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Pre-basic seed production of potato using tissue culture in Katibougou, Mali

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Abstract. *Abdoulaye M, Blay ET, Eleblu JSY. 2021. Pre-basic seed production of potato using tissue culture in Katibougou, Mali. Cell Biol Dev 5: 90-103.* In Mali, the main problem restraining the production and productivity of potatoes is the unavailability of quality seeds at affordable prices and in adequate quantities. This study proposes two experiments on the techniques adopted in Mali's IPR/IFRA plant biotechnology laboratory to meet quantity and quality improvements. That evaluation of the effects of three concentrations of coconut water and two of potassium nitrate on potato plantlets growth in vitro and of three substrates and two physiological ages on potato in vitro plants' establishment, mini tubers production in vivo, and post in vitro growth. The first experiment was a Completely Randomized Design (CRD) with 12 treatments replicated four times. The second was a factorial experiment with two factors (physiological ages: two levels and substrate: three levels) with six treatments replicated four times in a Randomized Complete Block Design (RCBD). The first experiment showed that the culture medium M7 (MS+40 mL/L of coconut water and 250 mg of potassium nitrate) had promoted all plant growth parameters (shoot emergence, number of nodes, leaves, and roots, plant height, and plant fresh and dry weight) after 30 days of in vitro culturing. After 30 days of coconut water (40 mL) and potassium nitrate (250 mg) per liter of MS medium, the lower concentrations had positive effects and significant on all the in vitro growth parameters. The second experiment showed that at 20 days after transplanting in vivo, the post-flask culture substrate S1 (only soil) and the plantlet weaning age of 25 days provided the best plant survival percentage. The substrate S2 (soil and cow dung 2:1) positively affects plant stem length, fresh and dry biomass formation, tuber yield, tuber numbers per plant, stem diameter, and tuber grading size B (diameter of less than 28 mm tubers). The composition S1 substrate (only soil) significantly affected the tubers' stored weight loss (12.50%) within eight weeks. The weaning age was 45 days, and the substrates S2 (soil and cow dung 2:1) and S3 (soil and cow dung 1:1) significantly reduced sprouts' number per eye on tubers and the number of sprouts per tuber. Moreover, the results indicate the coconut water concentration used as a supplement to MS medium should be 40 mL per liter of medium for the better and more rapid growth of potato plantlets in vitro culture. The substrate composition proportion of cow dung should not exceed the soil and cow dung ratio 2:1 for maximum post-transplanting plant re-establishment rate in vivo and rapid maturity of mini-tubers.

Keywords: Potato, *Solanum tuberosum*, tissue culture

INTRODUCTION

The potato (*Solanum tuberosum* L) is an important crop in the sub-Saharan region of Africa, a herbaceous tuberous plant originally from Latin America (MINRESI-IRAD 2012). Potato is the world's main non-cereal food commodity, with global production in 2016 of 19.2 million hectares at around 376.8 million tons, with a yield average of 20 tons/ha. Africa produced 24.5 million tons (FAO 2016). It grew in all Mali regions, with an estimated potato production in 2016 at 210,209 tons in an area of 10,525 hectares with an average yield of 20 tons/ha (FAO 2016).

Mali's market consumes 80% of the volume, and the rest by the sub-region, mainly Ivory Coast, Burkina Faso, Togo, Ghana, and Benin. Ivory Coast, the most important country, absorbed more than 90% of the exported potato (Diakité and Zida 2003). The potato is a cash crop and an important commodity because of its ease of production and high-energy content for millions of farmers in Africa, Latin America, and Asia. It is also important to urban agriculture, which provides employment and food security to 800 million people (FAO 2009). Moreover, it is important to note that in developed countries, particularly in Europe, production has declined in recent years, with a decline of nearly 20%. Still, Africa develops the most, increasing over 55% of production (Vanderhofstadt 2011).

Malian producers generally use two types of seeds: seeds produced by the non-certified farmers themselves and certified seeds imported from Europe; A farmer uses an average of 80% imported and 20% local seed (Diakité and Zida 2003). The timely supply of quality seed (certified seed) in Mali remains dependent on imports from France and Holland (Coulibaly et al. 2002). In Mali, Potato crop production has been increasing since 1973. The cultivated area increased by 4,843 ha from 2010 to 2014. It was estimated at 3,700 ha in 2010 and 8,543 ha in 2014 (Vanderhofstadt 2011). The potato seeds required are 1 to 2.5 tons/ha in Mali, with a minimum of 1,000 kg/ha (1 ton/ha) and an average price of 1,000 CFA francs/kg (BNDA 2014). The potato seeds used by Malian farmers in 2014 were estimated at 8,543 tons, with a turnover for potato seed companies of 8.5 billion CFA francs (around US$14.2 million).

Increased potato production will depend on quality seeds, i.e., varieties resistant to pests and diseases and capable to adapt climate change (FAO 2009). Therefore,

developing potato seeds and cultivation in Mali requires high-yielding varieties and local multiplicate to improve the quality and quantity involving plant biotechnology.

Potato is one of the first significant food crops where the virus has been successfully eliminated using plant biotechnology (Bajaj and Sopory 1986). In addition, tissue culture technology produced disease-free plants and micro tubers, disseminated to the field, and multiplied in many countries (Bajaj and Sopory 1986).

Establishing a seed supply chain requires the production of potato pre-basic seeds in the laboratory. The plant biotechnology laboratory at IPR/IFRA of Katibougou has initiated potato micropropagation since 2000. Some studies prefer to produce potato seed through tissue culture in a 4-year seed production scheme to limit pathogens importation from the in vitro plantlet through generations G0, G1, G2 to G3 seed (Coulibaly et al. 2002).

A study has investigated the coconut water effects on the growth of in vitro Desiree variety potato plantlets in Pakistan (Muhammad et al. 2015). In addition, coconut water has been used as a growth regulator by Overbeek (1941) in culture media for very young *Datura stramonium* embryo development. The cytokinin in coconut water promotes cell division, supplements the chemical components, and promotes plant growth (Jackson et al. 2004).

A study on four physiological ages (20, 30, 40, and 50 days after culturing) in Algeria of potato in vitro plants concluded that the 50-day-old plantlets produced a significantly higher number of mini tubers in vivo than the others (ITCMI 2012). The soil mixes nature used for transplantation could influence the re-establishment of in vitro plantlets in vivo (Anderson 1978). In addition, good aeration of the substrate used for transplanting the in vitro plantlets is important for some species' post-transplanting survival and growth (Gorst et al. 1978).

Unfortunately, the non-availability of quality seeds at affordable prices is the main problem limiting the productivity and production of potatoes in Mali. The seeds imported carry several pathogens, such as bacteria (*Ralstonia solanacearum*) and viruses (PVY and PLRV), that have affected potato production (Vanderhofstadt 2011). In addition, the quality seed supplied does not meet the quantities country's needs.

There is low production through tissue culture of potato pre-basic seed due to a low rate of multiplication of plantlets in vitro. In addition, a general lack of producing pre-basic seed at an identified-appropriate physiological age and a lack of research addressing problems of plantlets in vivo after transplantation associated with post-flask re-establishment under the current climatic conditions in Mali. Therefore, the general objective is to develop reliable protocols for producing potato pre-basic seeds via tissue culture in Mali.

MATERIALS AND METHODS

Study 1

The evaluation of coconut water and potassium nitrate effects on MS medium as supplement components on potato plantlets growth in vitro.

Experimental site

The IPR/IFRA Plant Biotechnology Laboratory, Mali, was the site for this experiment

Plant material

The variety of available potatoes (Sahel) was used as the in vitro plantlets plant material. Therefore, nodal stem cuttings were used and cultured for 30 days in vitro, and one-month-old potato in vitro plantlets was selected as explants (Figure 1).

