MICROTUBER INDUCTION AND PLANT REGENERATION OF POTATO (*Solanum tuberosum* L.) : A REVIEW

Abstract

Potato (*Solanum tuberosum* L.) is one of the most economically significant annual vegetable crops occupying the fourth position worldwide among all the food crops. The main drawbacks of the traditional seed production method are the seed tubers' high size, which drives up seed production costs, the ease with which viruses and other diseases can damage them, the slow rate of seed multiplication, and the lengthier time it takes to obtain breeder seed. There are various benefits to the invention and use of microtubers in potato farming. It is not necessary to acclimatize or move to a different medium when growing microtubers because they can be kept for up to a year and then transported straight to the market. By doing this, the field cycle needed to produce a sufficient amount of seed potatoes is greatly reduced, and a high quality of materials is guaranteed. Shoot tip and node or internodal explants, which have a survival probability of about 90%, are frequently used in the process of *in vitro* plant regeneration. Positive outcomes in stimulating shoot formation have been observed when Murashige and Skoog media enriched with benzyl amino purine (BAP) and kinetin is used. Furthermore, employing the MS medium has been reported to maximize the effectiveness of microtuber induction in potatoes. It's been found that exposing the plants to a 16-hour photoperiod and complete darkness can improve the effectiveness of microtuber induction even further. Ultimately, this review study offers an appropriate method for a successful pathway in the *in vitro* regeneration of potatoes by carefully examining prior scientific data.

Key words: Microtuberization, *Solanum tuberosum*, regeneration, plant growth regulators, microtubers.

Introduction

Potato (*Solanum tuberosum* L.) popularly known as 'The king of vegetables", belongs to the family "Solanaceae" and has emerged as the fourth most important food crop in India after rice, wheat and maize. Potato is a nutritionally superior vegetable as well as a staple food not only in our country but also throughout the world. "In Production, India is in second place after China with 50 mt. According to FAO, potato is consumed by more than one billion people the world over. It is a high-quality vegetable cum food crop and protein is produced by this crop 83% more efficiently than rice. Nutritional point of view, potato is a wholesome food and deserves to be promoted as a potential high-quality vegetable cum food crop in the country. It is one of the richest vegetable sources of vitamins from the B complex group, like pyridoxine (vitamin B6), thiamine, niacin, pantothenic acid, folate, and an excellent source of antioxidant and vitamin C" [21].

"It can be propagated either by botanical seed like true potato seed or by vegetative means of tubers. Seed potato tubers are utilized for multiplication and production [44, 38]. This conventional method has several disadvantages low rate of multiplication and high risk of various diseases and pathogens. To get disease-free plants particularly virus-free planting materials, farmers can use *in vitro* grown microtuber and plantlets obtained. Apart from mass clonal propagation and promoting healthy, virus-free plants, biotechnology will also contribute to improved potato breeding. Meristem culture and *in vitro* tuberization are the most common methods for *in vitro* regeneration of potatoes" [36].

"The microtuber has become an important mode of rapid multiplication for pre-basic stock in seed tuber multiplication as well as germplasm exchange" [51]. A micro tuber is a small potato tuber produced in tissue culture. It is easier to handle microtuber than *in vitro* plants. The micro tuber has advantages in storage, transportation, and production because of its small size and lightweight. They can be planted directly on land and can be produced every season. The characteristics of micro tubers like those found in field tubers make them a very important process to optimize and have a great impact on the world [41]. There have been studies using bioreactors for mass-producing microtubers for commercial production. However, the bioreactor has some disadvantages like high cost and a greater chance of microbial contamination. In this study, we briefly review research on *in vitro* regeneration of potatoes that has been conducted.

