



Optimizing Microtubers Production for Sustainable Potato Cultivation in Gujarat, India [†]

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Abstract: Gujarat is one of India's top potato-producing regions, making it one of the world's top producers of potatoes. The demand for potatoes is driven by the food processing industry, domestic consumption, and export opportunities. While potato production in India has been growing, there are several issues that affect the industry. The availability of high-quality potato seeds, as well as post-harvest losses due to improper handling and storage, are major challenges. The purpose of this study was to investigate the effects of various culture systems and nutrient supplements to establish and optimize a suitable system for in vitro shoot growth, microtuberization, and storage conditions. In vitro cultures of six different locally adapted potato cultivars were created and the shoot multiplication protocol was standardized. The microtubers protocol was optimized using four-week-old shoots, and a mean of four microtubers per shoot was observed on the Murashige and Skoog medium supplemented with 6-benzylaminopurine (0.88 μM) and sucrose (8%). Harvested microtubers were used to investigate storage conditions and shoot growth was evaluated from microtubers under in vitro as well as ex vitro conditions. All microtubers developed healthy shoots after 18 days of storage at 4 °C both in vitro and ex vitro, and the resulting plantlets showed >90% survival in the greenhouse. The distribution of high-quality potato seeds in Gujarat, which are in high demand, may benefit from the optimal microtuberization protocol. This study confirms the potential of long-term germplasm preservation and microtuber-based cultivation practices in the Gujarat.

Keywords: micropropagation; microtuber; liquid culture; sucrose; microtuber storage; germplasm



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1. Introduction

Potatoes, in addition to cereals, contribute largely to food security in India. In 2016, potatoes in India occupied an area of 2.13 million hectares, and total annual production reached almost 44 million tonnes with yields which averaged 20.5 tonnes per hectare. The potato is an important cash crop of Gujarat (ranked fourth) and leads the country for productivity with 125,000 hectares of land cultivation. The phenomenal increase in productivity and production of potatoes has been termed as the “Brown Revolution”, which placed India as the second major potato producer in the world [1]. With a projected population increase of 19% by 2050, India faces a tremendous challenge to increase its production of all food crops, including potatoes, to meet future demands.

Potato crops are vegetatively propagated using tubers, and farmers mainly receive seed tubers from the Central Potato Research Institute (CPRI), Himachal Pradesh, India. However, cultivars with improved resistances and tolerances to abiotic and biotic stresses are not able to solve the problem for potato farmers. There are significant crop losses due to bacterial and fungal diseases [2], and very limited resistant cultivars are in cultivation with limited planting material. Of the fungal diseases, late blight (caused by *Phytophthora*

infestans) is a major disease in potatoes and causes serious tuber losses globally [3,4]. The seed tuber cost contributes nearly 34% of the total cost of production, in addition to transportation and storage cost. A major constraint limiting the expansion of the potato production area and productivity in Gujarat is the scarce availability of resistant cultivars. Moreover, timely availability of planting materials, high transportation cost, and poor storage facilities increase the production cost.

There are many advantages to plant tissue culture (micropropagation) that are true to type plants for clean, disease-free, year-round production and easy transportation. Microtubers are small potato tubers produced through tissue culture using in vitro axillary buds [5–7]. Microtubers can be stored for longer durations in small spaces and used directly in the field for planting. Microtubers can be used for germplasm exchange and conservation. Microtubers can be used in the green house to produce minitubers or they can be used directly in the field. The microtubers used for direct field planting have high commercial potential, particularly in regions with warm and well-drained soils during planting seasons.

The objective of this study was to develop an efficient protocol for in vitro propagation of potato cultivars and microtuberization using a liquid culture system. The liquid-based culture system was compared with the semi-solid system for assessment of its efficiency for optimum microtubers development. Various sucrose concentrations and plant growth regulators were used to develop the microtubers. The effect of microtuber storage conditions and size were also evaluated for plantlets' development and acclimatization.

2. Materials and Methods

Plant Materials and In Vitro Culture Initiation

Tubers of six potatoes (*Solanum tuberosum* L.) cultivars—Kufri Badshah, Kufri Pukhraj, Kufro Mohan, Kufri Leema, Kufri Nilkanth and P-89400 T—were collected from the Potato Research Station, Gujarat, India (Figure 1A). Potatoes were stored in the refrigerator at 4 °C for 6–8 weeks. Potato bud sprouts were used as explants to initiate in vitro cultures (Figure 1B). Bud sprouts were surface sterilized with 0.1% HgCl₂ for 5 min followed by 8% Sodium hypochlorite for 5 min. They were then rinsed with autoclaved deionized water, four times for 3 min each wash. After sterilization, bud sprouts were cultured on a semi-solid MS (Murashige and Skoog) [8] basal medium supplemented with 3% sucrose and 0.8 g Agar. The pH of the medium was adjusted to 5.70 prior to autoclaving for 20 min at 121 °C and 118 kPa.

All the cultures were maintained in vitro on MS (Murashige and Skoog, 1962) medium supplemented with 3% sucrose under standard culture conditions (16 h light/8 h dark photoperiod from cool white fluorescent lamps with 40 μmolm⁻²s⁻¹ light intensity). Shoot multiplication medium was optimized using liquid and semi-solid MS medium supplemented with Benzyl aminopurine (0, 0.2, 0.5, 1.0 mg/L) and Gibberellic acid (0, 0.1 mg/L). Microshoots obtained from the cultures in shoot multiplication experiments were transferred to basal media for root development.