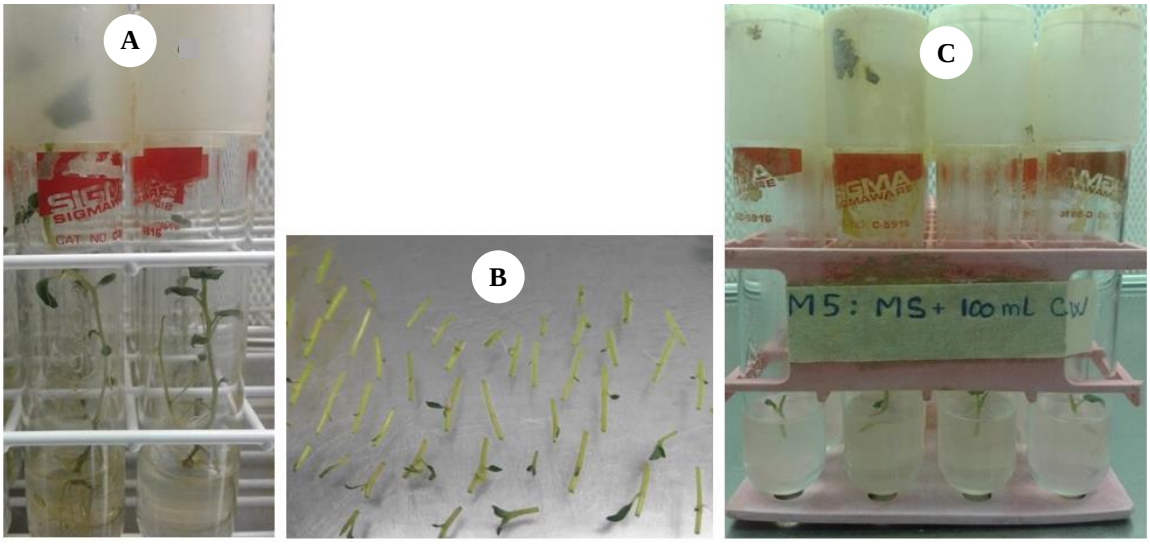

Figure 1. Plant materials. A. Plantlet used as explants; B. Nodal stem cuttings, and C. Explants on media

In vitro growth conditions

In vitro-derived plantlets of the Sahel variety were grown for 30 days at the growth temperature of 24 ± 1°C on 12 culture media and 16 hours of photoperiod. For the micropropagation study, the nodal explants of potato in vitro plants were used. They were obtained by cutting the plants at the internodes. The explant has an axillary bud and leaf, a stem portion 0.5 to 1 cm long, and is placed vertically with the solid culture medium in the culture tube in contact. Each tube contains 10 mL of solidified culture medium.

Culture media

The MS basic medium (Murashige and Skoog 1962) supplemented with 100 mg/L of Myo-inositol, and 30 g/L of sucrose was prepared. Various combinations of coconut water from the mature-dry fruit at two different potassium nitrate (250 mg and 1,000 mg per liter) and three different concentrations (40 mL, 100 mL, and 300 mL per liter) were used in combinations or singly to generate 12 different treatment media. MS medium with no coconut water and Potassium Nitrate (KNO_3) was used as the control.

The pH was adjusted to 5.7 ± 1 of each treatment medium before sterilizing; each treatment medium was solidified with two g/L of Gelrite. Then, the treatment media were sterilized by autoclaving at 115 ± 1°C for 30 minutes.

The twelve (12) media for the experiment were:

MS (control),
M2: MS+250 mg KNO_3;
M3: MS+1000 mg KNO_3;
M4: MS +40 mL/L Coconut Water (CW);
M5: MS+100 mL/L CW;
M6: MS+300 mL/L CW;
M7: MS +40 mL/L CW+250 mg KNO_3;
M8: MS +40 mL/L CW+1000 mg KNO_3;
M9: MS+100 mL/L CW+250 mg KNO_3;
M10: MS+100 mL/L CW+1000 mg KNO_3;
M11: MS+300 mL/L CW+250 mg KNO_3;
M12: MS+300 mL/L CW+1000 mg KNO_3.

In this study, the preparation uses different culture-media. First, the commercial bottles of pre-mixed powders, already available in the laboratory, were used. Then, the "MS Medium" bottle, as prescribed by Murashige and Skoog (1962), contained all the vitamins and mineral salts (macro and micronutrients) (Table 1).

Experimental design

The experiment was a Completely Randomized Design (CRD) with twelve (12) treatments replicated four times.

In vitro experiment layout

A sum of 240 culture tubes was used for the trial, each replication represented by five tubes containing 10 mL of media and one explant.

Data collection

A total of 5 plants were selected per replication per treatment as record plants, and data were collected as follows: (i) Shoot emergence taken ten days after propagating (DAP), (ii) Plant height measured 30 DAP; (iii) Number of leaves per plantlet was taken at 30 DAP; (iv) Number of roots per plantlet taken 30 DAP; (v) Number of nodes per plantlet taken 30 DAP, (vi) Average length of internodes calculated 30 DAP; (vii) Plantlet fresh and dry weight were taken 30 DAP.

Study 2

On evaluation, the weaning age of plantlets and substrate composition affect re-establishment, growth, and mini tuber production of the Sahel variety in vivo.

Experimental site

The experiment was conducted in vivo in a screen house at the IPR / IFRA Plant Biotechnology Laboratory, Mali.

Plant material

The plant material used was plantlets generated in vitro from the Sahel variety. In vitro plantlets at 25 and 45-days characteristics were as follows: (i) 5-7 cm height; (ii) with seven leaves; (iii) and at least four well-developed roots (Figure 2).

In vivo culture substrates preparation and composition

The substrates were composed of three varieties of a mixture of soil and cow dung in (1:0, 2:1, and 1:1). Then, the substrates were steam-sterilized for 30 minutes, cooling, and transferred to the plots and leveled at 7cm depth. The substrate was allowed to settle for 24 hours and well watered before transplanting.

Experimental design

The trial was a factorial experiment with two factors (two levels of physiological ages and three levels of the substrate) with six treatments replicated four times, laid out in a Randomized Complete Block Design (RCBD). A total of 24 plots, each with a plot size of 0.9 m x 0.8 m (0.72 m^2), were used (Table 2).

Table 1. Murashige and Skoog (1962) medium components

Components		**mg/L**
Macronutrients	$CaCl_{22}H_2O$	440
	MgSO42H2O	370
	KH2PO4	170
	NH2NO3	1650
	KNO3	1900
Micronutrients	KI	0.83
	H3BO3	6.20
	MnSO2H2O	22.30
	ZnSO27H2O	8.60
	Na2MgO22H2O	0.25
	CUSO45H2O	0.025
	CoCl26H2O	0.025
Iron	Na2EDTA	37.30
	FeSO47H2O	27.80
Vitamins	Myo-inositol	100
	Nicotinic acid	0.50
	Pyridoxine Hydrochloride	0.50
	Thiamine hydrochloride	0.10
	Glycine	2
Sugar	Sucrose	30000

Table 2. Cropping operations in vivo

Cultivation operations	Period	Dose / Quantity
Plots preparation	before planting	24 plots of 0.72 m^2
First mineral fertilizer application	before planting	300 kg/ha (NPK 17:17:17)
Furadan treatment	before planting	20 kg/ha
Transplantation	20-12-2017	10 cm x 10 cm
Watering	every day	-
Second mineral fertilizer application	15 DAT*	200 Kg/ha (NPK 17:17:17)
Third mineral fertilizer application	25 DAT	200 Kg/ha (NPK 17:17:17)
Fourth mineral fertilizer application	40 DAT	300 Kg/ha (NPK 17:17:17)
1st Earthing up	20 DAT	-
2nd Earthing up	35 DAT	-
3th Earthing up	50 DAT	-
Disease control	26 DAT	Ridomil (fungicide) 5.5 g/plot
Harvest	75 DAT	24 plots
Sorting, sizing, and counting of tubers	At harvest day	-

Note: *DAT: days after transplanting

Treatments

The treatment factors under consideration included the substrate composition on which weaning was carried out and the physiological age of the plantlet at weaning.

Two in vitro plantlet physiological ages at transplanting were evaluated [at 25 days (A1) and 45 days (A2)].

The three substrates used were:

S1 = Soil and Cow dung, 1:0;

S2 = Soil and Cow dung, 2:1;

S3 = Soil and Cow dung, 1:1.

The treatment combinations evaluated in this study are as follows:

T1 = Plantlets at age 25 days+soil and cow dung, 1:0 (A1S1);

T2 = Plantlets at age 25 days+soil and cow dung, 2:1 (A1S2);

T3 = Plantlets at age 25 days+soil and cow dung, 1:1 (A1S3);

T4 = Plantlets at age 45 days+soil and cow dung, 1:0 (A2S1);

T5 = Plantlets at age 45 days+soil and cow dung, 2:2 (A2S2);

T6 = Plantlets at age 45 days+soil and cow dung, 1:1 (A2S3).

The layout of in vivo experiments

This trial needed one screen house to be prepared. Therefore, to accommodate 56 plants were demarcated on each plot size of 0.9 m x 0.8 m. The spacing of 10 cm x 10 cm was adopted, so 1,344 in vitro plantlets were used for the trial (Figure 3).

Agronomic practices in vivo

The in-vitro plantlets were transferred from the culture chamber to the screen house on the same day (from 4 to 6 am) because the outside temperature was lower during this period. First, the in vitro plantlets were directly transferred to growing substrates already disinfected by steam sterilization from the in vitro culture tubes. Then, the plants were transplanted and immediately watered at a spacing of 10 cm × 10 cm.