In vitro regeneration of potato

Effect of explants on in vitro regeneration of potato

Explants play a vital role in the regeneration of potato crops through organogenesis and or somatic embryogenesis. Okazawa et al. [32] were the first to report that shoots could regenerate from large tuber explants. Khadiga et al. [16] reported by using the tuber segments as explants, the highest percentage of callus with 81% of the shoots survival rate was obtained. Haque et al. [14] concluded that "explants of leaf showed the best results in terms of callus length and weight, whereas explants of node or internode showed the best results in terms of shoot length and the number of leaves per plantlet". "Additionally, shoot tip explants produced better results for root length after 28 days of plantlet growth, whereas internode explants performed better for root length after 21 days of plantlet growth. The induction medium containing 10 mg/l BAP and 80 g/l sucrose favoured for production of more number of microtubers by using nodal explants" [9]. "The composition of MS with 2 mg/l BAP and 1 mg/l NAA formed 13.33% microtubers in potato cultivar Agrija. They also found that, the nodal segments as starting explants demonstrated higher efficiency compared to the sprouts" [19]. Liljana et al. [22] concluded that "explants from different cultivars treated with GA_3 gave good results in stimulating sprout formation and treated tubers had 100% effect on sprout growth". Gami et al. [12] reported that "when sprouts were used as an explant for initiation of culture with different combinations of growth hormones and the resultant shoots were divided and sub-cultured in the same environment for further multiplication". Molla et al. [28] reported that "explants from *in vitro*-grown potato varieties were derived from leaves and internodal explants demonstrated better shoot initiation results than leaf explants". In another study, Shahriyar et al. [40] reported "by using shoot tip and nodal segment explants from field-grown plants, the highest rate of shoot multiplication (73%) was obtained using MS medium with different hormone concentrations while the highest rate of root regeneration (96%) was obtained using MS medium without hormones". Dessoky et al. [7] reported that "*in vitro* propagation of potatoes, the highest percentage of survived nodal segments (explant) was obtained by surface sterilization with 0.2 % $HgCl_2$ fixative for five minutes". Mohamed et al. [26] concluded that "a significant increase in the density of single-node explants increases the number of microtubers and yields". Xhulaj and Gixhari, [48] found that "the sterilized sprouts were cut to isolate apical meristems which were cultured on shoot induction medium containing solidified MS media with vitamins and exogenous plant growth regulators and incubated at optimized culture conditions in room culture" (Table 1).

