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# Indirect *in vitro* Regeneration in Four Varieties of Potato (*Solanum tuberosum* L.) from Internodal Segments and Leaf Explants

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*Key words:* Indirect regeneration, Internodal segments and Leaf explants, Potato

## Abstract

Internodal segments and leaf explants from four varieties of potato (*Solanum tuberosum* L.) cultivated in Bangladesh, namely, Asterix, Diamant, Granola and Lady Rosseta were utilized for indirect *in vitro* regeneration of shoots through callus culture. The best responses regarding the reaeration of shoots were obtained when the explants were cultured on MS medium supplemented with 4.0 mg/l BAP and 1.0 mg/l IAA and 0.5 mg/l $GA_3$. The highest frequency of multiple shoot regeneration was recorded from internodal segments rather than from the leaf explants. Hormone free MS medium was found to be the most effective for healthy root induction from the *in vitro* raised excised shoots. Following adequate root induction, the *in vitro* regenerated plantlets were successfully transplanted and established to soil for further growth and development of tubers.

## Introduction

Potato (*Solanum tuberosum* L.) a common solanaceous crop ranks third among edible crops and top among non-cereal crops in terms of consumption. It is the fifth-most significant food crop in the world after wheat, maize, rice, and sugar cane (Dangol et al. 2018). About 1.3 billion people consume it regularly, and because of its nutrient-dense tubers, it is gaining popularity almost everywhere in the world (Stokstad 2019). It is assumed that by 2050, there will be 9.7 billion people on the planet, and the potato may be a crucial candidate crop for preventing a global food crisis (Dangol et al. 2018).

Total area of the world under potato crop is 18,132,694 hectares and total potato production is 376 million metric tons with average yield rate of 21 tons per ha (FAOSTAT 2023). In Bangladesh, it has been reported that the total potato cultivation areas is 1,147 thousand acres yielding a total of 10,144 thousand tons of potatoes (BBS 2022).

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Potatoes are still under scrutiny for their potential applications in managing health and wellbeing, including the avoidance of the onset of chronic diseases, in addition to their usage as a staple meal and source of energy. In comparison to many other vegetables, potatoes have a better overall nutrient-to-price ratio, and they are a significant staple food around the world. Potato is a crucial crop in the developing countries because it can generate significant yields on small plots of land and be marketed as a cash crop to support the incomes of small farmers (Ramani and Mouille 2019). Compared to other possible food crops, it can produce more carbohydrates, proteins, minerals and vitamins per unit of land and take less time to grow (Zaheer and Akhtar 2016). Vitamin C, potassium and dietary fiber are just a few of the essential elements that potatoes bring to the table (McGill et al. 2013). Nowadays, potatoes make up about half of all consumed root crops, making roots and tubers the third-largest source of carbohydrates in the world (International Potato Center 2018). Potato is widely utilized in industry to produce biofuels, alcohol, starch, animal feed, and processed food goods (Liang and McDonald 2014). Important dietary sources of starch, protein, vitamins, and antioxidants can be found in potato tubers (Burlingame et al. 2009). The production and quality of potatoes are constrained by several biotic and abiotic stresses. Its cultivation and output suffer significant harm from biological pressures such bacteria, viruses, fungi, and insects. One of the most significant causes of its poor yield is disease. Fungal, bacterial, and viral diseases that influence the development of potato crops are brought on by farmers' intensification and inexperience, which results in significant yearly economic losses. Typically, monoculture and haphazard crop rotation are the main causes of soil-borne diseases. Lack of disease-resistant clones and inadequate germplasm may cause some illnesses to spread. There have been 57 different potato diseases reported in Bangladesh so far (Hossain et al. 2008). The most significant diseases among them are late blight, stem rot, *Sclerotium* rot, wilt, common scab, potato leaf roll, and mosaic (Ahmed et al. 2000). Drought, salt, extremely high temperatures, chemical toxicity, and oxidative stress are only a few of the abiotic factors that pose major risks to agriculture (Wang et al. 2003). In recent years genetic transformation techniques have been used to develop biotic and abiotic stress tolerant crop plants. However, before embarking upon such a programme on genetic transformation it is necessary to establish *in vitro* plant regeneration system of that particular plant. Considering the importance of potato in the present investigation attempts were made to establish a genotype independent regeneration protocol for locally available potato varieties.

