J. Agrofor. Environ. 9 (1 & 2): 153-156, 2015 ISSN 1995-6983

# Acclimatization, field performance and minituberization of tissue cultured potato cultivars

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**Abstract:** The present study was conducted to evaluate the performance of *in vitro* calliclones and minituber formation of potato cvs. Diamant, Cardinal and Granula under the *ex vitro* condition. The interaction effects between cultivars and *ex vitro* performances showed significant differences for all the parameters used in the experiment. The *ex vitro* performance of cardinal calliclones showed the highest results over other two cultivars in terms of survival percentage (80) of plantlets, leaf area (151.3 $cm^2$), number of branches/plant (5.40), fresh weight of haulm (110g), dry weight of haulm (15.04g), number of tubers/plant (22.0) and weight of tubers/plant (375g). Finally it was found that the *ex vitro* field performances potentiality was highest in the variety of Cardinal followed by Diamont and Granula.
**Key words:** Acclimatization, calliclones, *ex vitro*, *in vitro*, minituber.

## Introduction

Potato is one of the most important food crops worldwide and is consumed as staple food in more than forty countries in the world. This crop ranks fourth amongst all global food crops after wheat, rice and maize (Moeinil *et al.,* 2011), while ranks first both in area and production among the vegetable crops grown in Bangladesh. In Bangladesh, about 8.6 million tons of potato were produced from nearly 0.444 million ha with an average yield of 19 t/ha during 2012-2013 (BBS, 2013). Demand for potato is rapidly increasing. However, production has to be increased even with the current rate of demand.

The true seed production of potato is difficult due to its heterozygosity and tetraploidity. So, the sexual production of potato seed is also difficult. Potato propagated vegetatively by means of tuber with some constraints such as, Potato production with seed tuber is constrained by the accumulation of pathogen, physiological declining and low multiplication rates. Seed tuber is most expensive input in potato production. At least 35-40% total cost of potato production is covered by seed tuber. The above major constraints can be overcome by using micro-propagation technique for rapid multiplication of plantlet production.

Micro-propagation offers an efficient and accepted method for rapid propagation and production of pathogen-free seed tubers. It is said that, the tissue culture or micro-propagation is the gateway of all biotechnological research. Tissue culture techniques have several advantages over traditional propagation methods. High frequency regeneration of plants from *in vitro* culture tissues is a pre-requisite for crop improvement and for engineering of this crop to supplement conventional breeding (Alsadon *et al.,* 1998). Thus improvement in the growth of such cultures could be benefit in both basic and applied plant biotechnology.

However a major limitation in large scale application of this technology is high mortality experienced by micropropagated plants during or following laboratory to land transfer. Micro shoots on being transferred to *ex vitro* conditions are exposed to abiotic (altered temperature, light intensity and humidity conditions) and biotic stress conditions i.e. soil microflora (Kumar *et al.,* 2007). So, the *in vitro* response of one variety will not same in case of field performance. Therefore, acclimatization is necessary for successful establishment and survival of plantlets. In order to increase growth and reduce mortality in plantlets at the acclimatization stage, research has been focused on the control of the environmental conditions (both physical and chemical) and to acclimatize the plants as well as to evaluate the field performance of different potato cultivars. The present study was therefore designed to identify the best performed cultivars of *in vitro* propagules under the *ex vitro* conditions.

## Materials and Methods

The field evaluation of *in vitro* calliclones of the three potato varieties were conducted at the ‘’Horticulture Germplasm Centre”, Patuakhali Science and Technology University, during December 2013 to March 2014. The experiment was laid out in the Randomized Complete Block Design (RCBD) with five replications. Each replication had three beds of 180cm x 60 cm. Plantlets were obtained from the *in vitro* grown plant with different concentrations and combinations of plant growth regulators (Plate 1). The uninodal cuttings of micropropagated plantlets were cultured on hormone free MS medium (Murashige and Skoog, 1962). After 45 days of culture when plantlets produced well developed roots and shoots, then these were used as source plant materials for field evaluation (Plate 2). After the soil was well pulverized, the beds were prepared with one part of sandy loam soil, one part of sand and one part of cow dung. Before few days of transplantation, the beds were treated with 1% formaldehyde solution and covered with polyethylene sheet for 7 days. After removing polyethylene sheet the beds were kept open for 4 days. On the other hand when the regenerated plantlets were of 30 days aged with well developed shoots and roots system, the test tube containing plantlets were taken out from the controlled environment of the growth chamber and kept at the room temperature for 5 days. After 5 days of acclimatization the plantlets were taken out from the test tube and the roots were washed under running tap water to remove medium. Then the roots were slightly removed and the plantlets were cut into 5 cm pieces. The cut pieces were wrapped with moist tissue paper and transported to the field for transplanting (Plate 3). Plantlets were transplanted in row keeping 15 cm space between two plants. The distantance between two rows were 30 cm (Gupta *et al.,* 2003). The plantlets were planted in soil by making an opening with the thumb pressing in soil. Care was taken to avoid damage to the roots and to ensure good contact between roots and soil. Then the entire field was covered with nylon net and regular spraying of water was done. Intercultural operation was done manually as

