Establishment of model system for efficient transformation of the egyption taro colocasia antiouorum by using gus reporter gene

Taha Mohamed Mohamed Abdel-rahman?

Taro [Colocasia esculenta t] is one of the most important tuber food crops in the tropics and sub tropics region notably in Egypt and the main source of income for many farmers comes from taro cultivation. In spite of its importance as a food and medicinal plant in the developing countries, taro still neglectable for many decades. Egyptian taro propagates vegetatively and unable to produce seeds, hence, the conventional breeding is unfit to improve it. Few attempts are being made to develop In vitro breeding approaches (such as gene insertion technologies) to overcome breeding barriers and to hasten the genetic improvement of food taro. Since taro is a monocotyledon, particle bombardment is most appropriate for transformation of taroThis study was conducted to:1-Establish a system for plantlet regeneration from callus of the Egyptian taro.2-Establish a transformation system by using Biolistic method.3-Confirm the occurrence of transformation in the resulted plants by using PCR and Histochemical GUS assay.This investigation was carried out through the period from 1999 to 2003 at the Plant Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), Menofiya University, Sadat city and Molecular Cytogenetic Laboratory (Prof. Dr. Said Hasanean Lab.) Faculty of Agriculture, Ain Shams University.This study was divided into two main parts, part I- Tissue culture techniques and part II- transformation system.Part I- Tissue culture techniquesMature corms of Baladi cultivar, of Egyptian taro were obtained from open field in Shanawan, a village in Menofiya governorate. In vitro stock plantlets of taro [Colocasia esculenta (L.) Schott] were obtained by culturing shoot tips and axillary buds (after surface-sterilization for 20 min in 2% (w/v) Na0C1 with 2 DROPs of Tween 20) on multiplication medium. The effect of many factors on shoot multiplication, callus formation and plantlet regeneration was studied.This part of study was carried out to select the best conditions (medium component and type of explant) for shoot multiplication, callus formation and plantlets regeneration, as a very important prerequisite for genetic transformation. So, this part included seven experiments to investigate:1-The effect of different number of In vitro shoots per cluster on shoot multiplication.2-The effect of decapitation of In vitro cormels on shoot multiplication.3-The effect of using half baby In vitro cormels instead of complete In vitro cormels.4-Callus induction from different type of explant excised from mature corm.5-Effect of different MS media with different explants on callus formation6-Effect of MS salt strength and taro extract on callus formation from leaf petioles sections7-Effect of different MS media with different explants on plantlet regeneration from callus.The obtained results showed thatI -Four type of explant (1, 2, 3 and 4 plantlets cluster) were used and explants were cultured on multiplication medium. After 4 weeks 1-3, 3-5, 5-8 and more than 8 shoots were obtained from 1, 2, 3 and 4 plantlets cluster, respectively. The largest number of shoots was obtained from four plants cluster and single plant gave the least mean. No significant difference between cluster which contain three shoots and which contain four shoots. So, using shoots cluster contain at least three plantlets gave the best results2-The effect of decapitation on shoot multiplication was highly significant and decapitated in vitro cormel produced 5-9 shoots while undecapitated in vitro cormel produced 1-3 shoots only. After second decapitation the cormel produced more than 30 shoots. Therefore, using decapitated in vitro cormel is better than using

