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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 locally adapted six different potato cultivars have been initiated and the shoot multiplication protocol has been standardized. The microtubers protocol was optimized using four-week-old shoots and a mean of 4 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 for 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 microtubarization 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, total annual production reached almost 44 million tonnes and yields averaged 20.5 tonnes per hectare. The potato is an important cash crop of Gujarat (ranked fourth) and leading the country for productivity with 125000 hectares' land cultivation. The phenomenal increase in productivity and production of potatoes has been termed as the "Brown Revolution" that 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 production of all food crops, including potatoes, to meet future demands.

Potato crops are vegetatively propagated using tubers, and farmers are getting seed tubers mainly from Central Potato Research Institute (CPRI), Himachal Pradesh, India. However, improved cultivars with resistances and tolerances to abiotic and biotic stresses, are not able to solve the problem of 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 potato 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 potato production area and productivity in Gujarat is 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 of plant tissue culture (Micropropagation) that are true to type plants, clean, disease-free, year-round production, and easy transportation. Microtubers are small potato tubers produced through tissue culture using *in vitro* axillary buds [5, 6, 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 the potato cultivars and microtuberization using 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 concentration and plant growth regulators were used to develop the microtubers. The effect of microtubers storage conditions and its size were also evaluated for plantlets development and acclimatization.

2. Materials and Methods:

2.1. 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 have been collected from Potato Research Station, Gujarat, India (Figure 1A). Potatoes were stored in the refrigerator at 4 °C for 6-8 weeks. Potato bud sprouts have been used as explants to initiate *in vitro* culture (Figure 1B). Bud sprouts were surface sterilized with 0.1% HgCl2 for 5 minutes followed by 8% Sodium hypochlorite for 5 minutes. They were then rinsed with autoclaved deionized water, four times, 3 min each wash. Bud sprouts after sterilization 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 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 have been 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 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%) used in the semisolid as well as liquid medium for microtubers development. Culture vessels with liquid medium kept on the rotary shaker (100 rpm) continuously. Total numbers of microtubers were recorded after 6 weeks of the cultures. Microtubers (>0.5 cm) were harvested and stored in the refrigerator at 4 °C in dark conditions. Shoot development from microtubers were evaluated under *in vitro* and greenhouse conditions after storage (0, 1, 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 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 the greenhouse (J and K) with more than 90% survival rate. .

3. Results and Discussion

The availability of locally adapted potato cultivars and their seeds 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 subculture cycle on the quality of the plants, and number and size of the tubers during potato shoot multiplication and tuber induction stages. Generally, protocol is genotype specific, the optimized protocol will be evaluated based on six potato cultivars to make the most common protocol.

A clean culture of six various potato cultivars have been established using bud sprouts as an explant (Figure 1C and D). All bud sprouts responded to MS basal medium for shoot development, however, nearly 22% got contamination 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 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.

An average numbers of microtubers (4 microtuber/shoot) were observed on the MS medium supplemented with 6-benzylaminopurine (0.88 μM) and sucrose (8%) which was significantly higher than 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 (4) were observed in liquid culture system compared to semi-solid medium. Temporary immersion based liquid culture system has been reported for microtuber development [12]. The size and numbers of the 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 harvested after 6 weeks of cultures.

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

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

In conclusion, *in vitro* cultures of locally adapted six different potato cultivars have been initiated and the shoot multiplication protocol has been standardized. The microtubers protocol was optimized using four-week-old shoots and a mean of 4 microtubers per shoot was observed on the Murashige and Skoog medium supplemented with 6-benzylaminopurine (0.88 μM) and sucrose (8%). Harvested microtubers have been used for storage conditions and evaluated 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. and H.A. participated in the conception and design of the study. S.R. executed the experiments, and collected, and analyzed the data. H.Z. and S.S. participated in the organization and management of the study. S.R. 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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