Effect of hormones on shoot formation in potato

The different types of cytokinins (BAP, Kinetin and GA_3) are used for shoot induction and multiplication of shoots in potato crops. Badoni et al. [4] reported that “a lower concentration of auxin (0.01 mg/l NAA) with GA_3 (0.25 mg/l) improved the shoot height in the MS medium when compared to the MS medium with Kinetin (Kin) and NAA”. Khadiga et al. [16] reported that “MS medium containing 5.0 mg/l TDZ had the highest days to shoot initiation, the highest percentage of callus with shoot (81%) and the highest number of shoots per callus (3.4). Shoots derived from callus, rooted most effectively in half-strength MS medium containing 0.5 mg/l IBA”. Haque et al. [14] confirmed “node and or internode explants produced better results when different concentrations of BAP and GA_3 were used. Similarly, internode explants produced better results for root length after 21 days of plantlet when a concentration of 1.0 mg/l IAA and 0.25 mg/l GA_3 was used. Shoot tip explants also produced better results in root length after 28 days of plantlet when concentrations 1.0 mg/l IAA and 0.25 mg/l GA_3 were used”. Khadiga et al. [16] reported that “the highest callus percentage was observed on MS supplemented with 2.0 mg/l 2, 4-D. MS medium supplemented with BAP and TDZ either alone or TDZ and BAP in combination with IBA and 2,4-D were employed for shoot regeneration. There was a 100% rooting response in all the media combinations as well as media without IBA”. Laboney et al. [20] stated that “the concentration of 2, 4-D is 2 mg/l has shown to be better for induction of callus (95.00%) within a minimum number of days (10.00), the maximum number (4.75), and the weight of callus (0.07 g) are also better results, respectively”. “Upon regeneration, the maximum number (3.75), as well as the highest shoot initiation (75.0%) was observed within a few days (15.50) in medium containing 1.0 mg/l BAP. MS medium supplemented with 60g/l of sucrose exhibited a better response than the other concentrations in mean values of microtuber number, diameter, and weight and was found optimum” [11]. Microtubers were successfully produced from *in vitro* grown plantlets using MS medium supplemented with IBA (0.5 mg/l). The best microtuberization rate was achieved when the MS/2 liquid media was supplemented with sucrose (80 g/l) in the Spunta cultivar. [24]. Gami et al. [12] concluded “by several experiments, that an MS medium supplemented with different concentrations of IBA, kin, NAA and 2,4-D were tested for initiation and multiplication of shoots showed good results differed according to the combinations of treatment: IBA 1.0 mg/l and NAA 1.0 mg/l + kinetin 2.0 mg/l were observed for a single shoot. The medium containing 1.0 mg/l IBA, 2.0 mg/l NAA, and 1.0 mg/l kinetin resulted in the observation of single shoots as well as branches. For combination multiple shoots, two combinations were observed: IBA 1.0 mg/l + NAA 1.0 mg/l + kinetin 1.0 mg/l + 2 4-D 1.0 mg/l, and the same combination except with 2 4-D 1 mg/l. Additionally, IBA 1.0 mg/l + NAA 1.0 mg/l + kinetin 2.0 mg/l and 2.0 mg/l IBA + 2.0 mg/l kinetin + 2.0 mg/l NAA + 1.0 mg/l 2,4-D were observed for combination multiple shoots as well as roots. Lastly, for microtuber production, a high level of sucrose (8%) yielded promising results”. Molla et al. [28] concluded that “MS basal medium supplemented with 0.5 mg/l IBA and 30 g sugar was used for *in vitro* potato plant development *via* nodal cuttings. The best result was obtained using MS medium supplemented with ZR (zeatin riboside) at a concentration of 5 mg/l from internodal and leaf explants in *in vitro* regeneration. Moreover, half-strength MS medium supplemented with 0.5 mg /l IBA produced well-developed roots within 21 days which gave the 100% survival at ex *vitro* condition”. Shahriyar et al. [40] reported that “the highest percentage of shoot multiplication (73%) was noticed in MS medium containing 2mg/l BAP with maximum number of shoots, and highest length of shoots. Higher concentrations of hormones showed a negative effect on root induction”. Teisson and Alvard, [45] and Yu et al. [50] obtained “the highest weight of the microtubers in the level of 6% sucrose and 1M BAP. The increase in BAP concentration significantly induced the maximum number of microtubers with the highest weight ($p < 0.05$)". Dessoky et al. [7] confirmed that "the explants are cultured show high levels of shoot initiation and proliferation (90%), node number (50%), and rooting (52%) on PS3 (MS medium containing 3 mg/l GA_3 and 0.1 mg/l kinetin) producing 14 shoots/magenta from 6 nodes, while the lowest percentage (15%) was observed on MS medium without growth regulators". "The medium containing 1 mg/l zeatin, 0.1 mg/l IAA and 7.0 mg/l GA_3 was considered the best for direct shoot regeneration and multiple shoot formation from different cultivars of Potato" was reported by Abeuova et al. [1](Table 1). Sota et al. [43] concluded that "the proliferation of the explants into plantlets was varied depending on the treatments. Related to shoot lets and root lengths, a slight efficiency of kinetin in comparison to BAP was observed". "The higher concentrations of BAP or kinetin (1 mg/l) caused a decrease in biometric parameters except leaves number. The best results for the number of roots per shoot (42.5) were obtained by using half-strength MS medium supplemented with IBA at 2.0 mg/l. The best concentration of BAP for microtuberization in potato was 1 mg/l BAP and Putrescine was 80 mg/l" [18]. Yagiz et al. [49] reported "the MS liquid medium containing 0.1mg/l TDZ most favoured for microtuber formation of plantets in the variety of Hermes". "The maximum number of nodes recorded on MS medium containing 0.25 mg/l BAP + 0.03 mg/l NAA + 0.05 mg/l GA_3 was 4-5. Additionally, the maximum number of leaves recorded on the same medium was 10" [15].