## Materials and Methods

Tubers of four varieties of potato (*Solanum tuberosum* L.) namely, Asterix, Diamant, Granola and Lady Rosseta used in this investigation were collected from Bangladesh Agricultural Research Institute (BARI), Joydevpur, Gazipur. Tubers were kept at 4°C refrigerator for 7 days to break the dormancy. After 7 days, tubers were placed in the

dark chamber for 15 days at 25 ± 2°C for the development of sprouts. Sprouts (2.0 cm) were then used as primary explants for the establishment of *in vitro* cultures.

For sterilization the sprouts were first washed three times with distilled water and surface sterilized with 0.1% (w/v) $HgCl_2$ for 7-8 mins inside a laminar flow cabinet. The surface sterilized sprouts were cultured on MS to obtain shoots. The desired explants of internodal segments and leaf were collected from 15 days old *in vitro* raised shoots. MS with various concentrations and combinations of BAP, IAA and $GA_3$ were used for indirect *in vitro* regeneration. The pH of the medium was adjusted to 5.8 before autoclaving. Cultures were maintained in growth room with a photoperiod of 16 hrs at 25 ± 1°C with light intensity of 2500-3000 lux. For induction of roots, regenerated shoots were excised and transferred to MS without plant growth regulator (PGR).

## Results and Discussion

MS medium supplemented with different concentrations of BAP, IAA and $GA_3$ were used to study their effects towards callus induction. It was observed that Asterix showed 79.0% responses towards callus induction when internodal segments were cultured on MS medium supplemented with 4.0 mg/l BAP and 1.0 mg/l IAA and 0.5 mg/l $GA_3$ (Table 1). In case of Diamant, 72.0% response was observed when explants cultured on the same media (Table 1). On the other hand, Granola and Lady Rosseta showed 65% and 54% responses, respectively on the same media composition (Table 2). Calli formed on this media were compact and green in nature. Callus induction and regeneration of shoots from callus were presented in Figs 1(a-f). Different concentrations and combinations of BAP, IAA and $GA_3$ were tried to observe their effect on shoot induction and their subsequent shoot development from leaf explants (Tables 1 and 2). Leaves were collected from 15 days old shoots cultured on MS medium. Leaf explants were placed on regeneration medium keeping the adaxial surface touched with the medium.

In case of Asterix, different concentrations of BAP (3.0-5.0 mg/l), (0.5-1.0 mg/l) IAA and 0.5 mg/l $GA_3$ were used. Best response towards shoot regeneration was observed on MS medium supplemented with 4.0 mg/l BAP, 1.0 mg/l IAA and 0.5 mg/l $GA_3$. In this case 66 % of the explants showed shoot regeneration and number of shoots per explants was 3.26 ± 0.15 (Table 1). In case of Diamant, different concentrations of BAP (3.0-5.0 mg/l), IAA (0.5-1.0 mg/l) and 0.5 mg/l $GA_3$ were used. Among these, best response towards shoot regeneration was observed on MS medium supplemented with 4.0 mg/l BAP, 1.0 mg/l IAA and 0.5 mg/l $GA_3$ (Figs 2 a-f). In this case 64% of the explants showed response and number of shoots per explants was 3.31 ± 0.15 (Table 2).

In case of Granola and Lady Rosseta, maximum number of regenerated shoots was found on same media combination (Figs 2 a-f). In this case, internodal segment explants of Granola and Lady Rosseta showed 53 % and 49 % regeneration. The number of shoots per explant was 2.35 ± 0.19 and 2.97 ± 0.13 (Table 2), respectively.

**Table 1. Effects of various combinations of BAP, IAA and GA3 on regeneration and proliferation of multiple shoots from internodal segments and leaf explants of potato (var. Asterix and Diamant)**

