IMPROVEMENT OF SALT TOLERANCE TO TRANSGENIC POTATO (*Solanum tuberosum* L.) BY ABUNDANT PROTEIN *(HVA1)* GENE TRANSFER

M. H. REFAAT[1,2], T. A. EL-AKKAD[1, 2], SHERIF, S. SALEH[3] AND B. A. ABDELGAWED[4]

1. *Department of Genetics and Genetic Engineering, Faculty of Agriculture, Banha University, Egypt*
2. *Moshtohor Research Park, Molecular Biology Lab., Banha University, Egypt*
3. *Tissue Culture and Germplasm Conservation Lab. Hort. Res. Inst. Agric. Res. Center, Egypt*
4. *Agriculture Genetic Engineering Research Institute (AGERI), ARC, Giza, Egypt*

Potato, (*Solanum tuberosum* L.) an economically vital crop species and the point of interest of a large agricultural enterprise. Also, potato is a model organism for genetic, developmental, and physiological research. Potato is a few of the most extensively consumed veggies in the global, and many of the compounds determined in potatoes have acquired a good deal interest in recent years for his or her capability health advantages. Potato (*Solanum tuberosum* L.) is the starchy, tuberous crop of *Solanaceae* circle of relatives and the arena fourth biggest meals crop after rice, wheat and corn (Bajaj, 1987).

Plants are subjected to numerous abiotic stresses together with low temperature, salt, drought, floods, warmness, oxidative stress and heavy metallic toxicity during their life cycle. Amongst all this, salinity is the most common abiotic stress (Mahajan and Tuteja, 2005). Salinity has terrible impact on agricultural productiveness affecting plant increase and restricting the usage of land. It's far expected that 6% of the arena's general land and 20% of the sector's irrigated areas are laid low with salinity (UNESCO Water Portal, 2007).

Potato plant increase and development are adversely tormented by salinity, that's a major environmental stress that limits agricultural manufacturing; inside the beyond, conventional breeding strategies developed various crop species with stepped forward environmental stress tolerance (Cullins, 1991). But, breeding development and crop improvement are time consuming and labour extensive. Novel breeding methods along with somaclonal variant, interspecific hybridization, somatic hybridization and gene transformation offer potential for improvement in critical characters required in lots of cultivated varieties.

Recently, plenty research has focused on pressure induced gene expression, that's concept to be a crucial indicator for the invention of recent genes responding to numerous plant stresses (Wang *et al*., 2003). Greater research were

Egypt. J. Genet. Cytol., 47:105-122, January, 2018
Web Site (*www.esg.net.eg*)

carried out on finding the motives in the back of drought and salt tolerance, and precise genes and transcription elements recognized and transferred into the genomes of inclined vegetation for drought and/or salt tolerance.

Transformation structures were established to circumvent environmental stresses using diverse genes. Such a genes code for proteins that manipulate the last of the leaf stomata to save you plant evaporation and to conquer cell dehydration (Somerville and Meyerowitz, 2002; Bahieldin *et al*., 2005; Oh *et al*., 2005). One of such proteins in barley is referred to as the past due embryogenesis plentiful three (LEA3), encoded by using *Hordeum vulgaris* abundant protein (*HVA1)* gene that has been confirmed to be precipitated underneath aba remedy (Hong *et al*., 1992; Dure *et al*., 1989).

They carry out diverse functions like appearing as hydrating buffers, sequestering ions, assisting in renaturation of proteins and appearing as chemical chaperones (Dure, 1993; Goday *et al*., 1994). It changed into located that overdue embryogenesis considerable (lea) protein gene *HVA1* from *Hordeum vulgares* L. upon transformation into rice confers salinity and drought tolerance to transgenic plants (Xu *et al*., 1996). Several different plants which had been genetically converted with the barley *Hva1* gene resulted of their increase in vegetative biomass and other symptoms associated crop drought and/or salt tolerance, tobacco (Lee *et al*., 2007), mulberry (Checker *et al*., 2012), maize (Nguyen and Sticklen, 2013) and oat (Maqbool *et al*., 2002).

A whole lot work has been carried out on callus induction and boom in potatoes (*Solanum tuberosum* L.). This has led to more than a few protocols and techniques being established by using researchers on the grounds that tissue way of life gained an importance in plant propagation, conservation and breeding (Ahloowalia, 1982; Wareh *et al*., 1989).

Previous researches confirmed that media used for callus induction and growth relies upon on the genotypes (Gonzalez *et al*., 2001; Alexeenko and Irkaeva, 1998) mentioned that advent of genes effecting the structure and kind of plant development into strawberry traces also motivated callus formation and shoot inducing *in vitro*. This depicts involvement of inheritance in callus growth.

Within the remaining two a long time, many transgenic vegetation were produced by using various transformation strategies; callus is used for most of those transformation methods inclusive of particle gun (Decima *et al*., 2010) and *Agrobacterium tumefaciens*-mediated transformation (Stiekema *et al*., 1988) as well as initiation of cell tradition.

The existing research were performed to acquire three most important objectives: (1) to investigate the regeneration capability of *Solanum tuberosum* genotype of commercial importance using distinctive callus induction and regeneration media; (2) to introduce the *HVA1*

gene into *Solanum tuberosum* by way of bombarding the callus using the biolistic bombardment approach and (3) to verify the integration of the *HVA1* gene in the genome of T_0 vegetation with the aid of specific molecular biology assessments.

