

Full Length Research Paper

Shooting responses of potato (*Solanum tuberosum* L.) varieties in liquid and solid media

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Potato seeds degenerate over time due to systemic diseases (viruses and bacteria wilt), whose detections are mostly evident during later stages of epidemiology. Potato varieties released by Institute of Agricultural Research for Development (IRAD) Bambui recorded a drop in yields of approximately 50% within 20 years indicating that the varieties had started degenerating and thus required cleaning. A study was conducted in 2012 and 2013 at IRAD Bambui (Cameroon) to evaluate the rate of shooting in four improved IRAD potato varieties: Cipira, Tubira, Mafo and Bambui wonder. Liquid and solid media stocks prepared according to the International Potato Center (CIP)'s protocol were used. A randomized complete block design with four replications was used. Number of shot out nodes and average shoot length were collected after 28 days. The average number of shot out nodes in liquid and solid media in all the varieties ranged from 87.5 to 100, with the solid medium yielding better results, 97.5 to 100% in all the potato varieties as compared to liquid medium, 8.75 to 97.5%. The average shoots length in the liquid and solid media ranged from 4.95 to 10.56 cm. The analysis of variance revealed that there were significant differences at $p = 0.05$ between potato varieties studied. The liquid medium showed better results (8.49 to 10.5 cm) in all the varieties compared to the solid medium (4.59 to 6.08 cm) but the plantlets suffered from vitrification and asphyxiation forming callus. From the results obtained, the following conclusions may be drawn: shoot proliferation of potato tissues are reduced in stationary liquid medium as a result of vitrification and asphyxiation; potato nodal cuttings have faster growth in liquid medium than in solid medium. It is therefore recommended that solid medium should be used over stationary liquid medium in potato micropropagation because the loss of plant tissues in the liquid medium as a result of vitrification and asphyxiation can be very high.

Key words: Potato, media, growth, shoots, Cameroon.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the fourth most important food crop in the world after wheat, rice and maize. Its

production represents almost half of the world's annual output of all roots and tubers with a production figure of

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321.69 million tons (FAOSTAT, 2013). Potato is eaten by 6475 million people worldwide and is part of the diet of half a billion consumers in developing countries (CIP, 1995). In Cameroon, potato is grown in the highland zones (5°26.51 to 6°01.6)N and (10°03.8 to 10°18.5)E between 1000 to 3000 m above sea level in six of the country's 10 regions (Njualement, 2010). The crop occupies the fifth range among the starchy staples and the third among the root and tuber crops. It occupies a relatively small land area (72.103 ha) compared to other staples. The North West and West regions are the highest potato producers in Cameroon accounting for more than 80% of the national production of 435.354 tons. The crop is one of the main sources of revenues for farmers of these regions. Although, the bulk of the harvest is eaten locally or sold in local markets, significant quantities are exported to neighboring countries such as Gabon, Equatorial Guinea, Central African Republic and Chad (Demo et al., 2000). Despite increases of potato production in the tropics, yields are generally low (Burton, 1989). Potato is attacked by 210 different diseases from the field to storage (Beukema et al., 1990). Many potato programs have failed due to degeneration generally caused by systemic diseases (viruses and bacteria wilt), whose detection are mostly evident only during later stage of the disease.

The Institute of Agricultural Research for Development (IRAD) has an ambitious potato seeds program at its Bambui Regional Center in Cameroon, which is a center of excellence within West and Central Africa, owing to its climatic conditions which is suitable for potato seed production. In 1992, the potato program of IRAD Bambui released potato varieties, six of which have been vulgarized (Cipira, Tubira, Mafo, Bambui wonder, Jacob and IRAD). These varieties were selected with major genes as the main sources of resistance to major diseases (Njualement et al., 2001). At the time of release, average yield ranged from 25 to 35 t/ha depending on the variety. In 2001, a yield drop of 15 t/ha was reported in some of these varieties (IRAD, 2006). The varieties had started degenerating and required cleaning.