| Varieties | Explants | Hormonal supplement (mg/l) | | | % of responsive explants towards multiple shoot regeneration | Mean no. of shoots/ explants after 40 days of inoculation (mean ± SD) | Mean length of shoot/plant (cm) after 40 days of inoculation (mean ± SD) |
|---|---|---|---|---|---|---|---|
| | | BAP | IAA | $GA_3$ | | | |
| Asterix | Internodal segments | 3.0 | 0.5 | 0.5 | 43 | 3.25 ± 0.14 | 6.19 ± 0.18 |
| | | 3.0 | 1.0 | 0.5 | 49 | 3.78 ± 0.13 | 6.39 ± 0.12 |
| | | 4.0 | 0.5 | 0.5 | 61 | 3.87 ± 0.15 | 5.99 ± 0.06 |
| | | 4.0 | 1.0 | 0.5 | 79 | 4.67 ± 0.11 | 5.68 ± 0.08 |
| | | 5.0 | 0.5 | 0.5 | 52 | 3.38 ± 0.12 | 6.11 ± 0.10 |
| | | 5.0 | 1.0 | 0.5 | 48 | 2.43 ± 0.13 | 5.98 ± 0.04 |
| | Leaf | 3.0 | 0.5 | 0.5 | 33 | 2.60 ± 0.13 | 6.71 ± 0.11 |
| | | 3.0 | 1.0 | 0.5 | 39 | 2.68 ± 0.17 | 6.12 ± 0.11 |
| | | 4.0 | 0.5 | 0.5 | 48 | 3.12 ± 0.14 | 5.41 ± 0.03 |
| | | 4.0 | 1.0 | 0.5 | 66 | 3.26 ± 0.15 | 5.38 ± 0.23 |
| | | 5.0 | 0.5 | 0.5 | 54 | 3.46 ± 0.12 | 6.34 ± 0.12 |
| | | 5.0 | 1.0 | 0.5 | 42 | 2.48 ± 0.13 | 6.54 ± 0.12 |
| Diamant | Internodal segments | 3.0 | 0.5 | 0.5 | 39 | 3.63 ± 0.11 | 6.12 ± 0.12 |
| | | 3.0 | 1.0 | 0.5 | 48 | 3.78 ± 0.12 | 6.04 ± 0.09 |
| | | 4.0 | 0.5 | 0.5 | 62 | 4.26 ± 0.29 | 5.85 ± 0.15 |
| | | 4.0 | 1.0 | 0.5 | 72 | 4.97 ± 0.13 | 5.63 ± 0.11 |
| | | 5.0 | 0.5 | 0.5 | 47 | 2.60 ± 0.16 | 6.07 ± 0.09 |
| | | 5.0 | 1.0 | 0.5 | 44 | 1.59 ± 0.17 | 5.85 ± 0.13 |
| | Leaf | 3.0 | 0.5 | 0.5 | 31 | 2.95 ± 0.17 | 6.23 ± 0.14 |
| | | 3.0 | 1.0 | 0.5 | 39 | 3.05 ± 0.16 | 6.02 ± 0.06 |
| | | 4.0 | 0.5 | 0.5 | 52 | 3.05 ± 0.18 | 5.15 ± 0.13 |
| | | 4.0 | 1.0 | 0.5 | 64 | 3.31 ± 0.15 | 5.43 ± 0.07 |
| | | 5.0 | 0.5 | 0.5 | 48 | 3.02 ± 0.16 | 5.52 ± 0.18 |
| | | 5.0 | 1.0 | 0.5 | 46 | 2.18 ± 0.18 | 5.64 ± 0.10 |

Between the two explants, internodal segments showed higher regeneration frequency. Sarker and Mustafa (2002) used leaf, nodal segments and inter-nodes of two local potato varieties for regeneration. They reported highest regeneration from leaf explants. Philip and Hampson (1995) showed high regeneration frequency from internode and leaf tissue explants of potato. Plant regeneration system in potato was either followed direct organogenesis using single media for all phases or indirect organogenesis (Hansen et al. 1997) where callus was initiated on one medium containing auxin and shoot formation on another medium containing cytokinins. For potato *in vitro* regeneration, MS medium was generally used as culture medium. Some researchers performed hormone free MS medium for *in vitro* plant production. On the other hand, studies were done on the effect of different concentrations and combinations of growth hormones for *in vitro* potato reproduction