MATERIALS AND METHODS

1. Plant Material

The 'Sponta" potato cv. was collected from disease free plant at the Experimental Potato Station in the Horticulture Researches Institute, Agricultural Researches Center, Egypt during 2016-2017 growth season and kept in cold for two weeks. After sprouting the micro nodes were thoroughly washed in tap water with a 5% detergent solution (Teepol) for 20 minutes, followed by 2-3 washes in sterile distilled water. The explants were cut into convenient sizes (2-3.5 cm in length) after removing the leaf sheaths. The cut pieces (micronodes) were surface sterilized with a 20% Colorex for 20 minutes, rinsed 4-5 times with sterilized double distilled water and then trimmed to 1.0-1.5 cm in length. The explants were transferred onto full strength MS free medium (Murashige and Skoog, 1962) as a growing and callus medium. The grown *in vitro* aerial stem explants (micronodes) were cultured on MS free medium, repeated cut into 1.0-1.5 cm in length and transferred to the same medium for obtaining a sufficient number of *in vitro* stems and callus. Explants were maintained in an air-conditioned room at 22±2°C and then kept in a photoperiod regime of 16 hr. light and 8 hr. dark with a light intensity of 3000 lux, provided by Philips cool white fluorescent tubes.

2. Methods

2.1. Callus Induction and Somatic Embryogenesis

Callus cultures derived from the aerial stem, leaves and roots explants were sub cultured onto MS medium containing 2,4-Dichlorophenoxyacetic Acid (2,4-D) or Naphthalene acetic Acid (NAA) at 2, 3, 4, 5 and 10 *mg*/l. These cultures were kept without sub culturing for 30 days to induce embryogenic calli. Plant regeneration was induced by transferring all the embryogenic calli which obtained on T_I and T_{II} callus induction media to the plant regeneration media which contained 6-Benzylaminopurine (BAP) and Kinetin (Kin) at 1.0, 2.0 and 3.0 *mg*/l.

2.2. Potato transformation

2.2.1. Plant expression vector

The plasmid *pABI* (kindly provided by Prof. Dr. Ahmed Bahieldin, Faculty of Agriculture, Ain Shams University and AGERI) was used for the transformation of potato calli. Plasmid map of *pABI* transformation vector is shown in Fig. (1). The plasmid contained the barley *HVA1* gene driven by the maize *ubil* promoter region (including the first exon and intron) and terminated by the *Nos* gene 3' non-translated region. The plasmid also contained the bar gene (under the control of the *35S* promoter and *Nos* terminator) which was used as a selective marker. The bar gene encodes phosphinothricin acetyl

transferase (PAT) enzyme which inactivates phosphinothricin, the active ingredient of the herbicide bialaphos.

2.2.2. Potato Transformation

As far as the biolistic approach was concerned, a mean of 3-4 (≈1.0 gm) potato somatic embryos per a 6 cm Petri dish (covering an approximate area of 9–10 cm^2) were placed on MS medium containing a 2.0 *mg/*l of 2,4-D and a 3 *mg/*l of NAA (Yadav and Sticklen, 1995) and supplemented with a 4% Mannitol. The somatic embryo explants were left on the above medium for 5 hours prior to bombardment.

Transformation was carried out using the biolistic particle acceleration device (PDS 1000/He, Bio-Rad). For microprojectile bombardment, plasmid DNA (1 µg/µl) was precipitated onto gold particles (1.0 µm in diameter) following the procedure described in the Bio-Rad instruction Manual. Each plate of potato calli was bombarded twice at a rupture pressure of 1100 and 1350 psi with a 5 µl of particle suspension mixture per bombardment.

2.2.3. Selection and recovery of transformed calli

Bombarded calli were incubated on the callus induction media for one week after bombardment. Then, selection of transformed cells was carried out by transferring the transformed calli to the best callus induction media containing a 1.5 *mg/*l of bialaphos for two weeks. The calli were, then, transferred onto fresh selective media containing a 3 *mg/*l bialaphos, with continuous subcultures every two weeks. All cultures were kept at 28°C in a dark growth chamber.

2.2.4. Regeneration and recovery of transformed plants

The Survival calli, which uniformly grew on the selection media, were transferred to regeneration medium supplemented with a 3 *mg/*l of bialaphos. Somatic embryogenic calli, capable of developing into green shoots within 2-4 weeks, were classified as putative transformants. The selected plantlets were transferred into Hoagland solution for 4-6 days and then transferred to soil in environmentally controlled growth chambers and biocontainment greenhouse conditions.

2.3. Evaluation of transformed plants

2.3.1. Polymerase Chain Reaction (PCR) analysis

Genomic DNA was isolated from leaf tissues of each putatively transformed plant as well as from untransformed plants (control) using DNeasy® Plant mini Kit.

Two sets of primers were used to detect the bar and *Hval* genes by PCR analysis. The sequences of the specific primers for the *Hva1* gene were; (5'- GGA GAT CTA ACA ATG GCC TCC AAC CAG AAC CAG GGG -3') for *HVA1a* and (5'- GGG ATA TCT AGT GAT TCC TGG TGGTGGTGG TG -3') for *HVA1b*.

While, the sequences of specific the primers for the bar gene were; (5'-TGC CAC CGA GGG GAC ATG CCG GC-3') for *bar-1* and (5'-CCT GAA GTG GAG GCC A TG GGG-3') for *bar-2*. The PCR reaction was carried out in a 25 µl reaction volume containing a 25 ng genomic DNA, a 20 *pmole*/ml of primers ,a 200 µM each of dATP, dCTP, dGTP and dTTP, a 50 µM of KCl, a 10 *Mm* of Tris-HCl, a 0.2 *mM* of $MgCl_2$ and a 0.2 unit of Taq polymerase.

The PCR amplification conditions were consisted of an initial denaturation cycle at 94°C (5min) followed by 35 cycles at 94°C (1min), 57°C (2 min), 72°C (2 min) and a terminal extension cycle at 72°C (7 min) for the bar gene. While, for the *HVA1* gene, the PCR amplification conditions were 94°C (4 min), followed by 35 cycles at 94°C (1 min), 59°C (40 sec), 72°C (1min) and a final cycle at 72°C (7 min). The PCR products were resolved by electrophoresis on a 3% agarose gel.

2.3.2. Southern Blot Hybridization

Genomic DNA (5 µg) isolated from leaves of each putative transformed potato plant was digested with *PstI* restriction enzyme, electrophoresed in a 0.7% agarose gel and transferred to a Hybond N+ nylon membrane (Roche) and then cross-linked to the membrane by UV irradiation. The hybridization probes were prepared by digesting the plasmid *pAB1* with *PstI restriction* enzyme to liberate the *bar* and *HVA*1 DNA. The desired inserts were labeled using random priming DNA labeling and detection kit (Roche). Further processing, prehybridization, hybridization and washes were carried out mainly according to the protocol described by Kreike *et al.* (1990)**.** The hybridization signals were detected by exposure of the membrane to X-ray films.

