REGENERATION AND TRANSFORMATION SYSTEM IN EGYPTIAN SWEET POTATO (*Ipomoea batatas* Lam.) CULTIVARS

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Sweet potato, (*Ipomoea batatas* L.) Lam.] is a dicotyledonous plant belonging to the family convolvulaceae. It is an important tuber crop grown in over more than 166 countries of the tropics, sub-tropics and warm temperate regions of the world (Mohanty *et al*., 2016). It ranks as the world's seventh most important crop with a total production of 103 million tons (FAOSTAT, 2015). Sweet potato is regarded as good for human health because of its beneficial protein composition as well as high content of antioxidants (Tumwegamire *et al*., 2011). Nowadays, sweet potato is preferred over other vegetables due to its multifaceted medicinal properties. The medicinal properties of sweet potato include anti-cancer, anti-diabetic, anti-inflammatory, anti-oxidant, anti-bacterial, anti-fungal, anti-viral, anti-ulcer, hepatoprotective, wound healing, and immunomodulatory (Parle *et al*., 2015). Sweet potato is hexaploid (6x=90), which makes it difficult to form seeds (Jin *et al*., 2015).

The potential benefits of using advanced agricultural biotechnology in Sweet potato genetic improvement have not yet been realized in Egypt, mainly because the successful utilization of plant biotechnology for plant improvement requires the development of an efficient shoot regeneration system from cultured cells or tissues. Thus, the lack of efficient system for regeneration has been a bottleneck for the application of biotechnology in sweet potato.

Many of the advances in plant biotechnology have been based on transformation; the ability to introduce DNA genes into plant cells to recover whole transgenic plants with high yield quality and quantity as well as resistant to biotic and abiotic stresses. Attempts over the last few years to produce transformed sweet potato plants utilized different gene transfer systems such as transformation by electroporation (Lawton *et al*., 2000), particle gun bombardment (Okada and Saito, 2008) or *A. rhizogenes* (Otani *et al*., 1993). Since the *Agrobacterium* mediated transformation system does not involve sophisticated equipment and frequently produces cleaner events (intact integrations and single copy) than other methods (Hansen *et al*., 1997), it remains to be the more favorable approach.

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MATERIALS AND METHODS

Plant material and explants type

Two Egyptian sweet potato cultivars (Abees and Mabrouka) were obtained from stock virus free germplasm bank in AGERI. The preliminary and the main experiments were conducted to establish an efficient regeneration and transformation system for sweet potato (*Ipomoea batatas* (L.) Lam) cultivars.

Agrobacterium via plasmid

Agrobacterium tumefaciens strain LBA4404 that harboring the plasmid pISV2678 was kindly provided by Dr. P. Ratet, Institute des Sciences Vegetales (ISV), Centre National de la Recherche Scientifique (CNRS), Gif-Sur-Yvette, France.

In vitro micropropagation of sweet potato

Sweet potato (*Ipomoea batatas* (L.) Lam) Abees and Mabruka cultivars were micropropagated *in vitro* using nodal cuttings technique as described by (Roca *et al.*, 1978). MS salts medium (Murashige and Skoog, 1962) was used as a basal medium supplemented with a 3% sucrose, a 0.4 mg/l of thiamin-HCl, a 2 mg/l of calcium pantothenate, a 1 ml of silver thiosufate solution STS (0.1 M sodium thiosulfate and 0.1 M silver nitrate, the ratio between silver and thiosalfate was 1:4), a 100 mg/l of myo-insoitol and a 1 mg/l of Giberellic acid (GA_3). The pH of the medium was adjusted to 5.6-5.7 by a 1 M of NaOH and HCl. A 2.2 mg/l of phytagel (Sigma product No. 46H0281) was added and the medium was cooked in a microwave for 6 min. The medium was poured into jars and autoclaved at 121°C (15 psi) for 20 min.

Callus initiation and shoot formation

Induced three weeks-old calli were subcultured into MS medium (MS salts and vitamins, a 30 g/l of sucrose and a 2.2 g/l of phytagel) supplemented with different growth regulators *i.e.* benzyl adenine (1, 3, 5 and 10 mg/l), kinetin (1, 3, 5 and 10 mg/l), and silver nitrate (2, 4, 8 and 12 mg/l) to enhance shoot formation or free from growth regulators as a control. The combination with naphthalene acetic acid and benzyl adenine was also used (a 0.01 mg /l of NAA + a 1 mg/l of BA) (Sivparsad and Gubba, 2012). Another concentrations from growth regulators were used such as MS basal medium for two weeks in dark then cultured on (MS + a 10 mg/l of BA) (El-Far *et al.*, 2009), [MS + a 0.5 mg/l of Indole-3-acetic acid (IAA)] ,(MS + a 1.0 mg/l of NAA + a 1.0 mg/l of paclobutazol (PBZ) (González *et al.*, 2008), (MS + 0.5 mg/l of 2,4-dichlorophenoxyacetic acid + a 1.0 mg/l of kinetine) (Qing and Guo, 1999) and (MS + a 1.0 mg/l of NAA + a 10 mg/l of BA). Subcultured callus were kept in the growth chamber under the same light and environmental conditions as previously stated for three weeks and subcultured once more for more three weeks. The clusters were incubated at 25 ± 2°C under a photoperiod of 16 h/8 h (light/dark) cycle and cool white fluorescent light (300

foot-candle) for 60 days. The number of regenerated shoots in each callus tissue was calculated and analyzed.

Rooting stage

When shoots reached 2-3 cm in length (after 70 days from culture), they were excised from shoot-clusters and placed into jars containing MS basal salt mixture supplemented with a 30 g/l of sucrose and a 2.2 g/l of Phytagel. The pH was adjusted to 5.6-5.7, then autoclaved at 121°C for 20 min. The cultures were incubated for 4-6 weeks at 25±2°C with 16/8 hr photoperiod.

Agrobacterium tumefaciens culture

A single colony from the *Agrobacterium* strain containing the pISV267 plasmid was grown in a 5 ml of LB liquid medium containing a 4 mg/l of bialaphos (glufosinate ammonium). The *Agrobacterium* cultures were grown over night at 28°C in shaking incubator at 200 rpm. At the next day a 300 µl from overnight bacterial cells was transferred to a 30 ml of LB liquid medium and incubated at 28°C for 4 hours at 200 rpm until OD reached 0.1, 0.3, 0.5, 0.7 and 1.0 at wave length of 600 nm.