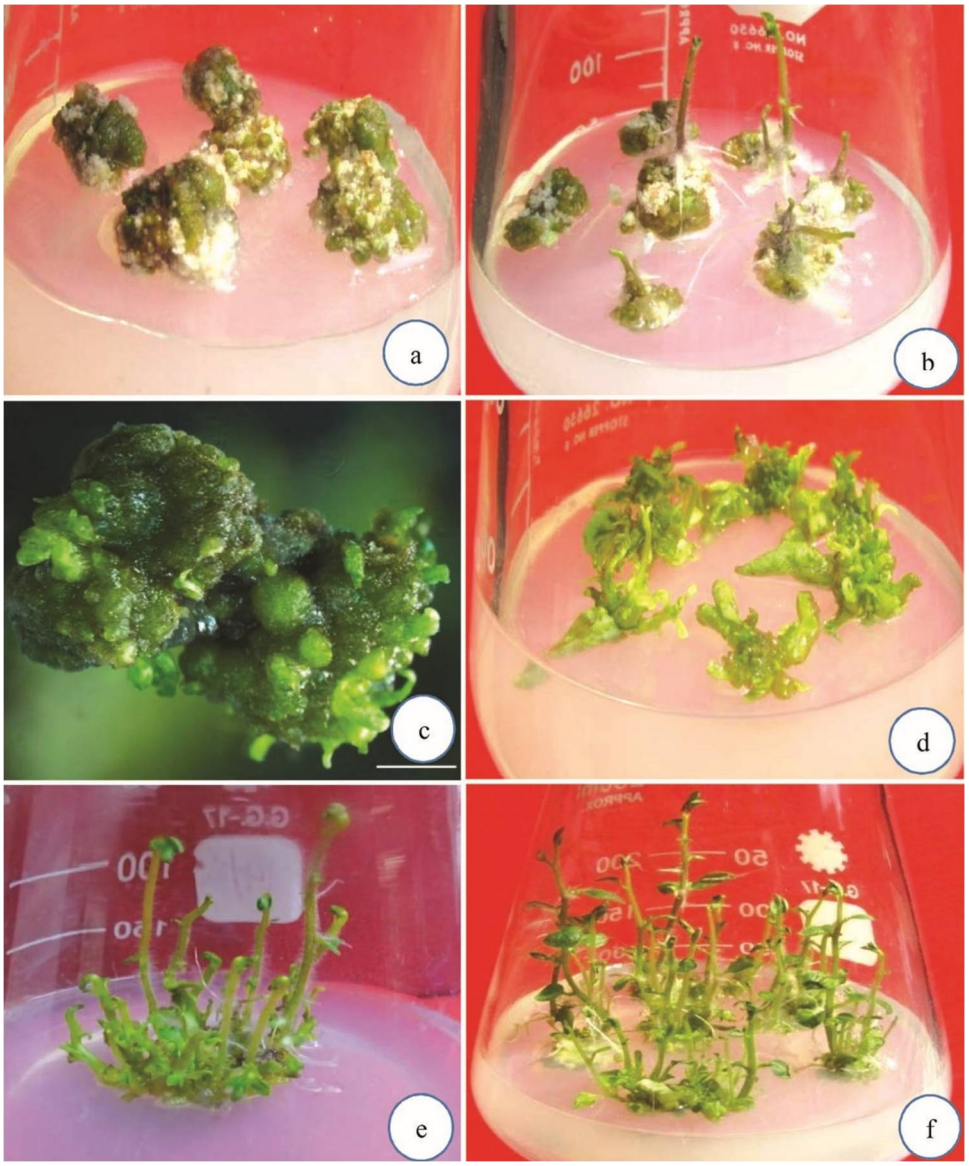


Fig. 1 (a-f): Different stages of indirect *in vitro* regeneration of shoots in Asterix and Diamant on MS supplemented with 4.0 mg/l BAP, 1.0 mg/l IAA and 0.5 mg/l $GA_3$. (a) Formation of callus in Diamant from internodal segments; (b) Initiation of shoots from internodal segment explants in Asterix:; (c) Stereomicroscopic view of initiation of shoots from green compact callus in Asterix (Scale bar = 2.0 mm); (d) Initiation of shoots from leaf of Asterix; (e) Multiple shoots formation in Asterix and (f) Elongated shoots of Diamant.

(Badoni and Chauhan 2009). Several researchers have reported about adventitious regeneration in potato using nodal, inter nodal, leaf disc, petiole, tuber disc explants via direct and indirect organogenesis (Biswas et al. 2010, Onamu et al. 2012 and Ghosh et al.

