*Proceedings of the Pakistan Academy of Sciences:*
*B. Life and Environmental Sciences* 57 (2): 75-82 (2020)

ISSN: 2518-4261 (print), ISSN 2518-427X (online)

Pakistan Academy of Sciences

Research Article

# *In vitro* Propagation of Potato Cultivar under Abiotic Stress

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**Abstract:** The research study was conducted to explore the abiotic effects on *in vitro* propagation of "Karuda" potato cultivar. Potato shoot tips were cultured on Murashige and Skoog medium (MS media) with varying pH (4.8-8.8). Best results were shown when explant was cultured on MS medium with adjusted pH 5.8 with numbers of nodes (8), shoot length (6.5 cm), numbers of leaves (7), numbers of roots (2), followed by numbers of nodes (3), shoot length (4.5 cm), numbers of leaves (2), numbers of roots (1) at pH 5.8. Different light conditions and temperature variations were also examined. The results declared that there was a significant difference in numbers of nodes (4), shoot length (4.9 cm), numbers of leaves (4.7), numbers of roots (1) were more at 25°C followed by numbers of nodes (3), shoot length (1.5), numbers of leaves (7), numbers of roots (3) at 30 °C while negligible growth was recorded at 40°C. The 2 mg/L BAP cytokinin+0.1 mg/L NAA showed maximum growth in potato. Moreover, this study also showed that light and dark conditions also affected plant growth. It is evident that environmental factors pose a great impact on plant growth.

**Keywords:** Potato, propagation, Environmental factors, Explant.

## 1. INTRODUCTION

Potato is the 4th important crop after wheat, maize, and rice in the world [1] Global climate change is a great challenge to sustainable crop production. With the changing climate, global warming poses a severe threat to plant growth and development and consequently poor crop yield [2]. Such negative effects of climate will increase abiotic stresses to crop plants in the future because of the continuous emission of greenhouse gases [3] Pakistan is already facing food security issues [4]. It has created a global challenge regarding food security, which demands that the productivity of major crops should be increased many folds for the next 50 years to meet the food requirements of the growing population [5]. Thus, to increase potato yield, we will have to develop suitable production practices and identify new promising potato genotypes that face changing climatic conditions without losing yield potential. Up till now, there are bottlenecks to get the precise mechanism of plants' resistance to abiotic stresses and the consequent ability to expect future results. The major focus is to mitigate the abiotic stresses which directly or indirectly influence on growth and development of potato plants, their yield, and potential adaptation approaches.

Potato (*Solanum tuberosum* L.) is one of the most important vegetables and a staple food crop in many countries [6]. Plants are the source of food for other organisms in an ecosystem but the growth and distribution of plants are limited due to environmental factors like water, nutrients, light, temperature, and pH. This study was designed to explore their promising effects on in vitro propagation of potato as a test plant. With the climate change potato is prone to different pests and diseases e.g. viral infections, (PLRV), late blight caused by a pathogen *phytophthora infestans,* [6] fungal wilts (*Fusarium* spp. and *Verticillium* spp.), powdery scab (*Spongospora subteranea*), black scurf (*Rhizoctonia solani*), and zinc deficiency. Pest includes aphids, cutworms, jassids, whiteflies, and mites. local "Desi" varieties are most vulnerable to virus infections [7]. Viruses infect potatoes

Received: February 2020; Accepted: June 2020
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crop naturally through infected tubers and cause significant yield loss [8]. These fungal and viral diseases are being controlled by the use of tissue culture. One of the major advantages is the eradication of different kinds of viruses that cause the drastic reduction of yield in terms of quality and quantity of the crop [9]. Micropropagation is the alternative to the conventional propagation of potatoes [10]. Propagation of potato by in vitro culture of axillary buds are commonly used for the production of disease-free plantlets, germplasm exchange, and seed tuber production. *In vitro* Propagation techniques possess numerous benefits that make them supreme propagules to get disease-free, high-quality seed tubers [11] in large quantity within a short period of the year [12].