Transformation of sweet potato shoot tip

In vitro shoot tip of plantlets (5-6 mm in diameter) were selected and collected in sterile Petri dishes under aseptic conditions. The shoot tips were transferred into a perti plate containing a 30 ml of an overnight *Agrobacterium tumefaciens* culture, and incubated for 5-15 and 30 min. After incubation, the excess bacteria was blotted on a sterile filter paper and the shoot tips were spread out onto the solid establishment medium (MS basal salt mixture + a 30 g/l of sucrose + a 1.0 mg/l of BA) for 2- 3 or 4 days at 25-28°C in the dark. After that, five shoot tips were transferred into a selection medium [establishment medium + a 200 mg/l of cefotaxime sodium salt + a 4 mg/l of bialaphos]. The antibiotics were added through a disposable filter (0.22 µm) to the autoclaved medium under sterile conditions. Well developed shoots were transferred into a rooting medium (MS basal salt mixture + a 30 g/l of sucrose + a 200 mg/l of cefotaxime and a 4 mg/l of bialaphos) and incubated for six weeks.

Detection of gus gene

Putative transformed shoot tip and leaves were placed in an eppendorff tube covered with X-Gluc solution buffer. The X-Gluc solution containing 1 mg/ml X-Gluc (5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid, cyclohexylammonium salt) fermentas® according to Jefferson *et al*. (1987). The tubes were immediately wrapped with aluminum foil (to prevent light effect) and incubated in incubator at 37°C overnight; 24 hrs later, the buffer was removed and explants were washed and placed in 20% bleach solution for 30 mins to remove any background color that may interfere with the resulted blue color of GUS. The blue color indicating the presence of GUS protein was then visualized under a microscope.

Herbicide bialaphos sensitivity

The herbicide resistance of non transformed sweet potato of Abees and Mabruka cultivars were determined by planting five shoot tip explants on MS medium supplemented with different concentrations of bialaphos i.e., 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l. Bialaphos was sterilized by filtration through disposable filters (0.22 µn) and incorporated into a precooled (45-50°C) autoclaved medium. After three weeks, the concentration of bialaphos which kill all explants was used in selection medium in the routine work of the plant transformation (Zang *et al*., 2009).

Genomic DNA isolation:

DNA was isolated from 21-25 days old leaves from *in vitro* cultures of transgenic and nontransgenic sweet potato cultivars.

Polymerase chain reaction (PCR)

PCR reaction was conducted to determine the presence or absence of GUS and *bar* genes in transformed plants. The amplification of DNA from putatively transformed and non transformed plants was carried out in a volume of 25 µl containing 2 µl of genomic DNA template, a 3 µl of primers (Table 1), a 0.5 µl of dNTP's, a 1.5 µl of $MgCl_2$, a 5 µl of 1X buffer and a 0.15 µl of Taq polymerase enzyme obtained from promega (REF 8305. LOT 0000077715, Madison, WI53711-5399, USA); amplification programmed was 94°C for 3 mins then (94°C for 30 sec + 58°C for 50 sec and 60°C for 1 min + 72°C for 1.5 min for 35 cycles) and the final cycle was at 72°C for 7 min. Genomic DNA and amplified PCR products were visualized using 1.5% agarose gels stained with ethidium bromide.

Statistical analysis

A completely randomized design with genotype, type of plant organ and type of plant growth regulator re-arranged as factors. Statistical analysis and analysis of variance (ANOVA) at 5% level of significance were performed. Mean values were compared using the least significance difference (LSD) test using SAS program at the 5% level of significance.

RESULTS AND DISCUSSION

Regeneration of sweet potato

In the present study, we investigated the possibility to establish a regeneration system for two Egyptian sweet potato cultivars (Abees and Mabruka), by using indirect and direct shoot formation. *In vitro* shoot tips explant was detached from plantlets (25 days old) grown on MS medium, and cultured under aseptic conditions on MS basal salt medium supplemented with 3% sucrose, and different concentrations of BA and Kin (1, 3, 5 and 10 mg/l) to initiate callus induction. There are several reports of in vitro regeneration of sweet potato plants via organogenesis. However, most of these protocols are limited to a few genotypes and must undergo a callus stage (Gosukonda *et al*., 1995). In our preliminary studies, it was demon-

strated that the callus was easily induced and proliferated, but the shoot was very difficult to regenerate from callus (Gong *et al*., 2005).

The explant was able to produce yellowish friable and compact callus on all concentrations of BA and Kin. Plants were regenerated from shoot tips explants within five months. Kreuze *et al*. (2008) found that cultivar Huachano took 12 months to regenerate and the same cultivar took five months to induce somatic embryogenesis (Sefasi *et al*., 2012). Time lapsed, non- embryogenic callus formed to embryogenic callus (Fig. 2A), therefore the adventitious shoots developed directly from these embryogenic callus after two or three weeks of culture (Fig. 2B). After four to five weeks, shoot clusters on the proximal region of the explants were seen (Fig. 2C, D). This was also confirmed by Sivparsad and Gubba (2012) who reported that the potential of producing adventitious shoots was restricted to the apical tips explants and that the meristem tissues from plants were the best explants for adventitious shoot induction and the developmental stage of explants was critical for the induction of adventitious shoots in sweet potato.

The inconsistencies in regeneration responses within the same cultivar may be due to variations in the developmental and physiological stage of in vitro plants, which affect the cultural behavior of explants (Triqui *et al*., 2008). For example, the sweet potato cultivar Duclos 11, which previously showed the ability to regenerate plants from protoplast-derived calli (Sihachakr and Dureux, 1987) showed no embryogenic response when lateral buds were used later. These result indicated that meristem tissues contain actively dividing cells that are responsible for length extension of the plant body and therefore have a greater capacity for regeneration.

