Production of potato microtubers by using tissue culture techniques

Alaa El-din Sayed Ewase*

Ali M. Blieblo**

Abstract

Production of potato microtubers was reviewed here. Microtubers production is an efficient method for obtaining a healthy material, through which the process of potato production is reduced with 3-4 years. In the same time, microtubers have a great importance because they could be produced in any period of the year, they are easy to be transported , handle, store and distribute. Also, their use is rapidly increasing world wide. Many chemicals are used for this process like 2,4-D, Coumarin, Kinetin and others under certain conditions of light and temperature , high sucrose content (90 g/l as a maximum) and through tissue culture techniques.

Key words

Potato – Microtubers – Tissue culture – Coumarin – 2,4-D- Kinetin. High sugar content.

Potato and its cultivated and wild relatives are classified in *Solanum L.* sect. *Petota* Dumort., which contains over 230 species **(Hawkes 1990)** or 199 species in the last taxonomy by **Spooner** and **Hijmans (2001).** These are further grouped into 21 taxonomic series **(Hawkes 1990)**, **(Nakagawa and Hosaka, 2002).**

* Botany department, Faculty of Science, Sirte University, Libya.
** Botany department, Faculty of Science, Benghazi University, Libya.

In addition to wheat, maize, and rice, potato (*Solanum tubersoum L.*) is the most important non-cereal world food plant, and considered to be the fourth important crop in the world , **(Khodeir 2005).**

Potato tuber is a source of food in almost every nation in the world. Potato is one of the most important vegetable crops grown in a large scale in Libya. In 2011, The Libyan production of potato occupied the first rank among all yields with a production of 352002 metric ton compared with potato production in the year of 2000 which was 190000 metric ton, **(FAO, 2013)** .

Potato tubers are important sources of the different substances such as carbohydrates, vitamins and trace elements important for human nutrition and for industrial processing, **(Marmiroli, 2000).**

Tubers contain around 75-80% water, 16-20% carbohydrates, 2.5-3.2 % crude protein, 0.8-1.2 % mineral nutrients, 0.1-0.2 % crude fats 0.6 % crude fiber and some vitamins **(Hooker, 1981).**

Potato is grown in a wide variety of climates and humidity ranges. Potato is from the economic point of view clearly an important food crop not only in terms of production but also in terms of nutrition and rural income. Also, it is considered as a good source of carbohydrates than any other food crop. However, its vulnerability to widely distributed diseases makes potato the heaviest user of chemical pesticides among the major crops **(Raman, 1994).**

The center of origin of potato in the high region of Anden in Lima, Peru, where the earliest attempts at cultivation were probably made 18 centuries B. C. **(Burton 1989).**

Between 1950 and 1998 potato production area increased at low latitudes and decreased at high latitudes, particularly around 53ºN (this zone includes parts of Belarus, Germany, Poland, Russia and Ukranie). The northern limit of potato production coincides with the boundaries of agriculture and the presence of human population. The peak between 23ºN and 34ºN coincides with the area of highest population density (per area of land and per area of arable land). About 25% of the global potato area is the highlands (above 1000 m), **Hijmans, 2001**.

Microtubers have a greet importance, and their use is rapidly increasing world wide, they are easier to handle, store and distribute **(Dodds, 1988,Wiersema *et al.*, 1987, Rosell *et al.* 1987).**

Microtuber production is an efficient method for obtaining a

healthy material, through which the process of potato production is reduced with 3-4 years. In the same time the microtubers are important because they can be produced in any period of the year, they are

easy to be transported and stored, **Nistor** et ***al.*** **(2010)**.

In addition, the use of tissue culture materials (microplants or microtubers) was estimated to be 26% less costly than that of seed tubers **(Wattimena, 1983).**

The use of 10 mg/l BA in the medium stimulated tuber development in 10 varieties for *in vitro* tuberization, **Kostrica *et al.*, (1985) .**

With the increased demand for healthy potao stock materials in Egypt, research need to be directed to maximize the technique of *in vitro* tuberization, at least to minimize the amount of imported seed potato and

to introduce these new materials to growers.However, methods of *in vitro* tuberization have not been well-defined, **Mohamed *et al.*, (1992).**

Mahfouze *et al.* (2012) showed that recently, plant tissue culture technology has become very popular and has a visible impact on the production of virus free pre-basic seed potatoes.