2014). During the present study, regeneration experiments were conducted using internodal segment and leaf explants via indirect organogenesis. When internodal segment and leaf explants were grown on MS media supplemented with various BAP and IAA concentrations, callus was also developed. With rising BAP and IAA concentrations, callus development frequency rose as well. Within 55 to 60 days on MS with 4.0 mg/l BAP, 1.0 mg/l IAA, and 0.5 mg/l $GA_3$, it was observed that shoot buds started to emerge from those calli. But in the present study, multiple shoot formation through callus was less in number than direct shoot regeneration from nodal segments

**Table 2. Effects of various combinations of BAP, IAA and $GA_3$ on regeneration and proliferation of multiple shoots from internodal segments and leaf explants of potato (var. Granola and Lady Rosseta).**

| Varieties | Explants | Hormonal supplement (mg/l) | | | % of responsive explants towards multiple shoot regeneration | Mean no. of shoots/ explants after 40 days of inoculation (mean ± SD) | Mean length of shoot/plant (cm) after 40 days of inoculation (mean ± SD) |
|---|---|---|---|---|---|---|---|
| | | BAP | IAA | $GA_3$ | | | |
| Granola | Internodal segments | 3.0 | 0.5 | 0.5 | 39 | 3.14 ± 0.12 | 6.25 ± 0.29 |
| | | 3.0 | 1.0 | 0.5 | 41 | 3.93 ± 0.07 | 6.08 ± 0.10 |
| | | 4.0 | 0.5 | 0.5 | 57 | 4.14 ± 0.13 | 5.79 ± 0.17 |
| | | 4.0 | 1.0 | 0.5 | 65 | 4.74 ± 0.06 | 5.48 ± 0.12 |
| | | 5.0 | 0.5 | 0.5 | 49 | 3.49 ± 0.06 | 6.25 ± 0.11 |
| | | 5.0 | 1.0 | 0.5 | 43 | 2.45 ± 0.08 | 5.90 ± 0.15 |
| | Leaf | 3.0 | 0.5 | 0.5 | 29 | 2.46 ± 0.07 | 6.12 ± 0.11 |
| | | 3.0 | 1.0 | 0.5 | 36 | 2.84 ± 0.07 | 6.05 ± 0.10 |
| | | 4.0 | 0.5 | 0.5 | 38 | 3.03 ± 0.07 | 5.47 ± 0.17 |
| | | 4.0 | 1.0 | 0.5 | 53 | 2.35 ± 0.19 | 5.45 ± 0.23 |
| | | 5.0 | 0.5 | 0.5 | 39 | 2.97 ± 0.03 | 5.62 ± 0.16 |
| | | 5.0 | 1.0 | 0.5 | 31 | 2.64 ± 0.17 | 6.07 ± 0.10 |
| Lady Rosseta | Internodal segments | 3.0 | 0.5 | 0.5 | 29 | 2.81 ± 0.12 | 6.21 ± 0.10 |
| | | 3.0 | 1.0 | 0.5 | 38 | 2.97 ± 0.13 | 6.28 ± 0.07 |
| | | 4.0 | 0.5 | 0.5 | 49 | 3.40 ± 0.17 | 6.49 ± 0.33 |
| | | 4.0 | 1.0 | 0.5 | 54 | 3.88 ± 0.10 | 5.77 ± 0.09 |
| | | 5.0 | 0.5 | 0.5 | 41 | 3.04 ± 0.19 | 6.03 ± 0.08 |
| | | 5.0 | 1.0 | 0.5 | 28 | 2.72 ± 0.19 | 7.16 ± 0.14 |
| | Leaf | 3.0 | 0.5 | 0.5 | 27 | 1.96 ± 0.13 | 6.18 ± 0.03 |
| | | 3.0 | 1.0 | 0.5 | 30 | 2.24 ± 0.11 | 6.11 ± 0.10 |
| | | 4.0 | 0.5 | 0.5 | 35 | 2.75 ± 0.17 | 5.10 ± 0.67 |
| | | 4.0 | 1.0 | 0.5 | 49 | 2.97 ± 0.13 | 5.10 ± 0.10 |
| | | 5.0 | 0.5 | 0.5 | 22 | 2.15 ± 0.13 | 6.16 ± 0.07 |
| | | 5.0 | 1.0 | 0.5 | 19 | 1.05 ± 0.18 | 6.75 ± 0.11 |

