

## Short Communication

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# PROLINE STATUS DURING GROWTH AND CALLUSING IN *SESAMUM INDICUM*

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Proline is an important amino acid present in plant tissues. Quantitative study was made for the various plantlet parts as well as for callus of *Sesamum indicum*. Proline content was found higher in cotyledons as compared to hypocotyl and seeds. The content was increased and decreased during callusing obtained by inoculation of hypocotyls on MS medium supplemented with BAP (1 mg/l) along with NAA (5 mg/l).

**Key Words :** Proline, callus, *Sesamum indicum*.

*Sesamum indicum* is a well known important oil seed crop. The crop was chosen for tissue culturing for not only its commercial and economical importance for yielding edible oil but also it provides raw material for lubricants, pharmaceuticals, cosmetics, illuminants etc. To know the various biochemical and metabolic changes occurring in the tissues while callusing, several biochemical estimations were done. However, present study is made up for proline estimation, which is very important amino acid present in free pool and as protein fraction of plant tissues. Proline status of plant organs and cell cultures is an active area of research in stress physiology (Aspinall & Paleg, 1981; Sheron & Nainawatee 1990) Elevated levels of proline are believed to protect plant tissues against stress by acting as nitrogen storage compound, osmolyte and hydrophobic protectant for enzymes and cellular structures (Rudulier *et al.*, 1984).

For the estimation of the content in cotyledon and hypocotyls, two weeks old seedlings were used which were grown aseptically on paper bridges. The callus was obtained when the hypocotyls were inoculated on Murashige and Skoog's medium (1962) supplemented with BAP (1 mg/l) in combination with NAA (5 mg/l). The changes in proline content in the callus were estimated for several subcultures. For the estimations Bates *et al.* (1973) method was used. Weighed plant material was homogenized and extracted in 3% sulphosalicylic acid and centrifuged. Supernatant was made to a known volume. 1 ml of this aliquot was taken in a test tube, 5 ml acid ninhydrin reagent and 5 ml

of glacial acetic acid were added. Stoppered tubes were boiled for one hour at 100°C on a water bath and the reaction was terminated in ice bath. The reaction mixture was extracted with 10 ml toluene. The absorbance of toluene extract was read at 520 nm against toluene blank.

Results for the proline content estimated at various levels from seed to seedling stage and during callusing are shown diagrammatically in a bar chart (Fig. 1). Proline content in the seed was not in a metabolically active form as in the seed, it lies only in storage form. However, after germination (two weeks) the content was found more in cotyledons as compared to hypocotyls. So proline activity can be considered to be higher in cotyledons. Similarly, estimation for callus showed higher content when it was two weeks old. However, there was a noticeable and sudden increase in proline level in the third week, when the nutritional requirement started depleting by that time of callusing. Hence, enhancement of the proline activity in stress conditions was obvious. In the fourth week the proline content again decreased because of the immediate requirement of nutrition. After subculturing in the fresh medium, proline content increased till the callus on the medium remained green, however, gradual browning of the callus once again led to decrease in the content.

The reason for the increase and decrease in the proline content could be summarised as follows: As according to earlier reports (Jain *et al.*, 1991) here also we came to know that higher levels of