The different concentrations of cytokinins (BA and kinetin) were tested to improve a reliable system for sweet potato regeneration using the shoot tips explants as presented in Table (2). The confirmative experiment was conducted by repeating the most succeeded concentration of both BA and Kin using the shoot tip explant. The results showed that the concentration of a 1 mg/l of BA was superior induced the highest rate of adventitious shoot formation from Abees cultivar and a 5 mg/l of Kin from Mabruka cultivar 26.3 and 13.3%, respectively. The results showed that the incorporation of cytokinin alone was able to generate growth and organs as shown in Table (3). Using regeneration media supplemented with BA or Kin, the explants were swelled and the shoot primordia were appeared after 3 months of culturing followed by the proliferation of small shoots at the explant cut edges. Meanwhile, using BA or Kin in combination with either auxins ex.; NAA, IAA or 2,4-D only callus with or without roots was produced. On the other hand, when BA was replaced by PBZ, the results showed that PBZ was significantly less responsive than BA for adventitious shoot formation.

It was shown by the reduction of shoot numbers per explant among all BA and Kin concentrations. BA ensured the explant vitality unlike PBZ which often caused browning of the explant. This was previously confirmed by (Mohamed *et al.*, 2007) who reported that PBZ induced about 6.7% of the cultured explants which showed multiple shoots per explants. However, the shoot buds were smaller than those obtained by BA which gave about 80% of multiple shoot per explants from 11 cultivars. While, 44 cultivars were totally ineffective and didn't elicit any morphological differences with using PBZ. He also suggested that the Cytokinin (BA) and cytokinin-like compound thidiazuron (TDZ) break apical dominance. Thus several shoot can proliferate from a preexisting initial shoot meristem/bud of the explants. In addition, the cytokinin (BA) as well as cytokinin-like compound (TDZ) was affected on cell division. Moreover, the most researchers who investigated effects of BA vs. TDZ had shown that TDZ was more effective at much lower concentrations to induce shoot multiplication than BA in many plant species (Sriskandarajah *et al.*, 2001). Sivparsad and Gubba (2012) reported that optimum shoot regeneration was achieved when shoot tip explants were cultured in medium containing 0.01 mg/L NAA and 1 mg/L BAP. The most we can talk about, we found in our study that the BA and Kin was produced root induction from callus in the two Abees and Mabruka cultivars. Moreover, Mabruka cultivar was the most responded which produced the highest frequency of roots from shoot tip explant with a 1.0 mg/l of BA 85.97%, but the highest root frequency from shoot tip explant of Abees cultivar was produced at a 3.0 mg/l of BA 23.96% as shown in the Figs (3 and 4). Therefore, the result indicated that with low range of BA concentration 1.0 mg/L which critical for the development of shoots and concentrations exceeding this threshold resulted in root formation. The distinctive limit of BA needed to promote shoot regeneration was probably due to the endogenous concentrations of auxin presented in this specific genotype. There results agreed with the classic report by Sivparsad and Gubba (2012), who demonstrated that how cytokinin and auxin interact to Produced different morphological responses, depending on the concentrations of endogenous hormones and the ratio of the concentrations of exogenous auxin to cytokinin.

On contrary, sweet potato regeneration had also been documented by using combined treatments of auxin and cytokinin (Gosukonda *et al.*, 1995; Xing *et al.*, 2008). These results may be regarded to that benzyl adenine medium probably had a significant assignment increased plant regeneration as which increased the cell potency towards differentiation. Similar result was reported by (Yi *et al.*, 2007) who produced shoot regeneration in sweet potato onto regeneration medium containing BA. Endogenous factors, such a level of growth regulators within plant explants, together with the interaction between endogenous and exogenous hormones may also influence the embryogenic response

(Jiménez, 2005). González *et al.* (1999) obtained root formation from CEMSA-78354 cultivar on hormone free medium but with very low frequency and indicated that it may due to the endogenous hormonal levels of tested cultivar.

Rooting stage

The well developed shoots (2-3 cm long) which obtained from in vitro culture were carefully transferred to the rooting media containing MS basal salt mixture supplemented with a 30 g/l of sucrose and a 2.2 g/l of phytagel. The cultures were incubated for 4-6 weeks which were observed during the earlier stages of sweet potato in vitro establishment to be most effective in root formation as shown in Fig. (5).

Sweet potato transformation via Agrobacterium-mediated

The *Agrobacterium*-mediated transformation system was used with shoot tip as explant for the transformation of the two Egyptian sweet potato cultivars (Abees and Mabruka). Shoot tip explants were inoculated for 5-15 and 30 min with *Agrobacterium tumefaciens* LBA4404 strain carrying the pISV2678 plasmid (the *Agrobacterium* culture was adjusted at 0.3 OD 600). After that, the explants were dried using filter paper followed by co-cultivation on regeneration media for two days at 28°C which gave the transformed cells which took enough time to begin the expression of introduced selectable marker gene allowing reliable selection. The inoculated explants were subcultured onto selection media which consisted of MS media, 3% sucrose, a 1 mg/l of BA with Abees cultivar and a 5 mg/l of Kin with Mabruka cultivar. A 4 mg/l of bialaphos as a selectable agent and a 200 mg/l of cefotaxime were used. Then, the explants were incubated in the light 16/8 hr at 25-28°C until starting the appearance of developed shoots.

The optimal OD was determined by using histochemical analysis; five different levels (0.1, 0.3, 0.5, 0.7 or 1) were tested by observing the number of blue color in shoot tip transformed explants by the *Agrobacterium* and also by the number of explants that exhibited this blue color for that particular OD measurement. The highest numbers of GUS expressing spots were observed at OD reading of 0.3. While, the higher OD levels (0.5 and 1) were very concentrated which caused hard damages and the explants were eventually necrosed. Therefore, the OD of 0.3 was used for this strain. Newell *et al.* (1995) reported that the optimal density (OD 600 nm) of LBA4404 strain is 0.5 nm. Xing *et al.* (2007) did not find a significant difference when various *Agrobacterium* OD concentrations (0.6, 0.8 and 1.0) at 600 nm were used for infection, although the best transformation was achieved when concentration of OD was 0.8. There was no significant difference between whole leaves and stem internode pieces in their responses to *Agrobacterium* concentration, co-culture duration and co-culture on filter paper (Sefasi *et al.*, 2014). Comparison between different concentrations of

bacteria was also carried out on apple transformation by De Bondt *et al.* (1994) who showed that the *Agrobacterium* concentrations for transformation were strain dependent. Gama *et al.* (1996) found that OD 600 at 0.6 nm was effective for sweet potato embryogenic callus. Besides that, OD 600 at 0.3-0.6 nm were used for other sweet potato cultures (Otani *et al.*, 1998). However, Xing *et al.* (2007) decided that OD 600 at 0.6-0.8 nm of EHA105 bacterial strain showed the highest transformation efficiency for sweet potato embryogenic callus.