and microtuber explants. Khatun et al. (2012) reported that leaf and internode showed best response on medium with NAA and BAP. It was observed in the present investigation that leaf explants had poor response towards shoot regeneration compared

to internodal segments. For further confirmation, different concentrations of $GA_3$ were also used in combination with BAP and IAA and observed their combined effect on regeneration and multiple shoot proliferation from leaf explants of four potato varieties. Among the hormones, $GA_3$ is well known to enhance shoot regeneration and elongation. Philip and Hampson (1995) showed high regeneration frequency from internode and leaf tissue explants of potato. Khatun et al. (2012) reported that leaf and internode showed best response on MS medium with NAA and BAP. Parveen et al. (2014) found best shoot regeneration from internode using 0.2 mg/l $GA_3$+0.5 mg/l IAA + 1.0 mg/l BAP.

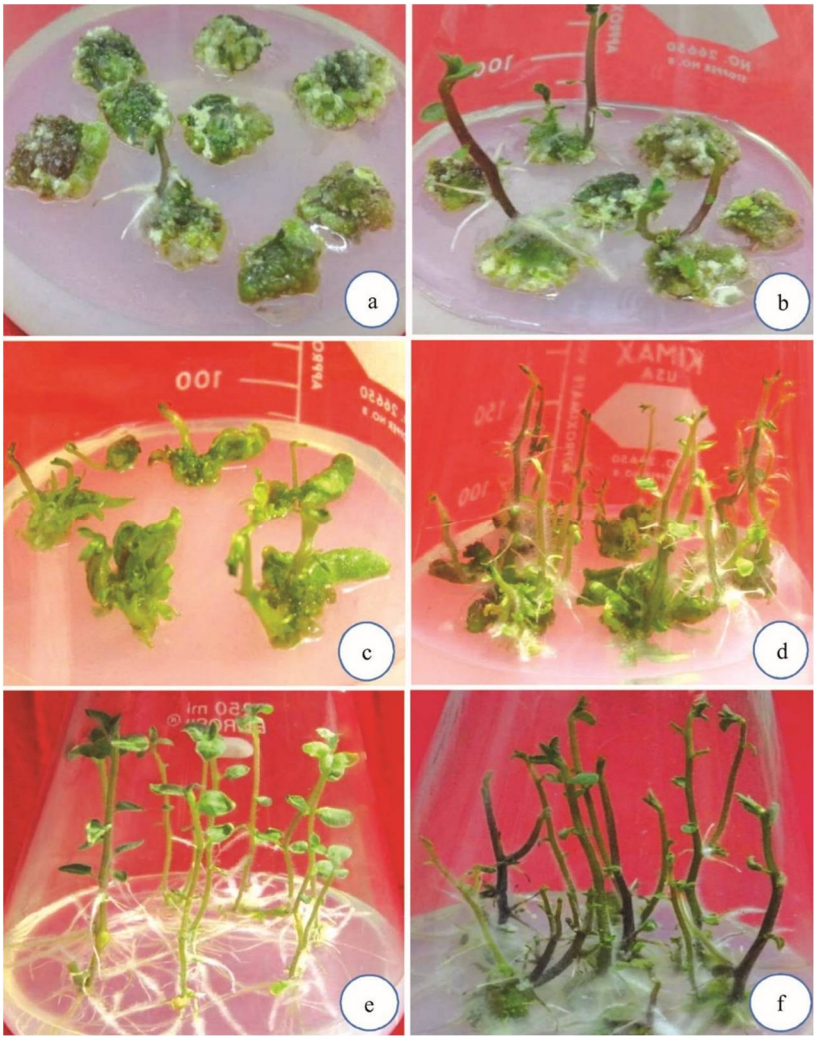


Fig. 2 (a-f): *In vitro* regeneration of shoots in Granola and Lady Rosseta on MS supplemented with 4.0 mg/l BAP and 1.0 mg/l IAA and 0.5 mg/l $GA_3$. (a-b) Formation of multiple shoots from callus of internodal segments of Lady Rosseta; (c-d) Development of multiple shoots from callus of leaf of Granola; (e) Elongated shoots of Granola and (f) Elongated shoots of Lady Rosseta.