Storage conditions

Tubers from the ten record plants were stored under natural room conditions, between 24-34°C.

Data collection of in vivo experiments

As record plants, ten plants were selected per treatment; the broader plants were excluded. Data were collected on the following:

Soil and cow dung analysis. Soil and Cow dung samples were seized before the mixture and sent to the laboratory to determine the cow dung's chemical properties (pH and nutrient status) and the physical and chemical properties.

Substrate samples analysis. Samples were seized after harvest from the three substrates used for the in vivo experiments and sent to the laboratory to determine the chemical status of the different spent substrates.

Plant re-establishment rate. The number of the surviving plant was used to calculate the re-establishment rate by counting plants that survived 20 days after transplanting (DAT).

Figure 2. In vitro plantlets at A. 25 days and B. 45 days at weaning age

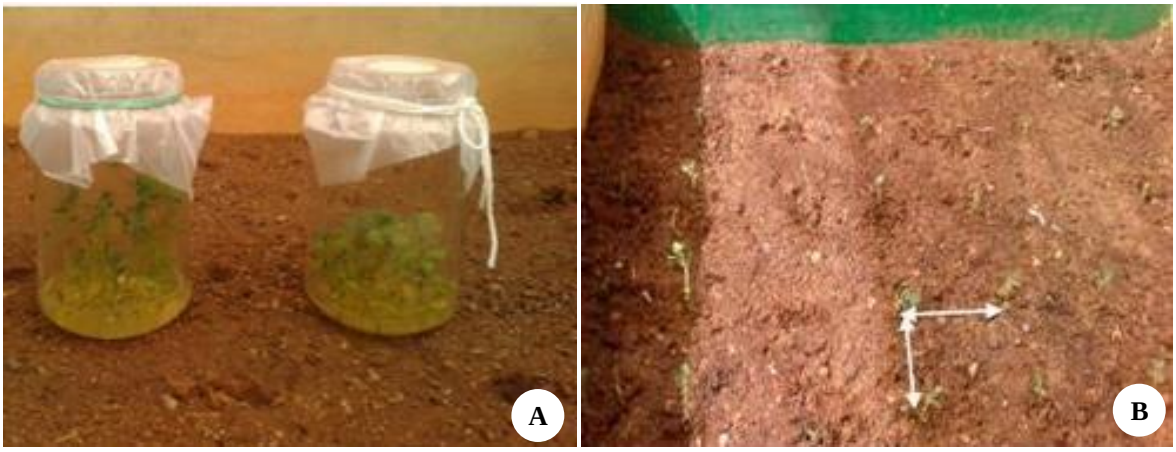

Figure 3. A. In vitro plantlets ready to be transplanted and B. Arrows showing plantlets transplanted at 0.1m x 0.1m spacing

Plant height measurements. Plant height was measured at 15, 40, and 60 DAT. Plant height was measured from the soil level to the top using a tape measure.

Stem diameter measuring. The stem diameter was measured at 15, 40, and 60 DAT; It was taken using a pair of calipers on the plant stem at the collar.

The number of leaves. The number of leaves per plant was taken at 40 DAT by counting the leaves on each record plant (10 plants per treatment) per replication.

Plant fresh weight at harvest. Plant fresh weight was recorded by weighing each of the ten (10) record plants at harvest (75 DAT) per treatment per replication.

Plant dry weight at harvest. The dry weight of the ten (10) record plants after oven drying at 70°C for 48 hours for each treatment was recorded. Before putting them in the oven, the plants were placed in an envelope.

Number of tubers per plant. The number of tubers produced per plant was recorded by calculating the average number of tubers at harvest from the ten (10) record plants per treatment per replication.

Tuber-weight per plant at harvest. Tuber weight per plant was recorded by weighing tubers at harvest for each of the ten record plants per treatment per replication.

Mean tuber weight. The mean tuber weight was taken by dividing the total plants' tuber weight by the number of tubers at harvest for each treatment.

Tuber grading size. The tubers were grouped into three (3) grading size groups from the ten record plants per treatment: (i) Grade A: tubers whose diameter was between 28 and 45 mm; (ii) Grade B: tubers with a diameter of less than 28 mm; (iii) Grade C: tubers with a diameter more than 45 mm. The percentage of each grade of tubers per treatment was recorded after the calculation.

Tuber weight loss in storage. Tubers were stored after harvest for eight weeks; the weight of tubers was taken in storage by weighing tubers from the ten record plants for each treatment every two weeks.

Number of sprouts per tuber in storage. After three months of storage, the number of sprouts per tuber was taken by counting the sprout on the ten stored tubers per treatment.

Sprout number per eye in storage. The number of sprouts per eye by dividing the number of sprouts per tuber by the number of eyes sprouted per tuber for each treatment was taken at three (3) months of storage.

Statistical analysis

Data were analyzed using ANOVA with GenStat (12th Edition). In addition, significant treatment means were separated using Fisher's LSD test at 5% significance.

RESULTS AND DISCUSSION

Effects of culture media on plantlets growth in vitro

Plantlets shoot emergence

The data collected ten days after culturing in vitro on shoot emergence (Table 3) showed significant differences ($p<0.01$) in culture media means. The maximum shoot emergence (100%) was observed in culture media M7 (MS+40 mL/L CW +250 mg KNO3), M8 (MS+40 mL/L CW+1,000 mg KNO3), M10 (MS+100 mL/L CW+1,000 mg KNO3) and M11(MS+300 mL/L CW+250 mg KNO3) after ten days of in vitro culturing. The lowest Shoot emergence (79.17 %), on the contrary, was observed in explants grown on medium M12 (MS+300 mL/L CW+1,000 mg KNO_3).

Plantlets height and internodes length

Data analysis on plantlet height and internode length 30 days after propagating is shown in Table 3, showing significant differences ($P<0.01$) in plant height and internode lengths between the different culture media means. The culture medium M7 (16 cm), with an internode length of 1.4 cm, shows the largest plantlet height. The M8 followed it with 13.5 cm and 1.4 cm internode length, then M4 and M2, respectively (12.8 cm of shoot length and 1.4 cm internode length, and 12.3 cm shoot length and 1.4 cm internode length). Finally, the shortest plant height of 4.8 cm was observed in the M12 with an internode length of 0.9 cm.

Number of leaves

Table 4 presented the data on the number of leaves at 30 days after propagating in vitro; in mean leaves numbers on the various culture media, highly significant differences ($p<0.01$) were observed. However, the M7 plantlets showed the highest mean number of leaves (13.45). M8 followed it with a mean of 11.7 leaves, then M4 of 11 leaves. There was no observed significant difference in mean leaves number between M2: MS+250 mg/L of KNO3, M5: MS+100 mL/L of coconut water, and M3: MS+1000 mg KNO3 with the respective leaf means of 10, 10, and 9.85. Finally, the least number of leaves was produced on M12: MS+300 mL/L CW+1000 mg KNO3 with 7.35 leaves.

Number of roots

Statistical analysis on plant root numbers after 30 days of in vitro propagation is presented in Table 4 and shows highly significant differences ($p<0.01$) between the 12-culture media. In culture media M7 and M8 observed the highest root number per plant, with nine roots each, and M3 recorded the least root number (1.2).

Number of nodes

Analysis of plant nodes number after 30 days of in vitro culture is presented in Table 4 and shows significant differences ($p<0.01$) between the culture media means. The M7 showed the highest mean number of nodes (11.45) per plant. M8 followed it with a mean of 9.65 nodes, next M4: MS+40 mL/L Coconut Water with nine nodes. On the contrary, the on M12 (5.35).

Plant fresh and dry weight

In mean plant fresh and dry weights between the different culture media, the data recorded 30 days after propagating (DAP) in vitro revealed significant differences ($p<0.01$). There were observed the highest plant fresh and dry weights (545.50 and 36.40 mg) on culture medium M7 (MS+40 mL/L of coconut water+250 mg of potassium nitrate), next by M2 (MS+250 mg/Liter of potassium nitrate) with the means of 364.50 mg for the fresh weight and 23.15 mg for the dry weight. While the M12: MS+300 mL/L of coconut water+1,000 mg/Liter of potassium nitrate with 99.20 mg for mean fresh and 13.20 mg for the mean dry weight (Figures 4, 5, 6, and 7) recorded the lowest.

According to Fisher's LSD test at 5% significance, letters represent significant differences among media

Plantlets weaning age and substrate composition effects on mini tubers production

Soil properties

The laboratory analysis shows that the soil has a sandy loam texture and a pH of 7.68, of the physical and chemical properties of the soil used as the basic substrate in the experiment (Table 5).

Cow dung properties

The laboratory analysis shows that the pH is 7.26, with a significant amount of NPK of the chemical properties of the cow dung used in the substrate composition for the experiment (Table 6).