Normally, 3-4 days are standard for co-cultivation in the most transformation protocols; also Xing *et al.* (2007) found that the optimal co-culture time was 4 days for cell suspensions of Xu55-2 cultivar. Some reports, Prakash *et al.* (1991) recommended co-culture of more than 3 days, while others, Otani *et al.* (1998) found that two days was sufficient. In this study, the results enriched that many of the injected shoot tip explants were transferred from the selection medium after two or three days. Moreover, the survival rate of 90% and greater was also maintained from co-cultivation for two days and a survival rate of 80% was achieved at three days. The explants began to become brown after four days from inoculation due to the overgrowth of the *Agrobacterium* on explants. Then, the explants were necrosed as a result of the highly condensed *Agrobacterium* cells which damaged the plant tissues. Thus, the suitable co-cultivation period with the *Agrobacterium* was two days which produced the best results in the GUS assay.

The putative transgenic shoots which passed from the bialaphos selection stage were excised and transferred to rooting media containing MS media supplemented (3% sucrose) with a 4 mg/l of bialaphos and a 200 mg/l of cefotaxime.

Selection of putatively transgenic sweet potato plants (Herbicide bialaphos sensitivity)

Prior to transformation it is necessary to study the bialaphos sensitivity of growth and differentiation for sweet potato plant, which was used in the establishment of transformation through selection of transformed shoots. To determine the optimum concentration of glufosinate ammonium that could be used for the selection of transformed sweet potato shoots, a kill curve experiment was carried out using non-transformed explants of sweet potato Abees and Mabruka cultivars. In this study, shoot tip explants were cultured under the same conditions which were used for regeneration. Six selective media were prepared by adding a filter sterilizing (1 mg/ml stock solution of glufosinate ammonium) to autoclave shoot regeneration medium to reach a final concentration of 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l. For each of the six selective media, three replicates were initiated by transferring five pieces of shoot tips for each replicate. The cultures were incubated in a continuous light at 25±2°C. After four weeks of growth, the survived healthy

shoots were obtained. The results showed that increasing in the bialaphos concentrations were accompanied by decreasing in the percentage of the survival explants. The lethal dose of bialaphos was determined on 4.0 mg/l of concentration. Where, 0.0, 1.0, 2.0 and 3.0 mg/l of bialaphos concentrations showed 66.6, 66.6, 46.6 and 6.6% of survival percentage, respectively as presented in Table (4). No survival plants were observed when 4.0 and 5.0 mg/l of bialaphos were used. The concentration of 4.0 mg/l of bialaphos was then chosen as a selection marker to select the transformed tissues in the sweet potato cultivars. This concentration of phosphinothrinicin (PPT) was lower than that reported by (Otani *et al.*, 2003) who suggested that 5 mg/l of PPT concentration was completely inhibited the growth of sweet potato embryogenic calli. Thus, sweet potato cell aggregates were more sensitive to PPT concentration than the embryogenic calli. This result will allow to confirm the selective medium which could be used in the present study. GUS histochemical assay

The shoot tip explants were tested for GUS activity according to Jefferson *et al.* (1987) to detect the putatively transformed plants. Photographic representations of these results are shown in Fig. (6). The stable GUS activity was detected in regenerated shoot tip explants which showed that none of the control plants gave positive result with the X-Gluc indicating the absence of any endogenous GUS activity. Whereas, the transformed plants showed the presence of the blue colour spots indicating the GUS expression. The average number of GUS spots was increased when OD reached to 0.3 nm with inoculated for 30 min compared to OD of 0.5 nm. Therefore, the OD of 0.3 nm was chosen to enhance the high recovery after inoculations which minimize the cell damage. Then, the positive samples were genetically analyzed and confirmed using the PCR analysis.

Molecular analysis of putative transgenic plants using PCR analysis

Total genomic DNA from leaf samples of putatively transgenic sweet potato plants resulted from the transformation experiments were analyzed by PCR using two primers specific to the coding region of the *bar* and *gus* genes which constructed on pISV2678 plasmid. Amplified DNA of five out of the 36 T_0 plants obtained from *Agrobacterium* transformation revealed the presence of the GUS DNA amplified fragment at 1050 bp for the cultivars as shown in Fig. (7) and the *bar* amplified fragment at 400 bp for the cultivars as shown in Fig. (8). Using the polymerase chain reaction technique (PCR) as an indicator for the presence of transgenes in the genomic DNA of putatively transgenic plants has been reported by many investigators (Otani *et al.*, 2003; Zang *et al.*, 2009).

Finally, the transformation and regeneration system described here is a simple and effective procedure, allowing routine introduction of target genes into (*Ipomoea batatas* (L.) Lam) Abees and

Mabruka cultivars, which could eventually open the door for genetic enhancement of sweet potato via modern biotechnology, which is a goal that has been addressed by many workers over the past.

SUMMARY

Genetic transformation is considered as one of the most favorable options for improvement of crop traits. In this study the regeneration frequency and transformation system were established on the Egyptian sweet potato (*Ipomoea batatas* (L.) Lam.) cv. Abees and Mabruka. The effect of different hormone combinations and type of explant on shoot regeneration was evaluated. The regeneration percentages from Abees and Mabruka cv. 26.3 and 13.3%, respectively were obtained on Murashige and Skoog MS basal salt mixture + 1.0 mg/l BA + 30.0 g/l sucrose + 2.2 g/l Phytagel with Abees cv. and the same media was used for cv. Mabruka with only cytokinin type different as 5.0 Kin and shoots were rooted on MS medium + 30 g/l sucrose and 2.2 g/l Phytagel. The *Agrobacterium*-mediated and microprojectile bombardement transformation system were successfully introducing the reporter *gus* and selectable *bar* marker genes in the sweet potato explants under pressure of 900 and 1100 psi and microcarrier travel distance (6 and 9 cm). Incorporation and expression of the *gus* and *bar* genes into sweet potato plants were confirmed using polymerase chain reaction (PCR) and GUS histochemical assay. Several factors were found to be important for regeneration and transformation in sweet potato. The most effective factors were plant genotype and the type of explants. Co-cultivation time and optical density of the *Agrobacterium* suspension were also critical for sweet potato transformation. This work is an attempt to open the door for further genetic improvement of sweet potato using important agronomic traits.

