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RESEARCH ARTICLE

In vitro Propagation of Potato under Different Hormonal Combinations

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Abstract

Potato (*Solanum tuberosum*) becoming a more important foodstuff in the world. The effect of hormones on *in vitro* micotuber formation and growth of potato were evaluated. The aim of this study was to establish a rapid protocol for *in vitro* propagation system of potato plants (*Solanum tuberosum* L.) cv. Diamant. The highest percentage of survived nodal segments was in surface sterilization with 0.2 % $HgCl_2$ solution for 5 minutes. *In vitro* stages of shoot initiation, multiplication, elongation and rooting were successfully achieved by using nodal explants. The highest percentages of shoot initiation and multiplication (90%), node number (50%) and rooting (52 %) were observed on PS3 (MS medium containing 3 mg/l GA3 and 0.1 mg/l Kin) producing 14 shoots/magenta from 6 nodes and the lowest percentage (15%) was on MS medium without growth regulators. The highest recorded values of tuberization percentage, weight (25%) and number of microtuber per shoot (29%) were obtained with 5mg/l benzyl amino purine (BAP), while increasing concentration of BAP more than 5 mg/l decrease the average number and weight of microtubers. Generally, we found that, the most efficient medium for microtuber induction was PM3, followed by PM2 medium, while PM1 medium was proved to be unsuitable for early induction of microtubers. Plantlets with roots of 4 to 5 cm in length were successfully transferred to pots containing sterile greenhouse (clay: sand) in equal ratio for acclimatization, and about 90% of plants were survived.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important crops in the world, it is grown in 100 countries worldwide. According to FAO statistical data (2012), the most potatoes are produced in Asia, then in Europe; South America; then in North and Central America. It considers the main vegetable crops grown in the Kingdom of Saudi Arabia. Potatoes have become an important food crop in Saudi Arabia in recent years Potato acreage has increased several times and this expansion was mainly due to KSA government policy of diversifying food production, Average yields vary between 20 and 40 t/ha (Abdelgadir et al., 2003).The estimated total annual potato production in Saudi Arabia varies between 400,000 MT 450,000 MT (most of which is suitable for processing into French fries). Imported potatoes account for 40,000 MT to 50,000 MT annually (Anonymous, 2012).

Many researchers used different growth regulators for in vitro induction of microtubers in potato (Tugrul and Samanci, 2001 and Hossain, 2005). A number of extensive physiological research has revealed that, in vitro

tuberization is controlled by several factors, such as hormonal combination, nutrient compositions etc. (El-Sawy et al., 2007 and Anoop Badoni and Chauhan, 2009) This technology has been used for disease- free potato seeds production in many countries (Wang and Hu, 1982; Islam and Chowdhury, 1998 and Khan et al., 2003).

Tissue culture techniques are used worldwide to produce pre-basic, virus-free seed potatoes known as microtubers. Tuberization process in potato is a very complex, but it can be induced under in vitro condition. Because of their small size and weight, microtubers have tremendous advantages in terms of storage, transportation and production practices. They can be directly sown into the soil and can be produced in bulk in any season. They have similar morphological and biochemical characteristics compared to field produced tubers. Therefore, mass production of potato microtuber is likely to revolutionize the world potato production (Kanwal and Shoaib, 2006).

Micropropagation is the methods to conventional propagation of potatoes (Rabbani et al., 2001). The application of tissue culture techniques for rapid propagation of potato became more widely used in many countries. Tissue culture used to increase propagation rates and to modify the germplasm of plants. Tissue culture has been applied to improve production of potato by germplasm conservation, pathogen free potato plants and micropropagation (Khalafalla, 2001).

