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Research Article

Micropropagation Potential of Roseval and Charlotte Varieties of Potato (*Solanum Tuberosum* L) from Nodal Segments During Subcultures

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Abstract

In Côte d'Ivoire, potatoes have become a key component of the local diet. To meet the rising demand, the government invests over 22 billion FCFA annually to ensure sufficient supply for the population. Despite its favorable climate, Côte d'Ivoire produces very little potatoes. The main constraint to potato cultivation is the lack of seed availability at the right time for producers. This study aims to develop a protocol for producing potato *in vitro* plantlets in Côte d'Ivoire. Nodal segments from 21-day-old *in vitro* plantlets were placed onto four basal media containing different combinations of growth regulators (MS0: MS + Morel and Wetmore vitamins; M1: MS + Morel and Wetmore vitamins + 0.25 mg/L BAP; M2: MS + Morel and Wetmore vitamins + 0.25 mg/L BAP+ 0.01 mg/L IAA; M3: MS + Morel and Wetmore vitamins + 0.25 mg/L BAP+ 0.01 mg/L IAA + 0.25mg/L GA_3). A series of subcultures was carried out on the medium expressing the best responses. The MS0 medium, lacking growth regulators, supported the highest seedling growth (11.33 cm) and healthy leaf development in all varieties. The Charlotte variety, during its fourth subculture, demonstrated the greatest multiplication capacity, producing plants with an average height of 9.21 cm, 12.1 nodes per leafy shoot, and 3.21 leafy shoots per explant. Beyond this stage, multiplication performance decreases sharply. These results will contribute to the development of a protocol for mass production of potato seeds in the form of *in vitro* plantlets.

Keywords

Propagation, Subcultures, Growth Regulators, Potato, Micropropagation

1. Introduction

The potato (*Solanum tuberosum*. L) is one of the world's most important crops. It is grown on almost every continent [1]. It is world's third most important food crop after rice and wheat. It stands out for its efficiency, offering superior yields and nutritional value more fiber, protein, and micronutrients [2]. Its consumption is now part of the culinary habits of the Ivorian population. To satisfy the population's need for this tuber, the State of Côte d'Ivoire spends nearly 22 billion FCFA a year [3]. To cope with this external dependence, several attempts were made to introduce its cultivation in Côte d'Ivoire

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through research programs in the Touba area in the north-west of the country [4]. All these initiatives ended in failure, mainly due to the unavailability of seeds at the right time for farmers. Also, the availability of quality seed to smallholder farmers for most crops in sub-Saharan Africa is a problem [5]. Yet it has been shown that seed is the most important agricultural input [6, 7]. According to Vanesse [8], the unavailability and cost of imported seeds force smallholders to use their harvests as seeds. This reduces tuber quality, encouraging the establishment and resurgence of diseases and lower yields from one season to the next.

Potato seedd is essentially made up of *in vitro* plantlets from developed countries [9]. The technology for producing this type of seed is difficult to adopt due to the technical skills required and the large investments to be made. Despite the difficulties of implementing this technology, it could solve the potato seed problem in sub-Saharan countries. Indeed, this technology makes it possible to rapidly obtain large numbers of sanitary-quality plants in a limited space, independently of the seasons, and genetically in line with the starting plant material [10]. Given the multiplication potential of this technique, it has been widely used to study potato seed production in technologically advanced agricultural countries. The literature review showed that the success of micropropagation depends on the composition of the environment and varies according to variety and species [11-13]. According to Mubeen *et al.* [14], a medium containing only the salts of Murashige and Skoog [15] would produce well-developed *in vitro* plants, while Zerbo et *al.* [16] found that a medium containing the salts of Murashige and Skoog supplemented with 0.5 mg/L IBA is better for the production of potato *in vitro* plants. However, multiplication potential is linked to the number of subcultures performed, which is unfortunately a source of somaclonal variation in the clones produced. The number of subcultures required to avoid somaclonal variation has yet to be determined.

The aim of this study is to develop a multiplication protocol to determine the number of subcultures required for efficient production of potato vitroplants under our growing conditions. The development of such a protocol will make it possible to provide local producers with potato seed in the form of vitroplants.

