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The Significance of Non-controlled Natural Light, Temperature and Humidity in the Commercial Micro-propagation of *Solanum tuberosum* L. Cultivar Diamant.

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Abstract

The aim of our study was to reduce the unit cost of *in vitro* micropropagation of the *Solanum tuberosum* cultivar Diamant widely cultivated in Bangladesh with the guarantee that the quality and quantity of the *in vitro* plants produced was not jeopardized. This was done by entirely replacing the conventional micropropagation conditions of maintaining the *in vitro* plants in a controlled room whose temperature varies between 25 and 30^0 C, its humidity between 60 to 70% and its light intensity of 20,238 to 20,409 for 19 hours; with a room whose roof was made of corrugated plastic sheets that allow a partial passage of natural light. Under this conditions the amount of light, temperature and humidity were not controlled. During the time work the temperatures in this non-conventional room fluctuate between 14 and 40^0C, the light intensities were between 20,017 to 20,687 Lux and the humidity between 40 to 90%. Experiments were initiated in May, 2009 through March, 2014 covering summer, rainy and winter seasons. After two years of laboratory research and two years of field studies, we have not found differences between yield production of the micropropagated plants grown under control and non-controlled conditions, very often the latter plants were robust and adapted faster when transfer to field conditions. All plants used in the field experiments were no more than seven *in vitro* passages. A RBCD yield trail of the plants was done during two seasons and it was no found any difference in yield between them. Moreover, a yield trail of the minitubers to produce breeder seed (second generation) was done during the season 2013-2014 and no differences were found between the controls and the tubers derived from the low cost.

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This paper shows a new approach of *in vitro* micropropagation of plants by reducing the total cost of electricity in the culture room by using non-controlled natural light, ambient temperature and humidity regimes. More importantly, this significant cost reduction (savings) is achieved without compromising the quality and quantity of the *in vitro* plants produced. Furthermore, the simplicity of this technology will allow application by small laboratories and progressive farmers with a minimal capital outlay.

Introduction

In Bangladesh, potato is a winter crop. Planting is done in October through November, and harvested in February through March.

In 2012 potato cultivation in Bangladesh occupied an area of 430,446 ha with a total production of 8.2 million tons and an average yield of 19 tons/ha, (http://faostat.fao.org/Desktop).

The shortage of good quality seed and its high cost are the two of the main factors limiting potato productivity in Bangladesh (Hossain el al. 2008).

Potato, being a semi-perishable crop, is susceptible to different viruses and virus like agents and insect pests. This brings a progressive accumulation of degenerative viral diseases in the potato tubers thereby posing a major threat to potato seed production (Sawyer 1979).

The availability of tissue culture technology for rapid multiplication of disease-free planting material has facilitated considerably potato seed production. Meristem culture is being successfully used to obtain virus free potato clones. These clones can be multiplied through micropropagation which is a tissue culture method used for rapid and true-to-type multiplication of plants grown under controlled artificial lighting and temperature regimes. (Roca et al.1978, Espinoza et al.1984).

The majority of laboratories involved in *"in vitro"* micropropagation use fluorescent tubes as a source of light, air conditioners for cooling purposes and dehumidifiers for maintaining the humidity (Kodym and Zapata-Arias 1999). As a result, the cost of production of *in vitro* is high, making these facilities often commercially unprofitable.

Present authors follow the concept that the room for micropropagating *in vitro* plants should have an optimum incubation temperature that is in the range between 26 - 30^0C, that the humidity should be between 60 to 70% and the optimum light intensities should be around 50 $\mu Mol.m^{-2}.s^{-1}$ between 13 to 19 hrs.

But, if any micropropagation program is going to succeed the cost of each *in vitro* plant should be less that the cost of plantlet raised under conventional procedures (Qaim 1999).

Presently in African and Asian countries brownouts happen every day and sometimes for long periods of time. Compounded to this the electricity and the chemicals used are expensive, making the whole process cost prohibitive (Kodyn and Zapata-Arias 2001).