necessary. The bed was fertilized with vermincompost three times followed by flood irrigation. Indofil MZ-72 WP was also sprayed @ 2 g/L wateras a preventive measure against late blight disease when the weather was cloudy. The crop was harvested after 95 days of transplanting at the full mature stage with 80-90% of leaf senescence. The haulm pulling was done before 7 days of harvesting. To assess the effects of different varieties, the data such as percentage of plant survived in the field, plant height (cm), leaf area ($cm^2$), number of branches/plant, fresh weight of the haulm, dry weight of the haulm, number of tubers/plant, grading of tubers/plant, weight of tubers/plants were recorded. The analysis of variance was performed and the means were compared by Least Significant Difference (LSD) test for interpretation of results. The significance of the difference between the pair of means was evaluated at 5% level of probability using the MSTAT-C computer package program (Gomez and Gomez, 1984).

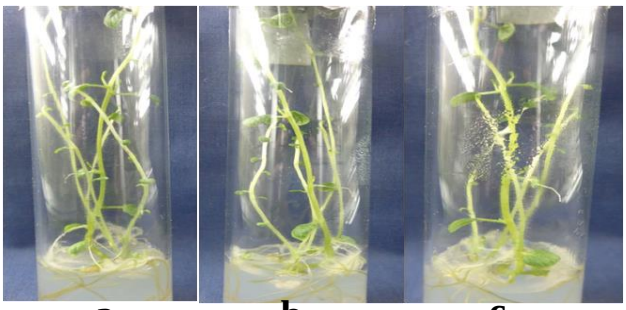


**Plate 1.** Prepared micro plant originated from the uninodal cutting of virus tested potato cvs. Diamant (a), Cardinal (b) and Granula (c)

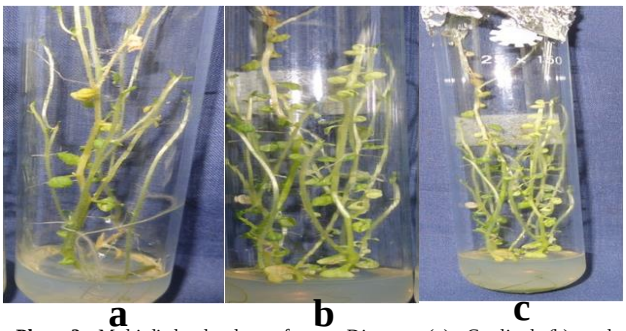


**Plate 2.** Multiplied plantlets of cvs. Diamant (a), Cardinal (b) and Granula (c)

## Results and Discussion

**Survival percentage of regenerated plants:** The percentage of survival rate of regenerated plants in Cardinal was 80, Diamant 69 and Granula 60 (Fig. 1).