Many approaches have been developed to clean potato seeds as soon as degeneration is apparent. Among these techniques is the meristem culture. A successful meristem culture usually starts with potato varieties that show promise of shooting (Kuria et al., 2008) in liquid and solid media. An investigation was conducted to ascertain the shooting performance of four IRAD improved potato varieties in liquid and solid media, so that the varieties are included in the IRAD potato seed production program.

MATERIALS AND METHODS

The experiments were conducted at IRAD Bambui plant tissue culture laboratory in 2012 and 2013. IRAD Bambui is one of the five Regional Centers of the Institute for Agricultural Research for

Development in Cameroon. Bambui is a village in the Northwest region of Cameroon located at 1600 m above sea level with an average annual rainfall of 1500 to 2000 mm and an average temperature of 21 to 24°C.

Plant material

Four improved Cameroon potato varieties: Cipira and Tubira released in 1992 through a collaborative research project between IRAD Cameroon and the CIP Lima, Peru (Martin et al., 1995); Mafo and Bambui wonder released in 2005 (IRAD, 2006) were used for the study. These varieties are resistant to late blight, tolerant to viruses and bacteria wilt, and adapted and high yielding in the Cameroon highland ecological conditions. The criteria of selection were based on yield, dry matter contents, disease resistance among others. The explants were derived from 32 days old mother plantlets of the four potato varieties selected from the growth chamber. The mother plantlets were selected according to the number of nodes (four or more nodes) and plant vigor (size and health).

Culture media

Two nutrient media were prepared under sterile conditions: 500 ml of liquid medium with no gelling agent (agar) and 500 ml of solid medium with agar. Both media were prepared using stock solutions according to International Potato Centre (CIP)'s laboratory manual (Toledo et al., 1998). Each culture medium was dispersed into 50 ml test tubes (Pyrex co.) (2 ml.tube⁻¹) and sealed with aluminum foil and autoclaved at 121°C for 15 min.

Experimental design

A complete randomized block design (CRBD) with four replications was used for the study. Each replication contained 10 culture node segments and forty nodes were cultured for each potato variety per medium type.

Cultural techniques

The tissues culture operations were carried out under aseptic conditions inside a laminar air flow chamber, previously cleaned with 70% alcohol. The mouths of the test tubes containing the mother plantlets were quickly flamed on a spirit lamp and the plantlets carefully removed from the test tubes using a pair of sterilized forceps. The plantlets were then placed on sterilized tissue paper and the stems cut into single node segments. All roots were removed. The average single node cutting was 3 to 5 mm long. Using the tip of a scalpel, the isolated nodes were carefully transferred onto the surface of the freshly prepared propagation media. The nodes were planted upright in the solid medium, taking care not to push them below the surface. In the liquid medium, the nodes were allowed to float taking advantage of the surface tension. The test tubes were re-sealed with aluminum foil. All test tubes were labeled according to potato variety, with date of culturing and replication numbers. The cultured nodes were then transferred to the growth chamber, protected from direct sun light and illuminated by fluorescent bulbs. The temperature within the growth chamber was maintained at 27°C with 16 h of light per day (80 $\mu\text{Mol.m}^{-2}.\text{s}^{-1}$). Humidity of 68% within the chamber was regulated with the aid of an air conditioner. The cultured nodes were allowed to grow for 28 days during which the following variables were collected on weekly basis: number of shot out nodes

Table 1. Average number of shot out of 4 potato varieties cultured in solid medium for 4 weeks.

Variety	Week 1	Week 2	Week 3	Week 4
Cipira	10.00 ± 0.00 ^a	10.00 ± 0.00 ^a	10.00 ± 0.00 ^a	10.00 ± 0.00 ^a
Tubira	9.50 ± 0.29 ^a	9.75 ± 0.25 ^a	9.75 ± 0.25 ^a	10.00 ± 0.00 ^a
Mafo	10.00 ± 0.00 ^a	10.00 ± 0.00 ^a	10.00 ± 0.00 ^a	10.00 ± 0.00 ^a
Bambui wonder	9.50 ± 0.50 ^a	9.75 ± 0.25 ^a	9.75 ± 0.25 ^a	9.75 ± 0.25 ^a

Means in a column followed by the same letter are not significantly different at $p = 0.05$.