In this paper authors are presenting a method to reduce the total cost per *in vitro* plant of the potato cultivar Diamant by the replacement of the conventional light room with one whose roof is made of corrugated plastic sheets that allow a partial passage of natural light in which the temperature variation as well as the humidity are not controlled. To study if there were any differences in their plant characteristics and in productivity, yield trails were performed at BRAC's farm situated at Gazipur, Bangladesh during the winter seasons of November 2012-March, 2013 and November, 2013 - March 2014.

Materials and Methods

Tubers of high yielding cultivar Diamant were obtained from the Bangladesh Agricultural Development Corporation (BADC) and grown under dark conditions for 30 to 35 days at 20 - 22^{0}C. Under these conditions the tubers sprout and the buds placed on a test tube containing 15 ml of MS and 30 g/l of sugar semi-solidified with 6 grams of agar with a pH adjusted to 5.8. Healthy growing plants were placed in fresh medium and heat shocked for 30 days at 38^{0}C. Shoot tips were isolated and grown into adult plants under controlled conditions of 16 hrs of light and constant temperature and humidity. Samples of *in vitro* plants were taken to the Bangladesh Agriculture Research Institute (BARI) and checked for the presence or absence of the following viruses: Potato Virus X (PVX), Potato Virus Y (PVY), Potato Leaf Roll Virus (LRV), Potato Virus M (PVM) and Potato Virus S (PVS) using the Enzyme Linkage Immunosorbent Assay (ELISA).

Four nodal stem cuttings with around 32 nodes were cultured in a magenta box containing MS major and minor salts, 30 g/l of commercial sugar, solidified with 6 g/l of agar and the pH adjusted to 5.8

The *in vitro* plants were maintained in the controlled room under the following conditions: the room temperature was kept between 26 to 30^{o}C; the humidity was maintained between 60 to 70% and the illumination was for 16 hrs (Figs 1 & 2). The illumination was provided by cool-white fluorescent tubes from Hitachi 36 watt pre-heat, F40T8/Day. The light intensities measured in Lux with a light meter 840006 from Sper Scientific, Taiwan. The humidity was measured in

percentage with a Hygrometer Super Scientific 736920, Germany. Finally, the temperature was measured in centigrade with a maximum and minimum thermometer from the company G. H. Zeal, London, England.

In the non-controlled room data on variation of light intensities were collected three times a day (morning, noon and afternoon), while in the controlled room only once in the morning. The observations began in June 2009 and finished in January, 2010.

In vitro plants growing under non-controlled conditions were maintained in a room (4.9 × 5.3 × 3 meters) with a roof made of corrugated plastic sheets that allow a partial passage of natural light with four windows two of 1.2 × 1.3 m and two of 1.27 × 1.50 m protected by a metal net of 1 mm of mesh. This net protected the room from insects and partially allowed the air to flow through; under these conditions; the room temperature varied depending on the season between 14.5 and 39.5ºC; the humidity from 25 to 90% and the light intensities (luminance) from 20,017 to 20,687 Lux.

The bottles were placed in a heavy duty 5-tier chrome plated wire shelving 60″ x 24″ x 76″ each shelf. In each level we could place up to 150 flasks (Fig. 3). A separation between the levels was of 30 cm and the total height was of 1.5 meters. They were divided into level 01 through level 06. Light intensities, room humidity and temperatures were measured at 9 a.m., 12 noon and at 4 p.m. at each level and data recorded.

In every flask containing 30 ml of fresh medium thirty two axillary buds in eight stem pieces with their tips cut were placed under sterile conditions. Between two to three weeks (Fig. 4) the total number of shoots as well as the total number of axillary buds were counted. Usually, half of the flasks were placed randomly under controlled and the other half under non-controlled conditions. The data collected after each subculture were to compare the conventional and the low cost plant characteristics and also their multiplication rates by counting the total number of nodes produced after two weeks.

Before transferring to the field *in vitro* plantlets were hardened for four days, thereafter placed for seven days in a pot filled with coco peat for rooting. After this procedure were planted in a field in single hill plots with 64 plants per replication with a total of 256 plants per treatment using an RCBD design (Fig. 5). The line to line distance was of 60 cm and the plant to plant distance of 15 cm.

