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BRIEF COMMUNICATION

Proline accumulation in *Tephrosia purpurea* Pers.

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Abstract

Tephrosia purpurea Pers. was found to accumulate high proline content in dry habitat. The proline content was higher in shoots, especially in leaves, than in roots. Pod walls and young seeds showed the highest proline content. The proline content of young leaves was higher than that of mature and old leaves. During leaf senescence *in vitro* proline content increased rapidly upto 6 h and further decreased in leaves as well as in leachate. High proline content seems to be positively related with 'survival capability' of this plant.

Key words: mature, old, senescence, young

Tephrosia purpurea Pers. belonging to the family *Fabaceae* is a common weed of field crops. Proline accumulation is a function of tissue water potential and it is involved in osmoregulation. When accumulation of proline was noticed in *Tephrosia purpurea*, we felt it worthwhile to know wheather this compound play any role in survival capability of this plant.

Tephrosia purpurea Pers. plants were raised from healthy seeds collected in Botanical garden in July 1993. Plants were grown in greenhouse (temperature 34/18 °C, relative humidity 56 %, natural photoperiod of *ca.* 13/11 h). Ten (60-d-old) plants were uprooted, brought to laboratory, washed, blotted-to-dry and sorted out for various plants parts: leaves, stems, roots, pod walls, young seeds and mature seeds. These were subjected to oven drying for 10 d at 85 °C. Dried material was powdered and subjected to proline estimation. At the same time, mature leaves (4^{th} to 8^{th} leaf from the top) of one-month old plants from two different soil moistures (high and low) were collected and subjected to proline estimation after oven drying.

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Further leaves of different insertion levels from the same plant were selected for analysis: young leaves (1st and 2nd leaf), mature leaves (4th to 8th leaf) and senescent leaves (12th to 16th leaf).

Fresh plant material was also taken and placed in distilled water for 4 h. Later, it was removed, blotted-to-dry and kept in oven at 80 °C for 4 d. The difference in mass was used to calculate moisture content.

Free proline content was determined according to Bates *et al.* (1973). Plant material was homogenised in sulfosalicylic acid and the filtrate alongwith glacial acetic acid and ninhydrin reagent was reacted for 1 h at 100 °C in water bath. The reaction was terminated by transferring the reactants to ice bath. The developed colour was extracted with toluene and the absorbance of toluene chromophore was measured at 520 nm colorimetrically; results are expressed as $\mu g\ g^{-1}$(dry mass) ± SE.

For *in vitro* senescence studies leaf discs of 0.24 cm^2 were floated in 15 cm^3 glass with distilled water for different time intervals (2, 4, 6, 24, 48 and 72 h) separately. These were incubated in dark at 25 °C. After the allotted time leaf discs and the leachate were subjected to proline estimation. The results are expressed as $\mu g\ g^{-1}$(fresh mass) ± SE or $\mu g\ cm^{-3}$(leachate) ± SE.

Low soil moisture (9.2 %) resulted in marked accumulation of proline [1326.5 $\mu g\ g^{-1}$(d.m.)] in leaf tissue but at high soil moisture (17.8 %) proline content was almost negligible [120.2 $\mu g\ g^{-1}$(d.m.)]. Thus a strong positive correlation was found between the nature of habitat of the plant and its proline content.

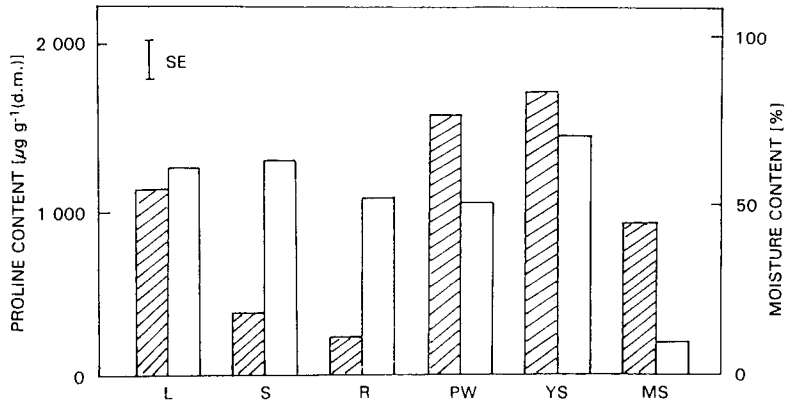

Fig. 1. Proline (*hatched columns*) accumulation and moisture content (*open columns*) in various plant parts (L - leaves, S - stem, R - roots, PW - pod wall, YS - young seeds, MS - mature seeds) of *Tephrosia purpurea* Pers.