Table 2. Average number of shot out of 4 potato varieties cultured in liquid medium for 4 weeks.

Variety	Week 1	Week 2	Week 3	Week 4
Cipira	8.00 ± 0.00 ^a	8.00 ± 0.48 ^a	8.75 ± 0.48 ^a	8.75 ± 0.48 ^a
Tubira	9.25 ± 0.25 ^b	9.75 ± 0.25 ^b	9.75 ± 0.25 ^a	9.75 ± 0.25 ^a
Mafo	9.75 ± 0.25 ^b	9.75 ± 0.25 ^b	9.75 ± 0.25 ^a	9.75 ± 0.25 ^a
Bambui wonder	7.75 ± 0.48 ^a	8.75 ± 0.48 ^a	8.75 ± 0.48 ^a	8.75 ± 0.48 ^a

Means in a column followed by the same letter are not significantly different at $p = 0.05$.

by counts and average shoot length by measuring the length of the longest shoot in centimeter with a meter rule.

Statistical analysis

Data obtained were analyzed using the SPSS-17 Statistical package and mean differences were separated using Duncan alpha multiple test ($p = 0.05$).

RESULTS

Number of shot out nodes

In solid medium

Table 1 represents the average shot out nodes of four potato varieties cultured in solid medium for four weeks. The average ranged from 9.50 out of 10.00 in Tubira and Bambui wonder within the first week, to 10.00 out of 10.00 in Cipira and Mafo in the fourth week (Table 1). One week after culture all the nodes of Cipira and Mafo had shot out (100%). Tubira and Bambui wonder each had an average number of shot out nodes of 9.50 out of 10.00 (95%). In the fourth week, all the nodes of Tubira had shot out while the average shot out nodes of Bambui wonder remained at 9.75 out of 10.00 from week 2 to 4. However, there were no significant differences at $p = 0.05$ between varieties (Table 1).

In liquid medium

Table 2 represents the average number of shot out nodes

of four potato varieties cultured in liquid medium for four weeks. It ranged from 7.75 out of 10.00 in Bambui wonder, within the first week, to 9.75 out of 10.00 in Tubira and Mafo in the fourth week (Table 2). One week after culturing, the average number of shot out nodes ranged from 7.75 out of 10.00 (77.5%) in Bambui wonder, to 9.75 out of 10.00 (97.5%) in Mafo. The average of Tubira and Mafo were significantly different at $p = 0.05$ from those of Cipira and Bambui wonder (Table 2). In the second week after culture, all varieties had reached their maximum number of shot out nodes which ranged from 8.57 out of 10.00 (85.7%) in Cipira and Bambui wonder, to 9.75 out of 10.00 (97.5%) in Tubira and Mafo. The averages of Tubira and Mafo were significantly different at $p = 0.05$ from those of Cipira and Bambui wonder (Table 2).

Shoot length

In solid medium

The average shoot length of nodes of the four potato varieties cultured in solid medium increased continuously from week 1 to 4 (Table 3). It ranged from 0.72 cm in Bambui wonder, in the first week, to 6.08 cm in Mafo in the fourth week. In the first week, the average shoot length varied from 0.72 cm in Bambui wonder to 3.28 cm in Mafo. The average shoot length of Mafo was significantly different at $p = 0.05$ from the averages of the other three varieties (Table 3). In the fourth week, the average shoot length of the nodes varied from 4.95 cm in Tubira, to 6.08 cm in Mafo, with no significant difference at $p = 0.05$ between varieties (Table 3, Figure 1A and B).

Table 3. Average shoots length (cm) of 4 potato varieties cultured in solid medium for 4 week.