Effect of hormones on in vitro tuberization in potato

Chichinska et al. [6] studied "the influence of several factors affecting *in vitro* tuberization in potato. They reveal that the highest tuberization was achieved when 80 g/l of sucrose was added to culture media. They also indicate that higher concentrations of sucrose cause a decrease of SuSy activities, and reduce consequently tuberization". Aboshama et al. [2] studied "the effect of auxin in the microtuberiztion were obtained the highest results at 1.5 mg/l NAA (1.66, 158, 8.2 and 5.1, microtuber number, weight, length, and diameter respectively). The effect of BAP proves that microtuberization can be induced *in vitro* in the absence of BAP when the medium is supplemented with 80 g/l sucrose. However, the addition of BAP increased the number of microtubers per shoot significantly at 2 mg/l given the best response in tuberization with short photoperiod". Zakaria et al. [51] concluded that "with addition of CCC Improved the microtuberization, Microtuber number increased with increased CCC, but the average weight decreased when CCC was at 500 mg/l and when it was absent, maximum numbers and average weight of microtuber were noted". "Light microtuberization efficiency increased when micropropagated source plants were grown under long days (16/8 h d/n) compared with short days (8/16 h d/n), followed by microtuber induction under short days or continuous darkness" [39]. Dwiati and Anggorowati [8] reported that "alar and dark photoperiod independently affected microtuber emergence, and the best Alar concentration for microtuber production was 10-3 mg/l with 10.67 microtubers/cutting. The dark period did not have a significant impact on the induction of potato microtuber". Ghavidel et al. [13] followed "the experiment results were the mean comparison for photoperiod shows that the highest productivity for whole traits is gained by this treatment 16 h photoperiod and utter darkness by using the combination of 2,4-D (2.26 μM) and BAP (22.19 μM), microtubers number, diameter and weight was increased" (Table 1). Emaraa et al. [10] reported that "the highest multiplication aspects were obtained by MS medium containing 0.2 mg/l NAA together with 0.2 mg/l kinetin". "Maximum number of microtuber (3.8) were obtained in MS basal salt containing 0.01mg/l BAP and 0.01mg/l NAA with 0.1mg/l GA_3" [27]. 2018). Samant et al. [37] stated that over the last decade, several researchers observed that

Jasmonic acid (JA), a growth regulator produced by plants exposed to stress seems to be involved in morphogenic events like tuberization and bulb formation and found that JA was by far the stronger *in vitro* promoter of stolon tuberization than Kinetin. In this study, they found that JA supplement at less than 5 µM in the plantlet multiplication medium generated plantlets with sturdier stems, better-developed root systems, and higher root/shoot biomass ratios compared to plantlets grown on conventional media. Sota et al. [43] reported that a higher percentage (53.3%) of tuberization was observed with the treatment which contained BAP 2 mg/l+ IAA 1 mg/l. However, the lower tuberization percentage (25.9%) was obtained on kinetin 2 mg/l + IAA 0.5 mg/l containing medium. On the other hand, it is observed that tuberization from under medium stolons was induced in a higher percentage for all treatments in comparison to the areal tuberization. The process of microtuberization in potato plants can be stimulated by increasing the concentration of sucrose in the MS medium. In this case, increasing the sucrose concentration from 40g/l to 60g/l and 80g/l has shown to promote microtuber formation. Furthermore, when it comes to promoting sprout proliferation in potato varieties, treating the tubers in vivo with 30mg/l of GA_3 has been found to be the most effective treatment. This treatment has resulted in the de novo sprouting of tubers, indicating its success in stimulating sprout growth [34]. The formation of microtubers was observed on potato seed tuber explants when they were grown on a MS medium supplemented with 0.5 mg/l of BA [46]. The optimum micro tuber number, heaviest weight and large size resulted from MS medium supplemented with 90 g/l sucrose plus 2.5 or 5.0 µg/l ABA. It was found that nutrient media, without growth regulator had higher yield and greater number of microtubers under both light (16 h) and dark conditions as compared to those cultured on media with growth regulator [30]. Sharma et al. [41] found that, the MS medium supplemented with BAP (1.5 mg/litre) and NAA (0.1 mg/litre) resulted in 100% shoot regeneration with an average of 3.75 shoots per explant. This combination of growth regulators promotes vigorous shoot proliferation. Vigorous shoot proliferation was achieved by fortification of calcium pentothenate (2 mg/litre) and gibberellic acid (0.25 mg/litre) in establishment medium. Pre-tuberization was done by the cultures were incubated for 28 days in liquid multiplication medium supplemented with NAA (0.5 mg/litre). It was also observed that the maximum number of microtubers (24) per culture flask was obtained within a period of 10 days when the tuberization medium was supplemented with 80 g/litre of sucrose. Additionally, the maximum diameter of 0.9 cm was recorded when a growth retardant called chlorocholine chloride was present at a concentration of 500 mg/litre (Table 1). Astarinil et al. [3] reported that, the MS medium containing 10 mg/l Kinetin and 2g/l Phytagel produced more number of microtubers and enhance microtuber growth in genotypes Viz., ATTX98468-5R/Y and ATTX98518-5P/Y. Mohamed et al. [26] found that, that the use of kinetin-induced medium has a slight effect on improving tuberization *in vitro*. However, when kinetin is combined with sucrose, this effect is enhanced, particularly when the concentration of sucrose is increased from 30 or 60 to 90 g/l. The highest percentage of tuberization after 8 weeks and the highest number of microtubers were obtained when a high concentration of sucrose (90 g/l) was used in conjunction with dark conditions.

