CHAPTER FIVE
SUMMARY

Maize is one of the world's three most widely cultivated crops (along with wheat and rice) and is arguably the most economically important cereal crop on a worldwide basis. That status is only likely to become more apparent in the next decade. Demand for maize is projected to increase by 50% to over 800 million tons per year by the year 2020 and will surpass both rice and wheat in global demand.

The present experiments were carried out at the Agricultural Botany Department, Biotechnology and the Tissue Culture Laboratory, Faculty of Agriculture, Saba Basha, Alexandria University, Egypt and Plant pathology Department (Genetics branch), Faculty of Agriculture, Damanhour University, Egypt. These studies were conducted during 2010 up to 2014 to evaluate the most suitable concentration of growth regulators for callus induction from mature and immature embryo explants of the maize hybrids, regenerate and high throughput callus for transformation, of *Anthranilate synthase* genes by particle bombardment to callus and finally, screening the genetic transformation of these genes on the end products.

The main objective of the present research is to:

1- Evaluate the most suitable concentration of growth regulators for callus induction from mature and immature embryo explants of the maize hybrids,

2- Regenerate and high throughput callus for transformation,

3- Transform of *Anthranilate synthase* genes by particle bombardment to callus,

4- Screening the genetic transformation of this gene.

Tow Egyptian single cross *Zea mays* L hybrids; namely, SC168, SC10 were used in the current study. Seeds of the hybrids 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 we used mature embryos for SC 168 and immature embryos for both hybrids. 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 40% SAVO. This was followed by three times rinse in sterile distilled water.

The immature embryos of single cross 168 and SC10 were when, thier length was 1.6-2.0 mm. The embryos were selected and cultivated in D culture medium then transferred to N6E culture medium, after three

times of cultivation (Figure1). The present results produced three types of calli, embryo genic, non-embryo genic and organogenic calli.

Every 21 days Type II was selected and transferred to fresh medium that when cultured *in vitro* can originate both Types embryogenic calluses. The results indicated clearly that, Type I callus have different morphological characteristics such as formed by hard, compact and yellowish tissue, these morphological characteristics effect of the viability to regenerate news plants, on the other hand the results showed that, Type II callus has different morphological characteristics such as soft, friable, highly embryogenic and able to regenerate a higher number of plants than Type I callus. Our results proved that only Type II embryogenic callus can regenerate.

Results showed that hybrid 168 is more efficient than SC10 in callus regeneration and response for sub-culture. The higher alive calli were (88.17 and 41.0) in 168 and SC10 hybrids, in respect. The number of dead calluses in variety SC10 was (26.67) compared with variety 168 was (7.17). The main conclusions from these results that variety 168 could be used for transformation more efficiency then SC10 based on their callus morphological characteristics.

Data showed the overall of alive and dead callus for the selected hybrids SC10 and 168 in respect. The highest values for alive callus were 147 and 51 in SC168 and 10, respectively. While the lowest values were 55 and 23. The overall of alive callus was 88.17 and 41.0 and dead callus was 26.67 and 7.17, in respect. SC168 was more efficient than SC10 and the percentage of dead callus was 8% compared with the other variety was 65%

The results showed that, callus was initiated on N6 medium supplemented also; results, induction of primary callus ranged between 0 and 93%. Generally, three types of calli were formed: embryo genic, non-embryo genic and organogenic callus. The embryo genic callus was formed within two weeks of culture in callus maintenance medium. Induction of embryo genic callus ranged between 0 and 5%. Somatic embryos were matured on N6 medium supplemented with 6% sucrose and 1 mg.dm-3 NAA.

Somatic embryogenesis is the process by which somatic cells, under induction conditions, generate embryogenic cells, which go through a series of morphological and biochemical changes that result in the formation of a somatic embryo. These characteristics have designated somatic embryogenesis into a model system for the study of morphological, physiological, molecular and biochemical events occurring during the onset and development of embryogenesis in higher plants.

Almost 50% after 40 days of the callus were died in SC10 and on the other hand in SC168 were nearly 90% of the callus were alive. The maximum number of alive callus were 28 in SC10 forwared by 26 for SC168.

For the second read (80 days) results in showed that the alive callus was increasing while the dead callus was deceasing. For example the highest number was 18.71 in SC168 compared with 11.65 in SC10. 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 15.01 in SC168 compared with 9.68 in SC10.

The high- expresser lines 9 and 12 contained > 12-16 times, line 1 show the Trp. level as wild type. In this report, we demonstrate that a feedback-insensitive ASA2 gene can be used as a selectable marker for the production of transgenic maize plants. Since maize expressing ASA2 resistant to 6MT, thus ASA2 may be an effective selectable marker gene for use with many different species.