2.3.3. Evaluating of transformed potato plants to salt tolerance

Transformed and non-transformed plants of Sponta cv. were used as two lines of potato plant materials to evaluate the effect of *HVA1* gene salinity tolerance in potato. The explants were subjected to salt stress by the addition of zero, 1000, 3000, 5000 and 7000 *ppm* NaCl and $CaCl_2$ to MS culture medium. Survival percentages were measured after four weeks for the two subcultures from starting salt treatment as well as, shoot number, shoot length and leaves number growth traits. Obtained data were statistically analyzed and mean comparisons were based on Duncan's multiple range tests by using the software according to Maxwell and Delaney (1989).

RESULTS AND DISCUSSION

Potato is one of the most studied higher plants because of its importance as a crop species, and of several advantages for genetical, molecular and physiological studies (Mccormick *et al.*, 1986). The *in vitro* morphogenic responses of cultured plant tissues are affected by the different components of the culture media, especially by the concentration of growth hormones, and it is therefore important to

evaluate their effects on plant regeneration. Development of an efficient protocol for potato transformation and its subsequent regeneration is a pre-request for the production of transgenic plants.

1. Somatic embryos production

In the present study, the establishment of an efficient regeneration system has been attempted for Sponta potato cv. to evaluate their potential for regeneration from callus cultures. Various concentrations of 2,4-D (T_1 media) and NAA (T_2 media) with different explants which were used to evaluate callus formation and somatic embryogenesis formation in potato. Embryogenic calli were identified by their yellowish white color and able to survive over repeated subcultures. While, non-embryogenic calli were discarded during further subcultures (Fig. 2 and Table 1)

According to the data in Table (1) the stem callus conversion to somatic embryos for the explants treated with 2,4-D at 4 mg/l gave the highest record (40.73%). Moreover, the highest percentage of somatic embryos conversion for callus induced from leaves was 85.15% at 3 mg/l of 2,4-D. Finally, the callus induced from roots gave a percentage of 14.81% for somatic embryos at 3 mg/l of 2,4-D. It was observed that the highest value (85.15%) of friable embryogenic calli were formed by the T_I medium from leaves which contained 2,4-D at 4 mg/l. These calli were grew fast in comparison to the embryogenic calli formed on T_{II} medium which contained the some concentrations of NAA with the some explants (Table1). This observation was in agreement with that reported by Bohorova *et al.* (1995) and Askari and Pepoyan (2015).

The frequency of embryogenic calli formed from the three different explants on T_I and T_{II} media which presented in Table (1) and Fig. (2) revealed that embryogenic calli formation was influenced by the explant type and chemical composition of the media. These results pointed out that the components and concentrations of the callus induction media had great influences on the production of embryogenic calli. All the tested explants showed better response in embryogenic calli formation when maintained on callus induction medium containing 2,4-D as compared to the medium supplemented with NAA.

2. Somatic embryos regeneration

Plant regeneration was induced by transferring all the embryogenic calli which obtained on T_I and T_{II} callus induction media to the plant regeneration media which contained BAP and Kin at 1.0, 2.0 and 3.0 mg/l. According to the data in Table (2) the somatic embryos gave the highest score of survival percentage (100%) for the control and BAP at 3.0 mg/l compared with the survival percentage (66.66%) for BAP and kin at 1.0 and 2 mg/l, respectively. Moreover, only BAP at 3.0 mg/l gave the highest percentage of regeneration (33%) compared with all treatments. While BAP and Kin at 2 and 3 mg/l, respectively gave lower percentage of regeneration (2.22%) but not a com-

plete growth and differentiation to whole plants.

3. Potato transformation

Transformed potato calli were achieved by particle bombardment using *pAB-1* plasmid harboring the *HVA1* gene. The putatively transformed calli were selected on T_I media containing bialaphos as a selective agent. RTI medium was used for regeneration of the putatively transformed plants (Fig. 2).

After growth and selection, the plantlets (two plantlets grew on regeneration media) were divided into small pieces and cultured on a multiplication media containing BAP at 0.25 mg/l. These results are in agreement with those of (Moghaieb *et al.*, 1999; Reda *et al.*, 1999) who reported that plant regeneration and somatic embryogenesis are genotype dependent. Also, Ashakiran *et al.* (2011) reported that the success in tomato regeneration response was largely depended on the genotype, explant and plant PGRs which used in the culture medium.

4. Salt tolerance screening

The transformed explants were more tolerant to salinity than the other non-transformed explants (Table 3 and Fig. 3). The explants showed the highest survival percentage (100%) for the non-treated explants under control in the first cycle of growth and this value gradually was decreased to 55.55, 48.14, 40.73 and 18.51% with increasing salinity concentrations. On the second cycle, this value was decreased to 37.03 under control as well as 11.51, 18.51, 12.21 and 22.18 for 1000, 3000, 5000 and 7000 ppm, respectively (Table 3 and Fig. 3).

The survival percentages in the transformed plants were a 100% for all explants under all salinity treatments in the two subcultures. Moreover, the growth of the transformed explants were more healthy than the other non-transformed explants as shown in Table (3) for shoot number (3.51) and shoot length (6.95) under 1000 ppm treatment and for leaves number (8.77) under 3000 ppm treatment. These values were increased after the first subculture to 5.29 for number shoot under 1000 ppm, 11.28 cm for shoot length under 3000 ppm and 6.58 for leaves under 1000 ppm.

These consequences are settlement with those discovered by Vitagliano *et al.* (1992) and Carceller and Ambrogio (1994) who stated that the *in vitro* boom of plant tissues became dramatically stricken by salt stress strains inside the media. In addition, consequences of Shibli *et al.* (2003) guided the performance of a microculture (*in vitro*) machine for the observe of salinity tolerance. Watanabe *et al.* (2000) stated that, the shoot tradition approach is one of the higher structures for testing and selecting for salt tolerance.