Table 3. Media effect on shoot emergence after 10 days, plant height, and internode length after 30 days of in vitro culturing

Culture media	Shoot emergence %	Plant height (cm)	Internode length (cm)
M1	83.33 ^{bc}	10.00 ^{d}	1.43 ^{b}
M2	91.67 ^{abc}	12.30 ^{c}	1.54 ^{a}
M3	95.83 ^{ab}	9.90 ^{d}	1.27 ^{c}
M4	83.33 ^{bc}	12.78 ^{c}	1.42 ^{b}
M5	95.83 ^{ab}	8.68 ^{e}	1.09 ^{de}
M6	95.83 ^{ab}	6.93 ^{g}	0.99 ^{ef}
M7	100 ^{a}	16.00 ^{a}	1.40 ^{b}
M8	100 ^{a}	13.53 ^{b}	1.40 ^{b}
M9	91.67 ^{abc}	8.95 ^{e}	1.18 ^{cd}
M10	100 ^{a}	7.60 ^{f}	1.18 ^{cd}
M11	100 ^{a}	5.45 ^{h}	0.92 ^{f}
M12	79.17 ^{c}	4.80 ^{i}	0.90 ^{f}
p-value	0.039	<.001	<.001

Note: Letters represent significant differences among means according to Fisher's LSD test at 5% significance. p-value= probability value

Table 4. Comparison of the effect of different media on No. of leaves, roots, and nodes of in vitro-grown potato plantlets after 30 days

Culture media	No. of leaves	No. of roots	No. of nodes
M1	9.00 ^{ef}	5.10 ^{d}	7.00 ^{ef}
M2	10.00 ^{d}	6.35 ^{c}	8.00 ^{d}
M3	9.85 ^{d}	6.95 ^{b}	7.85 ^{d}
M4	11.00 ^{c}	6.80 ^{b}	9.00 ^{c}
M5	10.00 ^{d}	4.00 ^{f}	8.00 ^{d}
M6	9.00 ^{ef}	1.60 ^{h}	7.00 ^{ef}
M7	13.45 ^{a}	9.00 ^{a}	11.45 ^{a}
M8	11.65 ^{b}	9.00 ^{a}	9.65 ^{b}
M9	9.60 ^{de}	4.00 ^{f}	7.60 ^{de}
M10	8.45 ^{fg}	4.50 ^{e}	6.45 ^{fg}
M11	7.95 ^{gh}	2.20 ^{g}	5.95 ^{gh}
M12	7.35 ^{h}	1.20 ^{i}	5.35 ^{h}
p-value	<.001	<.001	<.001

Note: Letters represent significant differences among means according to Fisher's LSD test at 5% significance. p-value= probability value

Table 5. Soil properties before the substrate mixture

Soil properties	Amount
Texture: Sand (%)	72.00
Silt (%)	26.00
Clay (%)	2.00
pH in water	7.68
pH in KCl	6.96
Organic carbon (%)	0.65
Nitrogen total (%)	0.05
Phosphorus assimilable (ppm/100g)	78.32
Potassium assimilable (mg/100g)	10.64

Note: Laboratory SEP-IER, Mali

Table 6. Chemical properties of the cow dung used

Cow dung properties	Amount
pH in water	7.26
pH in KCl	6.63
Nitrogen (N %)	0.81
Phosphorus (P2O5 %)	0.89
Potassium (K2O %)	0.25

Note: Laboratory SEP-IER, Mali

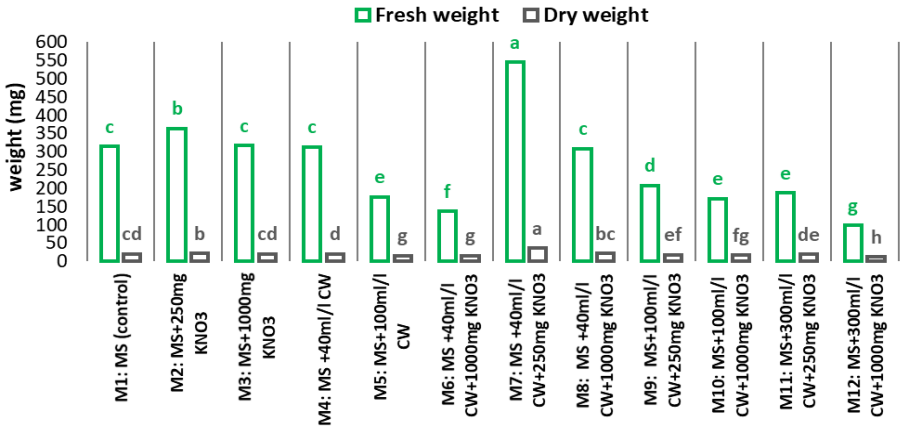

Figure 4. Effect of media on plantlet fresh and dry weights 30 days after culturing in vitro

Figure 5. Development of in vitro plantlets 30 days after culture on media M1, M2, M3, and M4

Figure 6. Development of in vitro plantlets 30 days after culture on media M5, M6, M7, and M8

Figure 7. Development of in vitro plantlets 30 days after culture on media M9, M10, M11 and M12

Chemical properties of spent substrates

The laboratory analysis of the three substrates of the physical and chemical properties used in the experiment shows that the pH of the three substrates tested was reduced compared to the initial state of the soil before a mixture of substrates and planting (Table 7).

Plant re-establishment

Table 8 presented the Statistical analysis from data on plant re-establishment at 20 days after transplanting, which indicated a significant difference ($p<0.01$) among plantlet weaning age. After 20 days of screen house culturing, weaning at 25 days produced the greatest percentage of plant re-establishment.

Between substrate mixtures, there were significant differences ($p<0.01$) were observed. Substrate S1 (soil and cow dung 1:0) recorded the highest plant survival percentage, while S3 (soil and cow dung 1:1) showed the lowest survival percentage.

The age and substrate interaction were also significant ($p<0.01$). The highest plant re-establishment percentage of 98.7 % and 97.8 % were recorded at treatment T1 (Seedlings ages 25 days and cultured on Soil and Cow dung 1:0) and T2 (age 25 days and cultured on Soil and Cow dung 2:1). Next by T3 (Seedlings ages 25 days: on Soil and Cow dung 1:1) with 91.5 % re-establishment. The treatment T6 (Seedlings ages 45 days: on Soil and Cow dung 1:1) observed the lowest with 59.8 % re-establishment after 20 days of culturing.

Plant height

After 15, 40, and 60 days of culturing in vivo, the data collected on plant height indicated significant differences between plantlet age and various substrates. In addition, plantlet age and substrate interaction also were significant (Table 9). The treatment T1 plants showed the greatest plant height with 7.7 cm and 28.3 cm, respectively, from 15 days to 40 days of culturing, but T2 recorded the highest plant height (49 cm) at 60 days of culturing. The lowest was at 60 days of culturing in vivo in T3 and T4 at 15 days, T4, T5, and T6 at 40 days, and T3, T4, and T6 at 60.

Stem diameter

Table 10 presents plant stem diameters recorded after 15, 40, and 60 days of culturing. Significant differences ($p<0.01$) were observed at plantlet weaning age at 15 days and 40 days after transplanting in stem diameters. However, there was no significant difference ($p>0.05$) at 60 days, and the largest plant stem diameters were formed in plantlets weaned at age 25 days, at 15 days, and 40 days after transplanting. Moreover, at 15 days, the substrate showed no significant difference ($p>0.05$) but at 40 days and 60 days after transplanting, highly significant differences ($p<0.01$) were observed. The substrate S3 recorded the largest stem diameters at 40 days and 60 days of culture, joined by the S2 at 60 days. Next, at 40 days and S1 at 60 days of transplanting, the smallest plant stem diameters were formed in substrates S2 and S1.

At 40 days and 60 days of culturing, there were significant differences ($p<0.05$) at 15 days and ($p<0.01$) with interactions between weaning age and substrate mixture. In T1 at 15 days (1.5 mm), in T3 (5.2 mm) and T1 (5.2 mm) at 40 days, and T6, T5, T1, and T3 at 60 days of culturing, respectively with 5.6 mm, 5.6 mm, 4.5 mm and 5.5 mm observed the greatest stem diameter. The lowest diameters at 15, 40, and 60 days respectively, with 1.1 mm, 3.2 mm, and 4.6 mm observed at treatment T4, while at 40 days, with 3.3 mm, was observed at treatment T5.

