In conclusions, from our results presented above we are prompted to speculate following findings:

- A complete protocol for sterilization of seeds with maximum efficiency was developed. Complete seed sterilization of C. intybus was obtained on HgCl₂ (0.1) for 10 min with 85% sterilization rate and 80% explant survival rate. Seed germination and complete seedling formation was noticed on MSx1/2 basal medium.

- Two different growth media were tested viz., MS (1962), Gamborg (1968). MS medium was found most suitable than B5 media. Shoot tips were more responsive than nodal explants.

- In order to trigger morphogenetic response in terms of dedifferentiation’s and proliferation, the presence of growth regulators was found to be mandatory. Use of auxins (IBA, IAA and NAA) alone did not show any multiple shoot regeneration except slight elongation of shoot tips and nodal explants.

- IAA/TDZ combination was found to be the best treatment of all the treatments for callus induction as well for callus biomass and among all the combination of auxins and cytokinins used, less callus biomass was obtained on 2,4-D/IAA, 2,4-
D/NAA, IBA/KN and IBA/KN combinations. However, BAP/IAA, BAP/NAA, BAP/IBA, Kn/IAA and Kn/NAA produced moderate amount of callus biomass.

✓ Both types of cytokinins (Kn and BAP) when used alone, were able to produce multiple shoots although BAP was more effective than Kn in terms of both the frequency of explants producing shoots and the mean number of shoots produced per explant.

✓ In respect of organogenetic potential for shoot induction TDZ/IAA combination was found to be more responsive. Significant increase was achieved in terms of both the percentage of explants forming shoots and the mean number of shoots produced per explants on. In an overall comparison, combinations of MS + TDZ (1µM) + IAA (5.5µM) using shoot tip as explants was more effective than all other combination of auxins and cytokinins used. The TDZ showed arrested elongation at higher doses, and produced stunted multiple shoot induction with lot of callus at the base.

✓ Kn (7.5µM) + IAA (5µM) was the second most suitable combination of phytohormones with 100% response with 54±2.26 mean number of shoots.

✓ Only non differentiating callus was produced by 2,4-D from various explants. 2,4-D/Kn and 2,4-D/BAP combination was least effective in terms of percentage response, callus formation and mean number of shoots.

✓ Direct shoot regeneration was achieved from nodal explants when cultured on the MS and B5 media augmented with different concentrations and combinations of Kn/IAA. However, indirect regeneration of shoots was noticed on increasing the concentration of phytohormones.

✓ IAA was more found more effective than the NAA and IBA for shoot regeneration when combined with the Kn, BAP or TDZ
Root induction was noticed on the MS medium without and with different concentrations of phytohormones (IBA, IAA and NAA) but IAA was noticed to best for rhizogenesis of microshoots. Direct rooting was observed in cultures supplemented with IAA and IBA. While NAA resulted in indirect rooting. The order of effectiveness of root induction was IAA>IBA>NAA.

Carbohydrates had an important significance in in vitro morphogenesis. The order of carbon sources which effectively induced regeneration is fructose (3 %) < maltose (3 %) < glucose (3 %) < sucrose (4 %) and glucose (3 %) followed by sucrose (3 %) was found to be more responsive in root induction process.

In vitro flowering response of C. intybus from shoot tip explants showed IBA (0.05μM) was most favorable concentration.

Use of autoclaved sand: soil: peat: vermiculite (1:1:1:1) mixture proved the best acclimatizing medium in transplanted plantlets.

The EMS has profound effect on seed germination. The percentage response for seed germination decreased at higher concentration of EMS.

Lower doses of EMS had stimulatory effect on cultures. The percentage of callusing was found to be maximum on lower EMS concentration. A marked difference in morphological appearance of callus was noticed with increasing EMS concentrations. The lower concentration of EMS also favored regeneration potential and root development as compared to control. The shoot and root length showed a sharp decrease with increase in EMS concentration. The results recommend low doses (0.2-0.8%) as stimulatory for increasing plant growth, development and metabolism as dose rates beyond it were detrimental for plant vigour and development.

The chlorophyll and carotenoid concentration determined in different tissues of C.intybus showed irregular distribution. Pigment content in naturally grown plants
was found higher compared to in vitro raised plantlets. At lower doses, EMS enhanced the production of pigments.

✓ In present investigation, the in vitro raised leaves contain maximum amount of soluble sugar content than leaves of field grown plants. Lower doses of EMS had stimulatory effect and favored high soluble sugar content in EMS treated in vitro raised leaves compared to EMS treated callus, untreated in vitro raised tissues and leaves of field grown plants.

✓ The proline content was found low in naturally grown plants. It showed comparatively different trend as its concentration increased with increasing EMS concentration. The maximum content of proline was found in EMS treated in vitro raised plantlets and callus at 1.6% v/v.

✓ This work presents for the first time, response in C.intybus to range of EMS treatments on the enzyme activities. In the study, Glutathione reductase (GR) activity, Ascorbate peroxidase (APX) activity and Superoxide dismutase (SOD) activity were performed to evaluate the oxidative stress in EMS treated in vitro raised tissues and leaves of field grown plants. The more research work needs to be carried out to study the mechanism for expression of genes under stress and to describe the potential of C.intybus L. as chemico-protectant for humans keeping in view genotoxicity of this plant.

✓ The variations in protein content were observed not only at different morphogenetic stages but also at different EMS treatments. The EMS treated regenerated shoots had sufficient amount of soluble protein content than regenerative callus and leaves of field grown plants. Intensities of expression of proteins were quite distinct and nearly all bands were prominent in all treatments as well in control used i.e. field grown leaves and in vitro raised shoot using SDS-PAGE. The protein expression in leaves of field grown plant material and in vitro raised shoot without treatment was less as compared with protein expression of all EMS treated tissues.
Conclusions

✓ A gradient reversed-phase high-performance liquid chromatography (HPLC) method using a C30 column was developed for quantification of esculin. Low dosage of EMS resulted in increment in production of secondary metabolites.

✓ Elicitation of secondary metabolites has applications in over-production of desired compounds, which is an area of commercial importance especially for high value low volume products. To this category belongs the production of anticancerous compounds like esculin which are of great medicinal value. Since the plant is exposed to a number of external stimuli, studies on signal transduction in plants for various physiological responses would receive focal attention to understand its adaptation.