The practical use of *in vitro* tubers was realized in the early eighties with a view to use as natural for international exchange of germplasm, especially to those countries where the expertise for handling *in vitro* plantlets is not available or to help transport germplasm under adverse conditions such as continuos darkness and variable temperatures and to use *in vitro* tubers for medium term.

The endogenous concentration of different cytokinins varies in different parts of the potato plants, **palmer** and **Smith (1969)** , **(1970)** and **Smith** and **palmer (1970).**

Forsline and **Langille (1975)** have confirmed the presence of four compounds with cytokinin activity in the potato plant and reported that the concentration of them changed in proportion to variation of environmental conditions such as day length and temperature which directly regulate the tuberization process.

Palmer and **Smith (1969)** for the first time reported that the cytokinin requirement in the tuberization of isolated stolons *in vitro* 2.5 mg /L Kn (6-furfuryl amino Purine) was more effective for tuber induction than other cytokinin.

Kn and SD 8339 [6-benzylamino-9-2 (tetra hydro propan-2-yl)-9H Purine] at all concentrations (0.25,2.2 and 25.0mg/L) and N 6

benzyladenine (BA) at 0.25 and 2.5mg/L induced 80-100% tuber formation in 90 days, although at 25 mg/L BA induced only 40% tuberization. They also found that no tuber initiation in cultures without cytokinin in the medium was observed.

Smith and **palmer (1970)** also reported that Kn was effective in stimulating starch accumulation in excised potato stolons grown *in vitro.*

Palmer and **smith (1970)** further found that Kn ($1.6x10^{-2}$ μM) induced 100% tuber formation when sucrose at 6 to 10% was present in the medium, however, sucrose without the presence of Kn did not induce tuberization reduced temperature was partially inhibitory to the Kn induced response a maximum of 50% tuber formation occurred on stolons incubated at 15°C for 30 days in the presence of Kn only. The response of Kn varied at different temperatures of incubation.

Palmer and **Barker (1972)** also reported that Kn 2.0mg/L induced tuberization at18°C in darkness.

In the presence of Kn the rate of shoot elongation declined substantially after 7 days, which coincided with onset of tuber initiation.

Stallknecht (1972) reported that Kn (2.5mg/l) supplemented to the medium induced smaller tubers than Coumarin induced tubers.

Mingo-Castel *et al.*, (1976) found that 5 mg/L Kn in the medium induced *in vitro* tuberization within 30 days in culture with 94% efficiency compared to 61% in the absence of Kn, the sucrose concentration was 6% in both.

Kin (1982) stated that 2.5mg /L BA in the medium induced tuberization, however, BA suppressed the effect of ABA on tuber induction. Zeatin did

not stimulate *in vitro* tuberization even when its concentration was increased (10^{-5} M). However it induced an increase in fresh weight of tubers when combined with high sucrose concentration above 4%, **Koda** and **Okasawa (1983).**

Mangat *et al.*, (1984) found that the degree of tuberization on stem segments cultured in media containing 10^{-7} M and 10^{-6} M 2,4-D was higher than the control stem segments. Mainly long stolons were observed on stem segments cultured in media containing 10^{-4} M and 2 x 10^{-3} M 2,4-D.

Mitten *et al.*, (1988) reported that using 2ip in the tuberization medium resulted in the greatest number of microtubers being initiated from single node cutting cv**.** Altanic and Norchip.

Mohamed *et al.*, (1992) found that there is a significant increase in the number and fresh weight of the harvested microtubers were obtained with the combinations 5mg/l BA + 6% sucrose or 5mg/l kinetin +9% sucrose. 3% sucrose reduced the number , weight and size of microtubers regardless of the cytokinin treatments. Sprouting was observed in many of the microtubers and increased more with higher KIN concentration.

Simko (1993) stated that kinetin alone at 2.5 mg/litre had no significant effect on tuber formation but significantly enhanced tuberization when applied with 0.001 mg/litre paclobutrazol. Paclobutrazol alone stimulated early tuber initiation and inhibited stem growth.