In vitro micropropagation methods using micro-tubers, nodal cuttings and, meristem tips are more genetic stability of the multiplied clones, (Wang and Hu, 1982). *In vitro* propagation of potato using single nodal cutting is the best method of rapid multiplication rates with maximum genetic stability (Chandra and Naik, 1993). Rapid production of pathogen free potato plants through meristem culture, micropropagation and elimination of virus free plants are likely using meristem culture (Jha and Ghosh, 2005). The limitations of microtubers production in many ways are based on the capacity to the components of the culture environment and photosynthetic ability of the plantlet. Microtubers production dependent on size and number of microtubers produced per cycle. Microtubers production methods are still less economical compared with *in vitro* rapid multiplication (Gebre and Sathyanarayana, 2001). Induction of microtubers in potato dependent on combination of growth regulators of cytokines and auxins (Tugrul and Samanci, 2001; Hossain, 2005).

MATERIALS AND METHODS

Sterilization of plant materials

The nodal segments buds were used as explants, their surface sterilized by immersing in 0.2% $HgCl_2$ solution for 5 min and then washed three times with sterile distilled water. The medium were autoclaved at 15 psi for 20 minute. The explants were cultured in MS medium (pH 5.8). After sterilization, explants were inoculated in MS medium supplemented with different hormonal combinations and shifted to culture growth room at 25±2°C and 16 h photoperiod under white light intensity (3000 Lux). Best combinations of GA3+ NAA and Kin + NAA with MS (Murashige, and Skoog, 1962). medium were selected on the basis of cultures growth performance i.e. shoot height, number of nodes, root length, shoot and root fresh weight.

***In vitro* multiplication:**

In vitro multiplication was carried out by nodal fragments on MS solid media containing different concentrations of GA3(1.2 and 3.0 mg/l) and concentration of 0.1 kin mg/l. Auxillary buds started to grow after 6-8 days of culture on MS medium. The medium were autoclaved at 15 psi for 20 minute. The explants were cultured in MS medium (pH 5.8). After sterilization, explants were inoculated in MS medium supplemented with different hormonal combinations and shifted to culture growth room at 25±2°C and 16 h photoperiod under white light intensity (3000 Lux).

Tuberization:

Stem segments about 1 cm in length each containing an auxiliary bud were prepared from in vitro shoot cultures and transferred onto the surface of solidified medium in culture jars and kept under complete darkness at 25±2^{o}C temperature for the duration of 60 days depending on the growth of microtubers. Media used for the induction of tuberization consist of 5 mg/l BAP and 0.2 mg/l Kin tuberization media

Experimental design:

Each treatment consists of 30 nodal cuttings containing auxillary bud explants for considering multiplication, elongation, rooting numbers and average weight of microtuber, aver experiments. All experiments were carried out in three replicates. The results were recorded from different experiments four weeks after culture.

Statistical analyses of data were carried out using different methods. analysis of variance (ANOVA) and mean separation were carried out using Duncan's multiple range test and significance was determined at the ($p < 0.01$) level. Data analysis was performed using SAS computer package.

RESULTS AND DISCUSSION

In vitro multiplication

In vitro stages of shoot initiation, multiplication, elongation and rooting of potato plant (Solanum tuberosum L.) cv. Diamant were successfully achieved from nodal explants (Fig.1). The cultures were multiplied on PS medium supplemented with different concentrations of GA3 (1, 2 and 3 mg/l) in combination with 0.1mg/l kin (Table 1, Fig.1). The highest percentage of shoot initiation and multiplication (90%) , node numbers (50%) and rooting (52%) were observed on PS3 (MS medium containing 5 mg/l GA3 and 0.1 mg/l Kin), producing 14 shoots/magenta from 6 nodes and the lowest percentage (15%) was on MS medium without growth regulators (Table 2, Fig1). The culture medium could be either growth regulator-free (Tavazza and Ancora, 1986) or containing GA 3 (Espinoza et al., 1986). Therefore nodal cuttings were cultured on two media, namely that developed by Espinoza et al., (1986) containing 0.25 mg /l GA3, and that developed by Shahin, (1984), which does not contain any growth regulator. Growth was more rapid on the medium of Espinoza et at., (1986) compared with the medium of Shahin (1984), probably due to the presence of GA3 in the medium which stimulates cell division and/or cell enlargement.