2. Materials and Methods

2.1. Plant Material

The plant material consisted of 21-day-old potato *in vitro* plantlets of the Charlotte and Roseval varieties. These seedlings were produced at the Laboratory of Biology and Improvement of Plant Production (LBAPV) of Nangui ABROGOUA University.

2.2. Media Composition and Culture Conditions

The basal medium used is that of Murashige and Skoog [15] salts without vitamins (MS0). To these salts were added the vitamins of Morel and Wetmore [17] and various growth regulators (BAP, IAA and GA_3). The pH of the medium was adjusted to 5.8 with NaOH (0.1 N) or HCl (0.1 N) after the addition of sucrose (3%) as a carbon source and gelrite (0.25%) as a gelling agent. The mixture was boiled to promote distribution into test tubes (10 ml) or jars (30 ml), then sterilized for 20 min in an autoclave, at a pressure of 1 bar and a temperature of 121 ℃. The four media formulated were as follows:

1) M0: MS + Morel and Wetmore vitamins;
2) M1: MS + Morel and Wetmore vitamins + 0.25 mg/L BAP;
3) M2: MS + Morel and Wetmore vitamins + 0.25 mg/L BAP+ 0.01 mg/L IAA;
4) M3: MS + Morel and Wetmore vitamins + 0.25 mg/L BAP+ 0.01 mg/L IAA + 0.25mg/L GA_3.

Cultures were incubated in a culture chamber at 25±2 ℃ with humidity fluctuating between 70 and 80% and a 16/8-hour photoperiod for 30 days.

2.3. *In Vitro* Plant Subculture Cycles

The *in vitro* plantlets obtained after each 30-day subculture cycle were used as explant sources for the following cycle. Under a laminar flow hood, 2 cm-long nodal segments bearing 1 or 2 nodes were harvested from the *in vitro* plantlets obtained. The explants thus obtained were transplanted onto a medium and transferred to a culture chamber.

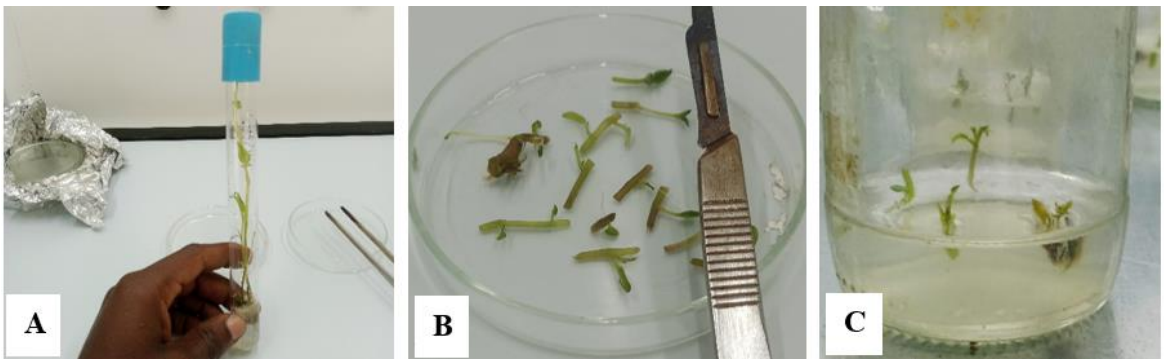

Figure 1. *transplanting process. A: 21 days-old seedlings, B: cuttings of nodal axes and C: transfer of cuttings on culture medium.*

2.4. Parameters Measured

Parameters were measured on 30 *in vitro* plantlets after 30 days of incubation. On each explant, the number of leafy shoots, the rooting rate and the number of roots were counted. The parameters taken from the leafy shoots were height, number of leaves and number of nodes.

2.5. Statistical Analysis

R software version 4.3.3 was used for data analysis. Analysis of variance tests (parametric or non-parametric) were performed to determine the responses of explants subjected to the different experiments. In the event of a significant difference, a Turkey test at the 5% threshold was performed to classify the means into homogeneous groups.