Leclerc *et al.*, (1994) stated that the layered shoots microtuberized more rapidly and produced significantly larger microtubers compared with nodal cuttings. The addition of coumarin or (2-chloroethyl)-trimetylammonium chloride and benzyladenine to microtuberization

medium either had no effect or significantly reduced microtuber weight per shoot compared with medium containing only 80 g/litre sucrose and minimally affected the number of microtubers per shoot. Increasing the incubation period from 28 to 56 days did not affect the number of microtubers but significantly increaesd the weight of microtubers per shoot and substantially increased the proportion, up to 20%, of microtubers heavier than 1g.

Khuri and **Moorby (1995)** found that a medium concentration of about 400mM with only sucrose was more suitable for microtuber production than media supplemented with maltose, glucose or fructose. However, a better microtuber yield was obtained when hexoses were added than with unsupplemented 4% sucrose media. When glucose was supplied at concentrations which had the same number of carbon atoms as 8% sucrose, the high osmolarity inhibited microtuberization. Therefore, it is cocluded that sucrose acts primarily as a suitable carbon source for uptake and utilization by the plantlets , but, at 8%, it also provides a favourable osmolarity for the development of microtubers.

Dobranszki (1996) demonstrated that tuberization was induced after culturing shoots for 4 weeks under long days. The dark treatment (11-12 weeks), applied after short days periods of 3-14 days, accelerated and synchronized tuber initiation and development and increased the number of tubers per plant. The best results were obtained when the dark period was continous.

Anjum and **Villiers (1997)** reported that the addition of BA (22.19 µM) to the medium speeded up the tuberization process, increased averaghe weight/tuber in *S.tuberosum* but reduced tuber number in both *S.tuberosum* and *S.commersonii*. When 2,4-D (2.26 µ M) was also added

to this medium, it further enhanced the tuberization process, increased the percentage of segments producing microtubers in *S.tuberosum* and *S.acaule* and also increased average weight/tuber in *S.tuberosum* with slight improvement in tuber number.A culture medium containing both BA and 2,4-D proved more successful for *in vitro* tuberization.

Sarkar and **Naik (1998)** demonstrated that reducing the total nitrogen supply increased the number but decreased the size of microtubers. The total availability of potassium in the medium influenced the effect of reduced nitrogen level on the rate of assimilate partitioning (harvest index) during cytokinin-induced microtuberization.

Dobranszki *et al.*, (1999) mentioned that light applied after the tuber induction phase delayed or inhibited tuber initiation (at proper photoperiods both at 111 and 55 umol m^2 $s\imath^1$ light intensities at 24/15°C day/night temperature). Darkness following the induction stage accelerated and synchronized tuber initiation after high light intensity.

Pelacho *et al.*, (1999) found that the addition of these organic acids (acetic, proponic, ascorbic, acetylsalicylic or salicylic acid) caused tuberization. The results with the acetic, proponic and ascorbic acids show that *in vitro* hormone-free tuberization can be easily and rapidly achieved.

Konstatinova *et al.*, (1999) reported that six potato cultivars were cultured on media with 0 or 1 mg IAA or kinetin/litre. IAA and kinetin increased with average yield of microtubers 1.5 to 2 times by weight compared with medium without growth regulators. IAA increased tuber size.

Romanov *et al.*, (2000) demonstrated that concerning microtubers production in seven different potato cultivars : auxin and cytokinin acted differently : IAA increased predominantly the the tuber size while kinetin increased the number of tubers and the degree of phytohormone effect on tuberization parameters depended on sucrose content of the medium and potato genotype.

Cui *et al.*, (2001) had cultured virus free potato varieties Jinguan and Mira *in vitro* on MS medium supplemented with 10% ordinary refined sugar or analytically pure sucrose under 0, 8 and 12-h photoperiods at ambient temperature (20±2°C). The results showed that culture in the dark was more favorable for the formation of microtubers, and subsitutuion of ordinary refined sugar or analytically pure sucrose as the carbon source did not affect microtuberization. It is, recommended that ordinary refined sugar be used in potato microtuber production to reduce cost.

Khodeir (2002) reported that best treatment for the production of potato microtubers was (0.1 mg/l 2,4-D), which gave an average weight of microtubers (411.8 mg), and number of microtubers per plant was (1.2), compared with control (342.6 mg and 0.6) and with Kinetin in all of its concentrations (1, 3 and 5mlg/l) which gave (344, 370 and 396.8 mg, and 0.73, 0.8 and 0.93 microtuber per plant respectively).