REFERENCES

De Bondt, A., K. Eggermont, P. Druart, M. De Vil, I. Goderis, J. Vanderleyden and W. F. Broekaert (1994). *Agrobacterium*-mediated transformation of apple (*Malus x domestica Borkh.*): an assessment of factors affecting gene transfer efficiency during early transformation steps. Plant Cell Reports, 13: 587-593.

El-Far, M. M., K. El Mangoury and H. E. M. Elazab (2009). Novel plant regeneration for Egyptian sweetpotato (*Ipomoea batatas* (L.) Lam.) Abees cultivar *via* indirect organogenesis stimulated by initiation medium andcytokinin effects. Australian Joural of Basic and Applied Sciences, 3: 543-551.

FAOSTAT, F. (2015). Agriculture Organization of the United Nations, 2011. FAO, Retrieved am from http://faostat3. fao. org/ faostat-gateway/go/to/ download/ Q/QC/S. Acceso 20.

Gama, M. I., R. P. Leite Jr, A. R. Cordeiro and D. J. Cantliffe (1996). Transgenic sweet potato plants obtained by *Agrobacterium tumefaciens*-mediated transformation. Plant Cell, Tissue and Organ Culture, 46: 237-244.

Gong, Y., F. Gao, K. Tang and P. Debergh (2005). *In vitro* high frequency direct root and shoot regeneration in sweet potato using the ethylene inhibitor silver nitrate. South African Journal of Botany, 71: 110-113.

González, R. G., D. S. Sánchez, J. M. Campos, E. P. Vázquez, Z. Z. Guerra, A. L. Quesada, R. M. Valdivia and M. G. González (1999). Plant regeneration from leaf and stem explants from two sweet potato (*Ipomoea batatas* L. Lam.) cultivars. Biotecnologia Aplicada, 16: 11-14.

González, R. G., D. S. Sánchez, Z. Z. Guerra, J. M. Campos, A. L. Quesada, R. M. Valdivia, A. D. Arencibia, K. Q. Bravo and P. D. Caligari (2008). Efficient regeneration and *Agrobacterium tumefaciens* mediated transformation of recalcitrant sweet potato (*Ipomoea batatas* L.) cultivars. Asia Pacific Journal of Molecular Biology and Biotechnology, 16: 25-33.

Gosukonda, R. M., C. Prakash and A. P. Dessai (1995). Shoot regeneration *in vitro* from diverse genotypes of sweetpotato and multiple shoot production per explant. HortScience, 30: 1074-1077.

Hansen, G., R. D. Shillito and M. D. Chilton (1997). T-strand integration in maize protoplasts after codelivery of a T-DNA substrate and virulence genes. Proceedings of the National Academy of Sciences, 94: 11726-11730.

Jefferson, R. A ,.T. A. Kavanagh and M. W. Bevan (1987). GUS fusions: *beta-glucuronidase* as a sensitive and *versatile* gene fusion marker in higher plants. The EMBO Journal, 6: 3901-3907.

Jiménez, V. M. (2005). Involvement of plant hormones and plant growth regulators on *in vitro* somatic embryogenesis. Plant Growth Regulation, 47: 91-110.

Jin, H. K., K. M. Kim and B. W. Yun (2015). Establishment of a one-step plant regeneration system in sweet potato (*Ipomoea batatas* [L.] Lam.). Global Journal of Biology, Agriculture and Health Sciences, 4: 48-55.

Kreuze, J. F., I. S. Klein, M. U. Lazaro, W. J. C. Chuquiyuri, G. L. Morgan, P. G. C. Mejia, M. Ghislain and J. Valkonen (2008). RNA silencing-mediated resistance to a crinivirus (*Closteroviridae*) in cultivated

sweetpotato (*Ipomoea batatas* L.) and development of sweetpotato virus disease following co-infection with a potyvirus. Molecular Plant Pathology, 9: 589-598.

Lawton, R., S. Winfield, H. Daniell, A. S. Bhagsari and S. K. Dhir (2000). Expression of green-fluorescent protein gene in sweet potato tissues. Plant Molecular Biology Reporter, 18: 139-139.

Mohamed, M., M. Mohamed and A. Ahmed (2007). Differential axillary-bud proliferation responses of two sweet potato cultivars to benzyl adenine and thidiazuron. Assiut University Bulletin Environment Research, 10: 21-30.

Mohanty, P., M. Rout and R. Behera (2016). Character association and path analysis in sweet potato (*Ipomoeabatatas* (L.) Lam.) genotypes. Advances in Life Sciences, 5: 441-448.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum, 15: 473-497.

Newell, C., J. Lowe, A. Merryweather, L. Rooke and W. Hamilton (1995). Transformation of sweet potato (*Ipomoea batatas* (L.) Lam.) with *Agrobacterium tumefaciens* and regeneration of plants expressing cowpea trypsin inhibitor and snowdrop lectin. Plant Science, 107: 215-227.

Okada, Y. and A. Saito (2008). Evaluation of resistance to complex infection of SPFMVs in transgenic sweet potato. Breeding Science, 58: 243-250.

Otani, M., M. Mii, T. Handa, H. Kamada and T. Shimada (1993). Transformation of sweet potato (*Ipomoea batatas* (L.) Lam.) plants by *Agrobacterium rhizogenes*. Plant Science, 94: 151-159.

Otani, M., T. Shimada, T. Kimura and A. Saito (1998). Transgenic plant production from embryogenic callus of sweet potato (*Ipomoea batatas* (L.) Lam.) using *Agrobacterium tumefaciens*. Plant Biotechnology, 15: 11-16.

Otani, M., Y. Wakita and T. Shimada (2003). Production of herbicide-resistant seetpotato (*Ipomoea batatas* (L.) Lam.) plants by *Agrobacterium tumefaciens*-mediated transformation. Breeding Science, 53: 145-148.