3. Results

3.1. Morphology of *in Vitro* Plantlets as a Function of Medium Composition

Generally speaking, whatever the variety, the leafy shoots obtained on M0 medium were morphologically the best, with green, full-blown leaves. These leafy shoots are more robust and have a greater number of nodes (Figures 2 and 3). On media M1, M2 and M3, the leafy shoots were small and slightly whitish, with signs of hyperhydricity.

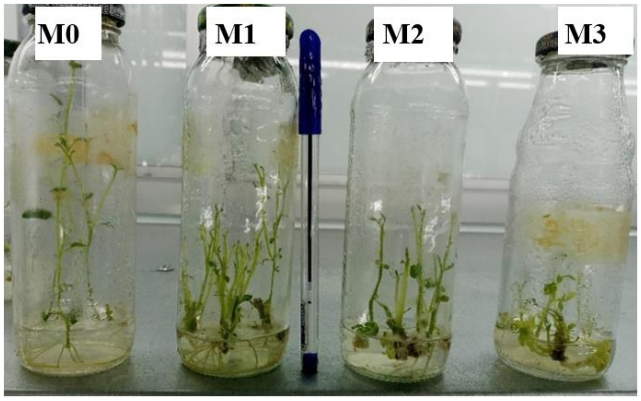

Figure 2. *Roseval in vitro plantlets at 1st subculture on M0, M1, M2 and M3 media.*

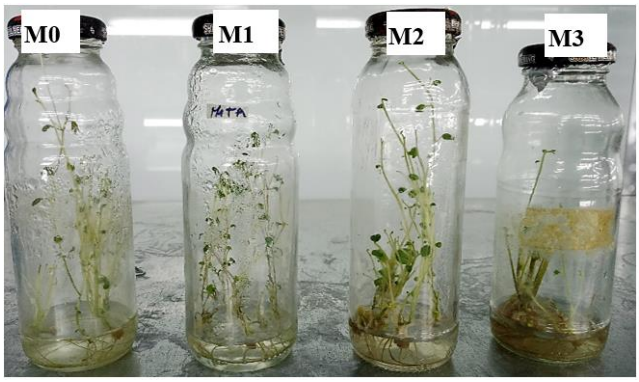

Figure 3. *Charlotte in vitro plantlets at 1st subculture on M0, M1, M2 and M3 media.*

3.2. Interaction of Variety Factors and Growing Medium Composition on the Growth of Potato *in Vitro Plantlets*

Table 1. *significance of factors according to parameters studied.*

Parameters	Variety (p)	Media (p)	Interaction Variety*Media (p)
Plantlet height	<0.001	<0.001	0.47
Number of nodes	0.017	<0.001	<0.001
Number of leafy shoots	0.014	<0.001	0.006

p: critical probability

Table 1 shows that the height of in vitro plantlets was very significantly influenced by variety (P<0.001) and growing medium composition (P<0.001). However, the interaction of these two factors had no effect on height. Number of nodes and number of leafy shoots were influenced by variety, growing medium and the interaction of these two factors.

3.3. Influence of Variety and Medium Composition on *in Vitro* Plant Height

The tallest *in vitro* plantlets were obtained with the Char-

lotte variety (10.82 cm). The Roseval variety (8.79 cm) produced small *in vitro* plantlets (Figure 4). With regard to culture media (Figure 5), the M0 medium produced large-sized *in vitro* plants (11.32 cm), while the lowest values were obtained with the M2 medium (8.37 cm).