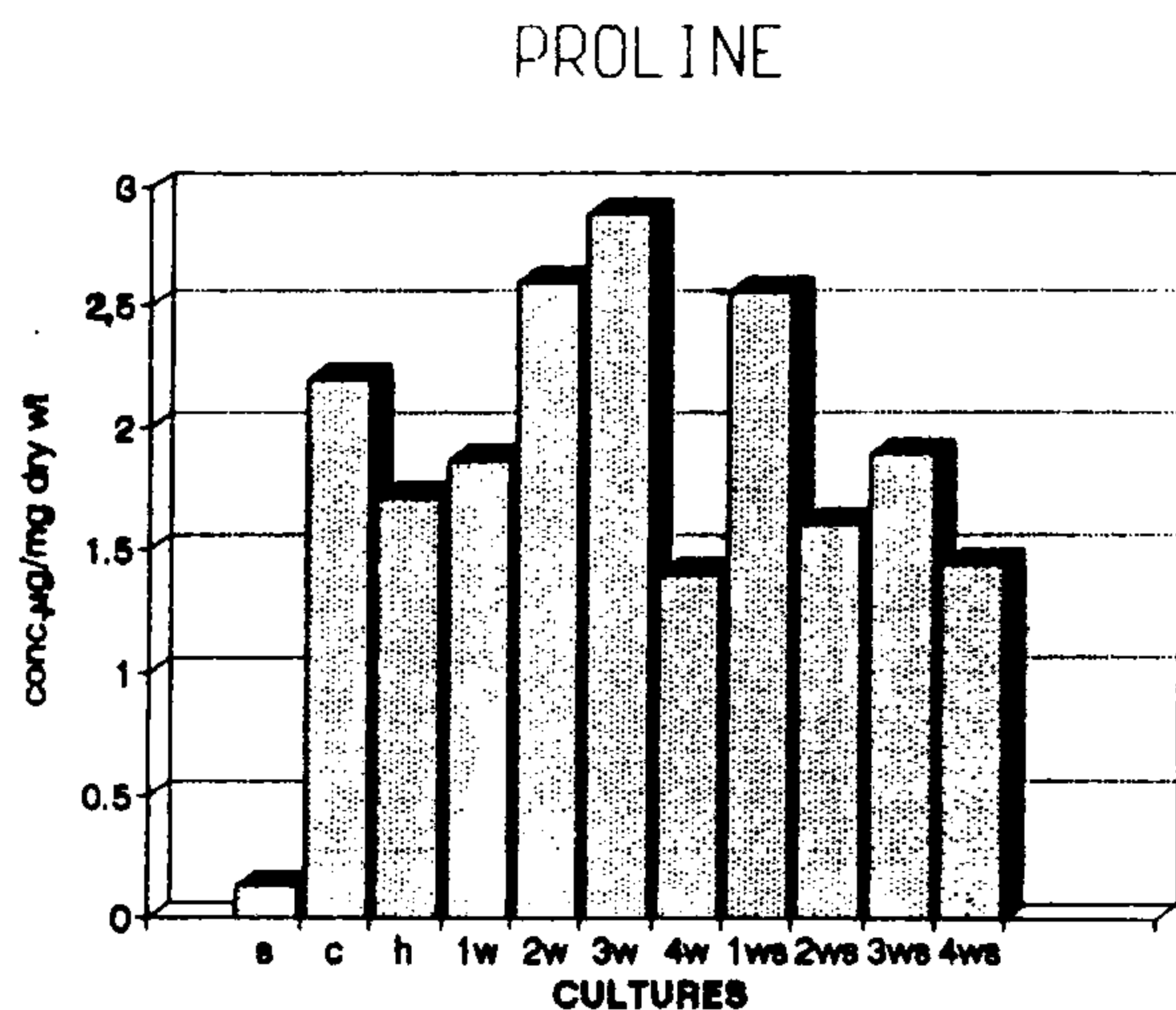


Figure 1. Histogram showing proline levels in seed/mg and callus of *Sesamum indicum*

proline can make cell proliferation and shoot regeneration feasible. The proline overproduction may result from a relaxed feedback inhibition of the regulatory step enzyme (Widholm, 1988), increased activity of the enzymes involved in the synthesis or inhibition of enzymes involved in degradation of proline (Kandpal *et al.*, 1981) or gene amplification (Wated *et al.*, 1983). Proline working as an osmoticum has been reported by several workers during water and salinity stress (Sudhakar *et al.*, 1987; Veeranjanyulu & Ranjitha, 1989; Corcuera *et al.*, 1989; Sreenivasulu & Veeranjanyulu, 1990). The physiological significance of accumulated proline during senescence is obscure but proline accumulation can be considered as an indicator of severity of senescence (Sreenivasulu & Veeranjanyulu, 1991). However, here when the nutrients of the medium provided to the callus started depleting, there was a sudden decrease in proline content.

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