Table 7. The three Substrates' properties after growing

Substrate	pH in water	pH in KCl	Organic carbon (%)	N total (%)	P assimilable (ppm/100 g)	K assimilable (mg/100 g)
S1	7.24	6.94	0.56	0.01	122.06	45.13
S2	7.38	6.94	1.12	0.04	142.16	62.35
S3	7.53	7.23	1.39	0.05	157.65	84.05

Note: Laboratory SEP-IER, Mali

Table 8. Effect of weaning age of in vitro plantlet and substrate mixture on plant re-establishment percentage at 20 days after transplanting

Age	Substrate (soil and cow dung)			Mean
	1:0	2:1	1:1	(age)
25 days	98.66 [a]	97.77 [a]	91.52 [b]	95.98 a
45 days	83.93 [c]	74.11 [d]	59.82 [e]	72.62 b
Mean (substrate)	91.29 a	85.94 b	75.67 c	
	Age	Substrate	Age*Substrate	
p-value	<.001	<.001	<.001	

Note: Letters represent significant differences among means according to Fisher's LSD test at 5% significance. p-value= probability value

Table 9. Plant height

Treatments		Plant height (cm)		
Age	**Substrate**	**15 days**	**40 days**	**60 days**
25 days	Soil: Cow dung 1:0 = T1	7.69 [a]	28.25 [a]	48.98 [b]
weaning	2:1 = T2	6.67 [b]	21.90 [b]	53.93 [a]
	1:1 = T3	6.04 [c]	22.68 [b]	40.15 [c]
45 days	1:0 = T4	6.10 [c]	15.65 [c]	39.10 [c]
weaning	2:1 = T5	6.67 [b]	14.88 [c]	46.03 [b]
	1:1 = T6	6.57 [b]	15.88 [c]	41.50 [c]
p-value		0.009	<.001	0.001

Note: Letters represent significant differences among means according to Fisher's LSD test at 5% significance. p-value= probability value

Table 10. Plant stem diameter after 15, 40, and 60 days of culturing in vivo

Treatments		Stem diameter (mm)		
Age	**Substrate**	**15 days**	**40 days**	**60 days**
25 days	Soil: Cow dung 1:0 = T1	1.472 [a]	5.18 [ab]	5.46 [ab]
weaning	2:1 = T2	1.277 [b]	4.99 [b]	5.18 [b]
	1:1 = T3	1.20 [bc]	5.24 [a]	5.45 [ab]
45 days	1:0 = T4	1.06 [bc]	3.15 [d]	4.55 [c]
weaning	2:1 = T5	1.11 [bc]	3.33 [d]	5.56 [a]
	1:1 = T6	1.13 [c]	3.93 [c]	5.57 [a]
p-value		0.026	<.001	<.001

Note: Letters represent significant differences among means according to Fisher's LSD test at 5% significance. p-value= probability value

Table 11. Mean number of leaves of different treatments at 40 days of culturing

Age	**Substrate (soil and cow dung)**			**Mean**
	1:0 (S1)	**2:1 (S2)**	**1:1 (S3)**	**(Age)**
25 days	8.43	9.73	9.35	9.17
45 days	8.40	9.75	9.00	9.05
Mean (substrate)	8.41 c	9.74 a	9.18 b	
	Age	Substrate	Age*Substrate	
p-value	0.410	<.001	0.498	

Note: Letters represent significant differences among means according to Fisher's LSD test at 5% significance. p-value= probability value

Table 12. Plant fresh and dry weights at 75 days of culturing

Age	**Substrate**	**Plant fresh weight (g)**	**Plant fresh weight (g)**
25 days	Soil: Cow dung 1:0 = T1	33.43 [c]	2.55 [a]
weaning	2:1 = T2	37.88 [b]	2.63 [a]
	1:1 = T3	25.75 [d]	1.99 [b]
45 days	1:0 = T4	16.70 [e]	1.38 [c]
weaning	2:1 = T5	37.78 [b]	2.45 [a]
	1:1 = T6	41.98 [a]	2.48 [a]
p-value		<.001	<.001

Note: Letters represent significant differences among means according to Fisher's LSD test at 5% significance. p-value= probability value

Stem diameter

Table 10 presents plant stem diameters recorded after 15, 40, and 60 days of culturing. Significant differences ($p<0.01$) were observed at plantlet weaning age at 15 days and 40 days after transplanting in stem diameters. However, there was no significant difference ($p>0.05$) at 60 days, and the largest plant stem diameters were formed in plantlets weaned at age 25 days, at 15 days, and 40 days after transplanting. Moreover, at 15 days, the substrate showed no significant difference ($p>0.05$) but at 40 days and 60 days after transplanting, highly significant differences ($p<0.01$) were observed. The substrate S3 recorded the largest stem diameters at 40 days and 60 days of culture, joined by the S2 at 60 days. Next, at 40 days and S1 at 60 days of transplanting, the smallest plant stem diameters were formed in substrates S2 and S1.

At 40 days and 60 days of culturing, there were significant differences ($p<0.05$) at 15 days and ($p<0.01$) with interactions between weaning age and substrate mixture. In T1 at 15 days (1.5 mm), in T3 (5.2 mm) and T1 (5.2 mm) at 40 days, and T6, T5, T1, and T3 at 60 days of culturing, respectively with 5.6 mm, 5.6 mm, 4.5 mm and 5.5 mm observed the greatest stem diameter. The lowest diameters at 15, 40, and 60 days respectively, with 1.1 mm, 3.2 mm, and 4.6 mm observed at treatment T4, while at 40 days, with 3.3 mm, was observed at treatment T5.

Number of leaves at 40 days of culturing

Table 11 presented the statistical analysis of the data on plant leaf numbers at 40 days of culture. Among in vitro plantlet weaning age means, there was no significant difference ($P>0.05$), while between substrate means indicated highly significant differences ($P<0.01$). The lowest in S1 (Soil and Cow dung 1:0) with 8.41 leaves, and the substrate S2 (Soil and Cow dung 2:1) showed the highest number of leaves (9.74). In the interactions between weaning age and substrate mixture means, no significant differences ($P>0.05$) were observed.

Plant fresh and dry weights at harvest

Table 12 presented the data analysis of plant fresh and dry weights at 75 days of culturing. No significant difference ($p>0.05$) was shown among plantlet weaning age in the fresh plant weight, but between the plant dry weight means there was a highly significant difference ($p<0.01$). The greatest plant dry weight was recorded by the weaning age of 25 days. For both fresh and dry weights, highly significant differences ($p<0.01$) were observed in the means of substrate mixture. The lowest in S1 (Soil and Cow dung 1:0) and the greatest plant fresh and dry weights were both recorded in substrate S2 (Soil and Cow dung 2:1). In the interactions between weaning age and substrate mixture, there were Highly significant differences ($p<0.01$). The highest plant fresh weight was observed in T6, with a mean of 42 g, next by T2 (37.9 mg) and T5 (37.8 mg). Furthermore, the greatest plant dry weights were obtained in T2 (2.6 g), T1 (2.6 g), T6 (2.5 g), and T5, with a mean of 2.5 g, next by T3 (2 g). T4 produced the lowest plant fresh and dry weights, with 16.7 g and 1.4 g, respectively.

Tuber size grading quality

Tables 16 and 17 present an analysis of the data on tuber size grading (A: tubers whose diameter is between 28 and 45 mm, B: tubers with a diameter of less than 28 mm, and C: tubers with a diameter greater than 45 mm). Between the weaning ages of in vitro plantlets for A, B, and C size grading, there were significant differences ($p<0.01$). Seedlings weaned for 25 days produced the highest percentage of tubers in grade A and C sizes than those weaned for 45 days, with the highest percentage in grade B, but on the substrate for grades A, B, and C, significant differences were observed ($p<0.05$). The substrate S1 (Soil and Cow dung 1:0) and S3 (Soil and Cow dung 1:1) produced the highest percentages of grade A tubers. The highest grade B tubers were produced on substrate S2 (Soil and Cow dung 2:1), while substrate S1 produced more tubers of grade C size than the others. Furthermore, between age and substrate ($p<0.01$) for grades A and B, there were significant differences in the interactions; and also significant differences ($p<0.05$) for grade C sizes. The highest percentage of tuber grade A recorded on treatment T1, while the treatments T4, T5, and T6 produced more grade B tubers than the others. On the other hand, in grade C, the treatment T1 mean was significantly higher (Figure 8).

Tubers weight loss in storage

Data recorded on tubers stored for eight weeks on weight loss (%) showed no significant difference ($p>0.05$) for ages 2, 4, 6, and 8 weeks of storage. There was no significant difference for substrate at 4 weeks ($p>0.05$), but significant differences occurred for substrate at 2, 6, and 8 weeks of storage ($p< 0.05$). From 2 to 8 weeks of storage, the interactions (age*substrate) were observed to be significant ($p<0.01$). The lowest weight loss percentages in stored tubers were obtained in substrate S1 and the highest in substrates S2 and S3. At two weeks, the lowest weight loss percentages were observed in the tubers in treatments T1, T2, T6, and T4. T1 and T6 at four weeks, T1 at six weeks, and T1 and T6 at eight weeks of storage. The highest was shown by treatments T3 at two weeks, T4 and T5 at four weeks, T5 at six weeks, and T2 tubers at eight weeks of storage (Table 16).