The objectives of this study include:

- Optimize the disinfectant (percentage and exposure time) for explants (potato).
- Study the effect of light, temperature, pH of culture medium, and growth regulators (Auxin and Cytokinin) on culture establishment of potato.

## 2. MATERIALS AND METHODS

This study was conducted in the tissue culture laboratory of BioResource Conservation Institute, NARC, Islamabad. To evaluate the effects of abiotic factors on *in vitro* propagation of potato initially the explant (apical & lateral parts) of potato plant (Karuda cultivar) was collected from the field of Potato Program NARC which was surface sterilized with tween 20 and a pinch of detergent dissolved in water. These were surface sterilized by washing underflow of tap water for 40-45 minutes. After washing, the clean bench (Laminar Air Flow chamber) was swabbed with ethanol, and sterilized tools were exposed to UV light for 10 to 15 min. Then explants were again surface sterilized in laminar airflow hood with different concentrations of Sodium Hypochlorite (NaOCl) i.e., 5%, 10%, 15% for 5, 3, and 2min respectively, multiple washing with autoclaved distilled water to remove extra NaOCl.

### 2.1 Media Composition

Murashige and Skoog (MS) [13] is the most reliable media used for basic tissue culture medium for plant regeneration from tissues and callus. For the preparation of MS media, stock solutions and sucrose were added in a beaker making the volume by adding distilled water. Sucrose was added in distilled water and placed on the stirrer before adding it into the stock solution. The pH of the solution was adjusted to 5.8 and finally, agar was supplemented before autoclaving. Media was transferred into test tubes and jars, 10 ml, and 50 ml media in each respectively. After transferring, the media was sterilized in an autoclave at 121°C for 17 -20 minutes in the case of jars whereas 7 minutes for test tubes.

### 2.2 Explant Culture and Incubation

The apical portion was further aseptically cut before being placed in the culture medium. The explants prepared from the above treatment were implanted vertically on culture medium individually in a glass test tube of size 25×190 mm, containing 10 ml of solidified media with the help of sterilized forceps. After inoculation, explant cultures were incubated at 25 °C under the light of white fluorescent tubes for 3 weeks. The cultures were always incubated at 25±1 ºC under 16 hours of light (2,000 lux) with a white fluorescent tube [14].

### 2.3 *In vitro* Subculturing

For *in vitro* propagation the multiplied shoots of uniform size were excised and transferred to MS media for different abiotic stresses and the data was recorded for the number of leaves, roots, nodes, shoots, and plant height,

### 2.4 Effect of pH

To check the effect of pH on *in vitro* propagation of potato, 750 ml MS media was prepared which was segregated and poured in five beakers with an equal volume (150 ml/beaker). The pH of the medium was adjusted individually i.e., 5.8, 4.8, 6.8, 7.8, and 8.8 before autoclaving. Finally, it was poured into glass jars (50 ml/jar).

### 2.5 Effect of Temperature

Temperature effect was studied on *in vitro* propagation of potato, after inoculation of explant under aseptic condition, they were kept under three different temperatures of 25 °C, 30 °C, and 40 °C.

### 2.6 Effect of Light

To see the effects of light on *in vitro* propagation of potato, after inoculation of explant under aseptic condition, they were kept under three different light conditions i.e., moderate, dark, and intense light conditions.

### 2.7 Effect of Growth Hormones

For studying the effect of growth hormones were supplemented in MS media (i.e. Auxins and Cytokinin: 2 mg/L BAP (cytokinin)+0.1 mg/L NA, auxin 2.5 mg/L BAP+0.1 mg/L NA) are used for explant inoculation under aseptic condition. Finally, they were placed in an incubator for three weeks.

### 2.8 Statistical Analysis

Randomized Complete Design with three replicates per treatment was observed. Data were recorded during the study. Statistics 8.1 and Microsoft Excel software was used for (ANOVA) and (LSD).

## 3. RESULTS

### 3.1 Optimization for Disinfectant

In the current study, different concentrations (5-15 %) of sodium hypochlorite (disinfectant) were used to evaluate the optimum concentration of disinfectant for surface sterilization of potato. Results showed that among the tested treatment, maximum plant survival (74%) was ascertained with 5% NaOCl (Table 1, Fig.1). However, the lowest % age survival (16%) of potato was obtained with a 15% concentration.