5. Molecular tests

The PCR assay confirmed the presence of both the *bar* and *HVA1* genes in the extracted DNA from these two transformed plantlets, which were absent in the

non-transformed plantlets control (Fig. 4A and B). Putatively transformed plants were subjected to Southern blot analysis to confirm the integration of the transgenes (*bar* and *Hva*1) in their genome (Fig. 5A and B). Comparable observations have been reported with the aid of Maqbool *et al.* (2002), Oraby *et al.* (2005) and kwapata *et al.* (2012) who confirmed a solid (100%) co-integration of two linked genes in the next transgenic progenies of oat, sorghum and commonplace bean, respectively.

Siviamani *et al.* (2000) recovered a ten transgenic wheat strains out of transformation experiments using *HVA1* gene. They analyzed a five of those traces through southern blot hybridization and observed that the transgene incorporated in only four of these lines, while the 5^{th} line did not longer show the appropriate of *HVA1* fragment which was shown by using gene expression analysis. Furthermore, Xu *et al.* (1996) and Lal *et al.* (2008) introduced *HVA1* gene into rice suspension cells and generated a huge variety of independent transgenic rice vegetation.

Askari and Pepoyan (2015) produced transformed potato (20 flora) by using reworking of *mtld* gene into *pbi* 121 plasmid, then transfered this gene in *Agrobacterium tumefaciens* lba4404 strains to three potato cultivars. Life of recombinant gene in transgenic flora became confirmed through PCR evaluation and characterized tolerance to salinity pressure of latest styles of GMO potatoes.

The correlation among accumulation of *LEA* institution three proteins and stress tolerance was well studied in wheat (Siviamani *et al.*, 2000; Bahieldin *et al.*, 2005), rice (Rohila *et al.*, 2002) and soybean (Liu *et al.*, 2010). Moreover, it is evident that genetic variability exists for pressure responses, and this can be occurred due to the differential expression and law of pressure responsive genes including *HVA1* gene, while the flowers were uncovered to pressure (Oraby *et al.*, 2005). Based on our experimental effects, the presence of *HVA1* gene in the genome of transgenic plant life under the manage of the *ubi 1* promoter indicated that constitutive expression of *HVA1* gene which could be enhance boom overall performance in transgenic flora under salinity stress situations. However, the precise characteristic of *LEA* proteins stays unsure.

Consequences of this take confirmed that a reproducible regeneration device for (*Solanum tuberosum* L.) ‘Sponta” potato cv. turned into correctly evolved of the use for the callus from leaf explant that produced maximum regeneration potentiality and transformation frequency.

SUMMARY

Potato, (*Solanum tuberosum* L.) is an economically crucial crop species and focus of a big agricultural industry. Drought and salt stresses are two predominant abiotic strain elements ensuing in potato, (*Solanum tuberosum* L.) and biomass yield losses.

In an attempt to produce salt tolerant potato plants, rapidly growing embryogenic calli were produced *in vitro* in various concentrations of 2, 4-D (T_1 media) and NAA (T_2 media). Different explants were used to evaluate callus formation and somatic embryogenesis formation on potato. Friable embryogenic calli which induced from leaves was 85.15% were formed in the T_I medium contained 2,4-D at 4 mg/l. These calli grew fast in comparison to embryogenic calli formed in T_{II} medium. Transformation was achieved with *pABI* plasmid which contained the barley *HVA1* (*Hordeum vulgaris* abundant protein) and the herbicide resistance (*bar*) genes using gene gun. After growth and selection, the plantlets (two plantlets grew on regeneration media supplemented with 3 mg/ml bialophous) were divided into small pieces and cultured on multiplication media contained BAP at 0.25 mg/l. The explants were subjected to salt stress by the addition of zero, 1000, 3000, 5000 and 7000 ppm NaCl: $CaCl_2$ to MS culture medium. Survival percentages was measured after four weeks for the two subcultures from starting salt treatments as well as shoot number, shoot length and leaves number. The survival percentages in the transformed plants were higher under salt stresses which were recorded 100% for all explants. Moreover, the growth of the transformed explants was more healthy than the other non-transformed explants. The polymerase chain reaction (PCR) and Southern blot hybridization confirmed the integration, of the transgenes.

ACKNOWLEDGEMENTS

This work was supported in part by Agriculture Genetic Engineering Research Institute (AGERI), ARC and Moshtohor Research Park, Molecular Biology Lab., Banha University, Egypt.

REFERENCES

Ahloowalia, B. S. (1982). Plant regeneration from callus culture in potato. Euphytica., 31: 755-759.

Alexeenko, T. V. and N. M. Irkaeva (1998). Investigation of callus formation and shoot-inducing processes in strains and F_1 interstrain hybrids of strawberry *Fragaria vesca* L. Genetika., 34: 1100-1105.

Ashakiran, K., V. Sivankalyani and M. Jayanthi (2011). Genotype specific shoots regeneration from different explants of tomato (*Solanum lycopersicum* L.) using TDZ. Asian J. Plant Sci. Res., 1: 107-113.

Askari, A. and A. Pepoyan (2015). Increasing salinity tolerance in three potato (*Solanum tuberosum* L.) cultivares by transferring *mtlD* gene. IJBPAS., 4: 1013-1019.

Bahieldin, A., H. T. Mahfouz, H. F. Eissa, O. M. Saleh, A. M. Ramadan and I. A. Ahmed (2005). Field evaluation of transgenic wheat plants stably expressing the *HVA*1 gene for drought tolerance. Physiologia Plantarum, 123: 421-427.

Bajaj, Y. P. S. (1987). Biotechnology and 21st century potato, In: Biotechnology in Agriculture and Forestry, Vol. 3: Potato, pp. 522, Springer-Verlag, Berlin, Heidelberg, New York, London, Paris, Tokyo.