Variety	Week 1	Week 2	Week 3	Week 4
Cipira	1.72 ± 1.57 ^a	3.05 ± 0.25 ^a	4.11 ± 0.28 ^{ab}	4.94 ± 0.30 ^a
Tubira	1.37 ± 0.09 ^a	3.58 ± 0.32 ^{ab}	4.59 ± 0.28 ^{ab}	4.59 ± 0.28 ^a
Mafo	3.28 ± 0.91 ^a	4.22 ± 0.49 ^b	5.37 ± 0.62 ^b	6.08 ± 0.72 ^a
Bambui wonder	0.72 ± 0.11 ^a	2.49 ± 0.36 ^a	3.97 ± 0.40 ^a	5.11 ± 0.41 ^a

Means in a column followed by the same letter are not significantly different at $p = 0.05$.

**Figure 1.** Mafo variety, four weeks after culture in solid (A) and liquid (B) media.

Table 4. Average shoots length (cm) of 4 potato varieties cultured in liquid medium for 4 weeks.

Variety	Week 1	Week 2	Week 3	Week 4
Cipira	1.48 ± 0.20 ^a	4.69 ± 0.64 ^a	7.52 ± 0.77 ^{ab}	9.13 ± 0.72 ^{ab}
Tubira	1.68 ± 0.54 ^a	5.53 ± 0.58 ^{ab}	5.88 ± 0.82 ^a	8.78 ± 0.73 ^{ab}
Mafo	3.05 ± 0.48 ^b	7.00 ± 0.42 ^b	9.35 ± 0.45 ^b	10.56 ± 0.62 ^a
Bambui wonder	0.84 ± 0.28 ^a	4.20 ± 0.46 ^a	6.72 ± 0.72 ^a	8.49 ± 0.38 ^a

Means in a column followed by the same letter are not significantly different at $p = 0.05$.

In liquid medium

Table 4 represents the average shoot length of 4 potato varieties cultured in liquid medium. All the 4 potato varieties showed a continuous increase in the average shoot length from week 1 to 4. It ranged from 0.84 cm in Bambui wonder in the first week, to 10.56 cm in Mafo in the fourth week (Table 4). In the first week, the average shoot length ranged from 0.84 cm in Bambui wonder, to 3.05 cm in Mafo. The average shoot length of Mafo was significantly different at $p = 0.05$ from the averages of the other three varieties (Table 4). In the fourth week, the average shoot length of the nodes ranged from 8.49 cm in Bambui wonder to 10.56 cm in Mafo. Cipira and Tubira were significantly different at $p = 0.05$ from Mafo and Bambui wonder, both of which were also significantly different from each other (Table 4).

DISCUSSION

A single node of an *in-vitro* plantlet placed in an appropriate medium will induce the development of an axillary bud (shoot) (Espinoza et al., 1992). These shoots could be produced in five to seven days (Kyazev, 1983); this was in agreement with the results obtained in the study after one week culturing where by all nodes (100%) of Cipira and Mafo had shot out while Tubira and Bambui wonder had a 95% average shot out nodes in the solid medium. These results were better than those obtained in the liquid medium in the range of 7.75 to 9.75 shot out nodes out of 10.00. The differences might have been due to varietal genetic make-up in the varieties. In the 4th week after culturing, all the nodes of three (Cipira, Tubira and Mafo) varieties cultured in the solid medium had shot out while all nodes in the liquid medium were in the range of 8.75 to 9.75 out of 10.00. These results differ from those of Sandal et al. (2001) who realized better shooting in many plant species in liquid than in solid media. The lower average number of shot out nodes in liquid medium could have been due to vitrification (or hyperhydricity) and asphyxiation of tissues (Pierik, 1997) which caused some of the cultured nodes to form callus (Figures 2, 3 and 4). Callus formation tends to stop shooting of the nodes in liquid medium.