**Table 1.** Optimization for surface Sterilization of Plant by NaOCl.

| T | Conc. | Time (min) | Plants | Survived | % Survival |
|---|---|---|---|---|---|
| **1** | 5% | 5 | 35 | 26 | 74% |
| **2** | 10% | 3 | 27 | 5 | 21% |
| **3** | 15% | 2 | 12 | 2 | 16% |

### 3.2 Effect of pH on *In vitro* Propagation of Potato

Surface sterilization of explant was done according to the optimization studies. The experiment was conducted in triplicates. After three weeks of inoculation, quantitative characters were recorded and data were statistically analyzed which revealed that best plant growth was obtained at 6.8 pH with 7 number of leaves, 8.6 number of nodes, 3 number of shoots, 2.6 number of roots, and 6.5 cm plant height followed by 5.8 pH with 2.6 number of leaves, 3 number of nodes, 1.6 number of shoots, 1.6 no of roots and 4.5 cm plant height. Results showed that pH significantly affected plant height, number of nodes, number of roots, number of shoots, and number of leaves which is shown in (Table 2; Fig. 2). The highest number of leaves ($7.00^{a}$) was recorded at $treatment_2$ followed by $treatment_3$ ($6.33^{a}$) whereas the lowest number of leaves (1.33c) was observed at Treatment4. Greatest number of nodes ($8.66^{a}$,) were observed at $treatment_2$ followed by $Treatment_3$ ($7.33^{a}$) whereas the smallest number of nodes ($1.00^{c}$) was observed at $treatment_4$. Each plant has shown almost similar response to root formation. The plant of $treatment_2$ with pH 6.8 has shown relatively better response. However there are insignificant results for the number of roots. The topmost number of shoots ($3.00^{a}$) was recorded at $Treatment_2$ followed by $treatment_3$ ($2.33^{ab}$) whereas the lowest number of shoots ($1.33^{c}$) was observed at $Treatment_1$ and $Treatment_4$. Hence pH 6.8 has proven to be very suitable for good shoot quantity and quality. Optimum plant height ($6.53^{a}$) was observed at $Treatment_2$ and $Treatment_3$ which has shown an almost similar response whereas the lowermost height was recorded at $Treatment_4$.

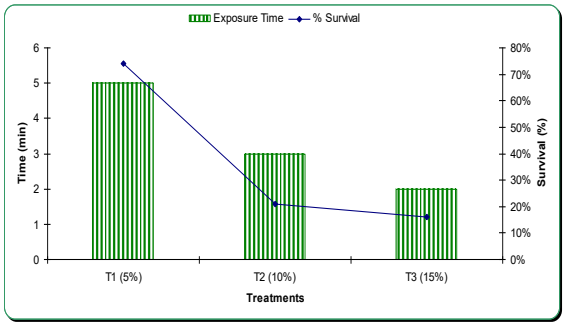


**Fig 1.** Optimization for chemical disinfectant.

### 3.3 Effect of Temperature on *In vitro* Propagation of Potato

Quantitative traits were noted during the incubation period. Data was statistically analyzed after three weeks which showed that best plant growth was obtained at 30 °C with 7 leaves, 3 nodes, 3 roots 2.3 shoots numbers and 5.7 cm plant height followed by


**Fig. 2.** Effect of temperature on *in vitro* propagation of potato (a) shows growth at 25°C, (b) at 40°C and (c) at 30°C.