Sidikou *et al.*, (2003) stated that that tuberization frequency increased with increasing concentrations of BA and sucrose . In the basal medium (10% sucrose without BA), the best tuberization was showed by cultivars Kennebic (80%), Desiree (75%), Spunta and Pamina (60%). The best microtuber yields were observed on Kennebic Spunta, Aida and Pamina.

Seabrook *et al.*, (2004) have excised single-node cuttings and grown them in high-sucrose tuberization medium in darkness. They demonstrated that leaf area did not affect the frequency, size, or weight of microtubers of cvs. Katahdin and Russet Burbank.The absence of leaves reduced microtuber diameter for Russet Burbank; wheeas Atlantic, Kennebec and Shepody were unaffected. Mean fresh weight ofr microtubers was reduced when leaves were removed for all cvs except Atlantic. No effect of the removal of the leaf was observed for mean dry weights of microtubers from all cvs, although microtubers from single-node cuttings without leaves accumulated significantly more percent dry matter than those with leaves.

Rafique *et al.*, (2004) stated that sucrose at 6% and BAP at 1 micro M recorded the maximum number of microtubers. Sucrose and BAP had also significant ($P<0.05$) effect on shoot and root lengths. MS medium supplemented with sucrose and BAP significantly induced microtubers in the 4 potato cultivars : Santa, Cardinal, Diamant and Desiree.

Paul *et al.*, (2004) had obtained potato microtubers weighing 0.5-1.0, 1.0-3.0 or 3.0-5.0 g, produced from desprouted (during storage) seed tubers of 6 cultivars (Kufri Chandramukhi, Kufri Jyoti, Kufri Bahar, Kufri Badshah, Kufri Lalima and Kufri Sindhuri).

Gopal *et al.*, (2004) , stated that Higher concentrations (60-80 g litre-1) of sucrose promoted biomass production, microtuber production and microtuber dry matter content in 3 potato cultivars (Kufri Badshah, Kufri Chandramukhi and Kufri Sindhuri).

KabSeog and **Leung (2004)** found that Upon transfer to a medium with an optimized level of sucrose (i.e. 8%, w/v) for in vitro tuberization, only

the plantlets previously grown in the sucrose-containing medium were capable of forming more microtubers of larger size (greater than 0.5 g).

Khodeir (2005) used Both virus-free and transformed potato plants were for microtubers production with different concentrations of 2,4-D . He found that the best treatment for microtubers production is that of (0.5) mg/l 2,4-D which gave 2.5 microtuber per plant with an average weight of 627 mg. While if the concentration of 2,4-D increases, both the number of microtubers per plant and their average weight , will decrease, until they reach 0.47 microtuber per plant with an average weight of 248 mg. Also, **Khodeir (2005)** studied the effect of silver thiosulphate (STS) on tuberization. He found that in case of the addition of 2mg/l silver thiosulphate (STS), both the number of microtubers per plant and their average weight decreased in comparative with the control treatment , where, the number of miocrotubers per plant was 0.75 and their average weight was 308 mg. So STS decreased microtubers yield, this is may be because of STS promote the total plant state (stem and leaves specially), and not microtubers.

EL-Sawy *et al.* (2007) found that sucrose is an important factor for micro-tubers formation. The highest tuber formation was achieved when 12% sucrose was added to culture medium. Among three tested levels of coumarin, 20 and 40 mg/L were more effective regardless of sucrose concentration. Concerning the potential of combination of sucrose and coumarin the highest percentage of tuberization (86.7%) was recorded with 6% sucrose + 20 mg L-1 coumarin. The highest number of microtubers per plantlet was detected when 9% sucrose was added to coumarin - induced medium. For molecular analysis, results refer that although microtubers derived plants of potato expressed a high degree of

polymorphism relatively to field-grown source, they were genetically similar. Slight molecular genetic differences were detected in plantlets derived from micro-tubers.