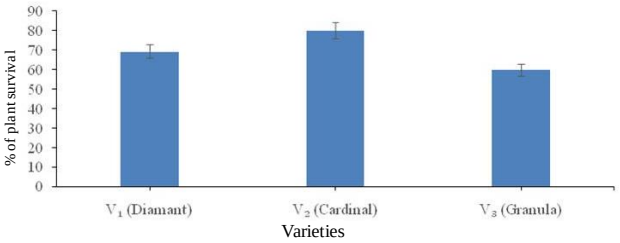


**Fig. 1.** Survival rate of regenerated plants after transferring to the soil

The survival rate of regenerated plants was maximum in Cardinal while lowest in Granula. *Ex vitro* establishment of Cardinal was quicker compared to plants of Diamant and Granula. This varied response towards acclimatization by three tested cultivars might be due to their genetic make-up. Different growth parameters and yield attributes such as plant height (cm), leaf area ($cm^2$), number of branches/plant, fresh weight of the haulm, dry weight of the haulm, number of tubers/plant, grading of tubers/plantand weight of tubers/plants showed significant differences among three potato cultivars. The plant height was significantly influenced by different cultivars at 60 DAP. The tallest plant (59.00 cm) was observed in Diamant followed by Cardinal (54.40 cm) but those were statistically similar with each other and the shortest plant (14.20 cm) was recorded in Granula. This finding is in accordance with the observation of Nagib *et al.* (2003) who also found significant difference in plant height. The highest leaf area (151.3 $cm^2$) was observed in Cardinal followed by Diamant (119.3 $cm^2$) while the lowest leaf area (9.578 $cm^2$) was recorded in Granula (Table 1).

**Table 1.** Field performance of the *in vitro* derived plantlets, morphological and tuber yield data recorded at 60 days after planting (DAP) and at the time of harvesting

| Varieties | Plant height (cm) at 60 DAP | Leaf area ($cm^2$) at 60 DAP | No. of branches per plant | Fresh wt. of haulm (g) | Dry wt. of haulm (g) | No. of tubers per plant | Wt. of tubers per plant (g) |
|---|---|---|---|---|---|---|---|
| Diamant | 59.00a | 119.3b | 4.80a | 102.0a | 12.64a | 21.20a | 288.0a |
| Cardinal | 54.40a | 151.3a | 5.40a | 110.0a | 15.04a | 22.00a | 375.0a |
| Granula | 14.20b | 9.57c | 0.40b | 14.42b | 1.26b | 3.60b | 9.36b |
| $LSD_{0.05}$ value | 7.53 | 13.82 | 1.69 | 29.03 | 4.63 | 4.77 | 192.5 |
| Level of sign. | ** | ** | ** | ** | ** | ** | ** |

In a column values having different letter (s) differ significantly at 5% level of probability, ** Significant at 1% level of probability

The maximum number of branches/plant was 5.400 in Cardinal followed by Diamant (4.800) but both were equal and the minimum number of branches/plant (0.400) was recorded in Granula(Table 1). This finding is in accordance with the observation of Nagib *et al.* (2003) who also found highest no. of branches (4.11) in Cardinal. The fresh weight of haulm was significantly influenced by different cultivars at the time of harvesting .The highest fresh weight of haulm (110 g) was observed in Cardinal followed by Diamant (102 g) but both are statistically similar and the lowest fresh weight of haulm (14.42 g) was recorded in Granula (Table 1). Although, plant height was maximum in Diamant, but the fresh weight of haulm was maximum in Cardinal because, cv. Cardinal produced the maximum number of branches which contained more number of leaves that ultimately gave maximum fresh weight of haulm.The highest dry weight of haulm (15.04 g) was observed in Cardinal followed by Diamant (12.64

g) but those ranked identical. The lowest dry weight of haulm (1.260 g) was recorded in Granula (Table 1). The highest dry weight of haulm in Cardinal and the lowest in Granula. That variation might be due to different genetic make-up of varieties tested.

Among the different cultivars, Cardinal produced the maximum number of tubers/plant (22.0) followed by Diamant (21.20) but ranked equaland the lowest (3.60) was observed in Granula (Plate 1, 2 and Table 1). The reasons of maximum number of tuber in Cardinal over two cultivars was due to the maximum number of branches/plant which contained more number of leaves with maximum leaf area, produced more amount of carbohydrate in plants and ultimately produced more number of tubers. Cardinal produced the maximum weight of tubers/plant (375.0 g) followed by Diamant (288.0 g) but statistically similar with each other and the lowest (9.369 g) was observed in Granula (Table 1). The result of the present study conformity with the findings of Nagib *et al.* (2003) and Karim *et al.* (2011) whom also found significant difference in weight of tubers/plant.

**Tuber grades:** Different cultivars varied significantly for the tuber grade per plant. No cultivar produced the large size i.e C-Grade (> 43mm in diameter) tuber. The maximum (8.800) B-Grade (28-43 mm in diameter) was obtained in Cardinal but the minimum (7.800) in Diamant which was statistically similar, while Granula did not produce B-Grade (28-43 mm in diameter) tubers. The maximum small size (13.40) A-Grade (< 28mm in diameter) was produced in Diamant followed by Cardinal (13.20) but statistically similar with each other while the minimum (3.600) was produced in Granula (Table 2). It has been previously documented that there were significant differences in tuber grades among the different cultivars of potato (Powell *et al.*, 1989).