Parle, M., M. M. Kadian and K. K. Sharma (2015). A review on psychosis and anti-psychotic plants. Asian Journal of Pharmaceutical and Clinical Research, 8: 24-28.

Prakash, C., U. Varadarajan and A. Kumar (1991). Foreign gene transfer to sweet potato (*Ipomoea batatas*). HortScience, 26: 492-492.

Qing, K. Y. and P. T. Guo (1999). Effects of salt stress on the ultrastructure of chloroplast and the activities of some protective enzymes in leaves of sweet potato. Acta Photophysiologica Sinica, 25: 229-233.

Roca, W. M., N. Espinoza, M. Roca and J. Bryan (1978). A tissue culture method for the rapid propagation of potatoes. American Potato Journal, 55: 691-701.

Sefasi, A., G. Ssemakula, M. Ghislain, K. Prentice, A. Kiggundu, R. Mwanga and S. Mukasa (2014). Transient expression of β-glucuronidase in recalcitrant Ugandan sweetpotato and putative transformation with two *cry* genes. African Crop Science Journal, 22: 215-228.

Sefasi, A., J. Kreuze, M. Ghislain, S. Manrique, A. Kiggundu, G. Ssemakula and S. Mukasa (2012). Induction of somatic embryo-genesis in recalcitrant sweetpotato (*Ipomoea batatas* L.) cultivars. African Journal of Biotechnology, 11: 16055-16064.

Sihachakr, D. and G. Ducreux (1987). Plant regeneration from protoplast culture of sweet potato (*Ipomoea batatas* Lam.). Plant Cell Reports, 6: 326-328.

Sivparsad, B. and A. Gubba (2012). Development of an efficient plant regeneration protocol for sweet potato (*Ipomoea batatas* L.) cv. Blesbok. African Journal of Biotechnology, 11: 14982-14987.

Sriskandarajah, S., S. Frello and M. Serek (2001). Induction of adventitious shoots *in vitro* in *Campanula carpatica*. Plant Cell, Tissue and Organ Culture, 67: 295-298.

Triqui, Z. E. A., A. Guédira, A. Chlyah, H. Chlyah, V. Souvannavong, R. Haïcour and D. Sihachakr (2008). Effect of genotype, gelling agent, and auxin on the induction of somatic embryogenesis in sweet potato (*Ipomoea batatas* Lam.). Comptes Rendus Biologies, 331: 198-205.

Tumwegamire, S., R. Kapinga, P. R. Rubaihayo, D. R. LaBonte, W. J. Grüneberg, G. Burgos, T. Zum Felde, R. Carpio, E. Pawelzik and R. O. Mwanga (2011). Evaluation of dry matter, protein, starch, sucrose, β-carotene, iron, zinc, calcium, and magnesium in East African sweetpotato [*Ipomoea batatas* (L.) Lam] germplasm. HortScience, 46: 348-357.

Xing, Y., Q. Ji, Q. Yang, Y. Luo, Q. Li and X. Wang (2008). Studies on *Agrobacterium*-mediated genetic

transformation of embryogenic suspension cultures of sweet potato. African Journal of Biotechnology, 7: 534-540.

Xing, Y., Q. Yang, Q. Ji, Y. Luo, Y. Zhang, K. Gu and D. Wang (2007). Optimization of *Agrobacterium*-mediated transformation parameters for sweet potato embryogenic callus using β-glucuronidase (GUS) as a reporter. African Journal of Biotechnology, 6: 2578-2584.

Yi, G., Y. M. Shin, G. Choe, B. Shin, Y. S. Kim and K. M. Kim (2007). Production of herbicide-resistant sweet potato plants transformed with the *bar* gene. Biotechnology Letters, 29: 669-675.

Zang, N., H. Zhai, S. Gao, W .Chen, S. He and Q. Liu (2009). Efficient production of transgenic plants using the *bar* gene for herbicide resistance in sweetpotato. Scientia Horticulturae, 122: 649-653.

Table (1): Synthetic primers designed for detection of *gus* and *bar* genes.

Primers	Sequence	Detection	Expected Product
GUS (F)	5`AGTGTACGTATCACCGTTTGTGTGAAC 3`	*gus* gene	1050 bp
GUS (R)	5`ATCGCCGCTTTGGACATACCATCCGTA 3`	*gus* gene	
bar (F)	5`TACATCGAGACAAGCACGGT 3`	*bar* gene	400 bp
bar (R)	5`ACGTCATGCCAGTTCCCGTG 3`	*bar* gene	

Table (2): Effect of a BA and Kin on shoot and root induction from shoot tips explants in two Sweet potato cultivars Abees and Mabruka.

Tretments	Abees				Mabruka			
	No. of regenerated shoots/ shooted callus	Regeneration frequency%	No. of roots per callus	Root frequency%	No. of regenerated shoots/ shooted callus	Regeneration frequency%	No. of roots/plantlet	Root frequency%
Control	0	0	0	0	0	0	0	0
BA(mg/l)								
1	**2.660^{a}** ± 0.352	**26.30**	**2.067^{bce}** ± 0.177	**20.64**	1.200^{cde} ± 0.133	12.64	**8.600^{a}** ± 0.964	**85.97**
3	2.100 ^{ab} ± 0.276	18.30	**2.401^{bcd}** ± 0.177	**23.96**	1.100^{de} ± 0.258	9.31	1.667^{cde} ± 0.484	16.64
5	2.300^{a} ± 0.334	20.96	2.023^{bce} ± 0.315	20.31	1.100^{cde} ± 0.314	9.98	1.632^{cde} ± 0.474	16.31
10	1.900abc ± 0.458	15.96	0.966^{de} ± 0.356	9.99	0.700^{de} ± 0.395	3.99	0.668^{de} ± 0.310	6.64
Kin (mg/l)								
1	0.900^{de} ± 0.314	10.64	0.700^{de} ± 0.291	2.99	0.500^{e} ± 0.166	8.32	2.801^{bc} ± 1.275	27.98
3	0.600^{de} ± 0.266	5.33	0.433^{e} ± 0.314	4.33	0.600^{de} ± 0.221	6.33	2.933^{bc} ± 1.332	29.32
5	1.100^{cde} ± 0.433	11.97	1.233^{de} ± 0.444	12.32	**1.900^{abc}** ± 0.276	**13.30**	3.633^{bc} ± 0.812	**36.30**
10	1.400^{bcd} ± 0.266	13.29	1.433^{cde} ± 0.355	14.31	1.200^{cde} ± 0.326	10.32	1.567^{cde} ± 0.555	15.65

Means with the same letters are not significantly different (LSD) at 0.05 (± SE, values).