Bohorova, E. N., B. Luna, M. R. Brito, D. L. Huerta and A. D. Hoisington (1995). Regeneration potential of tropical, subtropical, mid-altitude, and highland maize inbreeds. Maydica., 40: 275-281.

Carceller, M. and A. D. Ambrogio (1994). Growth and differentiation of callus cultures of sunflower *Helianthus annuus* L. in response to NaCl and proline. Phyton Buenos Aires, 55: 115-122.

Checker, V. G., A. K. Chhibbar and P. Khurana (2012). Stress-inducible expression of barley *HVA1* gene in transgenic mulberry displays enhanced tolerance against drought, salinity and cold stress. Transgenic Res., 21: 939-957.

Cullins, C. A. (1991). Breeding for resistance to physiological stress. In: Advanced Methods in Plant Breeding and Biotechnology, CAB International Redwood Press. UK, p. 340-351.

Decima, O. C., G. González and D. Lewi (2010). Biolistic maize transformation: Improving and simplifying the protocol efficiency. African Journal of Agricultural Research, 5: 3561-3570.

Delaney, F. J., J. M. Fletcher, S. E. Maxwell and P. Satz (1989). A structural model for developmental changes in the determinants of reading achievement. Journal of Clinical Child Psychology, 18: 44-51.

Dure, L. (1993). Structural motifs in lea proteins. Curr. Topics Plant Physiol., 10: 91-103.

Dure, L., M. Crouch, J. Harada, T. H. Ho and J. Mundy (1989). Common amino acid sequence domains among the LEA proteins of higher plants. Plant Mol. Biol., 12: 475-486,

Goday, A., A. B. Jensen, Macià F. A. Culiáñez, M. Albà, M. Figueras, J. Serratosa, M. Torrent and M. Pagès (1994). The maize abscisic acid-responsive protein Rab17 is located in the nucleus and interacts with nuclear localization signals. Plant Cell, 6: 351-60.

Gonzalez, J. M., E. Friero and N. Jouve (2001). Influence of genotype and culture medium on callus formation and plant regeneration from immature embryos of *Triticum turgidum* cultivars. Plant Breeding, 120: 513-517.

Hong, B., R. Barg and T. H. Ho (1992). Developmental and organ-specific

expression of an ABA- and stress-induced protein in barley. Plant Mol. Biol., 18: 663-674.

Kreike, C. M., J. R. Koning and F. A. Krens (1990). Non-radio-active detection of single-copy DNA-DNA hybrids. Plant Mol. Rep., 8: 172-179.

Kwapata, K., T. Nguyen and M. Sticklen (2012). Genetic Transformation of Common Bean (*Phaseolus vulgari*s L.) with the Gus Color Marker, the Bar Herbicide Resistance, and the Barley (*Hordeum vulgares* L) *HVA1* Drought Tolerance Genes. International J. of Agronomy, 2012: 1-8.

Lal, S., V. Gulyani and P. Khurana (2008). Over expression of *HVA1* gene from barley generates tolerance to salinity and water stress in transgenic mulberry (*Morusindica*). Transgenic Res., 17: 651-663.

Lee, Y. P., S. H. Kim, J. W. Bang, H. S. Lee and S. S. Kwak (2007). Enhanced tolerance to oxidative stress in transgenic tobacco plants expressing three antioxidant enzymes in chloroplasts. Plant Cell Rep., 26: 591-598.

Liu, Y., Y. Zheng, W. Wang and R. Li (2010). Soybean PM2 protein (*LEA3*) confers the tolerance of *Escherichia coli* and stabilization of enzyme activity under diverse stresses. Curr. Microbial., 60: 373-378.

Mahajan, S. and N. Tuteja (2005). Cold, salinity and drought stresses: an overview. Arch. Biochem. Biophys., 444: 139-58.

Maqbool, B. S., H. Zhong, Y. El-Maghraby, A. Ahmed, B. Chai, W. Wang, R. Sabzikar and B. M. Sticklen (2002). Competence of oat (*Avena sativa* L.) shoot apical meristems for integrative transformation, inherited expression, and osmotic tolerance of transgenic lines containing *Hva1*. Theor. Appl. Genet., 105: 201-208.

Maxwell, S. E. and H. D. Delaney (1990). Designing experiments and analyzing data: A model comparison perspective. Brooks/Cole Publishing; Pacific Grove, CA.

McCormick, S., J. Niedermeyer, A. Fry, R. Bamason, R. Horsch and R. Fraley (1986). Leaf disc transformation of cultivated tomato (*L. esculentum*) using *Agrobacterium tumefaciens*. Plant Cell Rpt., 5: 81-84.

Moghaieb, R., H. Saneoka and K. Fujita (1999). Plant regeneration from hypocotyl and cotyledon explant of tomato (*Lycopersicon esculentum* Mill.). Soil Sci. Plant Nutr., 45: 639-646.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 15: 473-497.

Nguyen, T. and M. Sticklen (2013). Barley *HVA1* gene confers drought and salt tolerance in transgenic maize (*Zea Mays* L.). Adv. Crop Sci. Tech., DOI: 10.4172/2329-8863.1000105.

Oh, S. J., S. I. Song, Y. S. Kim H. J. Jang and S. Y. Kim (2005) *Arabidopsis* CBF3/ DREB1A and ABF3 in transgenic rice increased tolerance to abiotic stress without stunting growth. Plant Physiol., 138: 341-351.

Oraby, H. F., C. B. Ransom, A. N. Kravchenko and M. B. Sticklen (2005) Barley *HVA*1 gene confers salt tolerance in R3 transgenic oat. Crop Sci., 45: 2218-2227.

Reda, E. A., H. Moghaieb, I. Saneoka and F. Kounosuke (1999). Plant regeneration from hypocotyl and cotyledon explant of tomato (*Lycopersicone esculentum* Mill.). Soil Sci. Plant Nutr., 45: 639-646.

Rohila, J. S., R. K. Jain and R. Wu (2002). Genetic improvement of Basmati rice for salt and drought tolerance by regulated expression of a barley *HVAl* cDNA. Plant Science, 163: 525-532.