Naik et *al*.(2008) studied Genetic parameters, character association and path analysis for yield components of microtuber production in vitro and their field performance were studied in 37 potato (Solanum tuberosum L.) genotypes. Among the microtuber yield components, average microtuber weight had maximum genotypic (or phenotypic) coefficient of variation, heritability and predicted genetic advance; however, the estimated values of these genetic parameters were maximum for tuber yield among the field yield components. The heritability estimates of field yield components were higher than that of their corresponding in vitro yield components of microtuber production. The highest correlation coefficients between average microtuber weight and microtuber yield suggested that microtuber weight was more important than microtuber number in determining microtuber yield potential in vitro. However, tuber number was found to be more important than tuber weight in determining tuber yield potential under field conditions. Average microtuber weight had maximum direct effect on microtuber yield, whereas tuber number had maximum direct effect on tuber yield under field conditions. The study showed that the relative importance of the components of microtuber production in vitro differed from that of corresponding field yield components. The expression of a genotype for microtuber production in vitro is different from that of tuber production under field situation i.e. the performance of a genotype in vitro is not a measure of its field performance

Hoque (2010) stated that MS medium supplemented with 4 mg/L of KIN showed best performance in respect of multiple shoot regeneration and microtuber formation. Simple MS medium was not able to produce

any micro tuber under in vitro condition. Dark condition better responded to tuberization than light condition. Among the three different explants (nodal segment, sprout and shoot apex) nodal cutting showed the best performance on days to microtuber formation and average weight of microtuber. MS + 6% sucrose + 4 mg/L KIN combination of treatment was best for in vitro tuberization among the parameters under study.

Nistor *et al*. (2010) had obtained microtubers of semi-early Romanian varieties (Christian and Roclas) and of early Dutch variety (Ostara) from potato micro-cutting cultures on Murashige-Skoog medium enriched with Cumarin and Kinetin. The sucrose was the most important stimulus for inducing the microtubers. Microtuber inducing and growing was achieved in cultures maintained in darkness, at 18- 20° C for 8-10 weeks.

Lê and **Thomas (2010)** demonstrated that The *in vitro* production of potato microtubers was carried out through several stages: at first microplant growth, then initiation of stolon followed by tuber formation (or tuberization). During the last stage, it is possible to develop high quality microtubers in terms of weight and size. However, after examination, the quality of plant material produced *in vitro* is so far better when the duration of tuberization is extended over 16 weeks culture.

Bolandi et *al*., (2011) studied sprouting potential and functions of microtubers gained from two commercial potato cultivars Agria and Marfona which categorize in three size groups <5mm, 5-10mm and >10mm which all had gone to dormancy for 3-5 months. Factorial

experiment using completely randomized block design in three replications. Sprouting, nonsprouting microtubers percentage along with weight, number and diameter of minitubers were recorded. There seems a significant and positive correlation between minitubers diameter and their sprouting percentage. Microtubers higher size show more efficient functionality than others with thin diameters. Results showed that Marfona with sprouting percentage 56.37% has better functionality in comparison to Agria with 48.87% while Agria has better functionality index. Among studied cultivar, greater diameter microtubers which spent most times in dormancy in comparison to thin diameter microtubers with less time in dormancy, in this research, show superiority in weight, number and minituber diameter.

Nistor *et al*. (2011) had studied The effect of inorganic nitrogen nutrition on production of microtubers in two potato genotypes Nicoleta and Christian. The objective of this study was to investigate whether reduction in total nitrogen level in the Murashige & Skoog medium would improve microtuberization. The effect of three levels of total nitrogen (30, 45 and 60 meq) on tuberization was studied at constant (20 meq K). Reducing the total nitrogen supply increased the number but decreased the size of microtubers. The weight of microtubers per vessel was the highest at the highest nitrogen concentration (60 meq): 1.91 g for Christian variety. A reduction in total nitrogen supply reduced the size of microtubers, the lowest weight being at the lowest nitrogen level – 30 meq: 1.01 g for Nicoleta variety, with a difference of -0.66 g, significant in a negative way. Decreasing the total nitrogen supply caused increasing the number of microtubers/ vessel which was the highest at the lowest nitrogen concentration (30 meq): 19.67 microtubers, with a difference of 4.34 microtubers, significant in a positive way, for Christian variety. The

number of microtubers per vessel was the lowest at the highest nitrogen concentration (60 meq): 15.33 microtubers, for both varieties.