Table (3): The combined effect of the different cytokinin and auxin concentrations on shoot induction of sweet potato for Abees and Mabruka cultivars which cultured for one to three months on MS media.

Growth regulators (mg/l)	Shoot tip explants
BA (1, 3, 5, 10)	Shoots (more)
Kin (1, 3, 5, 10)	Shoots
IAA (0.5)	Roots
NAA (0.01)+BA (1.0)	-
NAA (1.0)+PBZ (1.0)	Callus
NAA (1.0)+BA (10.0)	-
NAA (1.0)+BA (0.1)	-
NAA (1.0)	Callus
2,4-D (0.5)+Kin (1.0)	-
IAA (0.4)+BA (2.0)	-
$AgNO_3$ (2, 4, 8, 12)	Roots

Table (4): Determination of the lethal dose of herbicide bialaphos.

Concentration of bialaphos (mg/L)	No. of explants	No. of shooted explants after six weeks	Survival % after six weeks
0.0	15	10	66.6
1.0	15	10	66.6
2.0	15	7	46.6
3.0	15	1	6.6
4.0	15	0	0
5.0	15	0	0

Fig. (1): A diagram showing the physical map of the binary vector pISV2678 containing the gus-intron reporter and the bar selectable marker genes.

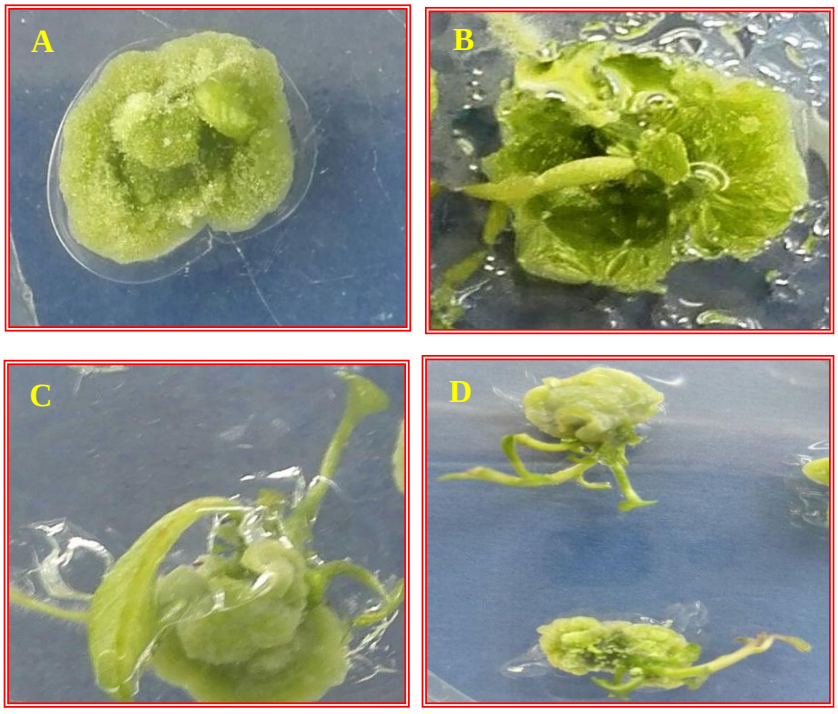

Fig. (2): Sweet potato regeneration steps: (A): The embryogenic callus from shoot tip explants. (B): Appearance of shoot primordia (green spots) after 4 months of culturing on the regeneration medium. (C, D) Shoot proliferation from the shoot primordia after 4 weeks of culturing on the same regeneration medium.

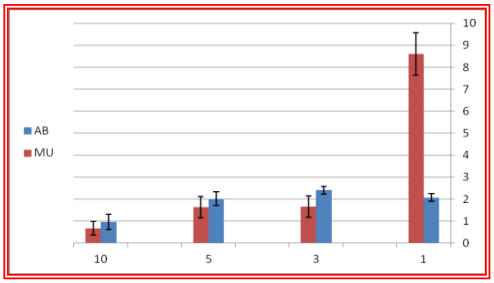

Fig. (3): Histogram illustrating means of the roots number for the two Sweet potato Abees (AB) and Mabruka (MU) cultivar from shoot tip explant with different BA concentrations (1, 3, 5 and 10 mg/l).

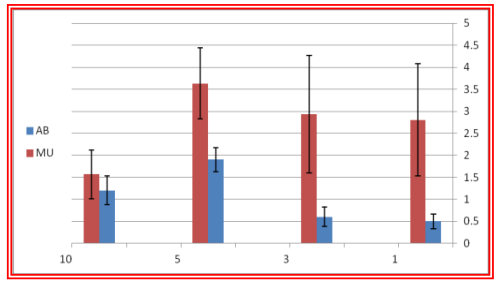

Fig. (4): Histogram illustrating means of the roots number for the two Sweet potato Abees (AB) and Mabruka (MU) cultivar from shoot tip explant with different Kin concentrations (1, 3, 5 and 10 mg/l).