Shibli, R. A., M. A. Shatnawi and I. Q. Swaidat (2003). Growth, osmotic adjustment, and nutrient acquisition of bitter almond under induced sodium chloride salinity *in vitro*. Communications in Soil Science and Plant Analysis, 34: 1969-1979.

Siviamani, E., A. Bahieldin, J. M. Wraith, T. Al-niemi, W. E. Dyer, T. D. Ho and R. Qu (2000). Improved biomass productivity and water use efficiency under water deficit condition in transgenic wheat constitutively expressing the barley *HVA*1 gene. Plant Science, 155: 1-9.

Somerville, C. R. and E. M. Meyerowitz (2002). Abscisic acid biosynthesis and signaling. In: The *Arabidopsis* Book, Rockville M. D., (Eds.). American Society of Plant Biologists.

Unesco, Water Portal (2007). http://www.unesco.org/water.

Stiekema, W. J., E. de Vries-Uijtewaal, L. J. W. Gilissen, E. Flipse, Ramulu K. Sree and B. de Groot (1988). Fate of introduced genetic markers in transformed root clones and regenerated plants of monohaploid and diploid potato genotypes. Theor. Appl. Genet., 78: 185-193.

Vitagliano, C., A. Minnocci, L. Sebastiani, A. Panicucci and G. Lorenzini (1992). Physiological response of two olive genotypes to

gaseous pollutants. Acta Hort., 474: 431-434.

Wang, W., B. Vinocur and A. Altman (2003). Plant responses to drought, salinity and extreme temperatures: Towards genetic engineering for stress tolerance. Planta, 218: 1-14.

Wareh, H. A., N. L. Trolinder and J. R. Gooding (1989). Callus initiation, shoot regeneration, and micropropagation of three potato cultivars. Hortscience, 24: 680-682.

Watanabe, S., K. Y. Kojima and S. Sasaki (2000). Effects of saline and osmotic stress on proline and sugar accumulation in *Populus euphratica in vitro*. Plant Cell Tissue and Organ Culture, 63: 199-206.

Xu, D., X. Duan, B. Wang, B. Hong, T. Davidho and R. Wu (1996). Expression of a late embryogenesis abundant protein gene, *HVA*1, from barley confers tolerance to water deficit and salt stressing transgenic rice. Plant Physiol., 110: 249-257.

Yadav, N. R. and M. B. Sticklen (1995). Direct and efficient plant regeneration from leaf explants of (*Solanum tuberosum* L.) cv. Bintje. Plant Cell Rep., 14: 645-647.

Table (1): Effect of various concentrations of 2,4-D and NAA on callus formation and somatic embryogenesis formation in (*Solanum tuberosum*) 'Sponta" potato cv.

Treatments		Stem		Leaves		Roots	
		Callus %*	SEF %**	Callus %*	SEF %**	Callus %*	SEF %**
T_I media	Control	3.70 ^{d}	0.00 ^{b}	1.11 ^{d}	0.00 ^{c}	3.70 ^{c}	0.00 ^{e}
	2,4-D 2 *mg*/l	22.22 ^{cd}	04.81 ^{b}	22.22 ^{bcd}	27.77 ^{b}	2.59 ^{c}	1.11 ^{de}
	2,4-D 3 *mg*/l	88.88 ^{a}	18.51 ^{ab}	88.88 ^{a}	85.18 ^{a}	49.99 ^{ab}	14.81 ^{a}
	2,4-D 4 *mg*/l	55.55 ^{abc}	40.73 ^{a}	96.29 ^{a}	11.48 ^{bc}	30.73 ^{abc}	04.44 ^{cde}
	2,4-D 5 *mg*/l	74.07 ^{ab}	03.70 ^{b}	40.73 ^{bc}	22.22 ^{bc}	33.33 ^{abc}	07.41 ^{bcd}
	2,4-D 10 *mg*/l	22.22 ^{cd}	18.51 ^{ab}	22.22 ^{bcd}	37.03 ^{b}	11.11 ^{c}	01.48 ^{cde}
T_{II} media	NAA 2 *mg*/l	44.43 ^{ad}	27.03 ^{ab}	85.18 ^{a}	1.85 ^{c}	22.22 ^{bc}	8.51 ^{abc}
	NAA 3 *mg*/l	66.66 ^{abc}	11.11 ^{b}	40.74 ^{bc}	24.07 ^{bc}	55.55 ^{ab}	12.22 ^{ab}
	NAA 4 *mg*/l	55.55 ^{abc}	4.80 ^{b}	37.03 ^{bc}	4.81 ^{c}	62.95 ^{a}	0.37 ^{de}
	NAA 5 *mg*/l	62.62 ^{abc}	3.70 ^{b}	48.14 ^{b}	0.74 ^{c}	29.62 ^{abc}	1.11 ^{de}
	NAA 10*mg*/l	37.03 ^{bcd}	1.11 ^{b}	14.81 ^{cd}	0.37 ^{c}	27.77 ^{abc}	1.11 ^{de}
LSD 5%		48.839	29.401	32.642	31.166	38.294	7.323

*Callus Formation% and **Somatic Embryogenesis Formation%.

Table (2): Effect of various concentrations of BAP and Kin on somatic regeneration of 'Sponta" potato cv. (*Solanum tuberosum*).

Cytokinines mg/l	Survival %	Regeneration %
Control	100.0	0.0
RT_{I} media		
BAP 1 *mg*/l	66.66	0.0
BAP 2 *mg*/l	33.33	2.22
BAP 3 *mg*/l	100.0	33.33
RT_{II} media		
Kin 1 *mg*/l	3.330	0.0
Kin 2 *mg*/l	66.66	0.0
Kin 3 *mg*/l	33.33	2.22
LSD 5%	6.433	1.433

Table (3): Effect of salinity treatments on growth of transgenic explants and non-transgenic explants on 'Sponta" potato cv. (*Solanum tuberosum*) during two subcultures.