**Table 2.** Mean performance of potato plant for variant quantitative traits

| No. of Treatments (pH) | No. of Leaves | No. of Nodes | No. of Roots | No. of Shoots | Plant Height |
|---|---|---|---|---|---|
| **Control (5.8)** | 2.6667$^{bc}$ | 3.0000$^{b}$ | 1.6667$^{a}$ | 1.6667$^{bc}$ | 4.5333$^{b}$ |
| **Treatment$_1$ (4.8)** | 3.0000$^{b}$ | 2.6667$^{b}$ | 2.3333$^{a}$ | 1.3333$^{c}$ | 5.2667$^{ab}$ |
| **Treatment$_2$ (6.8)** | 7.0000$^{a}$ | 8.6667$^{a}$ | 2.6667$^{a}$ | 3.0000$^{a}$ | 6.5333$^{a}$ |
| **Treatment$_3$ (7.8)** | 6.3333$^{a}$ | 7.3333$^{a}$ | 2.0000$^{a}$ | 2.3333$^{ab}$ | 6.5333$^{a}$ |
| **Treatment$_4$ (8.8)** | 1.3333$^{c}$ | 1.0000$^{c}$ | 1.3333$^{a}$ | 1.3333$^{c}$ | 2.3667$^{c}$ |
| **LSD** | 1.4092 | 1.4092 | 1.4854 | 0.9395 | 1.9316 |
| **CV** | 19.05 | 17.09 | 40.82 | 26.71 | 21.04 |

LSD: Least significant Difference, CV: Critical Value for Comparison

25 °C with 5 number of leaves, 4.6 number of nodes, 1 number of roots, 5.3 number of shoots and 5.6 cm plant height. Results showed that temperature significantly affected plant height, number of nodes, number of roots, number of shoots, and number of leaves which is shown in (Table 3; Fig. 3). Each plant has shown an almost similar response to the number of leaves. The plant at treatment$_1$ (7.00$^{a}$) which was as at 30°C has shown a somehow better response towards the number of leaves than Treatment$_2$ (5.33$^{a}$) and Treatment$_3$ (5.00$^{a}$). But they did not show statistically significant results for the number of leaves. The plants at treatment$_2$ (4.66$^{a}$) and treatment$_3$ (4.66$^{a}$) have shown relatively better responses towards the number of nodes than Treatment$_1$. However, there are statistically insignificant results for the number of leaves. The topmost number of roots (3.00$^{a}$) was recorded at Treatment$_1$ followed by Treatment$_2$ (2.00$^{ab}$) whereas the lowest number of roots (1.00$^{c}$) was observed at Treatment$_3$. Hence, 30 °C has proven to be very suitable for good root quantity and quality. Treatment$_3$ (5.33$^{a}$) gave a maximum number of shoots was followed by Treatment$_1$ (2.33$^{a}$) whereas the lowest number of shoots (1.66$^{b}$) was observed at Treatment$_2$. The greatest plant height (5.76$^{a}$) was observed at Treatment$_1$ followed by Treatment$_2$ (4.36$^{b}$) whereas the relatively smallest plant height (5.63$^{ab}$) was observed at Treatment$_3$.

### 3.4 Effect of Light on *In vitro* Propagation of Potato

After three weeks of inoculation, the quantitative characters of the plant were recorded and statistically analyzed. Results showed that best plant growth was obtained at intense light with 8.6 number of leaves, 3.3 number of nodes, 3.3 number of roots, 3

**Fig. 3.** Effect of light on *in vitro* propagation of potato (a) intense (b) moderate (c) Dark.

**Table 3.** Mean performance of potato plant for variant quantitative traits

| No. of Treatments | No. of Leaves | No. of Nodes | No. of Roots | No. of Shoots | Plant Height |
|---|---|---|---|---|---|
| **Treatment $_1$ (30°C)** | 7.0000$^a$ | 3.0000$^a$ | 3.0000$^a$ | 2.3333$^a$ | 5.7667$^a$ |
| **Treatment $_2$ ( 40°C)** | 5.3333$^a$ | 4.6667$^a$ | 2.0000$^{ab}$ | 1.6667$^b$ | 4.3667$^b$ |
| **Treatment $_3$ ( 25°C)** | 5.0000$^a$ | 4.6667$^a$ | 1.0000$^b$ | 5.3333$^a$ | 5.6333$^{ab}$ |
| **LSD** | 3.5240 | 2.2088 | 1.1535 | 1.1535 | 1.3452 |
| **CV** | 30.53 | 26.89 | 28.87 | 18.56 | 12.81 |

