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Effect of Immersion Time and Kinetin Growth Regulator on Micro Tuber Formation for Spunta Potato

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A B S T R A C T

This study was conducted at plant Tissue culture department which, belongs to Biotechnology research center located in Tripoli. In this study, micro tubers of Spunta potato variety were obtained through use of liquid culture medium by using temporary immersion system (RITA). The effect of liquid medium immersion time on plant growth was studied, as well as the effect of adding the kinetin growth regulator at 5 mg / l with 8% sugar on number and weight of formed tubers was also studied. The results showed that increase of immersion time from 1 minute to 2.5 minutes has a significant effect on increasing number and weight of formed tubers in each treatment. The average of number of tubers increased from 8.42 to 17.14 and 21.14 tuber in the vessel respectively. The total mean number of tubers in each pot was also increased when the kinetin growth regulator was added from 20.28 to 28.85 and 32.71 tuber per pot respectively. The average of tubers weight in each jar was also increased from 4.10 to 9.92g when the kinetin growth regulator was added.

Introduction

Potato plant (Solanum tuberosum L.) is considered one of the most import ant vegetable crops in the world in terms of production and cultivated area. Potato plant belongs to the Solanaceae family and include about 90 genera and about 2000 species (Al-Falah et al., 1999). The cultivated area globally in 2020 wasabout20 million hectares and production was about 359 million tons, while the cultivated area in Libya in 2020 was about 18 thousand hectares and productivity was about 366 thousand tons (FAO Stat, 2020).Potatoes are the fourth most economic crop in the world, alongside wheat, rice, and barley whereas, Potatoes are the fourth economic crop in the world, next to wheat, rice, and barley (Ferine, 2001). In addition, potatoes are one of the most important tuber crops and one of the most widely used vegetable crops, and they are consumed in relatively large quantities as they constitute an important source of many nutritional elements, as they contain a high percentage of starch, sugars, protein, amino and organic acids, vitamins, and mineral elements (Hassan, 1999) Potato plants are planted twice a year. The first plantation planted in spring season begins in January to mid-February, and the second one planted in autumn from August to September. On the other hand, potato can be propagated by seeds but, this method is used in breeding programs only because of the extreme diversity in the characteristics of the produced tubers resulting from the cultivation of potato seeds (Al-Safadi, 1995). Nevertheless, potato propagated vegetatively through

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tubers, which is a commonly used method. This method faces some problems such as lack of tubers, exposure to diseases and their deterioration generation after generation, which reflects on production quality and quantity. Particular there is an increasing demand for the potato crop due to its nutritional importance and its use as a key ingredient in preparing staple meals for many nations. Therefore, it is essential to consider effective methods for increasing potato production and obtaining larger quantities each year. Plant tissue culture is considered one of the best methods for propagation various plants, including potatoes. Plant tissue culture is a rapid method for mass-producing plants under sterile, controlled conditions. In vitro propagation of important plants can offer considerable benefits, including rapid cultivation of species that have limited reproductive capacity and exist in threatened habitats (Ziba et al.,2016). In vitro culture effectively produces uniform plants in key horticultural species. Therefore, to improve potato propagation new methods applied to avoid vegetative propagation. In vitro micropropagation is an effective technique for multiplying specific genotypes certain medicinal and aromatic plant species (Rout et al., 2001). Micropropagation of plants through use of plant tissue culture technology is one of the most widespread applications, as plants are propagated vegetative in large numbers over a short period of time by controlling apical dominance and stimulating lateral branching or encouraging callus cells to form somatic embryos that develop into vegetative growths (Al-RifaiandAl-Shubaki,2002). In fact, much research in the field of micro-tuber production has focused on the use of cytokinin in this field, especially the stimulating effect of kinetin (Al-Ziyara, 2004). However, addition of plant growth regulators to culture medium improves plant growth and gives a positive result. (Hamza I, 2013) found that the highest average of plant length and number of branches was obtained, after addition of 1 mg/liter of kinetin. Moreover, Ebadi et al, 2007 proved that addition of BA growth regulator to MS medium cultured by potato single nodes increase micro tuber formation. (Shojael, 2010) indicated that the MS supplemented with kinetin achieved the best multiplication, improves the vegetative growths of potatoes and helps in building starch necessary for the formation of fine tubers (Al-RifaiandAl-Shobaki,2002) and(Hones, 2003). Use of plant tissue culture technology in the production of micro-tubers is one of the most important programs for obtaining potato seeds free of viral diseases. In addition, potato seeds produced with this technique are small in size and the process of storing and transporting them is inexpensive compared to regular seeds. Those seeds can be grown in special fields with a high plant density and under controlled conditions to produce seeds in standard sizes desired by farmers (Al- Jubouri and Al-Salhi, 2009). Bioreactors have also been used to increase the ability to multiply and form micro- tubers through the differentiation and formation of branches directly from the plant explants (organogenesis), and the formation of vegetative embryos **(**Vasil & Levin, 1989; Preil, 1991) This system has been tried and has a high efficiency, especially in propagating many plants by obtaining vegetative embryos (embryogenesis somatic) such as bananas, pineapples, and coffee (Esclant and Teisson,1994). This system has also been tried in obtaining fine tubers of potato plants (Alvard and Teisson, 1999). Microtubes of potatoes were also obtained from the axillary buds growing from the branches using shaking liquid nutrient media, and using bioreactors, which showed that it was invaluable to increase the efficiency of tuber formation and reduce manual handling of the growths and tubers formed (McCown, 1991).Therefore, the aim of this study was produce fine tubers of potato plant Spunta using a temporary submersion system with the addition of the growth regulator kinetin.