Salinity Treatments ($NaCl: CaCl_2$)	First subculture							
	Non-transgenic explants				Transgenic explants			
	Survival %	Shoot No.	Shoot Length	Leaves No.	Survival %	Shoot No.	Shoot Length	Leaves No.
Control	100.00 ^{a}	3.77 ^{a}	2.34 ^{ab}	1.18 ^{d}	100	3.10 ^{a}	5.12 ^{ab}	3.25 ^{d}
1000 ppm	55.55 ^{b}	2.16 ^{b}	2.90 ^{ab}	3.45 ^{bc}	100	3.51 ^{a}	6.95 ^{a}	3.40 ^{d}
3000 ppm	48.14 ^{b}	1.33 ^{bc}	2.37 ^{ab}	6.22 ^{a}	100	2.88 ^{ab}	3.30 ^{b}	8.77 ^{a}
5000 ppm	40.73 ^{b}	1.33 ^{bc}	3.32 ^{a}	4.66 ^{b}	100	1.66 ^{bc}	6.82 ^{a}	5.71 ^{c}
7000 ppm	18.51 ^{b}	1.16 ^{c}	1.93 ^{b}	2.84 ^{c}	100	1.36 ^{c}	4.53 ^{b}	7.08 ^{b}
LSD 5%	38.349	0.871	1.165	1.511	NS	1.4145	1.827	1.294
Second subculture								
Control	37.03	4.40 ^{a}	20.92 ^{a}	9.97 ^{a}	100	1.52 ^{d}	19.27 ^{a}	7.22 ^{a}
1000 ppm	11.51	1.92 ^{c}	6.83 ^{b}	3.48 ^{b}	100	5.29 ^{a}	11.18 ^{b}	6.58 ^{a}
3000 ppm	18.51	1.21 ^{d}	6.18 ^{b}	1.11 ^{c}	100	3.10 ^{b}	11.28 ^{b}	1.38 ^{b}
5000 ppm	12.21	2.66 ^{b}	5.52 ^{b}	4.14 ^{b}	100	2.12 ^{cd}	6.11 ^{c}	1.58 ^{b}
7000 ppm	22.18	1.22 ^{d}	2.10 ^{c}	10.29 ^{a}	100	2.25 ^{c}	1.51 ^{d}	2.03 ^{b}
LSD 5%	NS	0.2596	2.267	0.9696	NS	0.6314	1.1373	0.9129

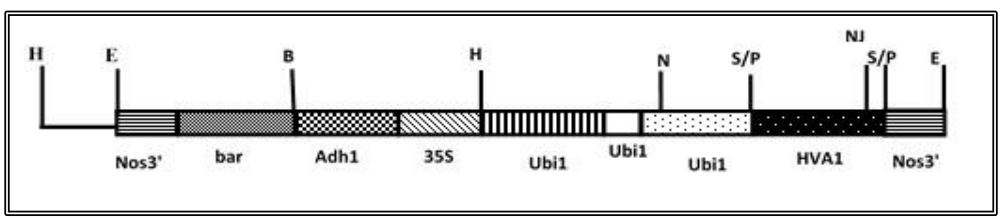

Fig. (1): Plasmid map of *pAB1* transformation vector. Abbreviations for the restriction sites: B: *BamHI*, P: *Pst1*, S: *Sal1*, E: *EcoRI* H: *HindIII* and N: *Ncol*.