**Table 2.** Different graded tubers in potato cultivars

| Varieties | Grading of tuber | | |
|---|---|---|---|
| | No. of A-Grade tubers (< 28mm in diameter) | No. of B-Grade tubers (28-43mm in diameter) | No. of C-Grade tubers (> 43mm in diameter) |
| Diamant | 13.40a | 7.800a | 0 |
| Cardinal | 13.20a | 8.800a | 0 |
| Granula | 3.60b | 0.000b | 0 |
| $LSD_{0.05}$ value | 1.705 | 3.854 | |
| Level of significance | ** | ** | |

In a column values having different letter (s) differ significantly at 5% level of probability, ** Significant at 1% level of probability, A-Grade indicate small size; B-Grade indicate moderate size; C-Grade indicate large size


**Plate 3.** Prepared plantlets wrapped with tissue paper(a), prepared bed(b), planting of plantlets in bed (c), plants after 60 days of planting(d), haulm pulled plant(e) and harvesting of tuber(f)

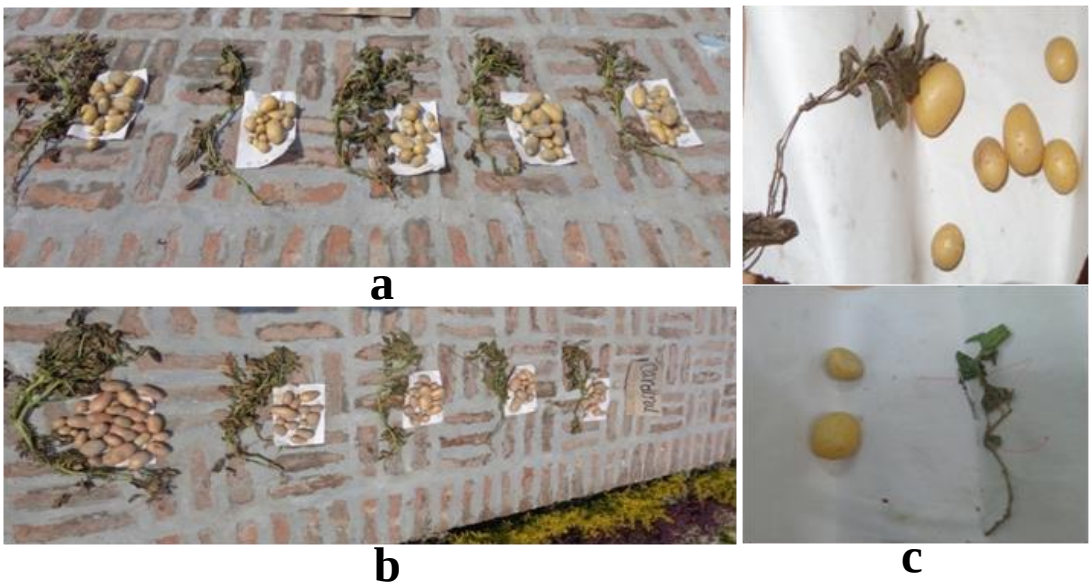


**Plate 4.** The tuber yield of potato cultivars Diamant(a), Cardinal (b), and Granula (c)

Micro propagated plantlets suffer high mortality when transferred from *in vitro* to *ex vitro* conditions (Plate 3). Plantlets should be slowly acclimatized to *ex vitro* conditions with high light intensity and low humidity conditions. The potato mini-tuber production by direct transplanting of plantlets in field though well established but still requires more research on the production and pre-treatment of the *in vitro* plantlets before planting as well as on the management of the crop grown from these *in vitro* plantlets to further increase the number of mini-tubersper*in vitro* plantlet. Since, one of the important factors that affect plant potential for tuber formation is the cultivars from where *in vitro* plantlets were grown (Plate 4). From the study it was revealed that, the potato cultivar Diamant performed best in case of acclimatization and mini tuber formation from the *in vitro* plantlets followed by cv. Cardinal and Granula. It is therefore imperative to undertake the study on all of the existing recommended cultivars of potatofor maximizing the number of mini-tubers per*in vitro* plantlet and improving the size ofmini-tubers while reducing the proportionof extremely small sized mini-tubers for successful multiplication in open fields.

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