Materials and Methods

Sample collection and preparation

Potato tubers variety of Spunta were taken from the local agricultural company which, imports potato tubers from outside of Libya. Spunta Potato tubers directly moved to plant tissue culture laboratory for the next stages of the experiments. The tubers were washed well by water, then with distilled water, and dried on filter paper. After that, the samples were placed in the dark at room temperature to obtain buds. After buds grew to a lengthof1to cover a period of four weeks, the buds were separated from the tubers.

Surface sterilization

The surface sterilization processes of obtained buds were started by immersing it in 70% ethyl alcohol for one minute, then immersing it in a 2% commercial Clorox (NaOCl) solutionfor20 minutes, and immer sing it three times in sterile is tilled water, each time for five minutes.

Stage of obtaining a contamination-free tissue culture.

The sterilized buds were cultured in 250 ml glass jars containing MS (Murashige and Skoog, 1962) medium supplemented with0.7% agar and3% sucrose, with four buds per jar. were placed in a growth chamber under conditions of 16 hours of light per day and 8 hours of darkness and a temperature of 25 °C ±. Potato samples were incubated in the growth chamber for two to six weeks until the cultured plants reached the desired growth.

Branch formation stage

This stage was conducted through separating the obtained growths into (single nodes) and used as plant explants and planted in a temporary immersion (RITA) system (Automatic Temporary Immersion Recipients) with individual nodes in each pot, at immersion times of 1, 2, 5 minutes for two weeks to form branches in liquid MS medium supplemented with 3% sucrose without adding he growth regulator and under the same incubation conditions as before. After that samples were transferred to MS nutrient medium supplemented with the growth regulator kinetin at a concentration of 5mg/L for two weeks. The goal of this stage is to obtain a sufficient quantity of vegetative branches for the stage of micro-tuber formation in the temporary submersion system.

The temporary immersion system (RITA) consists of a number of heat-resistant plastic containers with a capacity of one liter. Each container consists of two parts, an external part and an internal part. Figure (1) In which the plant explants are placed on a plastic barrier that prevents the plant explant from reaching the liquid medium that is placed in the container with a volume of 200 ml. To enable the nutritional medium to reach the plant extracts present on the plastic barrier for the feeding Process, a stream of sterile air is pumped in by passing it through fine filters with holes with a diameter of 0.2 micrometers. To ensure the passage of a sterile stream of air to the bottom of the container, which in turn leads to the liquid nutrient medium rising to the top to reach the plant explants on the barrier, so that the feeding process takes place through the phenomenon of membrane diffusion of plant tissues, and the time and times of immersion per day are controlled via an electronic panel prepared for this purpose.

Formation of fine tubers stage

The potato samples obtained from the previous stage were transferred to the tuber formation stage. Potato tubers formation stage was conducted by using MS supplemented with 8% sucrose at immersion times of1, 2, 5minutes for six weeks. Samples incubated a temperature of 18°C in 24-hour dark. In this stage, the effect of adding the growth regulator kinetin (kin) at a concentration of 5 mg/L and the immersing time on the number and weight of the fine tubers was measured.