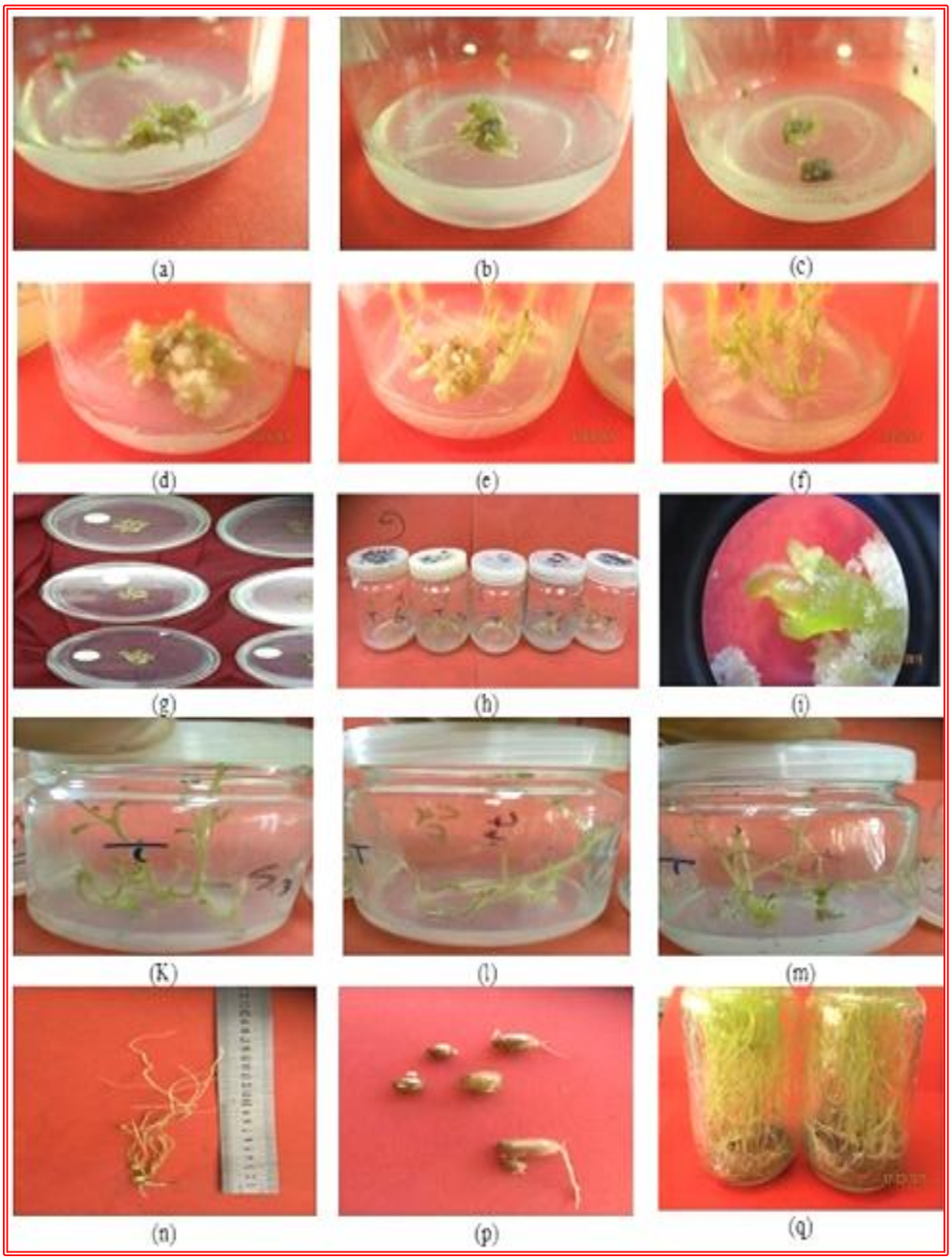

Fig. (2): [a, b, c] Callus formation from various types of explants (stem, leaf and root) , [d] organogenesis callus , [e] regenerated shoot lets from callus, [f] shoot proliferation, [g] somatic embryos on MS media containing a 40 g/l of mannitol before shooting, [h, i] somatic regeneration and shoots formation, [k, l, m] transformed plantlets,[n, p] microtubers formation after incubation periods for 40 days, [q] transformed regenerated plants.

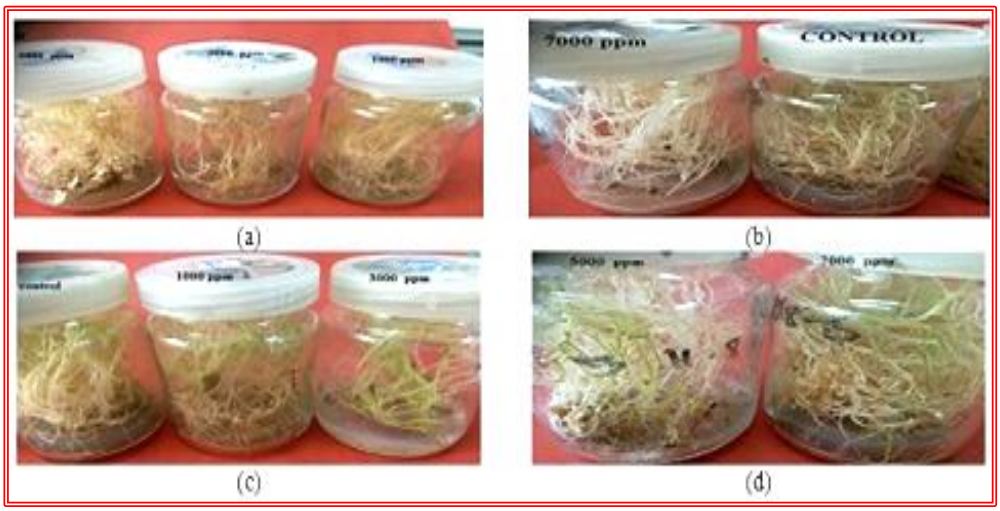

Fig. (3): [a, b] Non-transgenic explants culture on MS medium containing NaCl: $CaCl_2$ at 0.0, 1000, 3000, 5000 and 7000 ppm; [c, d] Transgenic explants cultured on the same medium and concentrations.

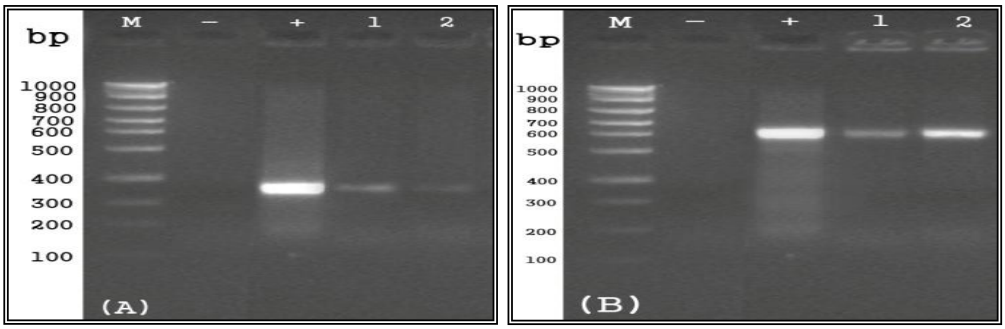

Fig. (4): PCR amplified DNA using the bar gene specific primers (A) and the *HVA1*gene primers (B) untransformed potato plants (-), *pABI* plasmid (+) and putatively transformed potato plants (1-2), (M) 100 bp DNA ladder marker.

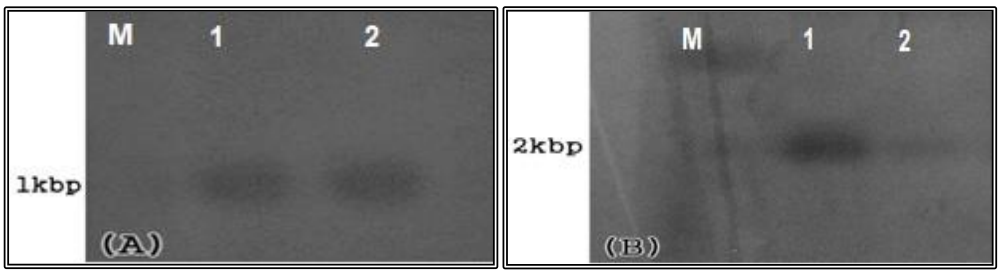

Fig. (5): DNA hybridization analysis of putatively transformed potato plants for the *HVA1* gene (A) and the *bar* gene (B). Lanes (1-2): putatively transformed potato plants. (M): 100 bp DNA ladder marker.