6. Summary

Mungbean (*Vigna radiata* (L.) Wilczek) is one of the important grain-legume crop and a good source of dietary protein. In the present study, *in vitro* techniques were applied to standardize the regeneration protocols. This would have a tremendous application in improvement of this crop through selection and transformation. In the light of these, the present study was undertaken. The following are the important highlights of the present investigation.

6.1. Multiple shoot regeneration

(i). Multiple shoot regeneration from cotyledon explants of 12 hrs imbibed seeds were achieved.

(ii). Shoot tip, nodal explants from 7 day old *in vitro* raised seedlings on MS medium containing BAP have produced multiple shoots.

(iii). Cotyledonary node explants from 4 day old *in vitro* raised seedlings produced maximum number of shoots on MS medium containing BAP (1.0mg/l).

(iv). Maximum response of shoot multiplication was noticed with 3% sucrose.

(v). GA₃ was found suitable for optimum elongation of shoots than GA₃ + BAP combination.

(vi). Optimum rooting was achieved on MS half strength medium containing 1.0 mg/l IBA.
6.2. Callus induction and plantlet regeneration

In the present investigation, a protocol for induction of callus was standardized from various explants such as cotyledon (only the distal half), hypocotyl, epicotyl and leaf. In mungbean there are no previous reports on regeneration of plants from cotyledon and hypocotyl derived callus and the protocol was standardized to regenerate plantlets from the calli from cotyledon and hypocotyl.

(i) Among the explants (cotyledon, hypocotyl, epicotyl and leaf) tested, the cotyledon and hypocotyl explants responded well in the induction of maximum percentage of organogenic callus.

(ii) The nature and texture of the callus varied with the explant type and hormone type and concentration.

(iii) Among the various combinations and concentrations of phytohormones tested, 2, 4-D and NAA had produced friable calli in all the explants.

(iv) Among the auxins and cytokinins combinations tested, MS medium containing NAA (2.0mg/l) + BAP (2.0mg/l) was found to be essential for organogenic callus production.

(v) Better regeneration of shoots was achieved on MS medium fortified with NAA (0.5mg/l) + BAP (2.0mg/l) and further supplemented with casein hydrolysate (200mg/l) from cotyledon and hypocotyl derived callus.

(vi) MS medium with GA3 (1.0mg/l) was found best for shoot elongation.

(vii) MS half strength with IBA (1.0mg/l) induced good rhizogenesis.
6.3. Somatic embryogenesis

In the present study, protocol for somatic embryogenesis was standardized through suspension culture using immature cotyledon derived callus. Although the previous reports have documented about the somatic embryo induction, there is no report on plantlet regeneration from somatic embryos. In the present study a protocol has been standardized for somatic embryogenesis.

(i) NAA (5.0mg/l) had produced the maximum percentage of embryogenic callus induction from immature cotyledons.

(ii) The embryogenic callus when transferred to MS liquid medium containing 2,4-D (1.5mg/l) with L-proline (50mg/l) produced the maximum number of globular, heart and torpedo shaped embryos.

(iii) The torpedo shaped embryos when transferred to MS medium containing BAP + ABA (each 1.0mg/l) had enhanced the maturation of somatic embryos after torpedo shaped and produced a tiny plantlet with distinct shoot and root.

(iv) The tiny plantlets when transferred to half strength MS solid medium had enhanced further growth of the plant.

6.4. Selection of NaCl tolerant cell line

Mungbean being the salt sensitive plant, application of tissue culture technique to evolve salt tolerant mungbean plantlets would help to improve this crop. So in the present study, a protocol has been standardized to evolve NaCl tolerant cell line. The following are the key steps involved in the production of NaCl tolerant cell line in the present investigation.
(i) The inhibitory concentration of NaCl has been screened by exposing the fast growing callus from cotyledon explants to different concentrations (25mM – 300mM) of NaCl.

(ii) For the selection of NaCl tolerant cell line the one month old fresh calli of approximately 200 mg were transferred to MS medium containing 250mM NaCl.

(iii) When the calli were transferred to NaCl containing medium most of the calli died and only 10 % of culture showed a surviving pockets of cells, and they were named as selected tolerant callus.

(iv) Ionic accumulation studies showed more amount of Na⁺, Cl⁻ in the non selected callus than the selected callus The K⁺ and Ca²⁺ decreased with increase in NaCl concentrations in non selected callus whereas in the selected callus the decreasing trend was observed only at the higher concentrations of NaCl.

(v) The accumulation of proline was more in selected callus than in the non selected callus.

(vi) The leaf callus derived from the *in vitro* regenerated shoots of tolerant callus was transferred to MS medium containing 250mM NaCl showed normal growth without prior selection. This showed the retention of tolerance from the regenerated shoots.

The protocols standardized in the present study would have tremendous application in improvement of this important pulse crop by biotechnological means.