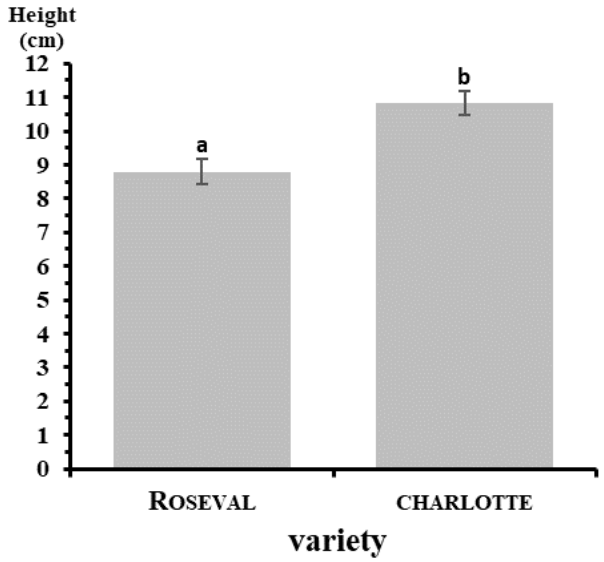

Figure 4. *Height of potato variety in vitro plantlets under in vitro culture conditions. Bars topped with the same letters indicate statistically identical values at the 5% threshold.*

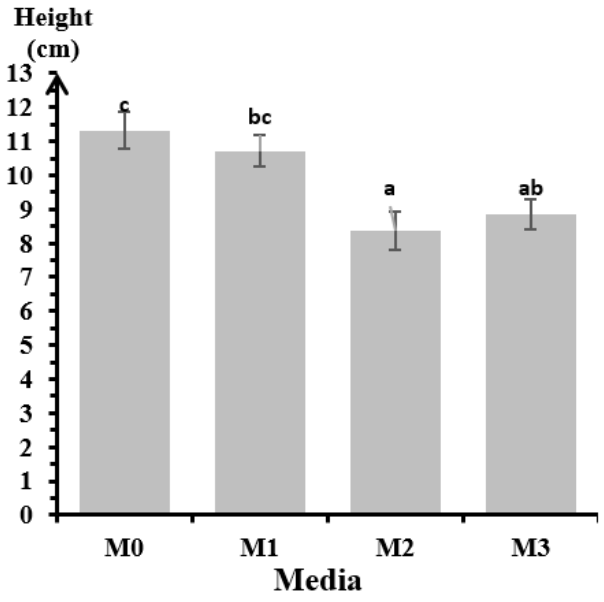

Figure 5. *Height of potato in vitro plantlets as a function of culture medium composition. Bars topped with the same letters indicate statistically identical values at the 5% threshold.*

3.4. Combined Effect of Variety and Medium Composition on the Number of Leafy Shoots and Knots in *in Vitro* Plantlets

Charlotte *in vitro* plantlets grown on M1 medium produced a high number of nodes, while a low number was obtained with Charlotte *in vitro* plantlets grown on M2 and M3 media (Table 2). In terms of the number of leafy shoots, the highest values were obtained with the Roseval variety on M2 and M2 media. The lowest values were observed with the Charlotte variety on medium M2.

Table 2. *number of nodes and leafy shoots depending on variety and media.*

Variety	Media	Number of nodes	Number of leafy shoots
Roseval	M0	19.67 ± 1.47^{bc}	1.80 ± 0.18^{ab}
Roseval	M1	17.43 ± 1.30^{abc}	2.87 ± 0.28^{bc}
Roseval	M2	14.97 ± 1.61^{ab}	3.37 ± 0.47^{c}
Roseval	M3	19 ± 1.23^{bc}	3.83 ± 0.43^{c}
Charlotte	M0	15 ± 1.14^{ab}	2.03 ± 0.25^{ab}
Charlotte	M1	22.10 ± 1.43^{c}	2.63 ± 0.28^{abc}
Charlotte	M2	11.97 ± 0.99^{a}	1.53 ± 0.10^{a}
Charlotte	M3	12.47 ± 1.07^{a}	3.07 ± 0.30^{bc}
	P	<0.001	0.006

In the same column, means followed by the same letter are statistically identical at the 5% threshold. Mean ± standard error, p: critical probability

3.5. Interaction of Variety and Subculture Factors on the Growth of Potato in Vitro Plantlets

The effect of the interaction of variety and subculture factors was studied on *in vitro* plantlets height, number of nodes, number of roots and number of leafy shoots. The significance of the analysis of variance for these parameters is shown in Table 3. The variety*subculture interaction was highly significant ($P<0.01$) for number of leaves and significant ($P<0.05$) for *in vitro* plantlets height and number of roots. The interaction of these factors had no effect on the number of nodes and the number of leafy shoots ($P>0.05$).