In vitro tuberization

In vitro tuberization was obtained after propagation; the effect of BAP and Kin concentrations with MS medium was studied for microtuber formation and development. Data showed that, the average of maximum number of microtubers per shoot (29%) and average weight of microtubers (25%) were produced when propagated shoots were subcultured on PM3 medium supplemented with 7 mg/l BAP and 0.2 mg/l Kin (Table 2, Fig. 1), whereas MS medium without growth regulators gave the lowest average of microtubers per shoot (3%) and average weight of microtubers (1.7%) (Fig.1). In general, medium PM3 was more efficient for microtuber induction, followed by medium PM2,while medium PM1 proved to be unsuitable for early induction of microtubers. Wang and Hu (1982) did not add any growth inhibiting substance to the medium but used a higher concentration of BAP (10 mg/l). Kwiatkowski et al., (1988) reported that 10 mg/l kin with 0.1 mg/l IAA were used to induce microtubers in vitro from single nodal cuttings of 43 potato clones and two wild species (S. microdontom and S. pinnatisectum). Mangat et al., (1984) demonstrated also that increased numbers of tubers on nodal segments cultured in vitro on media containing low concentrations of 2, 4-D (0.2 - 2 mg /l). Microtubers induced under eight hours photoperiod had a shorter dormancy period than when induced in complete darkness (Tovar et al., 1985; Estrada et al., 1986). Microtubers were also produced from the stems of the plantlets on the shoot culture medium of Espinoza et al., (1986), which contained 0.25 mg/l GA 3 and 2 mg/1 pantothenic acid, after 8 weeks of culture under a 16 h photoperiod. Gibberellins promote vegetative growth and inhibit tuberization and tuber development (Bottini et al., 1981; Koda and Okazawa, 1983; Hussey and Stacey, 1984). Garner and Blake (1989) found that, the complete darkness or short photoperiod promoted an initial acceleration in the rate of microtuber formation but there was no discernible effect on the number of microtubers produced. Wang and Hu (1985) suggested that a short photoperiod of low light intensity or complete darkness should be used, and the addition of cytokines to the medium stimulate the tuberization process. On the other hand a long photoperiod at a relatively high light intensity should be used if cytokines are not added to the medium.

CONCLUSION

In conclusion the results of the present study showed that the highest record of tuberization percentage, weight and number of microtubers per shoot were obtained with 5mg/L benzyl amino purine (BAP), established a rapid protocol for in vitro propagation system of potato plants (*Solanum tuberosum* L.) cv. Diamant, significantly improve the potato plant growth and microtuber induction. This protocol will help for mass propagation for horticulture, production of virus free plants and *in vitro* germplasm conservation of potato plants *Solanum tuberosum* L. cv. Diamant.

Table 1: Effects of different hormonal combinations with MS media on shoot height, node number, and root length of potato cultivar Diamant.

Treatment GA3 + Kin (mg/l)		Shoot height (cm)	Node number	Root length (cm)
C	0.0+0.0	1.500 d	1.16 a	2.50 c
Ps1	1 + 0.1	4.00 c	2.60 c	2.53 c
Ps2	2 + 0.1	5.800 b	3.20 b	4.53 b
Ps3	3 + 0.1	9.00 a	5.00 a	5.26 a

Means with the same letter are not significantly different ($p < 0.01$) according to Duncan's.

Table 2: Effects of different concentrations of BAP and Kin on *in vitro* percentage of tuberization, number of microtubers / shoot and weight of microtubers /shoot of potato cultivar Diamant.

Treatment BAP + Kin (mg/l)		*In vitro* tuberization %	No of microtubers / shoot	Average weight of microtubers /shoot (gm)
C	0.0+0.0	11.50000 e	0.33000 c	0.19000 c
PM1	1 + 0.2	20.76667 d	1.23333 b	0.17500 c
PM2	3+ 0.2	43.56667 c	2.43333 a	0.25667 b
PM3	5 + 0.2	66.43333 a	2.86667 a	0.33333 a
PM4	7 + 0.2	56.10000 b	2.90000 a	0.25933 b

Means with the same letter are not significantly different ($p < 0.01$) according to Duncan's.