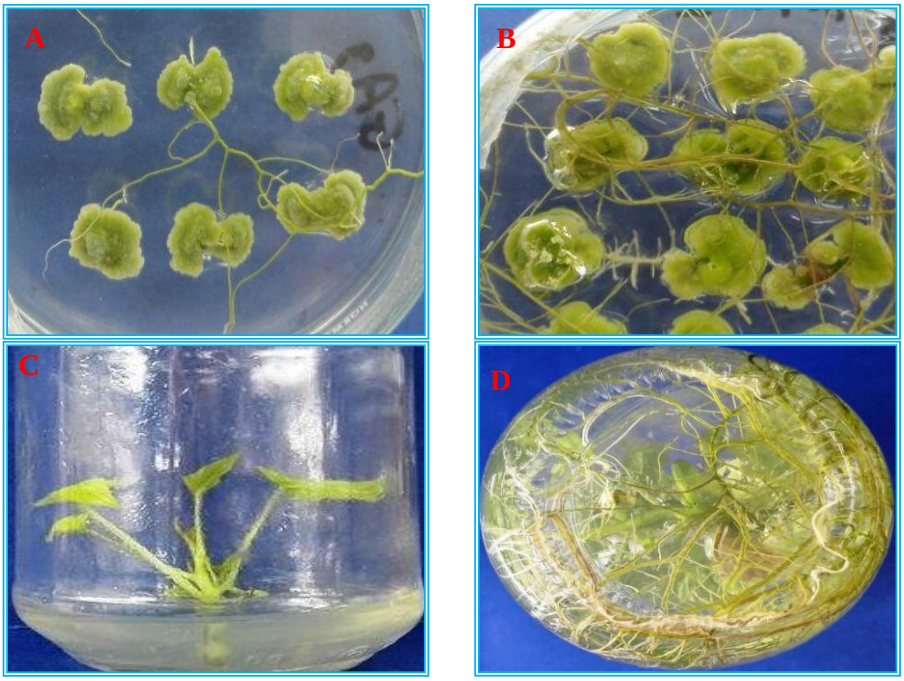

Fig. (5): Sweet potato rooting steps. (A & B) Direct root induction from callus. (C) Sweet potato plantlets on rooting media. (D) Root initiation of plantlets.

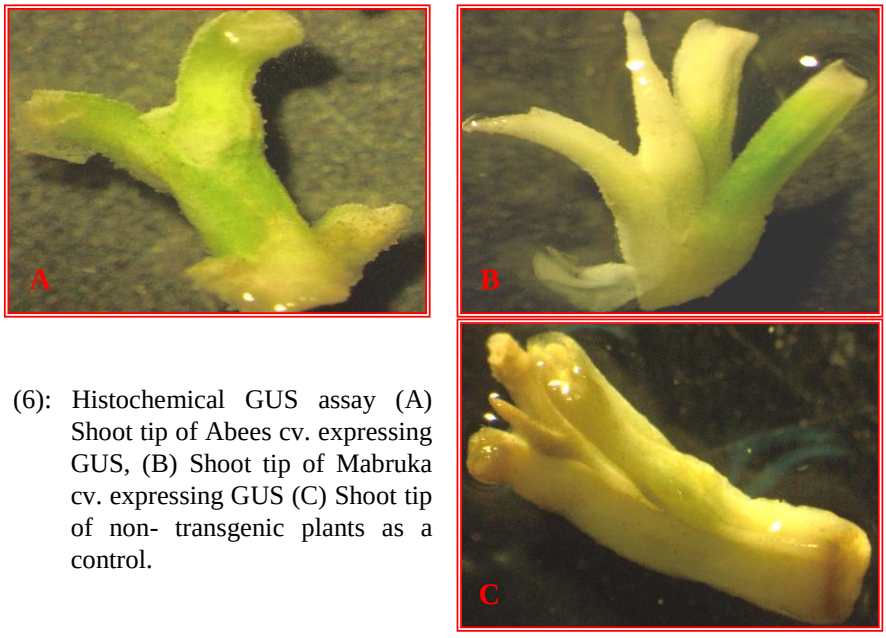

Fig. (6): Histochemical GUS assay (A) Shoot tip of Abees cv. expressing GUS, (B) Shoot tip of Mabruka cv. expressing GUS (C) Shoot tip of non- transgenic plants as a control.

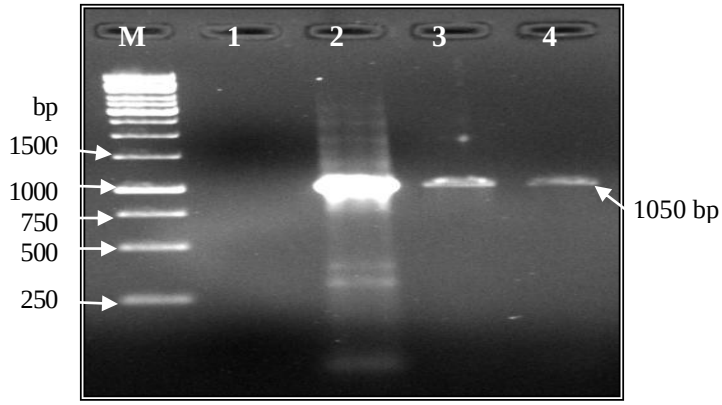

Fig. (7): Verification of *gus* gene presence and incorporation into putative transgenic sweet potato plants *via* PCR analysis for the two cultivars. Lane M: A 1 kbp ladder. Lane 1: Negative control (non-transformed sweetpotato). Lane 2: A plasmid containing the DNA constructed from pISV2678 plasmid as a positive control. Lane 3: A transformed plant from Abees cultivar. Lane 4: A transformed plant from Mabrouka cultivar.

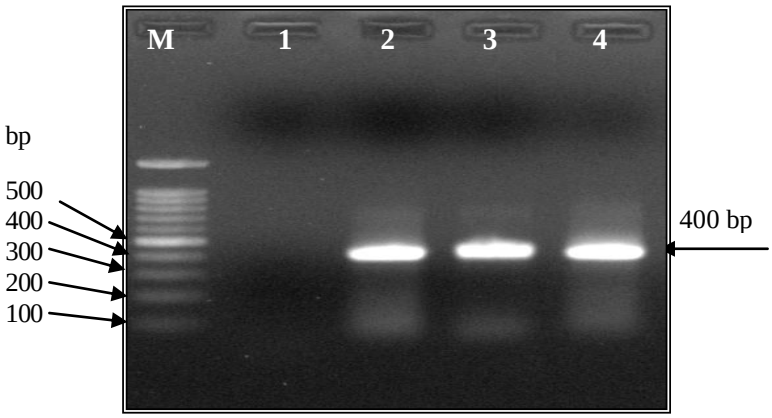

Fig. (8): Verification of bar gene presence and incorporation into putative transgenic sweet potato plants via PCR analysis for the two cultivars. Lane M: A 100 bp ladder. Lane 1: Negative control (non-transformed sweetpotato). Lane 2: A plasmid containing the DNA constructed from pISV2678 plasmid as a positive control. Lane 3: A transformed plant from Abees cultivar. Lane 4: A transformed plant from Mabrouka cultivar.