The field for the yield studies was prepared as follows: 150 of urea, 110 of TSP, 150 of MP, 50 gypsum, 4 of zinc sulfate, 50 of magnesium and 6 of boric acid (all in Kilograms per acre). Mini-tubers were harvested after approximately 100 days when the yield data were calculated (Fig. 6).

Results and Discussion

The maximum light intensities expressed in Lux in the controlled room were of the order of 20,566 and the minimum ~~of~~ 20,238. In the non-controlled room the maximum light intensities at 9.00 AM gave a reading of 20,666 with an average of 20,315 and a minimum of 20,018 with an average of 20,015 during the month of August 2009. At noon the maximum light intensities gave a reading of 20, 687 with an average of 20,541; this was in January 2010 and a minimum of 20,021 with an average of 20,015. The reading at 4.00 PM gave a maximum of 20,566 with an average of 20,409 and a minimum of 20,238 with an average of 20,173.

Since the light in the controlled room illuminates the culture bottles from top to bottom, whereas the light in the non-controlled room light illuminates them in all directions the *in vitro* plants in the controlled room were taller than in the non-controlled room. In spite of this difference, the number of buds was not statistically significant.

Moreover, *in vitro* plants growing under non-controlled conditions were stronger and shorter and the color of the leaves were deep green. These findings demonstrate that the quality of light is of extreme importance (Table 1).

Table 1. Average number of buds produced by the cultivar Diamant per rack level and its ANOVA test. A comparison between controlled and non-controlled conditions after sixteen days in culture.

Replication	Shelf level number					
Number	01	02	03	04	05	Control
1	97	114	111	99	116	118
2	100	108	104	105	98	114
3	112	100	98	106	100	98
4	98	98	108	93	98	102
5	110	95	112	98	102	112
6	120	112	98	102	90	95

The ANOVA test using Microsoft Excel showed that there was no significance difference between the *in vitro* plants grown during the months of September-October. The following values are the result of the ANOVA test: F (5,30) = 0.69, MSE = 61.98, n.s.

All *in vitro* plant propagation studies consider the range of 19 to 28^{0}C as the ideal room temperature. Generally, temperatures below 17^{0}C and over 32^{0}C are considered not suitable for growth of plant tissue cultures, while tropical and subtropical plants are cultured at slightly higher temperatures than temperate species (George and Sherrington 1984).

Figs 1 - 6: 1. Control room. 2. Ten days old plants grown under controlled conditions. 3. Non-controlled room. 4. Ten days old plants grown under non-controlled conditions. 5. Yield trail at Gazipur, Bangladesh. 6. First generation of potato production from low cost plants.

In present experiments contrary to the common belief that the day- and night- temperature in the growth room must be strictly controlled at an even level, we found that *in vitro* plants can tolerate wide fluctuations in temperature. During the day the maximum temperature was 39ºC that occurred during the

months of July and August. The lowest temperature was 14ºC and was in the month of January. These wide variations of temperature did not have any visible effect on the growth and rate of multiplication of the *in vitro* plants.

Table 2. Yield comparisons in tons/ha on the production of minitubers of *in vitro* plants derived from low cost and conventional methods of the potato cultivar Diamant (2012-2013).

Replications	Low cost	Conventional
1	22.56	19.95
2	17.52	19.65
3	19.11	19.38
4	17.23	20.33

The ANOVA test using Microsoft Excel showed non-significance and the following values are the result of the test: F (1,6) = 0.34. MSE = 3.08, n.s.

Table 3. Yield comparisons in tons/ha on the production of mini-tubers of *in vitro* plants derived from the low cost and conventional methods of the potato cultivar Diamant (2013-2014).

Replications	Low cost	Conventional
1	16.65	20.17
2	15.14	19.69
3	18.16	15.63
4	18.41	12.36
5	18.15	15.13
6	17.15	15.89
7	17.15	20.40
8	16.98	17.65

The ANOVA test using Microsoft Excel showed non-significance and the following values are the result of the test: F (1,14) = 0.01. MSE = 4.66, n.s.