Table 3. *significance of the analysis of variances for the factors and parameters studied.*

Parameters	Variety (p)	Subculture (p)	Interaction Variety* Subculture (p)
Number of leaves	0,366	<0,001	0,005
Number of roots	<0,001	<0,001	0,036
Number of leafy shoots	0,228	<0,001	0,079
Plantlet height	<0,001	<0,001	0,016
Number of nodes	0,965	<0,001	0,052

p: critical probability

3.6. Effect of Subcultures on the Number of Leafy Shoots and Nodes

The highest number of leafy shoots and nodes was obtained in the 4th subculture (G4) with an average of 3.21 leafy shoots and 12.10 nodes (Figures 6 and 7). All other subcultures gave statistically significantly lower values the number of leafy shoots, while the lowest number of nodes (6.30) was obtained in the 3rd subculture (G3).

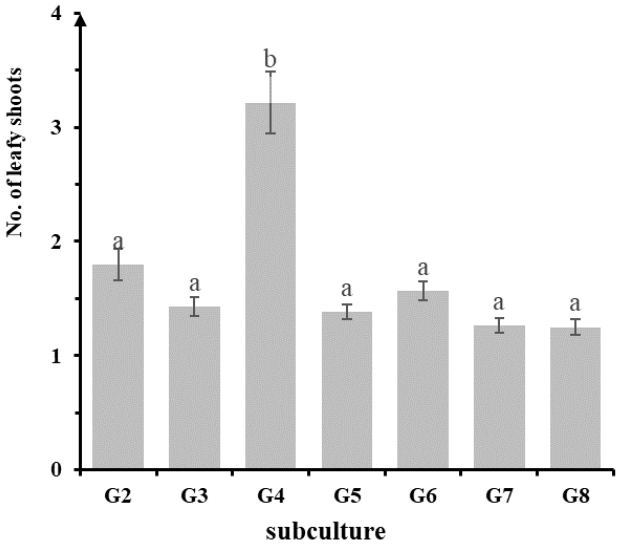

Figure 6. *number of leafy shoots according to subcultures. Bars topped with the same letters indicate statistically identical values at the 5% threshold.*

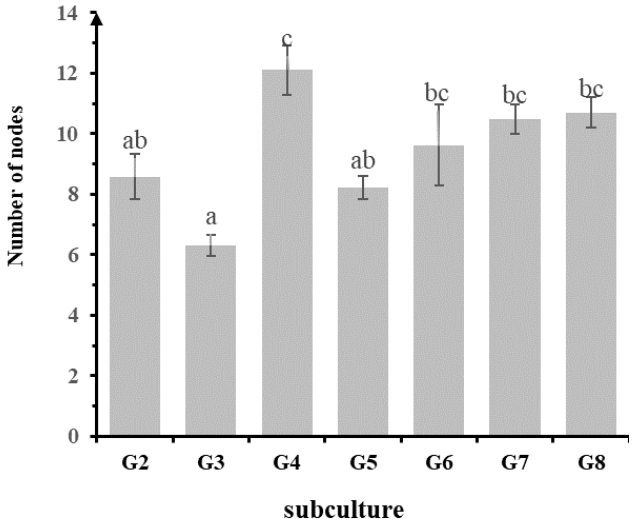

Figure 7. *number of nodes according to subcultures. Bars topped with the same letters indicate statistically identical values at the 5% threshold.*

Generally speaking, during subcultures, a progressive loss of green stem coloration and a reduction in *in vitro* plantlets leaves were observed. At initiation (G0), the *in vitro* plantlets showed robust stems with good green pigmentation and broad, well-spreading green leaves. (Figure 8a). From the first to the fifth subculture (G1-G5), the in *in vitro* plantlets showed less robust stems, reduced leaves and poor green stem pigmentation (Figures 8b to 8f). Finally, from the sixth to eighth subcultures (G6-G8), the *in vitro* plantlets showed fragile stems, very small leaves and stem pigmentation that turned from green to white (Figures 8g to 8i).