Tuber sprout numbers after three months in storage

The potato-tuber sprout number results after three months in storage are presented in Table 17, Figures 9 and 10. Significant differences ($p< 0.01$) occurred for the weaning age of in vitro plantlets. The lowest tuber sprout number was observed on plantlets weaned for 45 days, while plantlets at 25 days recorded the highest number of sprouts.

For the substrate used for transplantation, significant differences ($p<0.01$) were observed. The number of sprouts on a tuber on substrates S2 (soil and cow dung 2:1) and S3 (soil and cow dung 1:1) significantly reduced than to S1 (soil and cow dung 1:0), which produced the highest number of sprouts on a tuber. On Age and Substrate interaction, no significant difference ($p>0.05$) was observed.

Table 13. The yield of tubers in tons per hectare

Age	Substrate (soil and cow dung)			Mean (age)
	1:0 (S1)	2:1 (S2)	1:1(S3)	
25 days weaning	58.12 [a]	59.28 [a]	44.03 [c]	58.81 a
45 days weaning	33.08 [d]	47.08 [b]	46.03 [bc]	42.06 b
Mean (Substrate)	45.60 b	58.18 a	45.03 b	
	Age	Substrate	Age*Substrate	
p-value	<.001	<.001	<.001	

Note: Letters represent significant differences among means according to Fisher's LSD test at 5% significance. p-value= probability value

Table 14. The average weight of one tuber

Age	Substrate (soil and cow dung)			Mean (age)
	1:0 (S1)	2:1 (S2)	1:1(S3)	
25 days weaning	11.98 [a]	7.83 [b]	7.25 [c]	9.02 a
45 days weaning	5.56 [e]	5.54 [e]	6.14 [d]	5.75 b
Mean (substrate)	8.77 a	6.69 b	6.69 b	
	Age	Substrate	Age*Substrate	
p-value	<.001	<.001	<.001	

Note: Letters represent significant differences among means according to Fisher's LSD test at 5% significance. p-value= probability value

Table 15. Number of tuber per plant at harvest

Age	Substrate (soil and cow dung)			Mean (age)
	1:0 (S1)	2:1 (S2)	1:1(S3)	
25 days weaning	4.85 [d]	7.58 [b]	6.08 [c]	6.17 b
45 days weaning	5.95 [c]	8.50 [a]	7.50 [b]	7.32 a
Mean (Substrate)	5.40 c	8.04 a	6.79 b	
	Age	Substrate	Age*Substrate	
p-value	<.001	<.001	0.009	

Note: Letters represent significant differences among means according to Fisher's LSD test at 5% significance. p-value= probability value

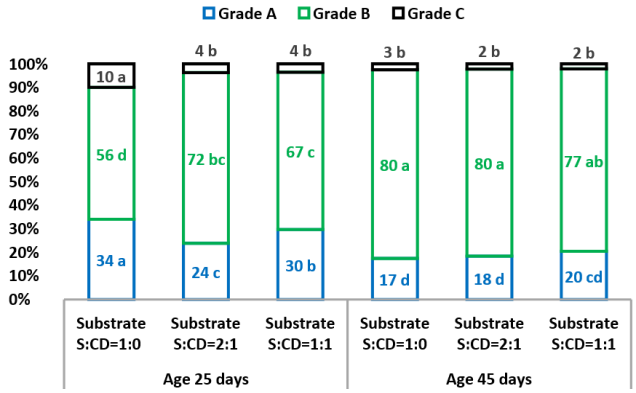

Figure 8. Tuber size grading. Letters represent significant differences among means according to Fisher's LSD test at 5% significance. Grade A (p-value: <.001), Grade B (p-value: 0.001), Grade C (p-value: 0.043)

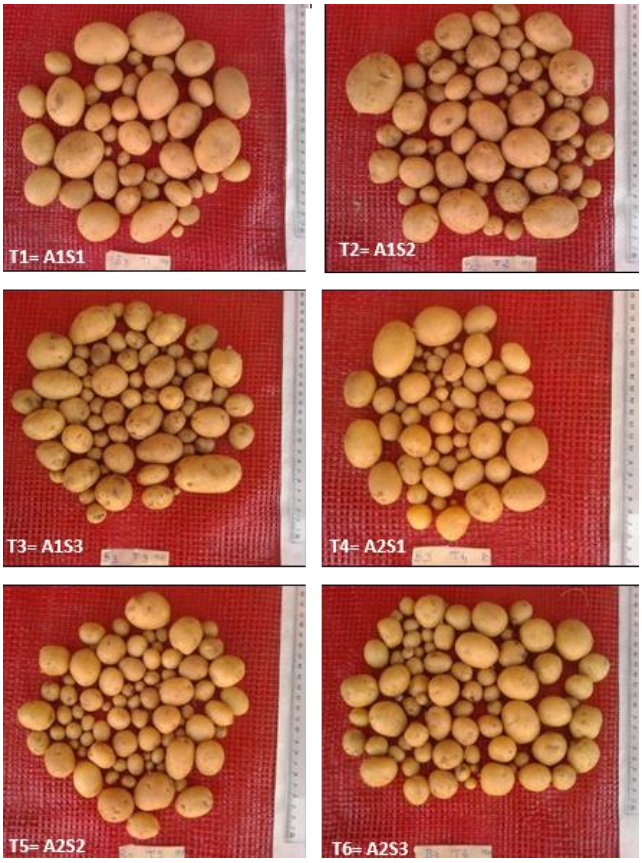

Figure 9. Tubers produced at harvest by 10 plants under the 6 treatment conditions. A1= plantlet weaning age 25 days; A2= plantlet weaning age 45 days. S1= soil and cow dung 1:0; S2= soil and cow dung 2:1 and S3= soil and cow dung 1:1

Table 16. Tubers' percentage weight loss from harvest to 8 weeks in storage

Treatments		Tubers weight loss (%)			
Age	**Substrate**	**2 WIS**	**4 WIS**	**6 WIS**	**8 WIS**
25 days	Soil: Cow dung 1:0 = T1	4.46^{a}	6.76^{a}	9.37^{a}	12.50^{a}
weaning	2:1 = T2	4.84^{a}	7.46^{b}	10.87^{bc}	18.48^{d}
	1:1 = T3	6.50^{c}	8.50^{c}	12.54^{d}	17.75^{cd}
45 days	1:0 = T4	5.05^{ab}	8.26 ^{c}	10.73^{bc}	15.08^{b}
weaning	2:1 = T5	5.53^{b}	8.27^{c}	11.58^{c}	15.86^{bc}
	1:1 = T6	4.43^{a}	7.07^{ab}	10.31^{b}	13.60^{ab}
p-value		<.001	<.001	<.001	0.003

Note: Letters represent significant differences among treatments according to Fisher's LSD test at 5% significance. The comparison's direction is ascending. WIS: week in storage. p-value= probability value

Table 17. Tuber sprout number after 3 months in storage

Age	Substrate (soil and cow dung)			Mean (age)
	1:0 (S1)	2:1 (S2)	1:1(S3)	
25 days weaning	9.45	7.72	7.40	8.19 b
45 days weaning	6.92	6.50	5.75	6.39 a
Mean (Substrate)	8.19 b	7.11 a	6.58 a	
	Age	Substrate	Age*Substrate	
p-value	<.001	0.004	0.287	

Note: Letters represent significant differences among means according to Fisher's LSD test at 5% significance. The comparison's direction is ascending. p-value= probability value

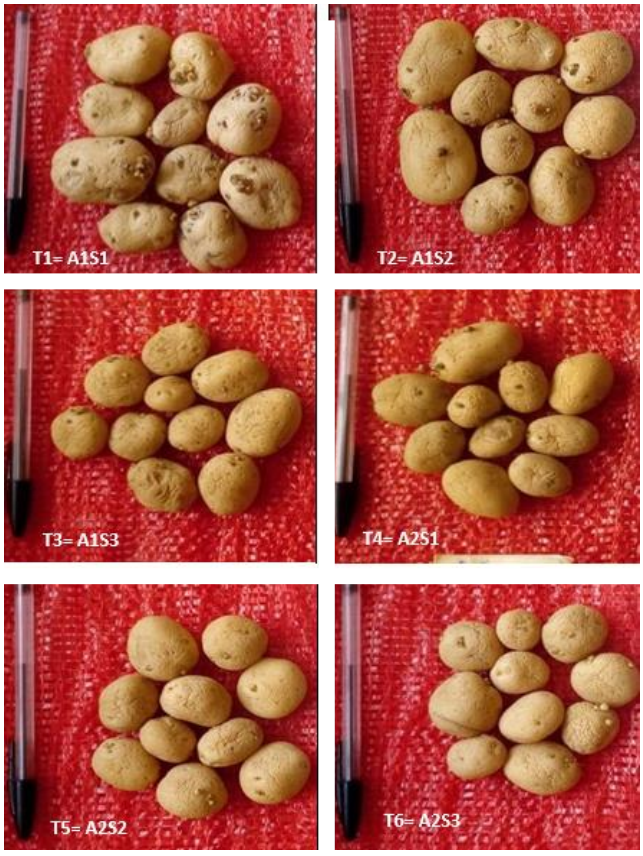

Figure 10. Tubers produced under the 6 treatment conditions 3 months after storage. A1= plantlet weaning age 25 days; A2= plantlet weaning age 45 days. S1= soil and cow dung 1:0; S2= soil and cow dung 2:1 and S3= soil and cow dung 1:1

