo et al.¹⁴ to reduce proline content and, in turn, inhibit bolting—the fast stem elongation occurring immediately after flower transition in many flowering species. In addition these antisense transgenics showed morphological alterations in leaves and an overall bushy appearance.¹⁴ Incidentally, in a screen aimed to identify early targets of CONSTANS (CO), a transcriptional factor regarded as a major flowering promoter of the photoperiodic pathway, *P5CS2* was identified among the four earliest targets induced by CO,¹⁵ further corroborating a possible crosstalk between proline and flowering.

Besides bolting, there are other developmental processes in which proline has been associated to rapid elongation, such as the elongation of the pollen tube,^{4,16,17} the elongation of the hairy roots in dicotyledonous plants infected by *Agrobacterium rhizogenes*,^{18,19} the elongation of the maize primary root at low water potential,^{20–22} suggesting that proline might be generally exploited by the plant cell in developmental programs involving rapid cell growth.

As already mentioned, the functional significance of proline accumulation in these tissues may be that of providing the cell enough energy to sustain rapid growth. A positive correlation between proline and cell elongation exists, however, might also be explained in term of protein synthesis, as hydroxyproline-rich glycoproteins (HRGPs, extensins and arabinogalactan proteins), are important structural constituents of the plant cell wall thought to play a key role in the regulation of cell division, cell wall self assembly and cell extension.^{23–25} In support of this hypothesis Nanjo et al.¹⁴ found decreased proline and hydroxyproline content in the cell wall protein fraction of antisense-*P5CS1* transgenic Arabidopsis impaired in bolting.

Proline and Flower Transition

The possibility that the time of flowering may be affected by proline, either developmentally- or stress-induced, is an old idea supported by a limited number of reports and based on the belief that stress can induce flowering. An involvement of proline in flower transition, for example, was suggested in *Sinapis alba*,²⁶ kiwifruit,²⁷ tobacco,^{19,28,29} tomato³⁰ and *Vigna aconitifolia*.³¹ However, although a crosstalk between stress and flowering clearly exists, a positive correlation between stress and flowering has been convincingly demonstrated only for salicylic acid-mediated stresses, such as a few pathogen infections and UV-C stresses,³² and for mild thermal stress under short day conditions.³³ On the contrary, salt stress and the stress-related phytohormone abscisic acid (ABA), among the most powerful inducers of proline accumulation, have been shown to delay flower transition.³⁴

Two recent papers by Mattioli et al.^{35,36} however, raised the possibility that modulations of low proline concentration localized

in apical meristems may signal optimal conditions for the plant to flower, while higher concentrations of proline might be interpreted by the plant as a stress signal and induce adaptive responses, including late flowering. In a similar way glucose has been reported to trigger in yeast different developmental programs at different glucose concentration: growth stimulation at low concentration and growth repression at high concentration.³⁷ Indeed, a relationship between flower transition and proline was recently reported by Mattioli et al.³⁵ who found that transgenic Arabidopsis harboring a *35S-P5CS1* construct behaved as early flowering, both in long- and short-day conditions, and exhibited a transient peak of *P5CS1* overexpression and proline accumulation prior to visible flower transition. Furthermore, Arabidopsis *p5cs1* knock-out mutants, containing a T-DNA insertion into *P5CS1* exhibited reduced proline levels and were late-flowering.³⁵ While *P5CS1* and *P5CS2* are generally thought to have non-redundant pattern of expression and functions,³⁸ in the case of flower transition the two genes seem to play overlapping roles, as inferred by their pattern of expression and by the analysis of the double mutant *p5cs1-1^{-/-} p5cs2-1^{+/-}*. Both *P5CS1* and *P5CS2* are expressed at similar levels and with the same pattern of expression in vegetative and floral shoot apical meristems as well as in axillary meristems, and, most importantly, double mutants *p5cs1-1^{-/-} p5cs2-1^{+/-}* showed a stronger late-flowering phenotype than *p5cs1* single mutants.³⁶

Role of Proline in Embryo Development and Concluding Remarks

Recently, Székely et al.³⁸ characterized Arabidopsis mutants defective in either *P5CS1* or *P5CS2* and found that knockout mutations of *P5CS1* result in the reduction of stress-induced proline synthesis, hypersensitivity to salt stress and accumulation of reactive oxygen species, implying that *P5CS1* is required for proline accumulation under osmotic stress. Mutations in *P5CS2*, in contrast, caused embryo abortion during late stages of seed development, pointing to an involvement of *P5CS2*, and, in turn, of proline, in embryo development.^{36,38} These findings are consistent with the high levels of proline previously found in the siliques and developing seeds of many plant species, and further support of a role of proline in embryo development.

Arabidopsis *p5cs2* mutants were also studied by Mattioli et al.³⁶ who essentially confirmed that embryo lethality is associated to *P5CS2* disruption, and found alterations of cellular division planes in most of the aberrant embryos. Intriguingly these authors found that exogenous proline accelerated organ growth and meristem formation, and stimulated expression of the cell cycle-related protein CYCB1;1 suggesting an involvement of proline in cell division.³⁶ Since it is believed that stimulation of cell division is associated to, or precedes, flower transition³⁹ a putative involvement of proline in cell division could reconcile the effects of proline on meristem stimulation with those on flower transition, and we propose that this amino acid may act both as a metabolic substrate to sustain the needs of rapidly dividing cells and, in turn, as a feedback signal molecule to fine-tune developmental processes such as flower transition.

Tight coordination between cell cycle and developmental processes has been well described in yeast and mammals, where is normally achieved by the TOR pathway^{40,41} which perceive metabolic signals, usually sugars or amino acids, to coordinate nutrient availability with cell cycle progression. The TOR pathway seems to be conserved in plants,⁴² and the level of expression of *AtTOR* has recently been correlated with root and stem growth in *Arabidopsis*,⁴³ although little is known on the role of amino acids in the regulation of this pathway. It is tempting to speculate that proline may act as a

signal molecule in regulating the TOR pathway in plants, playing the same role exerted by leucine in animals and by glutamine in yeast.⁴⁰

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