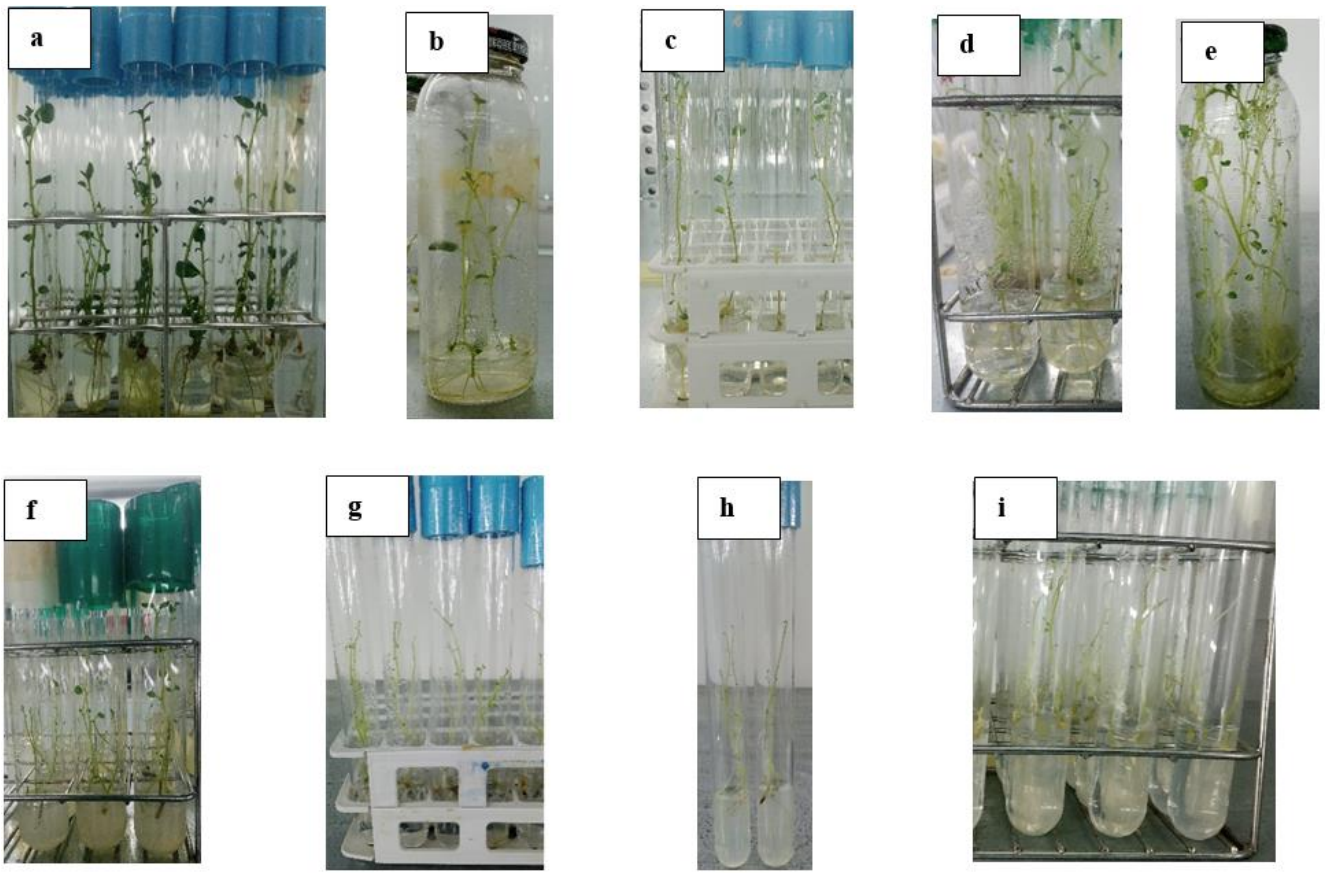

Figure 8. *in vitro plantlets from different subcultures. (a): initiation; (b): 1st subculture; (c): 2nd subculture; (d): 3rd subculture; (e): 4th subculture; (f): 5th subculture; (g): 6th subculture; (h): 7th subculture; (i): 8th subculture.*

A high number of leaves was obtained with the Roseval variety in the 4th subculture (Table 4). As for the number of roots, the Charlotte variety gave a high number of roots at the 7th subculture. The highest heights were observed at 4th subculture with the Charlotte variety. Whatever the growth parameter studied, the lowest values were obtained with the Roseval variety at 3rd subculture.