The results of the study show that the average shoot

length of nodes for all potato varieties cultured in liquid medium were better than those in the solid medium. The averages after four weeks of cultured ranged from 8.49 to 10.56 cm in liquid medium and 4.59 to 6.08 cm in solid medium. These results agree with those of Kuria et al. (2008) who reported higher biomass accumulation in liquid media than in solid media. The availability and ease of uptake of water and nutrients as well as the close contact between the explants and the medium could have led to faster growth of plantlet (Mbiyu et al., 2012). However, not all plant species will grow normally on stationary liquid media as soaking or hyperhydricity may result. Generally, Mafo had the longest average shoot lengths from week 1 to 4 in both liquid and solid media while Tubira and bambui wonder had the shortest lengths after four weeks of culture in solid and liquid media, respectively. The significant difference in shoot length between the different potato varieties could be explained by the fact that varieties respond differently to shooting due to genetic variation among them.

Conclusion

Based on the foregoing study, the following conclusions may be drawn:

1. The four Cameroonian improved potato varieties (Cipira, Tubira, Mafo and Bambui wonder) responded positively to *in vitro* culture. Mafo variety had the best performance in culture in both liquid and solid media in terms of number of shot out nodes and shoot lengths.
2. Shoots proliferation of potato tissues is reduced in stationary liquid medium probably as a result of vitrification (hyperhydricity) and asphyxiation.
3. Potato nodal cuttings have faster growths in liquid medium than in solid medium hence subculturing can be done more frequently with liquid media.

Conflict of interests

The author(s) have not declared any conflict of interests.

Recommendations

From the research results, the following are recommended:

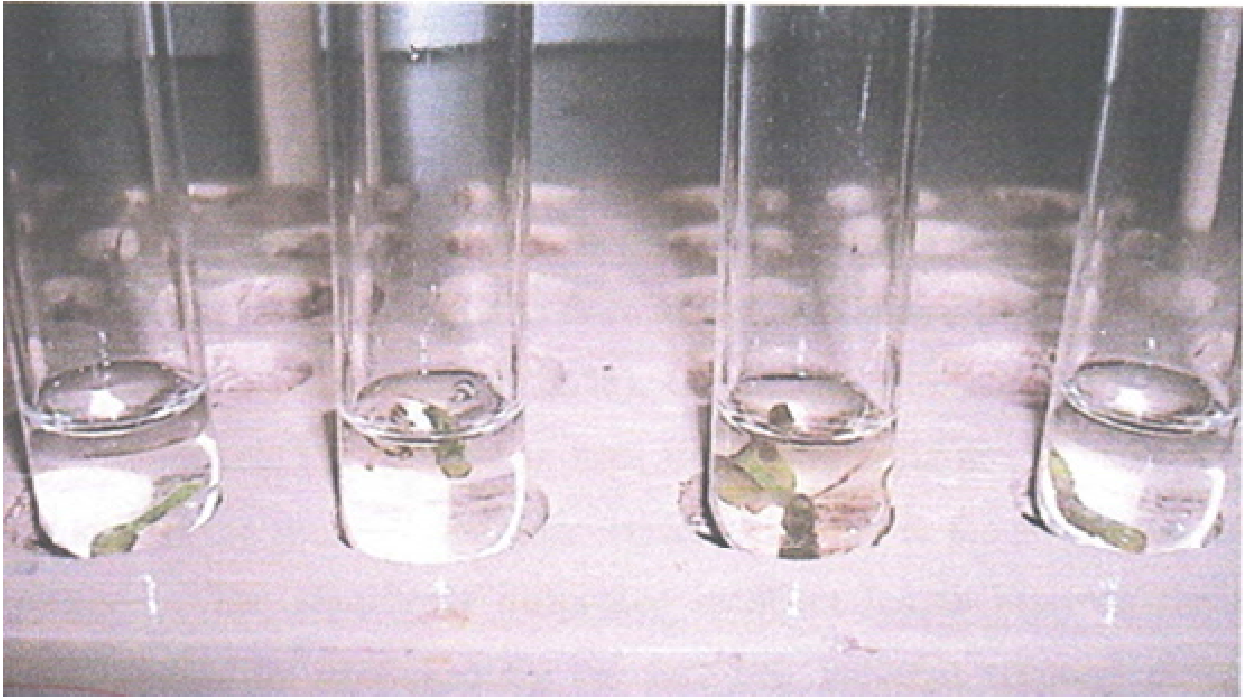


Figure 2. Asphyxiation of culture nodes of Cipira in liquid medium.