number of shoots, and 6.7 cm plant height followed by moderate light with 7 number of leaves, 3.3 number of nodes, 3 number of roots, 2.6 number of shoots and 5.7 cm plant height. Results showed that light intensity significantly affected plant height, number of nodes, number of roots, number of shoots, and number of leaves. The effect of different light intensities during plant growth is represented in Table 4: Fig. 3. The highest numbers of leaves (8.6667$^a$) were recorded at Treatment$_2$ followed by Treatment$_1$ (7.00$^b$) whereas the lowest number of leaves (1.33$^c$) was observed at Treatment$_3$.The treatment Treatment$_2$ (7.00$^a$) shown a maximum number of nodes followed by Treatment$_1$ (3.33$^b$) and Treatment$_3$ (2.66$^c$). The topmost number of roots (3.33$^a$) was recorded at Treatment$_2$ followed by Treatment$_1$(3.00$^b$) whereas the lowest number of leaves (1.00$^b$) was observed at Treatment$_3$. Each plant has shown an almost similar response to the number of shoots. The plant at treatment Treatment$_2$ (3.00$^a$) which was as in intense light has shown better response to some extent towards the number of shoots than Treatment$_1$ (2.66$^a$) and Treatment$_3$ (2.33$^a$). Though there are no significant results for the number of leaves. The maximum number of nodes (6.53$^a$) was recorded at Treatment$_2$ followed by Treatment$_1$ (5.766$^a$) and the lowest was recorded at Treatment$_3$ (3.56$^b$).

### 3.5 Effect of Growth Hormones on *In vitro* Propagation

Plant hormones are natural or synthetic chemical compounds that are used to promote or inhibit plant growth and development or alter specific physiology or metabolic factors. To examine the influence of growth initiating hormones on *in vitro* propagation of potato, MS media was supplemented with synthetic Auxin (NA) and Cytokinin (BAP) under controlled conditions (Table 5). The experiment was conducted in triplicates. After three weeks of inoculation, the quantitative characters of the plant were recorded and records were statistically analyzed which revealed that best plant growth was obtained at Treatment$_2$ (2 mg/L BAP cytokinin + 0.1 mg/L NA) with 4.6 number of leaves, 5 number

**Table 4.** Multiplication of plant under different light conditions.

| No. of Treatments | No. of Leaves | No. of Nodes | No. of Roots | No. of Shoots | Plant Height |
|---|---|---|---|---|---|
| **Treatment 1 moderate)** | 7.0000[b] | 3.3333[b] | 3.0000[a] | 2.6667[a] | 5.7667[a] |
| **Treatment 2 (intense)** | 8.6667[a] | 7.0000[a] | 3.3333[a] | 3.0000[a] | 6.5333[a] |
| **Treatment 3 (dark)** | 1.3333[c] | 2.6667[b] | 1.0000[b] | 2.3333[a] | 3.5667[b] |
| **LSD** | 1.4891 | 1.4891 | 0.6660 | 1.4891 | 1.0821 |
| **CV** | 13.15 | 17.20 | 13.64 | 27.95 | 10.24 |

**Table 5.** Mean performance of potato plant for variant quantitative traits.

| No. of Treatments | No. of Leaves | No. of Nodes | No. of Roots | No. of Shoots | Plant Height |
|---|---|---|---|---|---|
| **$T_1$ (1.5 mg/L BAP cytokinin + 0.1 mg/L NAA auxin)** | 2.3333[b] | 4.3333[a] | 1.3333[b] | 2.6667[b] | 2.6667[b] |
| **$T_2$ (2 mg/L BAP cytokinin+0.1mg/L NAA)** | 4.6667[a] | 5.0000[a] | 2.6667[a] | 4.3333[a] | 3.6667[a] |
| **LSD** | 1.3088 | 0.9255 | 1.3088 | 1.3088 | 0.4139 |
| **CV** | 16.50 | 8.75 | 28.87 | 16.50 | 5.77 |