Table 4. *growth parameters of in vitro plantlets of varieties during subcultures.*

Variety	Subculture	Number of leaves	Number of Roots	Height (cm)
Roseval	G2	12.63 ±1.57 ^{bcde}	3.60 ±0.59 ^{ab}	8.18 ±0.77 ^{de}
Roseval	G3	7.70 ±0.60 ^{a}	1.87 ±0.42^{a}	5.40 ±0.32^{a}
Roseval	G4	16.20 ±1.52 ^{e}	4.30 ±0.51^{bc}	6.31 ±0.30^{abcd}
Roseval	G5	10.87 ±0.60 ^{abcd}	4.5 ±0.44 ^{bc}	5.85 ±0.29^{abc}
Roseval	G6	10.47 ±0.72 ^{abcd}	3.30 ±0.36 ^{ab}	5.51 ±0.14^{ab}
Roseval	G7	11.27 ±0.61 ^{abcd}	3.73 ±0.42 ^{ab}	6.19 ±0.34^{abcd}
Roseval	G8	11.80 ±0.85 ^{abcd}	4.60 ±0.31 ^{bc}	7.60 ±0.31^{bcde}
Charlotte	G2	8.79 ±0.70 ^{ab}	4.86 ±0.57 ^{bc}	7.86 ±0.95^{cde}
Charlotte	G3	9.13 ±0.60 ^{abc}	4 ±0.36 ^{abc}	6.37 ±0.41^{abcd}
Charlotte	G4	13.40 ±1.06^{de}	5.03 ±0.64 ^{bc}	9.21 ±0.59^{e}
Charlotte	G5	9.83 ±0.61 ^{abcd}	4.13 ±0.41 ^{bc}	6.95 ±0.37^{abcd}

Variety	Subculture	Number of leaves	Number of Roots	Height (cm)
Charlotte	G6	10.19 ±0.59 abcd	5.07 ±0.32 bc	7.39 ±0.40abcde
Charlotte	G7	13.30 ±0.86cde	6.10 ±0.45 c	7 ±0.29abcd
Charlotte	G8	13.03 ±0.79bcde	5.10 ±0.48 bc	8.33 ±0.27de

In the same column, means followed by the same letter are statistically identical at the 5% threshold. Mean ± standard error.

4. Discussion

The results of this study highlight significant differential responses of potato *in vitro* plantlets according to variety, culture medium composition and number of subcultures. These observations confirm the complex, multifactorial nature of *in vitro* micropropagation, largely influenced by genotype, hormonal conditions and transplanting cycles. The height of Charlotte *in vitro* plantlets was higher than that of Roseval. This behavior can be explained by Charlotte's specific genetic vigor and greater tolerance to *in vitro* environmental stress. Many authors confirm the inter-varietal variability in response to micropropagation [18, 19]. Similary, Zakaria *et al*. [20] observed distinct *in vitro* salt stress responses across potato genotypes, attributing these to hormonal and genetic factors. This diversity is thought to result from differential expression of genes linked to potato growth or tuberization [21]. The observed responses could be specific to each of these varieties. Hajare *et al.* [10] also showed that the potato varieties Gudiene and Belete have variety-specific responses. They concluded that this could be due to a genotypic difference between the two varieties. These findings align with those of Bairu *et al*. [22], who discussed the role of somaclonal and genotypic variation in tissue culture-based propagation systems, further supporting the necessity of variety-tailored micropropagation protocols.