Moisture content in various plant parts was similar but proline content was higher in shoots, especially in leaves. At the same time, maximum proline content was observed in young seeds followed by pod wall. In mature seeds, proline content and also moisture content were lowest (Fig. 1). High proline content found in leaves is obvious because leaves are the site of proline synthesis. High proline content in reproductive parts suggests that a great portion of proline synthesized in leaves might

be diverted towards seeds where it might be accumulated or converted into *i.e.* ornithine and arginine. This sounds logical because by virtue of its being a legume, *Tephrosia* seeds are rich in nitrogen and ultimately in proteins; moreover, they possess significant amount of proline (Murumkar 1993). This may help to survive under unfavourable conditions during germination.

Young leaves showed the highest proline content, but with aging leaf proline content decreased (Fig. 2*B*). Moisture content also declined with leaf insertion. In senescent leaves both proline content and moisture content were the lowest. Nevertheless in senescent leaves considerable proline content was noticed. *In vivo* proline was translocated to younger parts and so its accumulation is higher compared to that in older ones (Singh *et al.* 1973). Our findings are in agreement with this report. Relatively high proline content in senescent leaves may be attributed to slow rate of translocation as well as enhanced proteolysis resulting in release of 'bound' proline. *In vitro* studies also justified these results.

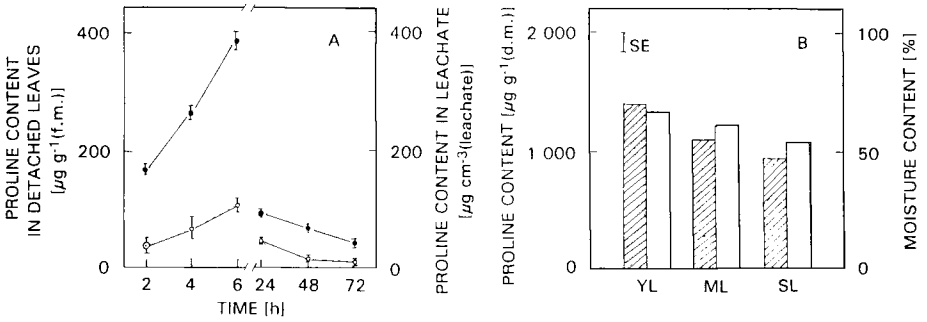

Fig. 2*A*. *In vitro* senescence studies in *Tephrosia purpurea* Pers. leaves. Proline content in detached leaves (*closed circles*) and in leachate (*open circles*).

Fig. 2*B*. Proline (*hatched columns*) and moisture (*open columns*) content in *Tephrosia purpurea* Pers. leaves of different insertion level (YL - young leaves, ML - mature leaves, SL - senescent leaves)

In leaf segments proline progressively accumulated during the first 6 h (Fig. 2*A*). During this, proline also leached out from the leaf discs as there was a consistant rise of proline in leachate. But after 24 h proline content was substantially decreased in leaves as well as in the leachate and the decline continued upto 72 h. By this time proline from the leachate was partially reabsorbed by leaf discs. There was no regulatory mechanism to withhold accumulated proline as this was reflected in the release of synthesized proline in the surrounding medium, *i.e.* leachate. This might be due to break down in the subcellular compartmentalisation. But once the senescence had set in, proline content in leaf segments decreased progressively suggesting that proline catabolism had taken over and at the same time uptake of proline from the leachate also took place as there was a progressive decline in proline content in the leachate as well. This finding claims that as a result of early shock, plasma membrane and tonoplast might have been disrupted, resulting in leaching of proline from the cell. Both, the plasma membrane and tonoplast, had been gradually

recovered, especially the plasma membrane, because it had again assumed the role of solute uptake from the medium. This signifies the role of plasma membrane in regulating the cell to be in tune with the surrounding.

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