Sharma *et al.*.(2011) studied Micro-tuber production behaviour of six commercially important cultivars of potato (Solanum tuberosum L.) was studied under standard medium and culture conditions using ten double node cuttings per flask. Significant differences were observed among the different potato genotypes for most of the characters during in vitro tuberization. Per cent micro-tuberization and number of stolons per nodal cutting were found to be maximum in Kufri Badshah and minimum in Kufri Pukhraj. Shoots weight/flask was maximum in Kufri Anand, followed by Kufri Badshah and Kufri Pukhraj, whereas, roots weight was higher in Kufri Badshah and minimum in Kufri Surya. The number of micro-tubers/flask was maximum (14.0) in Kufri Anand, which was almost same and statistically at par with three other varieties, viz Kufri Badshah, Kufri Bahar and Kufri Chipsona 1. Micro-tubers were found to be minimum in Kufri Pukhraj (7.5 tubers/flask). Total yield of micro-tubers/flask and harvest index were also maximum in Kufri Anand (3.7g and 0.29 respectively). Average weight of micro-tubers was maximum (0.27g) in Kufri Anand, whereas, tubers were of lighter weight in Kufri Surya (0.114g). The dry matter content of freshly harvested micro-tubers was maximum in Kufri Chipsona 1(19.65%) and minimum in Kufri Anand (14.75%). The proportions of normal shaped micro-tubers were significantly higher in Kufri Badshah (99%), at par with Kufri Chipsona 1, Kufri Bahar, Kufri Anand and minimum in Kufri Pukhraj (79.2%). The proportion of micro-tubers with burst lenticels was maximum in Kufri Pukhraj (43.5%) and minimum in Kufri Badshah (0.2%).

Sherin A Mahfouze *et al*. (2012) produced virus free microtubers *in vitro*, to investigate the stimulating effects of low doses of gamma irradiation on microtuber mean number, mean fresh weight and size. Among the gamma radiation doses tested (1.5, 2, 2.5, 5 and 10 Gy), the 5 and 10 Gy doses gave the highest number of microtubers, had signifi cant effects on microtuber weight increase and also generated the highest size microtubers (180 cm3). Additionally, nine potato unique markers were identifi ed among the 45 polymorphic bands, as analyzed by random amplifi ed polymorphic DNA-polymerase chain reaction profi les, with one marker detected for the 5 Gy gamma radiation dose and none for the 10 Gy. The highest number of markers (4) was obtained with the 2.5 Gy dose.

Motallebi-Azar and Kazemiani (2012) that mannitol was more effective on microtuber related traits than sorbitol. Microtubers length and diameter were minimum with different concentrations of sorbitol. In medium containing mannitol, microtuber fresh weight was significantly decreased with adding mannitol above than 0.11 mol/l. For mannitol, increasing concentration up to 0.11 mol/l had raising effect on eyes number per tuber but further increase in mannitol concentration negatively affected this trait. Eyes growth was decreased when nodal explants were cultured in higher concentrations of either mannitol or sorbitol and this trend shows that alcohol sugars had inductive effects on microtubers dormancy. In total, suitable amounts of sugar alcohols improved the microtuberization and its related traits and may be a feasible practical approach in microtuber production industry.

Kawakami and Iwama (2012) examined the effect of the size of the potato microtuber (MT) produced *in vitro* on the posterior field

performance, we examined the growth and yield of the late maturity cultivar Norin 1 using four sizes of MT: 0.3–0.5 g, 0.5–1 g, 1–3 g and 3–5 g, and conventional seed tubers (CT) (approximately 50 g). The experiment was conducted at Hokkaido University, Sapporo Japan in 1998 and 1999. The tubers were planted in May of each year, in a randomized block design with three replications. Plants from MT lighter than 0.5 g showed a slower initial leaf and tuber growth than heavier MT, but around the full flowering stage there was no significant difference with the MT size in leaf or tuber growth. CT plants showed higher initial leaf and tuber growth compared with MT plants, especially in 1999. No differences in growing period, number of tubers, and tuber fresh and dry yield were observed with the MT size. However, in 1999, the growing period was longer and tuber fresh and dry yields at harvest were higher in CT plants. MT of all sizes used in the study can be used for direct field planting, but more studies are needed to increase the yield
stability of MT plants.

References

Anjum-MA. and Villiers-TA. (1997). Induction of microtubers *in vitro* from stem segments of *Solanum tuberosum L., S.commersonii Dun.* and *S.acaule Bitt.* Scientica Horticulturae.1997, 70: 2-3, 231-235.

Burton, W. G. (1989). The potato, third edition longman Group. U. K . Limited, PP. 1-27, 233-236 .