The results were recorded after sixteen weeks of culture. The tubers were harvested, which included the number and weight of the tubers formed in each pot. The results were statistically analyzed using a Completely Randomized Design (CRD), with 10 replicates for each treatment, and the averages were compared using the Duncan test at the 0.05 level.

Results

This study was conducted to evaluate the efficiency of the temporary immersion system (RITA) in obtaining potato tubers. The study demonstrated that using this system was successful in producing potato tubers. For evaluation, the number of formed tubers and their weight were recorded, and the results were as follows:

Number of tubers.

Effect of immersing time and addition of kinetin growth regulator on number of formed potato tuber was studied. The results showed in Figure (1) that by increasing the immersion time during the tuber formation stage to 5 minutes, the number of tubers formed in each pot increased significantly to 21.14 in the nutrient medium (MS) free of growth regulator. Adding the growth regulator kinetin at a concentration of 5mg/L, Figure (1) increased number of formed tubers during the immersion timeof5minutes whereas, the number of formed tubers reached to 32.71 tubers per pot. Tuber formation in potatoes depends greatly on the concentration of sugar in the nutrient medium and the surrounding environmental conditions. Micro tubers are obtained using a temporary immersion system in MS medium in the dark without adding any growth regulator. As found (Wang, and Hu,1985). However, increasing the concentration of sugar in the liquid MS medium from 3 to 8% led to an increase in the number of tubers formed in each container (200 ml

conicalflask) from11.5 to 24.7 tubers, respectively, and by increasing the sugar concentration to 9% the number of tubers formed decreased to 15.4 tubers this, recorded results proved the positive effect of sugar on tuber formation. Cultivation in liquid media had a clear effect in increasing the number and size of tubers produced by Spunta potatoes (G. Rosll et al, 1987).The results demonstrated that increasing the immersing time from oneminuteto2and 5minutes led to a significant increase in the number of tubers formed from15.7 tubersto27.78 tubers and 27.6 tubers in the nutrient medium devoid of kinetin, andfrom19.4 tubers to 30.70 tubers when adding the growth regulator kinetin at 2 minutes and 5 minutes, respectively. Adding kinetin to the nutrient medium led to a non-significant increase in the number of tubers formed in each pot, and this is consistent with what was found by (Rosll et al, 1987). On the other hand, PO-jen Wang (1982) and Ching-yehHu (1982) found that use of MS liquid medium supplemented with BA at a concentration of 10 mg/L plus 8% sucrose to the liquid MS medium led to an increase in the number of tubers formed in each pot from 2.1 to 48.6 tubers.

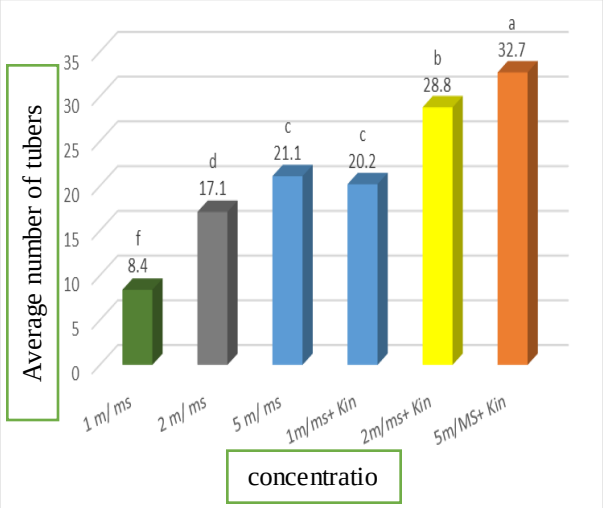

Figure 1. The effect of immersing time and the growth regulator kinetin 5 mg/L on the number of tubers in the pot.

Weight of formed tuber.

