CHAPTER FIVE
SUMMARY

The present researches were carried out at the Agricultural Botany Department, Biotechnology and the Tissue Culture Laboratory, Faculty of Agriculture, Saba Bash, Alexandria University and Plant pathology Department (Genetics branch), Faculty of Agriculture, Damanhour University, Egypt. The study were conducted during 2010 up to 2014 to detect the most suitable concentration of growth regulators for callus induction from immature embryo explants of the two inbreed maize lines namely, SD7 & SD34 and callus for transformation, of COMT gene by particle bombardment to callus and finally, screening the genetic transformation the gene.

Biofuels are commonly defined as fuels derived from renewable biological products and are often regarded as an attractive, "green" alternative to fossil sources of energy due to their potential contribution to lowering carbon dioxide emissions

Biofuels crops have been identified and are at various levels of domestication and cultivar selection, while genetic and genomic resources for these species, including draft genome sequences and transformation protocols, are currently being developed. Major breakthroughs on the understanding of lipid metabolism and plant cell wall biosynthesis and structure are still needed to overcome low oil yields and the recalcitrance of lignocellulose, respectively, for efficient and cost-competitive conversion to biodiesel and other liquid fuels. However, despite its abundance and potential environmental benefits, the efficient and sustainable use of plant biomass for energy purposes remains a challenging endeavor, requiring major investments in science and technology

Maize biologists have long awaited the development of an effective and efficient transformation method. Few plants share maize's importance to both agronomy and basic biology, and the ability to create transgenic maize easily and rapidly would be a tremendous advantage for both those trying to improve the agronomic characteristics of maize and those hoping to use transformation as a tool to explore fundamental questions about maize genetics and development, therefore, the main objective of the present research is to:

Evaluate the most suitable concentration of growth regulators for callus induction and plant regeneration from maize embryo,

Regenerate and high throughput callus for transformation, Transform of COMT- antisense gene by particle bombardment to callus, Screening the genetic transformation of this gene on the end products.

The results of the present study indicated that two inbreed line of Zea mays L; namely, SD7, SD34. Seeds of the lines were grown in experimental field (Faculty of Agriculture Saba Basha, farm research station); ears were harvested between 16 and 20 days after pollination then transferred to the laboratory. In this experiment immature embryos were used. Size of immature embryos was 1-4 mm. The ears were surface sterilized for 5 min in 70% ethanol and then for 20 min in 10% Clorox ,followed by three times rinse in sterile distilled water in order to remove excess of the chemical.

The immature embryos of SD7 and SD34 were used those length was 1.6-2.0 mm. Immature embryo was selected from ears of pure line. The embryos were selected and cultivated in two different culture medium (D and N6E). After three times of cultivation two types of callus were produced.

Every 21 d when cultured in vitro can originate Type I or Type II embryo genic calluses, Type II was selected and transferred to fresh medium The results indicated clearly that, Type I callus is formed by hard, compact and yellowish tissue, usually unable to regenerate plants, on the other hand Type II callus is soft, friable, highly embryo genic and able to regenerate a higher number of plants.

Data showed the overall of alive and dead callus for the selected varieties SD7 and 34 in respect. The highest values for alive callus were 260 and 180 in SD7 and 34, respectively.

While the lowest values were 137 and 120. The overall of alive callus was 190 and 148 and dead callus was 76 and 2.17, in respect.

Although varieties were inbred lines, SD34 was more efficient than SD7 and the percentage of dead callus was 2% compared with the other variety was 40%.

Every 40 days for three times we calculated the dead and alive callus as shown in Table xx. Almost 40% after 40 days of the callus were dead in SD7 and on the other hand in SD34 were nearly 80% of the callus were alive. The maximum number of alive callus were 28in SD7 forwared by 23 for SD34.

For the second read (80 days) results in Table xx showed the alive callus increasing while the dead callus were deceasing.

For example the highest number was± 11.44in SD34 compared with± 14.44in SD34

Finally, after 120 days of the callus cultured the results achieved the same trend, although both dead and alive callus increasing. The overall of alive callus after the 120 days were ±10.44 in SD34 compared with± 13.99 in SD7.