Cui-Cui; FengFa-He; JiChun-Wang; QingYuan-Zhou; YuanXin-Huang; and DaoBin-Tang. (2001). Effects of photoperiod and carbon sources on the formation of microtubers of potato *in vitro*. Journal of Southwest Agricultural University. 2001, 23: 6, 547-548.

Dobranszki-J. (1996). Effects of dark treatment on tuber initiation and development of induced potato plantlets cultured *in vitro.* Acta Agronomica Hungarica. 1996, 44: 4, 377-486. {c.f. Horticulture abstracts}.

Dobranszki, J; Tabori, K.M and Ferenzy,A.(1999). Light and genotype effects on *in vitro* tuberization of potato plantlets.Potato Research 42, 483-488.

Dodds, J. H. (1988). Tissue culture technology : practical application of sophisticated methods. Am. Potato. J. 65: 167- 180.

Forsline, P.L. and A.R. Langille (1975). Endogenous Cytokinin in *Solanum tuberosum L.* as influenced by photoperiod and temperature.

Gopal,.J; Chamail. A and Sarkar,D.(2004). In vitro production of microtubers for conservation of potato germplasm: effect of genotype, abscisic acid, and sucrose. In-Vitro-Cellular-and-Developmental-Biology-Plant. 2004; 40(5): 485-490

Hawkes, J.G. (1990). The Potao-Evolution, Biodiversity and Genetic Resources. Belhaven Press, London.

Hijmans, R.J. (2001). Global distribution of the potato crop. Amer. J. of Potato Res.(2001) 78 : 403-412.

Hooker, U.G. (1981). Compendium of Potato diseases. The American Phytopathological Society, St. Paul, Minnisota.

KabSeog, Y. and Leung,D.W.M. (2004). Relative importance of maltose and sucrose supplied during a 2-step potato microtuberization process. Acta-Physiologiae-Plantarum. 2004; 26(1): 47-52

Khodeir, A.E.S.S.E. (2002). Potato seeds production through plant tissue culture techniques. M.Sc. thesis. Faculty of Science, Menoufiya University, Egypt.

Khodeir, A.E.S.S.E. (2005). Biotechnological studies on potato (Solanum tuberosum L.) plants. . Ph.D.Sc. thesis. Faculty of Science, Cairo University, Egypt.

Khuri-S. and Moorby-J. (1995). Investigations into the role of sucrose in potato cv. Estima microtuber production *in vitro*. Annals of Botany.1995. 75 : 3, 295-303.

Kin, Y.C.(1982). *In vitro* tuber formation from proliferated shoot of potato (*Solanum tuberosum L*.) as a method of aseptic maintenance .Ph.D.dissertation , Jean Buk, National University, South Korea.

Koda, Y. and Okasawa (1983). Influence of environmental hormonal and nutritional factors on potato tuberization *in vitro*. Jap. Journal of Crop science. 52 : 582 – 591.

Kostrica, P.; B. Polreichova and J. Domkarova (1985). The use of *in vitro* tuber formation for the maintenance of potato genetic resources. Genetika a Slechten. , 21 : 269 – 278.

Leclerc-Y.; Donnelly-DJ. and Seabrook-JEA. (1994). Microtuberization of layered shoots and nodal cuttings of potato : the infleunce of growth regulators and incubation peroids. Plant Cell, Tissue and Organ Culture, 1994, 37: 2, 113-120.

Mangat, B. S., G. Kerson and D.Wallace (1984). The effect of 2,4–D on tuberization and starch content of potato tubers produced on stem segments cultured *in vitro* Am. Potato Journal., 61: 355 – 361.

Marmiroli, N.; Agrimonti, C.; Visoli,G.; Colauzzi,M.; Guarda,G. and Zuppini, A. (2000). Silencing of G1-1 and A2-1 genes. Effects on general plant phenotype and on tuber dxormancy in *Solanum tuberosum L.* Potato Research 43 (2000) : 313-323.

Mingo – Castel, A.M.; R. E. Young and O.E. Smith (1976). Kinetin-induced tuberization of potato in vitro on the mode of action of kinetin. Plant and Cell Physiology, 17: 557-570.

Mitten, D.H.; C. Boyes and J. Cucuzza (1988). *In vitro* produced microtuber of potato. Am. Potato Jour., 65:492.