Provides an excellent indicator of the quality of the propagation method used. In this study weight of formed tubers was measured in order to evaluate

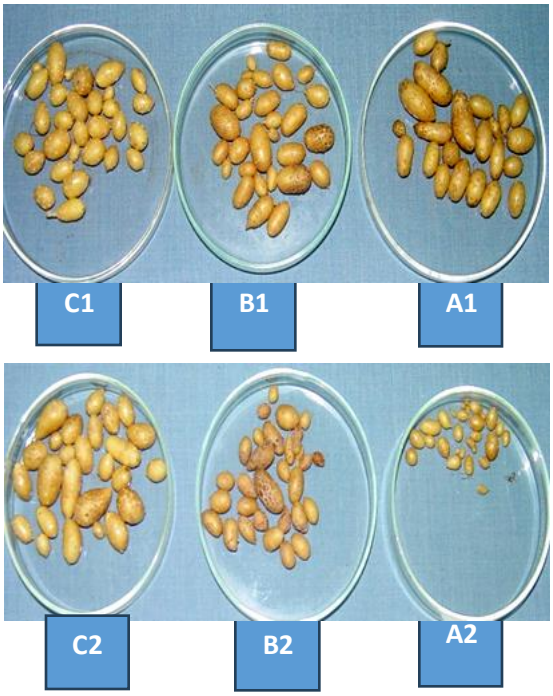

Figure 5. Microtubers of potato cultivar (spunta) A = 1 minute, B = 2 minutes, C = 5 minutes. 1 = With the growth regulator kinetin at a concentration of 5 mg/L

2 = Without added growth regulator

quality of RITA system for producing potato tubers. The results of this study figure (2) showed that the total weight of the formed tubers increased significantly when the immersion time increased from 1 to 2 and 5 minutes while, the results showed also that the increase in the immersion time led to a significant increase in the total weight of the formed tubers in each pot at the 5 minutes immersion time over the rest of the immersion times, as the weight of the tubers formed in each pot increased from 4.15g to 9.92g. Moreover, adding the growth regulator kinetin had an adverse effect on the total weight of tubers and the average weight of one tuber, as the total weight of tubers decreased significantly at immersing time of one minute. The recorded results showed that there was a little increase in tuber weight due to addition ofkinetin12.53gramswhenaddingthegrowthregulator kinetin at a immersing time of 5 minutes, Figure (2). Particularly the results of this study proved that treatment of MS supplemented with 5mg kinetin at 5minutes immersing time gave the best results and the

highest average of potato tubers was obtained in this treatment followed by treatment of 2menutes immersing time. The average weight obtained by 5mg kinetin at 5 minutes immersing time was significantly higher than other treatments whereas, these results proved the key role of kinetin in plant growth improvement. The results also showed that the best immersing time was 5 minutes in terms of the number and weight of fine tubers when planting in both treatments in liquid MS medium free of growth regulators and when adding the growth regulator kinetin at a concentration of 5 mg/L.

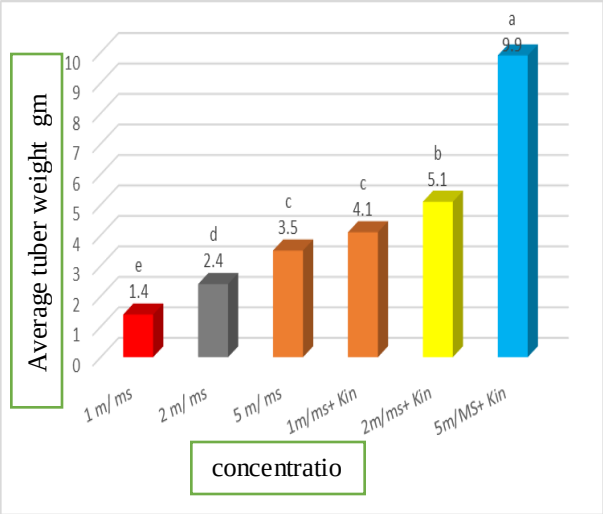

Figure 2. The effect of immersion time and the growth regulator kinetin at a concentration of 5

Figure 3. Growth of potato var Spunta in the temporary system.

Conclusion

The local production of potato tubers for cultivation is a crucial factor in reducing the annual import of potatoes. This study has demonstrated the success of the RITA system in producing potato tubers that can be relied upon in the future. The results also showed that using a liquid medium with temporary immersion for five minutes in the presence of kinetin hormone yield sexcellent results, facilitating the production of an acceptable number of tubers with potential for future improvement.

Conflict of interest: The authors declare that there are no conflicts of interest

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