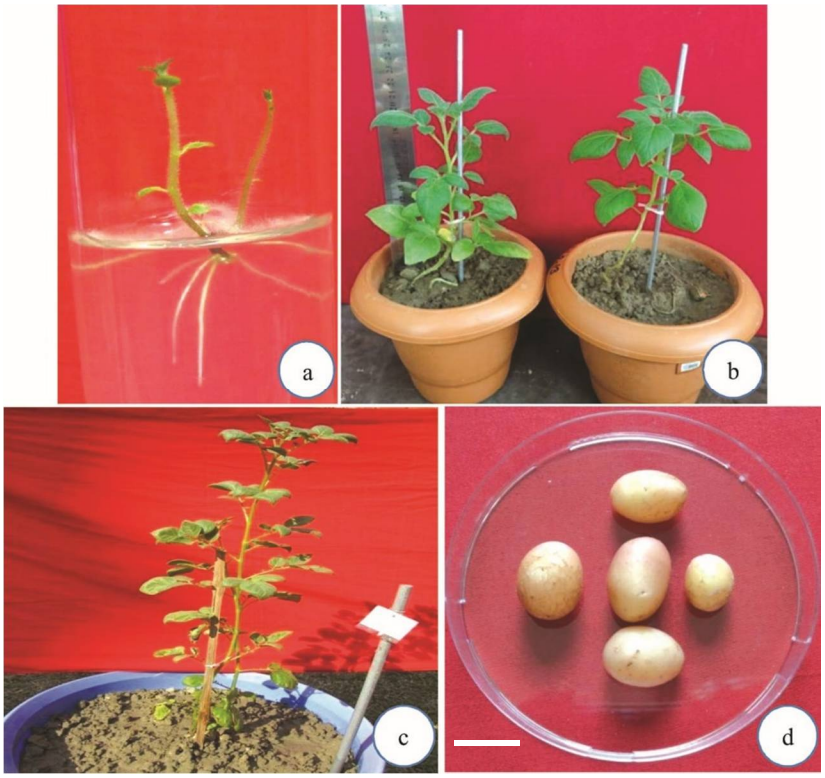


Fig. 3 (a-d): Transplantation of tissue culture derived plantlets of Diamant. (a) Fully developed rooted plantlet on MS medium; (b) Plantlets transferred to small pot containing soil; (c) Regenerated plant of Diamant after two months following transplantation and **(**d**)** Fully developed matured minitubers (Scale bar = 2.0 cm) from tissue culture derived Diamant.

Induction of roots from regenerated shoots is considered to be very important to obtain complete plantlet. In the present study most of the explants produced high number of roots on shoot induction medium (MS supplemented with BAP and IAA) and subsequently these roots were found to produce healthy effective root system when they were transferred to MS basal medium. After 3-4 weeks, well rooted shoots were carefully taken out from the culture tube and were successfully transplanted to plastic pots containing soil. About 95% transplanted plantlets survived which produced phenotypically normal minitubers.

Agar solidified full and half strength of MS medium devoid of any hormonal supplement were used as root induction medium. About 15 days old (2-3 cm in length) single shoots were used for root induction at the base of *in vitro* grown shoot. Four varieties of potato showed identical response towards root induction on MS medium and it took 6-8 days for root induction (Figs 3 a-d).

Healthy root induction at the base of *in vitro* regenerated shoots is a crucial step in the development of plantlets. Throughout the current study, it was observed that a

number of roots spontaneously formed from the *in vitro* grown shoots. However, it was found that spontaneous roots were less successful at transferring *in vitro* developed plantlets to the soil. Hormone free MS was found to be the most successful treatment for root induction in regenerated shoots in all four varieties of potato.

Plantlets that had developed strong roots enough to be transplanted to soil did well in the field. However, Sarker and Mustafa (2002) found best response for root formation on half strength of MS medium supplemented with 0.1 mg/1 IAA. Borna et al. (2010) reported that MS medium containing 0.2 mg/1 IBA showed best response in developing roots of Diamant, Cardinal and Granola.

Overall results of present study indicated that potato varieties used in present investigation were very responsive towards regeneration. Therefore, these materials may also be suitable for the incorporation of desired genes through *Agrobacterium*-mediated genetic transformation to improve their agronomic or qualitative properties.

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