Mohammed F.H.; Sanaa, A. Awny and Mohamed Moursy (1992). *In vitro* tuberization of potato (*Solanum tuberosum L.*) I. The combined effects of susscrose, Kinetin and NAA. Bull. of Suez Canal univ. Appl. Sci, vol. 1, Mar., 1992.

Nakagawa,K. and Hosaka,K. (2002). Species relationships Between a Wild Tetraploid Potato Species, *Solanum acaule* Bitter, and Its Related Species as Revealed by RELPs of Chloroplast and Nuclear DNA. American J. of Potato Res. (2002) 79: 85-98.

Naik, P. S.; Sarkar ,D. and Gaur, P.C. (2008). Yield components of potato microtubers: in vitro production and field performance. Annals of Applied Biology . 06/2008; 133(1):91 - 99.

Palmer, C.E and E. Smith (1969). Cytokinin and tuber initiation in the potato (*Solanum tuberosum L.*). Nature, 221: 279-280.

Palmer, C.E. and O.E. Smith (1970). Effect of kinetin on tuber formation on isolated stolons of (*Solanum tuberosum L.*). cultured *in vitro.* Plants cell physiology, 11: 303-314.

Palmer, C.E. and W.G. Barker (1972). Changes in enzyme activity during elongation and tuberization of stolons of (*Solanum tuberosum L.*). cultured *in vitro*. Plant and cell physiology 13 : 681- 688.

Paul, V.; Ravichandran,.G and Ezekiel,,R. (2004).

Field performance of different grades of little tubers produced on physiologically old potato tubers. Journal-of-the Association. 2004; 31(1/2): 85-89

Pelacho, A.M.; Closas-Martin and Sanfeliu, J.L.I. (1999). *In vitro* induction of potato tuberization by organic acids. Potato Research 42, 585-591.

Rafique, T.; Jaskani,M.J; Hasnain,R. and Mazhar,A.. (2004). *In vitro* studies on microtuber induction in potato. International-

Journal-of-Agriculture-and-Biology. 2004; 6(2): 375-377.

Raman, K. V. (1994). Potato pest management in developing countries. In: Advances in Potato pest. Biology and Managsement Aps press, PP. 583-596.

Romanov-GA; Aksenova-NP; Konstatinova-TN; Golyanovskaya-SA; Kossmann-J; and Willmitzer-L. (2000). Effect of indole-3-acetic acid and kinetin on tuberisation parameters of different cultivars and transgenic lines of potato *in vitro*. Plant-Growth-regulation.2000, 32: 2-3, 245-251.

Rosell, G.; De- Bertoldi, F. G.; Tizio, R. and Bertoldi, F.G.(1987). *In vitro* mass tuberization as a contribution to potato micropropagation. Potato Res. 30: 111-116.

Sarkar, Debabrata and Naik, Prakash S.(1998). Effect of inorganic nitrogen nutrition on cytokinin-induced potato microtuber production *in vitro*. Potato Research 41, 211-217.

Seabrook,J.A.E; Douglass,L.K. and Arnold,D.A. (2004). Effect of leaves on microtubers produced from potato single-node cuttings *in vitro*. Amer. J. of Potato Res. (2004) 81 : 1-5.

Sidikou-RDS; Sihachkar-D; Lavergne-D; Nato-A; Ellisseche-D; Jouan-B and Ducreux-G. (2003). Contribution of microtuberisation to the adaptation of potato culture in the Sahel. Cahiers-Agricultures.2003, 12 :1, 7-14.

Simko-I. (1993). Effects of kinetin, paclobutrazol and their interactions on the microtuberization of potato stem segments cultured *in vitrio* in the light. Plant-Growth-Regulation.1993, 12 : 1-2, 23-27.

Smith, O.E. and C. E. Palmer (1970). Cytokinin induced tuber formation on stolons of *Solanum tuberosum L.* Physiol. Plantarum, 23: 599-606.

Stalknecht, G.F. (1972). Coumarin induced tuber formation on excised shoots of *Solanum tuberosum L.* culture *in vitro*. Plant Physiol., 50: 412-413.

Wattimena, G.B. (1983). Micropropagation as an alternative technology for potato production in Indonesia. Dissertation Abstract. 44-07 : 2040.

Wiersema, S. G.; Cabello, R.; Tovar, P. and Dodds, J.H. (1987). Rapid seed multiplication by planting into beds microtuber and *in vitro* plants. Potato Res. 30: 117-120.