Conclusion

This limitation usually means that potato production begins or continues with deteriorated seed material. The complex physiological process of tuberization is influenced by a number of variables, including the environment, genotypes, growth nutrients, growth regulators, photoperiods, potato cultivar, sucrose content, temperature, nitrogen availability, and the explants source. It is easier to handle and store *in vitro* potato microtubers made from single-node cuttings than from sprouts. Compared to a normal multiplication approach, the generation of seed potatoes *via* micropropagation offers further advantages such fast multiplication, disease-free mother stock, and reduced field exposure risks. The modern, high-tech method of growing seed potatoes by micropropagation is preferred by seed potato growers because of these many benefits.

Table 1. ***In vitro*** **regeneration of potato**

Sl. No	Method	Explant	Medium compositions	Reference
1.	Microtuber induction	Single node	MS+10 mg/l BAP + 80 g/l sucrose	Ebadi and Iranbakhsh et al. [9]
2.	Micropropagation	Sprouts and Nodal	MS+ 0.5 mg/l IBA +80 g/l sucrose	Mani et al [24]
3.	Microtuberization	Nodal segments buds	MS + 3 mg/l GA_3 + 0.1 mg/l kinetin MS + 5 mg/l BAP + 0.2 mg/l kinetin	Dessoky et al.[7]
4.	Microtuberization	Sprouts	MS + 0.2 mg/l BAP + 0.1 mg/l NAA MS + 1.0 mg/l 2,4-D; MS + 2.0 mg/l BAP	Aboshama et al.[2]
5.	Microtuber induction	Nodal cutting	MS + 10 mg/I BA +500 mg/I CCC.	Zakaria et al.[51]
6.	Callus formation & shoot and root formation	leaf, Node, Internode, and shoot tip	MS + 1.0 mg/l 2,4-D + 0.25 mg/l kinetin MS + 1.0 mg/l IAA + 0.25 mg/l GA_3	Haque et al.[14]
7.	Callus induction and shoot regeneration	Tuber segments	MS + 5.0 mg/l TDZ MS + 0.5 mg/l IBA	Khadiga et al.[16]
8.	Microtuberization	Single node microcuttings	Modified MS + 120 g sucrose +100 ml Coconut milk	Dwiati and Anggorowati [8]
9.	Microtuberization	Nodal segments	MS + 2.26 µM 2,4-D + 22.19 µM BAP	Ghavidel et al.[13]
10.	Microtuberization	Sprouts	MS + 2.0 mg/l IBA + 2.0 mg/l kinetin + 2.0mg/l NAA + 1.0 mg/l 2,4-D	Gami et al.[12]
11.	Shoot tip culture	Shoot tip explants	MS + 2.0 mg/l 2, 4-D. MS + 1.0 mg/l BAP + 0.5 mg/l GA_3	Laboney et al. [20]
12.	Microtuberization	Sprouts	MS + 8% sucrose + 4 mg/l kinetin + 1 mg/l BAP	Momena et al. [29]
13.	Microtuberization	Nodal segments	Ms+ 6g/l Sucrose	Fufa and Diro, [11]
14.	*In vitro* shoot regeneration	Internodal explants	MS + 2-5 mg/l ZR.	Molla et al. [28]
15.	Multiple shoots regeneration	Shoot tip nodal segment	MS + 0.1 mg/l GA_3 MS without hormone	Shahriyar et al. [40]