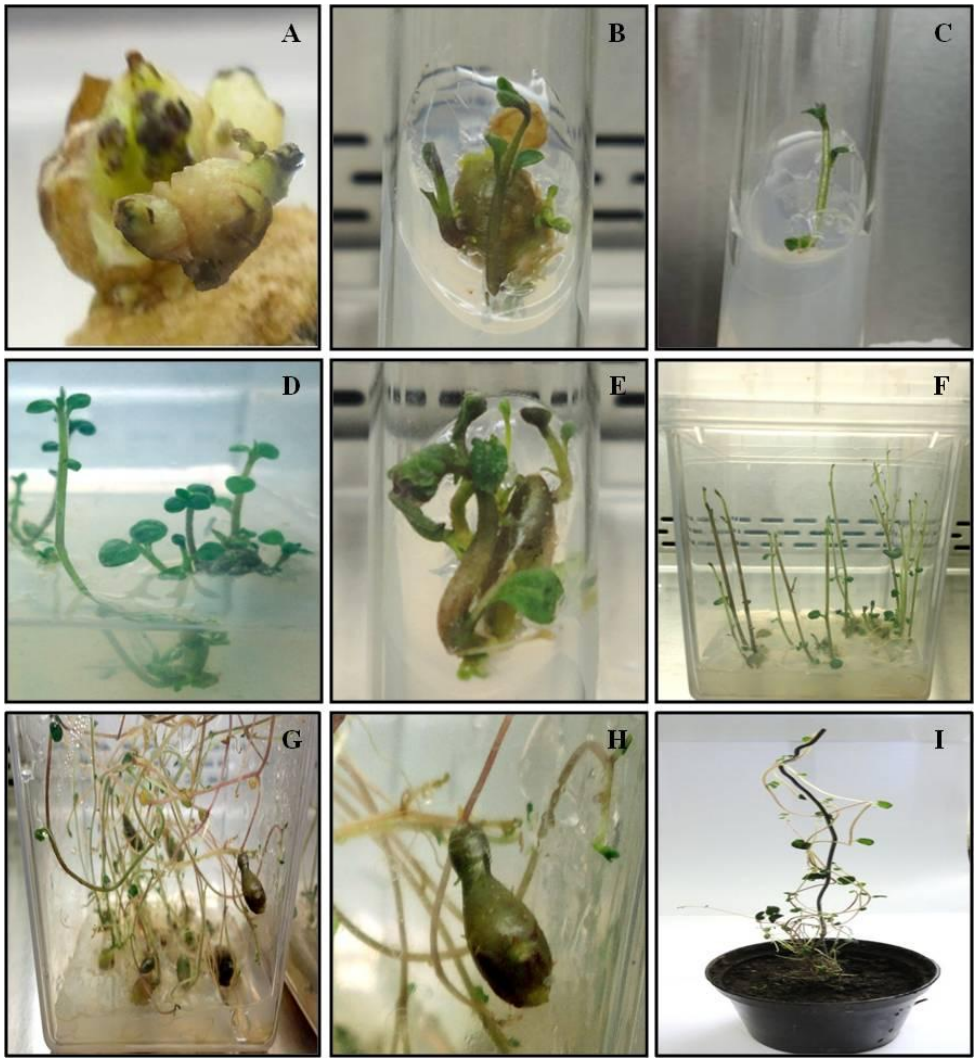

Figure 1. Stages of *in vitro* propagation of Potato (Solanum tuberosum) plant. A, Nodal cutting explants harboring auxillary buds. **B**, Shoot initiation on start media. **C**, Shoot elongation on Ps3 media. **D**, Nodal cutting explants harboring auxillary buds appear on shoot grown on Ps3 media with 0.3% (w/v) sucrose. **E**, Shoot initiation from nodal explants. **F**, and **G**, shoots harboring auxillary buds and micro tubers. **H**, Microtubers initiation from nodal explants. I, Plantlets with six to seven roots of 5 to 7 cm length were successfully transferred to pots for acclimatization.

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