Microtuberization and storage: four-week-old in vitro shoots with 5–6 internodes were used for microtuberization experiments. Different levels of sucrose (3, 6, 8, 10%) were used in the semi-solid as well as liquid medium for microtuber development. Culture vessels with liquid medium were kept on a rotary shaker (100 rpm) continuously. The total numbers of microtubers were recorded after 6 weeks of the culture. Microtubers (>0.5 cm) were harvested and stored in the refrigerator at 4 °C in dark conditions. Shoot development from microtubers was evaluated under in vitro and greenhouse conditions after storage (0, 1, and 2 weeks). Rooted shoots from microtubers and directly from nodal explants were transferred in the tray with soil mixture and covered with plastic for 10 days. All experiments were repeated twice with a minimum of 3 replications. Means were compared using Tukey's test and values for $p < 0.05$ were considered statistically significant.



Figure 1. In vitro shoot multiplication and microtubers development in various potato cultivars. Potatoes of various cultivars were collected from the Potato Research Station, Gujarat, India, (A) and bud sprouts (B) were used to initiate the in vitro cultures (C,D). In vitro shoots were multiplied on optimal Murashige and Skoog basal medium (E) and individual shoots with 5–6 internodes were used for microtuber development on semi-solid (F) and liquid (G) medium. The microtubers were collected and stored in the refrigerator at 4 °C (H) and all microtubers developed healthy shoots (I) after 18 days. All the shoots developed from microtubers were transferred to a greenhouse (J,K) with more than a 90% survival rate.

3. Results and Discussion

The availability of locally adapted potato cultivars and their seed tubers, transportation, and storage loss are major constraints for potato cultivation. The aim of the present study was to establish a low-cost production system for microtubers and to investigate the liquid culture systems, nutrient medium, plant growth regulators, and number of sub-culture cycles on the quality of the plants, as well as number and size of the tubers during potato shoot multiplication and tuber induction stages. Generally, the protocol is genotype specific, and the optimized protocol was evaluated based on six potato cultivars to construct the most common protocol.

A clean culture of six various potato cultivars was established using bud sprouts as an explant (Figure 1C,D). All bud sprouts responded to MS basal medium for shoot development; however, nearly 22% became contaminated after 10 days. Single nodal segments were used for shoot multiplication and development. Shoot height (8.5 cm) and internodes per shoot (5) were observed to be highest in the MS medium supplemented with BA (1.0 mg/L) and GA3 (0.1 mg/L) in semi-solid medium compared to other levels of BA and control treatments.

The average number of microtubers (four microtuber/shoot) observed on the MS medium supplemented with 6-benzylaminopurine (0.88 μ M) and sucrose (8%) was significantly higher than that observed with other sucrose concentrations. Carbon source, in the form of sucrose, is the key factor in the medium for microtubers development [9,10]. Similarly, cytokinin-induced potato microtubers were observed under in vitro conditions [11]. One of the higher numbers of microtubers (four) was observed in the liquid culture system compared to the semi-solid medium. A temporary immersion-based liquid culture system was reported for microtuber development [12]. The size and numbers of tubers varied by cultivars; however, the cultivar response was not significantly different. Similar observations were recorded in the study conducted by [13]. In the present study, an average of 15 microtubers per flask was harvested after 6 weeks of culture.

Microtubers (>0.5 cm) were harvested and stored in a refrigerator at 4 °C in dark conditions. We found that 100% of the microtubers developed shoots under in vitro conditions as well as in the greenhouse after 18 days of storage. The uniformity of microtubers and size were important factors for shoot development [14]. All shoots from in vitro microtubers survived in the greenhouse conditions. In 10 days, rooting was observed in all shoots from the microtubers and nodal explants when transferred to half strength MS basal medium. Three-weeks-old rooted shoots from nodal explants and microtubers acclimatized successfully, with a survival rate of more than 90%.

The major issue of the lack of healthy seed tubers of tolerant cultivars can be resolved by mass producing microtubers and supplying them at low cost. The optimized protocol can be used to mass-produce disease-free microtubers at commercial scale. Various potential germplasms, as well as their microtubers, can be maintained under in vitro conditions using the standardized protocol, showing potential for farmers and breeders.

In conclusion, in vitro cultures of six different locally adapted potato cultivars were established, and the shoot multiplication protocol was standardized. The microtubers protocol was optimized using four-week-old shoots, and a mean of four microtubers per shoot was observed on the Murashige and Skoog medium supplemented with 6-benzylaminopurine (0.88 μ M) and sucrose (8%). Harvested microtubers were used to evaluate storage conditions and shoot growth coming out from microtubers under in vitro as well as ex vitro conditions. This optimized protocol emphasizes the importance of living germplasm conservation under in vitro conditions.

Author Contributions: S.R.S. and H.M.A. participated in the conception and design of the study. S.R.S. executed the experiments and collected and analyzed the data. H.N.Z. and S.D.S. participated in the organization and management of the study. S.R.S. prepared the manuscript and all authors read and approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

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