Concerning the effect of media on morphogenesis and growth, the M0 medium containing Murashige and Skoog salts and the vitamins of Morel and Wetmore, without any exogenous hormone supply, enabled optimum growth of *in vitro* plantlets. These *in vitro* plantlets have well-developed leaves, a robust stem and a high number of nodes. The results reported here suggests sufficient autotrophy of the explants, probably linked to a prior accumulation of sufficient endogenous hormones to support early cell division and tissue differentiation. This finding corroborates the observations reported by Mubeen *et al.* [14], who demonstrated the efficacy of basal Murashige and Skoog medium without growth regulators on the development of *in vitro* plantlets. These data support the idea that varieties such as Roseval and Charlotte may possess a higher intrinsic morphogenetic potential under aseptic culture conditions. In contrast, the work of Badoni and Chauhan [23] reported a low node number (9.4 nodes) in the cultivar Kufri Himalini.

The M1, M2 and M3 media, presumably containing additive phytohormones, induced signs of hyperhydricity (whitish leaves, watery appearance), suggesting a hormonal imbalance. This phenomenon could be accentuated by overconcentration of cytokinins or poor aeration of the agar medium [24-26]. These variations cannot be predicted in advance [27]. The M0 medium containing salts of Murashige and Skoog and vitamins of Morel and Wetmore, without exogenous hormones, was selected to determine the effect of subcultures on the growth and development of *in vitro* plantlets.

Repeated subcultures (from G0 to G8) enabled us to monitor the morphological evolution of *in vitro* plantlets. It is interesting to note that maximum performance (number of shoots, nodes, leaves and height) was recorded at the 4th subculture (G4), with stability relatively maintained until G5-G6, before a qualitative decline beyond G6. The number of shoots (3.21) and nodes (12.10) were highest in the 4th subculture. For each variety, the highest number of leaves was obtained during 4th subculture. Generally speaking, the highest values were obtained from 4th to 8th subculture, but the morphological quality of the *in vitro* plantlets decreased with the number of subcultures. This could be explained by the fact that the plants tend to adapt to their new lifestyles. Also, epigenetic factors linked to subculture cycles play a role in this progressive degeneration. Hundayehu *et al.* [28] have shown that stresses linked to artificial environments (medium, light,..) can induce epigenetic modifications responsible for morphological variation. Similar observations have also been reported by Astarini *et al.* [29] with four potato genotypes. They concluded that *in vitro* development depends on the explants and their interaction with the environment. They therefore stressed the importance of developing specific protocols for each genotype, as each responds differently to the environment.

5. Conclusion

The aim of the present study was to investigate the influence of subcultures on *in vitro* plantlets growth and development, and to develop a protocol for *in vitro* plant multiplication. The study showed that the hormone-free medium allowed production of *in vitro* plantlets displaying best growth and development. The 4th subculture gave the best values for multiplication rate. Beyond this rank of subculture, the *in vitro* plantlets showed a different aspect (fragile stems, very small

leaves and stem pigmentation that turned from green to white) from those produced by previous subcultures. This implies that it will be necessary to limit subculturing to four subcultures in the conduct of the micropropagation protocol of the two varieties tested.

Abbreviations

BAP	6-Benzylaminopurine
GA_3	Gibberellic Acid
HCl	Hydrochloric Acid
IAA	Indole-3-acetic Acid
IBA	Indole-3-butyric Acid
LBAPV	Laboratory of Biology and Improvement of Plant Production
MS	Murashige and Skoog
MS0	Murashige and Skoog Basal Medium Without Vitamins
NaOH	Sodium Hydroxide
NLS	Number of Leafy Shoots
NN	Number of Nodes
p	Critical Probability

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Author Contributions

Pyminnan Traoré: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft

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Brahima Ouattara: Data curation, Formal Analysis, Investigation, Methodology, Writing – review & editing

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Tchoa Koné: Data curation, Formal Analysis, Investigation, Methodology, Writing – review & editing

Mongomaké Koné: Conceptualization, Formal Analysis, Investigation, Methodology, Supervision, Validation, Writing – review & editing

Conflicts of Interest

The authors declare no conflicts of interest.

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