SUMMARY
What we call the beginning is often the end. And to make an end is to make a beginning. The end is where we start from.

T. S. Eliot (1888-1965)
Four Quartets “Little Gidding” (1942) pt. 5.
Summary

Until quite recently, Mendel’s laws and their direct consequences formed an adequate base on which to establish plant genetic improvement programmes. A sustained effort in research and innovation in all branches of agriculture and biotechnology is essential for the improvement of agricultural production. This effort should also aim at reducing the interval before the results of research are applied within the farming world.

Biotechnology has the potential to provide new technological approaches and to find solutions to a range of problems like pest management, biotic and abiotic stresses and increase in productivity to meet the requirement of the growing population. It can shorten the period of normally required very many years of conventional breeding to evolve newer strains. The ability to revolutionize seed production and genetically enhance food and fibre crops through the manipulation of their isolated, cultured cells holds great promise for those crops that are difficult to improve by conventional means (Litz & Gray 1995).

The rapid propagation of plant material, the production of pathogen-free plants and the conservation and exchange of germplasm are some of the examples of possible achievements in plant biotechnologies. It is not utopian to envisage the creation of new cultivars with higher nutritive qualities and resistance to pathogens and parasites, that could grow in harsh environments and would not need nitrogen fertilizers because they could fix atmospheric nitrogen (Linton 1983). Finally growers and consumers must be convinced that agricultural products obtained as a result of transfer of alien genes are both safe and necessary (Litz & Gray 1995).

Breeders have used a range of conventional techniques to improve the chickpea crop and produce varieties for increased yield and/or resistance to some pests. However, there are several constraints, such as the Fusarium wilt and pod-borer, for which there is no known source of resistance or only low-level resistance in the cultivated germplasm. Biotechnological techniques provide the opportunity to solve some of these problems to improve the existing resistance and to explore alternatives to conventional breeding which is time consuming.

The wild species of Cicer are a valuable source of resistance to most major diseases and pests, but the cross-incompatibility barrier has deterred the production of hybrids with the useful traits. Transfer of these agriculturally important genes from
these wild species to *C. arietinum* by asexual means would be advantageous, because a gene is introduced into a related nuclear and cytoplasmic background of the cultivated species than is the case with sexual hybridization.

For this, the development of efficient *in vitro* regeneration protocols via somatic embryogenesis and/or organogenesis is a major pre-requisite. Regeneration protocols via somatic embryogenesis were existent when the present endeavour was initiated, but the established regeneration protocols were limited to immature explants, the availability of which, was dependent on the seasons. Another major disadvantage of using immature explants is that they are responsive only at a specific developmental stage. The present work was therefore initiated with the aim of developing regeneration and transformation protocols from explants which are available throughout the year.

With this main objective, regeneration protocol was standardized via somatic embryogenesis from mature embryo axes explants of chickpea. Somatic embryogenesis and plant regeneration has been achieved from mature embryo axes explants. Different concentrations and combinations of auxins were evaluated for their potential to induce somatic embryos from mature embryo axes of five cultivars of chickpea. Different basal media, gelling agents, organic supplements, pH were scored to optimize the conditions for high frequency somatic embryogenesis.

Somatic embryogenesis was achieved from mature embryo axes of cultivar PG12 when cultured on MS basal medium supplemented with 3.0% sucrose, 0.6% agar and 3.0 mg/l 2,4,5-T (solid T3 medium) after 12 weeks of culture in Petri dishes. Further development of these embryos warranted their subculture onto the same medium in test tubes. Cotyledonary-stage somatic embryos were obtained with a frequency of 2.3 % after 6 weeks of culture. To reduce the time taken for the induction and development of somatic embryos from mature embryo axes on agar media, the embryo axes were cultured in MS basal medium supplemented with 3.0% sucrose and 3.0 mg/l 2,4,5-T lacking a gelling agent but supported on Whatman No. 1 filter paper bridges (liquid T3 medium). Cotyledonary-stage somatic embryos arose directly from the cotyledonary node and shoot tip region of the explant within 4 weeks without the need for a subculture against a two step procedure of obtaining cotyledonary-stage somatic embryos on agar media within 18 weeks. Histological sections confirmed the direct origin of embryos from the explant without an intervening callus phase.
Maturation and conversion of cotyledonary-stage somatic embryos was a two step protocol. Incubation of these embryos in half-strength MS basal medium supplemented with 0.1 mg/l ABA for 10 days was mandatory for the maturation of these embryos in our system irrespective of their origin on agar or on liquid media. Conversion of these embryos was on half-strength MS medium supplemented with 1.0 mg/l zeatin. The regenerated plants were morphologically normal, grew to maturity and set viable seeds.

The standardized regeneration protocol could be successfully exploited for genetic transformation experiments because 1) the origin of embryos from the explant is without an intervening callus phase and leads to the production of genetically uniform plants and 2) higher number of regenerants could be obtained from a single explant.

This regeneration protocol represents the first ever report of direct somatic embryogenesis from mature embryo axis explants. This work has been published in the journal “**Plant Science**” (see Author’s Publications Section).

Alongwith normal somatic embryos, embryos with aberrant morphologies were also observed. These embryos failed to convert normally to give rise to plants thereby resulting in low conversion rates. The reasons for the non-conversion was probed histologically. The lack of meristematic cells at the shoot apical meristem of the aberrant somatic embryos was implicated as the reason for their non-conversion. These studies could be useful for selecting embryos of desirable types for use in genetic transformation studies and in synthetic seed technology. The results obtained were reported in the journal “**In Vitro Cellular and Developmental Biology**” (see Author’s Publications Section).

The different developmental stages of both zygotic and somatic embryos has been studied with the help of scanning electron microscope. Studies reveal that they follow similar pattern of development. Since somatic and zygotic embryos follow similar pattern of development, and zygotic embryos are difficult to isolate at early stages of development, somatic embryos could be used to carry out gene expression studies. The technique of isolation of zygotic embryos at early stages of development could be extrapolated to the isolation of hybrid embryos in interspecific crosses and their in vitro culture. Aberrant somatic embryos show varied phenotypes and a few of them lack the shoot apical meristem. Normal as well as aberrant somatic embryos
development was also studied using scanning electron microscope. (Paper communicated).

Genetic transformation studies using Agrobacterium was initiated using tobacco as a model system. Viable transformants were obtained and their transgenic nature was confirmed by GUS staining. The transgenic plants were grown to maturity and the collected seeds had the inheritance of the transgenes in the ratio 3:1. This protocol was then extended to chickpea. The ability of mature embryo axis explants to give rise to multiple shoots from the shoot tip and cotyledonary node region on BAP containing medium was exploited for this purpose. Putative transformants were confirmed at different developmental stages with GUS staining. Southern analysis also confirmed their transgenic nature. However, the transformation frequency is very low (0.196-1.458 %) and needs further improvement before it can be exploited for the transfer of desirable genes of interest (Paper communicated).

The present study describes the development of a somatic embryogenesis system from mature embryo axes explant which could be used in synthetic seed technology and genetic transformation studies. The developed genetic transformation protocol after refinement, could be exploited for the transfer of genes of desirable traits for the improvement of chickpea crop.