Table 4. Yield comparisons in tons/ha on the production of 2nd of generation between minitubers derived from low cost and conventional methods of the potato cultivar Diamant (2012-2013).

Replications	Low cost	Conventional
1	19.97	17.71
2	15.55	17.44
3	16.96	17.26
4	15.29	18.04

The ANOVA test using Microsoft Excel showed non-significance and the following values are the result of the test: F (1,6) = 0.38. MSE = 5.99, n.s.

By maintaining *in vitro* cultures at a regulated temperature with air conditioners adds to the cost but does not contribute to specific plant quality. Partial exclusion of this factor significantly contributes to reduction in electrical costs.

In the case of humidity we only studied its effect on the *in vitro* grown plants such as plant height, leaf color and the rise of humidity inside the culture bottle.

We collected data three times a day (morning, noon and afternoon) on variations of this parameter. We began in June, 2009 and finished in January, 2010. The maximum humidity in the controlled room was of 74.5% and the minimum of 62.2%. In the non-controlled room the maximum at 9 a.m. was 90% with an average of 83.4% and the minimum of 40% with an average of 47.5%. At noon the maximum humidity gave a reading of 85% with an average of 77% and a minimum of 40% with an average of 44.7%. At 4.00 p.m. the maximum humidity was ~~of~~ 90% with an average of 71.5% and a minimum 29% with an average 41.6%.

We observed that in the lower rack the levels of the natural light experiment the *in vitro* plants were elongated while at the top levels they were shorter but their color was always deep green. As against this, the plants under controlled conditions at all rack levels were elongated and pale. Water drops formed inside the bottles under both conditions. We overcame this problem of high humidity inside the culture bottles by using cotton plugs instead of plastic cups. Usually the plants grown under controlled conditions needed hardening, while the ones grown under natural conditions did not.

In general there were no significant variations between the average total number buds per bottle produced by the *in vitro* plants of the potato cultivar Diamant grown under conventional and low cost conditions as well as on the yield trails for two season**s** between the conventionally and non-conventionally grown plants.

Our experiments have shown that the micropropagation of *in vitro* plants of potato can withstand extreme changes in light intensities, temperature and humidity without important changes in their overall bud multiplication rate, characteristics of the plants and field trails. We have obtained similar results in banana, strawberry, turmeric, gerbera, etc.

We can conclude that our innovation is repeatable, ecologically friendly, economically feasible and socially impartial and could be used by small laboratories and/or progressive farmers.

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References

Espinoza N, Estrada R, Bryan J and **Dodds JH** (1984) Tissue culture micropropagation, conservation, and export of potato germplasm. Specialized Technology Document, International Potato Center, Lima. Perú

George EF and Sherrington PD (1984) Plant Propagation by Tissue Culture. Exegetics Ltd., Basingstoke, UK.

Hossain M A, Hasan MK and Naher Q (2008) Assessment of technical efficiency of potato producers in some selected areas of Bangladesh. J. Agric. Rural Dev. **6**(182): 113-118.

http://faostat.fao.org/Desktop

Kodym A and Zapata-Arias FJ (1999) Natural light as an alternative light source for the *in vitro* culture of banana (*Musa acuminata* cv. "Grande Naine"). Plant Cell, Tissue and Organ Culture **55**:141-145

Kodym A and Zapata-Arias FJ (2001) Low cost alternatives for the micropropagation of banana. Plant Cell, Tissue and Organ Cult. **66**: 67-71.

Murashige T and Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. **15**: 473-497.

Qaim M (1999) A socioeconomic outlook on tissue-culture technology in Kenyan banana production. Biotechnology and Development Monitor Nº 40: 18-22

Roca WM, Espinosa N, Roca MR and Bryan JE (1978) A tissue culture method for the rapid propagation of potatoes. Am. Potato J. **55**: 691-701.

Sawyer R (1979) Annual report. International Potato Center. Lima, Perú .In Micropropagation for production of quality seed in Asia-Pacific.