16.	Regeneration, root induction	Tuber segments	MS + 5 mg/l BAP + 1 mg/l IBA.	Khadiga et al. [16]
17.	Microtuberization	Seed tubers	MS without hormone	Mohamed et al. [26]
18.	Microtuberformation	Shoots	MS + 0.2 mg/l NAA + 0.2 mg/l kinetin.	Emaraa et al. [10]
19.	Microtuberization	Nodal segments	MS + BAP + JA (1 and 5 mg/l	Samant et al. [37]
20.	Microtuberization	Nodal segments	MS basal salt + 0.01mg/l BAP + 0.01mg/l NAA + 0.1mg/l GA_3 + Sugar 80g/l	Mohapatra et al. [27]
21.	Minituber production	Sprouts	MS + 2 ppm Ca pantothenic acid + 0.25 ppm GA_3.	Xhulaj and Gixhari, [48]
22.	Microtuberization	Long sprouts	MS + 4.0 mg/l BAP + 1.0 mg/l IAA.	Borna et al. [5]
23.	Microtuberization	Axillary bud	MS without PGRS	Naqvi et al. [30]
24.	Microtuberization	Buds (Sprout)	MS + BAP 2 mg/l + IAA 1 mg/l.	Sota et al. [43]
25.	Microtuberization	Shoots	MS+ BAP 1 mg/l + 80 mg/l Putrescine	Khorsandi et al. [18]
26.	Microtuberization	Whole plant	liquid MS + 0.1/mg/l TDZ	Yagiz et al. [49]
27.	Micropropagation	Nodal explant	MS + 0.5 mg/l 2-iP	Pradana et al. [35]
28.	Microtuberization	Seed tubers	MS + 0.4 mg/l kinetin	Oves et al. [33]
29.	Microproagation	Seed tubers	MS+ 10 mg/l BAP + 10 mg/l NAA	Khan et al. [17]
30.	Microtuber induction	Nodal explant	MS+ 10 mg/l Kinetin + 2g/l Phytagel	Astarinil et al. [3]
31.	Microtuber induction	Seed tubers	MS + 40 g sucrose + 0.5 mg/l GA_3	Makau et al.[23]
32.	Microproagation	Nodal explant	MS + 7.5 g/l sucrose without hormone	Karyanti et al. [15]
33.	Microproagation	Seed tubers	MS + 0.25 mg/l BAP + 0.03 mg/l NAA + 0.05 mg/l GA_3.	Venat et al. [47]
34.	Microproagation	Seed tubers and nodal explant	MS + 30 mg/l GA_3.	Petrova and Gudeva, [34]
35.	Minituber	Seed tubers	MS + 0.5 mg/l BA.	Toma [46]
36.	Microtuber induction	Single Node	MS+ 0.04 mg/l kinetin and 1.0 mg/l IAA.	Mohamed et al. [26]
37.	Microtuber induction	Nodal segment	MS+ 1.5mg/l BAP+ 0.1 mg/l NAA	Sharma et al.[42]

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