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Interaction of simulated stress with calcium and exogenous amino acids in the accumulation of free proline content in *Phaseolus vulgaris* seedlings

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SUMMARY
Stress studies were conducted to check the effect of calcium on the accumulation of free proline. It was observed that stress treatment showed marked increase in the accumulation of proline (0.665 mg/g dw). Calcium treatment further leads to the significant accumulation of proline (0.828 mg/g dw) at PEG -4 level of stress. Application of exogenous amino acids like glycine, alanine, proline, lysine, glutamic acid and threonine were tested for the accumulation of this metabolite. Exogenous lysine and threonine caused more accumulation of proline. This accumulant ameliorate the effect of stress. Present study showed that calcium play an important role in increasing the level of proline so, during drought their accumulation in crops proves to be fruitful.

Key words : Calcium, Drought and Proline.

Drought is one of the main environmental limitations responsible for reduced growth and productivity. One of the critical cellular responses to water deficit is accumulation of osmolytes. Several fold increase in free amino acid pool in stressed plant is well documented Thakur and Rai (1982), Singh *et al*. (1985) and proline accumulation has also been correlated to drought resistance (Singh *et al.*, 1972; Raggi, 1994). Rajagopal and Sinha (1980) and Thakur and Rai (1985) showed that various amino acids applied to the root medium could maintain better water balance under osmotic stress conditions. Thus, it becomes clear that amino acids could play adaptive role under drought conditions and proline also ameliorate the effect of stress.

Exogenously supplied amino acids including proline, differentially reduce the permeability of *Vinca* petal membranes (Rai and Kumari, 1983). They also inhibit efflux of betacyanin from beet root disc. Hellebust (1980) proposed that the accumulation of osmotic solutes in tissue subjected to various environmental stresses might be triggered by certain membrane bound signal transducers like calcium. Shah *et al*. (1990) reported the influence of calcium ion on the proline content, which enhance the tolerance in salt stressed plant tissue. So, to check the involvement of calcium and exogenous amino acids in the alleviation of moisture stress present investigation was carried. Present paper reports the role of calcium and specific amino acids including proline in total free proline contributing towards the endogenous pool and which could provide drought tolerance, as these are osmoregulatory.

MATERIALS AND METHODS

Seeds of *Phaseolus vulgaris* cv. Kentucky wonder, procured from Directorate of Agriculture, Shimla, were surface sterilized (0.1 % $Hgcl_2$) and germinated in incubators with continuous fluorescent light (11 Wm^{-2}) at 25$\pm$ 2 ^{0}C and 90 -95 % RH and 12 – 14 day old seedlings were used for the experiment. The Solution of PEG-6000 was used to create osmotic stress. Solution of PEG were made as per calculation given by Wiggans and gardner(1958). Plants were initially treated with different levels of stress for 48 hrs (-2, -4, -6, -8 bars). Then treatment was prolonged for another 24 hrs along with test solutions in presence of PEG – 6000. The seedlings were placed in calcium nitrate (5.0 mg of calcium/20 ml) with or without amino acids (exogenous amino acids ie. 10^{-3} to 10^{-5} M were checked but 10^{-3} M was found to be effective) given to root for 24 hrs each treatment comprised of three seedlings with three replications. Data was stastically analysed by calculating standard error. Free proline was estimated by the method of Bates *et al*. (1973). Total amount of free proline was calculated in stressed plants and in stressed plants with different level of amino acids and calcium.

RESULTS AND DISCUSSION

It was observed that with increase in stress levels there was about three fold increase in proline accumulation. Calcium application to the stressed seedlings further increased proline accumulation. Marked promotion was observed at PEG-4 (Table 1).

Analyses of effect of individual amino acid on