undecapitated in vitro cormel.3-The effect of using half in vitro cormel on shoot multiplication was high significant. So that, using half cormel will increase the number of resulted shoots compared to using complete cormel.4-All explants (meristem tip, corm segments, fleshy leaves and axillary buds) formed callus but they gave different type of callus, meristem tip, axillary buds and the tissue near meristem or fleshy leaves around meristem tip formed white to yellowish friable calli. On the other hand, the corm segments formed yellow granular and hard masses of amber, semispherical tissue . 100% of meristem tip 66.7% of fleshy leaves, 64 % of axillary buds and 4.8- 2.4% of corm segments under meristem tip formed callus.5-The effect of media and explants on callus formation were high significant. All types of explants (in vitro leaf petioles sections, corm segments, in vitro root plantlet sections andin vitro etiolated stem sections) formed callus. The highest callus fresh weight was obtained from leaf petioles with medium 2 containing (half strength MS containing 100 ml /1 Taro extract + 5 mg/1 NAA + 5 mg/I BA + 30g/1 sucrose) and the least mean of callus fresh weight was obtained from root plantlets sections on medium 4 containing (MS + 2 mg/I NAA + 2 mg/1 2i P + 30g/l sucrose). Axillary buds and corm segments gave the best response with medium 1 containing (full strength MS + 2 mg/I NAA + 3 mg/1 2,4 D + 30g/1 sucrose). Root plantlet sections gave the best response with medium 2 and medium 3 containing (Half strength MS +25 m1/1 Taro extract + 2 mg/1 2,4 D + 200 mg/1 glutamine + 30g/1 sucrose) was the best medium for induction of callus from shoot tip. Data also show that etiolated stem gave the best response on medium 2. Moreover, calli from leaf petiole sections, etiolated stem sections and shoot tip gave friable white to pale yellow callus. On the other hand the corm segments formed yellow granular and hard calli. Therefore, leaf petiole is considered the best explant for callus induction by using the medium which consisting of (half strength MS containing 100 ml /1 Taro extract + 5 mg/I NAA + 5 mg/I BA + 30g/1 sucrose).6-There are highly significant difference between callus fresh weights by using different concentration of taro extract. On the other hand, there was no significant difference between callus fresh weight by using different MS strength. The concentration of 100 m1/1 TE with half MS salt strength recorded the highest callus fresh, MS full strength with 25 ml TE gave the least weight of callus. So that, the callus induction medium from leaf petiole should have from 75-100 ml /1 taro extract.7-The effect of media and explants on plantlets regeneration from callus were highly significant. There was no significant difference between means of number ofregenerated plantlets on medium 2 containing (Half MS + 25 m1/1 TE Taro Extract) and medium 1 containing (Half MS + 25 rn1/1 TE taro extract +100 m1/1 coconut water). In spite of medium 1 containing (Half MS + 25 m1/1 TE taro extract +100 m1/1 coconut water), it gave the highest number of regenerated plantlets, elongation of shoots were limited and if regenerated plantlets transferred to medium 2 elongation will exceed. Callus of leaf petioles sections gave the highest number of regenerated plants. Etiolated stem callus came in the second rate Part (II): Establishment of transformation system.Transformed plantlets are produced by introducing foreign genes into either single cells (callus) or intact tissue (leaf petiole sections) using biolistic delivery system. Several variables [explants type, target distance (between the stopping screen and the target tissue) and pressure] were tested to determine the optimum conditions for transient expression in taro. Transient expression of the GUS gene with X-Gluc assay is used as the simplest indicator of success at particle delivery and putative expression of transferred genes. Also, transformation was confirmed by PCR technique.This part of study was carried out to select the best conditions (pressure, target distance and type of explant) for transformation of the Egyptian taro. So, this part included three experiments to investigate:1-Effect of bombardment condition (pressure and target distance) on Gus-expressing blue spots on callus and leaf petioles2-Effect of culture duration after chopping the callus.3-Confirm the occurrence of transformation by histochemical assay and PCR technique.The obtained results showed that1-The highest number of blue spots were obtained when callus bombarded at pressure 1100 psi. and at 9 cm target distance. Pressure 1100 psi. and distance 9 cm gave the highest number of blue spots with callus and leaf petioles, twelve cm target distance at pressure 1100 psi. gave the least average number of blue spots on callus2-The effect of duration after chopping of callus was high significant. Average number of blue spots was highest at 5 days after chopping.3-PCR detection:PCR was utilized in this investigation for the rapid screening of GUS gene explants. For screening, total genomic DNA was isolated from explants of two transformants. A same band (about 1700 by was

founded in the transformants and positive control. DNA of non-transformed explants was used as a negative control and DNA of pAB6 was used as a positive control.S