Table 18. Sprout numbers per eye after 3 months of storage

Age	Substrate (soil and cow dung)			Mean (age)
	1:0 (S1)	2:1 (S2)	1:1(S3)	
25 days weaning	1.90 ^{b}	1.42 ^{a}	1.35 ^{a}	1.56 b
45 days weaning	1.31 ^{a}	1.20 ^{a}	1.25 ^{a}	1.25 a
Mean (Substrate)	1.60 b	1.31 a	1.30 a	
	Age	Substrate	Age*Substrate	
p-value	0.001	0.008	0.047	

Note: Letters represent significant differences among means according to Fisher's LSD test at 5% significance. The comparison's direction is ascending. p-value= probability value

Sprout number per eye after three months of storage

Table 18. shows the effect of the weaning age of substrate and in vitro plantlet for transplanting on sprout numbers per eye after three months of storage. There was a significant difference (P<0.05) among the means for weaning age. The lowest number of sprouts per eye on tubers grew at a weaning age of 45 days and the highest in tubers from 25 days.

Furthermore, depending on the substrate used for transplanting, significant differences (p< 0.05) were observed g. The tubers with the lowest sprouts per eye produced on substrates S2 (soil and cow dung 2:1) and S3 (soil and cow dung 1:1) and the highest in tubers on S1. Also, for age and substrate interaction, there were

significant differences ($p<0.05$). The lowest number of sprouts per eye was shown by treatments T2, T3, T4, T5, and T6, in which only T1 showed the highest.

Discussion

Effects of culture media on plants grown in vitro

The study showed that after 30 days of in vitro, culturing growth and development parameters stimulated by the culture medium M7, 40 mL of coconut water, and 250 mg of potassium nitrate per liter of MS medium were used. This stimulation may be due to adequate quantities of coconut water and potassium nitrate. Coconut water contains several organic compounds such as sucrose; vitamins of B group: nicotinic acid, biotin, and folic acid; phytohormones: auxin, cytokinins, gibberellins, and abscisic acid; and mineral nutrients: potassium, sodium, calcium, phosphorous (Yong et al. 2009; Mullukattil 2013; Reddy and Lakshmi 2014). The in vitro explants growth could be stimulated on a medium supplemented with 5% to 15% coconut water (CIDES 1999; Prades et al. 2011), which is the quantity of 5% close to the concentration of 40 mL of coconut water per liter medium (4% of culture medium volume). The coconut water at 40 mL combined with 250 mg of potassium nitrate per liter MS medium played a role in the plant growth stimulation observed.

The media M7 (MS + 40 mL/L of coconut water + 250 mg of potassium nitrate), M8 (MS + 40 mL/L coconut water +1,000 mg of potassium nitrate), M10 (MS+100 mL/L coconut water + 1,000 mg of potassium nitrate) and M11 (MS+300 mL/L coconut water +250 mg of potassium nitrate) observed to promote faster shoots emergence than the other media. Those media resulted in 100 % emergence, compared to M1 (MS medium, the control), which recorded shoot emergence at 83 %.

Furthermore, after 30 days of culturing, the greatest in vitro plantlet height (16 cm) was shown by the culture medium M7 (MS + 40 mL/L of coconut water + 250 mg of potassium nitrate) than the MS medium used as the control, with a maximum of 10 cm shoot length. The adequate quantity of growth-promoting substances may explain that in coconut water, such as N6-Furfuryladenine (Kinetin), which promotes cell division in plants (Yong et al. 2009) and the effect on the cultured explants of the nitrogen contained in potassium nitrate. Nitrogen is an essential element for photosynthesis (chlorophyll) and cell formation. Therefore, the main factor of plant growth and quality influences plants' protein content (UNIFA 2005).

After 30 days of in vitro culturing, the highest number of nodes (11.5) was observed in culture medium M7 as a supplement to MS medium containing 40 mL of coconut water and 250 mg of potassium nitrate per liter. However, M1 (MS), the control medium, recorded only seven nodes. The number of nodes per plant refers to the plant multiplication rate. The culture medium M7 recorded the highest mean number of leaves (13.5). But the control, M1 (MS), recorded fewer leaves than M7 containing 40 mL/L of coconut water, and 250 mg/L of potassium nitrate were used as supplements to the MS medium. Another study stated that cytokinin (kinetin) promotes bud formation in many in vitro cultured organs, such as leaves and node numbers reported by Afshin et al. (2011).

The root number per plant was greatest in M7 and M8 (9 roots) versus M1 (MS), which recorded 5.1 roots, which may be due to the coconut water's indole- 3-acetic acid (auxin). The IAA plays a significant role in growth regulation and plant root emergence (Muhammad et al. 2015). Even though this study realized that M6, M11, and M12, 300 mL of coconut water per liter, revealed the least root numbers per plant, 1.6, 2.2, and 1.2 roots, respectively. The higher cytokinin concentration can explain that in 300 mL of coconut water per liter. The CIDES (1999) reported that cytokinin at higher concentrations in a culture medium is responsible for multiple shoots and callus formation.

The highest plantlet fresh and dry weights (545.5 and 36.4 mg) after 30 days after propagating in vitro were observed in culture medium M7 (MS + 40 mL/L of coconut water and 250 mg of potassium nitrate). Moreover, 50 mL/L of coconut water in a culture medium in Calanthe hybrids increased plant fresh and dry weights (Abdullahil et al. 2011). In this study, the maximum fresh and dry potato plantlets were in M7, where MS was supplemented with 40 mL/L of coconut water and 250 mg of potassium nitrate. This coconut water concentration is comparable to the 50 mL/L cited before.

The lower concentrations of coconut water (40 mL) and potassium nitrate (250 mg) per liter of MS medium interaction after 30 days of culturing have significantly affected all the in vitro growth parameters. Conversely, plant growth in height and root emergence were blocked by the higher concentrations of coconut water (300 mL per liter of MS medium).

Physiological age and substrate effects on mini tubers production in vivo

The pH in the experiment was reduced on the three substrates used compared to the soil's initial state before the substrates' mixture and planting. Applying cow dung or mineral fertilizer (NPK) to all three substrates could decrease pH levels in all substrates. This result is consistent with Monirul et al. (2013) and Suh et al. (2015). However, the reduction was shown more with the substrate S1 (soil and cow dung 1:0), with no cow dung but had the application of mineral fertilizer (1,000 kg/ha of NPK 17:17:17), with a reduction of 5.7% of the observed initial pH (7.68). The treatments T1 (age 25 days and substrate-only soil) and T2 (age 25 days and substrate 2:1 Soil and Cow dung) recorded the maximum plant re-establishment percentage.

The growing substrate pH may affect plantlets' in vivo survival and growth (Conner and Thomas 1982). The laboratory analysis showed that the maximum plant survival percentage was observed at the lower pH in substrate S1 (7.24). On the other hand, S2 (soil and cow dung 2:1) promoted significantly higher plant stem length, stem diameter, and fresh and dry biomass formation. Moreover, the effect of plantlet weaning age was insignificant for plant stem diameter and fresh and dry weight. The highest yield of tubers was recorded on the treatments T2 (59.28 t/ha) and T1 (58.12 t/ha). That is

possibly due to the level of organic carbon and the pH in S2. However, S3 presented a higher amount of organic carbon compared to S2 but showed a higher level of alkaline pH than others, which could be a disadvantage for tuber formation and plant growth. According to Mimouni (2011), the soil's alkaline pH can block the absorption of phosphorus, copper, iron, manganese, boron, and zinc.

Furthermore, in treatment T5 (weaning age 45 days and substrate 2:1 Soil and Cow dung) have the maximum number of tubers per plant, which may be due to the ability to increase nutrient availability through high biological activity and the high nutrient level of the two substrates (Pengthamkeeratia 2011). Plantlets with a weaning age of 25 days produced significant numbers of grade A, while the substrates S1 and S3 (soil and cow dung 1:1) means were significant in grade A.