of nodes, 2,6 number of roots, 4.3 number of shoots and 3.6 cm plant height followed by $Treatment_1$ (1.5 mg/L BAP cytokinin + 0.1 mg/L NA) with 2.3 number of leaves, 4.3 number of nodes, 1.3 number of roots, 2.6 number of shoots and 2.6 cm plant height. The $Treatment_2$ ($4.66^a$) shown a maximum number of leaves as compared to $Treatment_1$ ($2.3333^b$). They showed statistically significant results for the number of leaves. Plants at both treatments had shown somehow similar responses but $Treatment_2$ ($5.00^a$) had shown a slightly better response than $Treatment_1$ ($4.33^a$). Therefore, statistically, they had shown insignificant results. The $Treatment_2$ ($2.6667^a$) had shown the highest number of roots as compared to $Treatment_1$ ($1.33^b$). Statistically significant results were found for the number of roots. Plants at both treatments had shown different responses for the number of shoots i.e. $Treatment_2$ ($4.33^a$) had shown a maximum number of shoots than $Treatment_1$ ($2.66^b$). The highest plant height was observed at $Treatment_2$ ($3.66^a$) whereas the lowest height was observed at $Treatment_1$ ($2.66^b$). They showed statistically significant results for the number of leaves. All abiotic factors that were experimented with within this research had a significant effect on the number of potato plants.

### 3.6 Multiplication of Plan

Four explants were used for multiplication which produced 36 plants as shown in Table 6.

## 4. DISCUSSIONS

The lowest % age survival (16%) of potato was obtained with 15% concentration. From the experimental results, we can conclude that a high concentration of NaOCl has a severe side effect [15] The NaOCl is both an oxidizing and hydrolyzing agent. The lower NaOCl concentration gives rise to lower fungal and bacterial contamination and is not toxic enough for plant cells [16]. A balance between concentration and time must be determined

**Table 6.** Mean performance of potato plant for variant quantitative traits.

| Explant used | Parameters | Treatments | Replicates | Explants Multiplied | Explant used |
|---|---|---|---|---|---|
| **4 (1 for each Parameter)** | pH | 4 | 3 | 4×3=12 | 12+9+9+6= **36** |
| | Temperature | 3 | 3 | 3×3=9 | |
| | Light | 3 | 3 | 3×3=9 | |
| | Hormones | 2 | 2 | 2×3=6 | |

empirically for each type of explant because of its phytotoxicity [17].

Overall results of the pH parameter are in agreement with [18] who reported that pH values above 7.5 cause iron, manganese, copper, zinc, and boron ions to be less available to plants, and pH values below 6 cause the solubility of phosphoric acid, calcium and magnesium to drop. Numerous studies have noted that the yield decline associated with high pH levels is usually attributed to pH-related deficiencies of Phosphorus and iron. In areas with acidic soils, both the number of tubers infected and the area of the tuber surface damaged tend to increase at high pH i.e., above 7.0 [19]. The optimum pH range for potatoes is 5.5 to 7.5 [20].

Similar observations have been reported earlier that the rate of plant development is influenced primarily by temperature and that flower development increased as temperature increased [21, 22]. High temperatures show an adverse effect on the growth of many plant species because the rate of photosynthesis (the basic process plants use to make sugar) shows rapid decline after a critical high temperature [23].

Willson et al. [24] recorded earlier that the plants or populations grown in strong light are often capable of greater maximum photosynthesis than the same plants or populations grown in the weak light. Leaves from stronger light tended to be thicker than those from weak light. Nizamuddin et al. [25] also recorded similar results while using growth regulators in different combinations for potatoes.

## 5. CONCLUSION

Environmental conditions such as light, temperature, and pH of medium significantly affect the growth and development of potato explants. However application of 2 mg/L BAP cytokinin+0.1mg/L NAA + MS media considerably reduced the negative effects of temperature, light, and pH on explants of Karuda genotypes under *in vitro* condition, consequently, explants showed high root shoot growth and the number of leaves and roots.

**Conflict of Interest.** The authors declare that there is no conflict of interest.

## 6. ACKNOWLEDGMENTS

Authors acknowledge the support from Bio-Resource conservation Institute and potato program from the National Agriculture Research Centre, Islamabad, Pakistan.

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