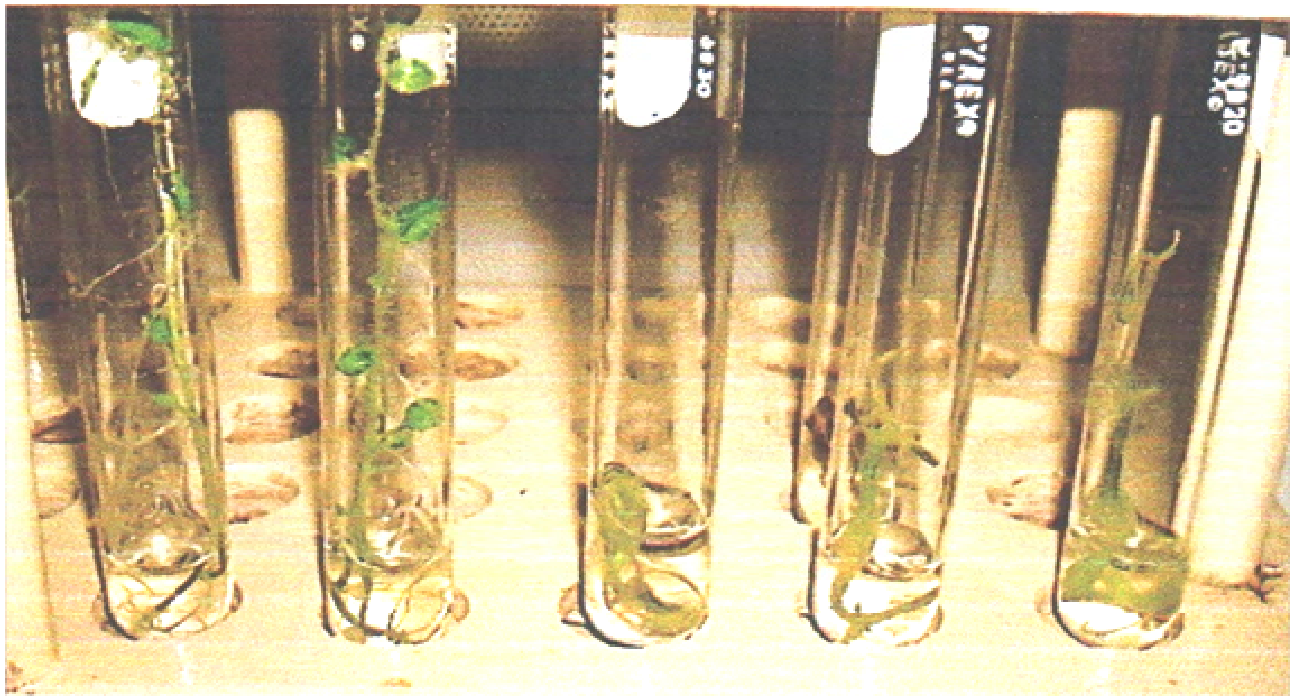


Figure 3. Vitrification of Tubira in liquid medium (left tubes).

1. The four improved varieties (Cipira, Tubira, Mafo and Bambui wonder) should be used for meristem culture to

clean the plant varieties for Cameroon.

2. Solid medium should be preferentially used than in



Figure 4. Callus formation on plantlets of Bambui wonder variety in liquid medium.

stationary liquid medium in potato micropropagation

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