The substrate composition positively affected the weight loss (%) of stored tubers after eight weeks, and the lowest percentages were obtained in substrates without cow dung. The Substrate S1 (only soil) showed the same trend of tubers' low weight loss from the 2nd week to the 8th week of storage, and the highest weight loss percentages in stored tubers were obtained in S2 (Soil and Cow dung 2:1), and S3 (soil and cow dung 1:1) that the substrate contained cow dung. The highest percentages of weight loss in Substrates S2 (Soil and Cow dung 2:1) and S3 (soil and cow dung 1:1) can be the lower immaturity of tubers' skin. Moreover, at harvest time was observed in Substrate S1 (only soil) plots, all plants had reached the senescence stage (physiological maturity). Generally, the senescence stage progressively changes the leaves' color to yellow from the base to the top, with subsequent drying out. Even though plants in all plots were still growing in substrates S2 (Soil and Cow dung 2:1) and S3 (soil and cow dung 1:1), that can lead to immature tubers harvested from these treatment plots. A study by Paris IV University in collaboration with ARVALIS showed that water loss in tubers was proportional to Vapor Pressure Deficit (VPD) between the tuber and the ambient air and also showed a significantly higher loss in case of the immaturity of the skin or injury of tubers (Martin 2006).

Furthermore, for the weaning age of 45 days at the substrates S2 (soil and cow dung 2:1) and S3 (soil and cow dung 1:1), the number of sprouts per tuber and sprouts number per eye on tubers was minimized significantly. These results differ from the weaning age of 25 days and substrate S1 (soil and cow dung 1:0) after three months of storage, which promoted early emergence and higher sprouts number on the tuber. One of the most important factors in the deterioration of quality during storage is tuber sprouting (ARVALIS /Institut-du-végétal 2013). The tuber dormancy time for the small size grade is longer than that of larger sizes reported by Reust (1982). Moreover, the weaning age of 45 days and the substrates S2 (soil and cow dung 2:1) conditions that the maximum quantities of small-size grade tubers were produced, revealed in this current study.

In conclusion, the study's results showed the highest plantlets multiplication rate on the culture medium M7 (MS+40 mL/L of coconut water +250 mg of potassium nitrate) after 30 days of in vitro culture, with an average number of 11.45 nodes per plant. Furthermore, on all the in vitro growth parameters, the interaction between lower concentrations of coconut water (40 mL) and potassium nitrate (250 mg) per liter of MS medium has significantly positive effects. Also, during 30 days of culturing, the higher concentrations of coconut water (300 mL) potassium nitrate (1,000 mg) per liter of MS medium adversely affect plant growth parameters. Moreover, at 20 days after transplanting in vivo, the Plantlet weaning age of 25 days and the post-flask culture substrate S1 (only soil) appears to give the best plant survival percentage. On all plant in vivo growth and tuber yield parameters, substrate S2 (soil and cow dung 2:1) was positively affected. Finally, in storage three months after harvest, the weaning age of 45 days and the substrates S2 (soil and cow dung 2:1) and S3 (soil and cow dung 1:1) conditions produced significantly reduced mini tubers quality loss.

REFERENCES

Abdullahil BS, Yun K, Elshmari T, Lee EJ, Paek KY. 2011. Effect of light quality, sucrose and coconut water concentration on the microporpagation of calanthe hybrids ("bukduseong" × "hyesung" and "chunkwang" ×' hyesung'. Austral J Crop Sci 5 (10): 1247-1254.

Afshin AH, Kaviani B, Tarang A, Zanjani SBI. 2011. Effect of different concentrations of kinetin on regeneration of ten weeks (*Matthiola incana*). Plant Omics J 4: 236-238.

Anderson WC. 1978. Rooting of tissue cultured rhododendrons. Proc Intl Plant Prop Soc 28: 135-139.

ARVALIS /Institut-du-végétal. 2013. Rapport d'activités pommes de terre 2012, 94.

Bajaj YPS, Sopory SK. 1986. Biotechnology in Agriculture and Forestry, Vol2, Crops. Springer, Bferlin Heidelberg, New York, Tokyo. DOI: 10.1007/978-3-642-61625-9.

BNDA. 2014. Fiche Technique - Pomme de terre/Banque Nationale de Développement Agricole du Mali, 5.

CIDES. 1999. Micropropagation pour l'entreprise serricole - Cahier de références techniques, 44.

Conner AJ, Thomas MB. 1982. Re-establishing plantlets from Tissue culture: A review. 342-357.

Coulibaly M, Dembélé D, et Vanderhofstadt B. 2002. Unité pilote de production de plants de pomme de terre au Mali, SOC International - AMATEVI., 82.

Diakité L, Zid M. 2003. Etude diagnostique de la filière pomme de terre dans trois pays de l'Afrique de l'Ouest. CILSS-INSAH, II, 58.

FAO. 2009. New Light on a Hidden Treasure. Review, 148. Rome.

FAO. 2106. The State of Food and Agriculture 2016 in Africa. Rome.

Gorst JR, Bourne RA, Hardaker SE, Richards AE, Dircks S, de Fossard RA. 1978. Tissue culture propagation of two *Grevillea* hybrids. Proc Intl Plant Prop Soc 28: 435-445.

ITCMI. 2012. Bulletin Mensuel d'information nº 06 Novembre 2012, 2.

Jackson JC, Gordon A, Wizzard G, McCook K, Rolle R. 2004. Changes in chemical composition of coconut (*Cocos nucifera* L.) water during maturation of the fruit. J Sci Food Agric 84: 1049-1052. DOI: 10.1002/jsfa.1783.

Martin M. 2006. Lutter contre la déshydratation des tubercules, 10-13.

Mimouni A. 2011. Effet de la fertilité des sols et de la fertilisation sur les jeunes plants de l ' arganier transplantation en milieu réel, 109-118.

MINRESI-IRAD. 2012. Contribution à l'augmentation de la productivité et de la production de la pomme de terre par la création et la diffusion de matériel végétal performant, (Projet C2D Pomme de terre Cameroun), 24.

Monirul MI, Akhter S, Majid NM, Alam MS, Jannatul F. 2013. Integrated nutrient management for potato (*Solanum tuberosum* L.) in grey terrace soil. Aust J Crop Sci 7 (9): 1235-1241.

Muhammad K, Gul Z, Jamal Z, Ahmed M, Rehman A, Khan ZU. 2015. Effect of coconut water from different fruit maturity stages, as natural

substitute for synthetic PGR in in vitro potato micropropagation. Intl J Biosci 6: 84-92. DOI: 10.12692/ijb/6.2.84-92.

Mullukattil LR. 2013. The chemical composition of tender coconut (*Cocos nucifera* L.) water and coconut meat and their biological effect in human body. Intl J Green Herbal Chem 2 (3): 723-729.

Overbeek VJ, Conklin ME, Blakeslee AF. 1941. Factors in coconut milk essential for growth & development of very young *Datura* embryos. Science 94: 350-351. DOI: 10.1126/science.94.2441.350.

Pengthamkeeratia P, Motavalli PP, Kremer RJ. 2011. Soil microbial activity and functional diversity changed by compaction, poultry litter and cropping in a claypan soil. Appl Soil Ecol 48: 71-80. DOI: 10.1016/j.apsoil.2011.01.005.

Prades A, Dornier M, Diop N, Pain JP. 2011. Coconut water-uses composition and properties: a review. Fruits 67: 87-107. DOI: 10.1051/fruits/2012002.

Reddy EP, Lakshmi T. 2014. Coconut water-properties, uses, nutritional benefits in health and wealth and in health and disease: A review. Clin Med Lab Biochem 2 (2): 6-18.

Reust W. 1982. Contribution à l'appréciation de l'âge physiologique des tubercules de pommes de terre (*Solanum tuberosum* L.) et étude de son importance sur le rendement (thèse de doctorat. Ecole Polytechnique Fédérale, Zurich.

Suh C, Meka SS, Ngome AF, Neba DA, Kemngwa IT, Sonkouat AD, Njualem D. 2015. Effect of organic and inorganic fertilizer on the yield and. J Agric Soc Res 5 (2): 5. DOI: 10.7537/marsnsj14021607.

UNIFA. 2005. Principaux éléments fertilisants Parlons fertilisation. Infa 1-6.

Vanderhofstadt B. 2011. Etude de faisabilité de la mise en place d'une filière "plant de pomme de terre" au Mali, (Ref : RMMAPCDA1111vc), 1-77.

Yong JW, Ge L, Yan FNg, Tan SN. 2009. The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water. Molecules 14 (12): 5144-5164. DOI: 10.3390/molecules14125144.