Evaluation of the effects of four growth media on potato (Solanum tubérosum L.) plantlets production in Burkina Faso

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Abstract

Aims: To identify an appropriate culture medium suitable for micropropagation of plantlets with good root proliferation and stem growth of potato.

Study design: In Burkina Faso, potato growers face difficulties in obtaining potato seeds. In general, most of the imported seeds partially meet the quality requirements of potato production. Furthermore, these seeds are not accessible in terms of cost and are unavailable in time. In vitro culture can remedy this problem by providing quality seeds.

conducted in the laboratory of

Place and Duration of Study: This study was ~~conduction in the *in vitro* culture~~ laboratory of the Institut de l'Environnement et de Recherches Agricoles (INERA) at Kamboinsé in Burkina Faso, between August to September 2022.

the explants were cultured

Methodology: Explants from in vitro sprouts (1 cm) of the same generation were used as ~~plant material and transplanted onto four~~ different media. The media used were as follows: M0= 4.4 g l^{-1} MS medium + 7 g l^{-1} agar; M1= M0 + 30 g l^{-1} sucrose; M2= M0 + 0.5 mg l^{-1} 3 indole butyric acid (IBA) and M3= M0+30 g l^{-1} sugar + 0.5 mg l^{-1} IBA.

those in

Results: this study indicated that M1 and M3 regenerated ~~the~~ in vitro plantlets better than M0 and M2 did. Sucrose ~~likely~~ had a positive effect on root length, stem diameter, number of nodes and number of opened leaves. These parameters strongly differentiated M1 from the other media. IBA had a positive effect on the ~~number of~~ root proliferation of in vitro plantlets in the M2 medium. The combined effect of sucrose and IBA had even greater effects on stem height, number of roots, number of leaves open and weight of in vitro plantlets, which strongly differentiated medium M3 from the other media.

Conclusion: M3 proved to be the best medium for in vitro plantlet production.

in the 10 cultivars of potato.

Key words: *Micropropagation, sugar, auxin IBA, in vitro culture, seeds.*

INTRODUCTION

Seed technology is a set of applied sciences, technologies, and socioeconomic aspects that contribute to the production and availability of good quality seed (Turner 2010). The potato seed is not excluded from this technology. Potatoes are considered the fourth most important food crop in the world after maize, rice and wheat (FAO 2022). Globally, potato production has increased at a much higher rate in Asia and Africa while Europe is showing its decline (FAO 2022). Half of its current global production occurs in China and India (FAO 2022; (Devaux and al, 2014). The observed increase is thought to be due to the use of in vitro cultivation technology, which is used in Asia for seed production (Devaux and al., 2014). According to (P. Wang and Hu 1982), this technology has been used to produce disease-free seeds. This methodology is also used in sub-Saharan African countries including Burundi, Ethiopia, Kenya, Malawi, Rwanda, Tanzania and Uganda and has led to a large increase in minituber production although this increase is still small (Harahagazwe et al., 2018; Campos and Ortiz 2019). Despite this increase in potato production, the majority of developing countries, particularly those in West Africa, face difficulties obtaining seeds (Amina. Belguendouz 2011; Abdoulaye 2018). Burkina Faso is not an exception to this constraint (Zerbo et al. 2022). According to statistics from the Ministry of Agriculture, Animal Resources and Fisheries, most of the seeds used by producers come from outside the country. Two types of potato seeds are used in Burkina Faso: farm seeds that consist of small caliber tubers from previous production and certified seeds imported from outside the country, especially from Europe. However, most of the imported seeds partially meet the required quality criteria and these imported seeds are not available on time (Harahagazwe and al. 2018; Zerbo et al. 2022). As a result, local seed production needs to be developed. In vitro culture technology through micropropagation can help alleviate this problem by providing growers with quality seeds. This technique requires the use of

MS salts

appropriated cultural media. Various culture media composed of MS medium (Murashigue and Skoog 1962), sucrose and the auxin IBA have been tested. MS medium is generally used as a basic medium in many in vitro cultures (Dubuc 2010; Rahman et al. 2010; Chen et al. 2020). For this reason, it is essential to test culture media with different compositions for the production of in vitro plantlets. The purpose of our study was to identify an appropriate culture medium suitable for micropropagation of plantlets with good root proliferation and stem growth of potato. Specifically, we aimed to evaluate: i) the effect of media on the root proliferation of in vitro plantlets; ii) the effect of media on the growth parameters of in vitro plantlets; and iii) the Performance of cultivars in culture media.

MATERIALS AND METHODS

Materials

The plant material consisted of a total of two certified cultivars (Sahel and Spunta) commonly grown in Burkina Faso and eight cultivars received from in vitro plantlets from International Potato Center (IPC) of Peru (Table 1). These cultivars are adapted for cultivation during the dry season (November to March) under irrigation in the Coastrian West Africa zone.

Table 1: Cultivars list List of cultivars used in the experiment

Numbers	Cultivars	Origins
1	**CIP 393371.58**	**Peru/Lima**
2	**CIP381381.13**	**Peru/Lima**
3	**CIP 393079.4**	**Peru/Lima**
4	**CIP 393385.39**	**Peru/Lima**
5	**CIP 398208.704**	**Peru/Lima**
6	**CIP 398208.29**	**Peru/Lima**
7	**CIP 398208.505**	**Peru/Lima**
8	**CIP 392797.22**	**Peru/Lima**
9	**Sahel**	**INERA**
10	**Spunta**	**INERA**

Methodology

The study was carried out at the INERA station of Kamboinse, in the in vitro culture laboratory. The tubers of the improved cultivars underwent initiation, then multiplication before being used as plant material. The sprouts of approximately 1 cm, were used as explants and transplanted onto each of the four cultures media at a rate of 10 ml of culture medium per explant. each was inoculated in 10ml medium.

The culture medium consisted of the following:

M0= 4.4 g l^{-1} MS (Murashigue and Skoog 1962) + 7 g l^{-1} agar, M0 is the base medium;

M1= M0 + 30 g l^{-1} sucrose;

M2= M0 + 0.5 mg l^{-1} auxin IBA and

M3= M0+30 g l^{-1} sucrose + 0.5 mg l^{-1} auxin IBA.

The media used were formulated based on previous studies (Chen et al. 2020; Demo et al. 2008). The pH of each medium was adjusted to 5.7±0.1 with 1 N NaOH. A 909 ml cylindrical glass jar was used as the culture vessel. The prepared media were dispensed into the containers and then autoclaved for 20 minutes at a pressure of 120 bar before subculturing the explants are cultured in the jars in the jars under a laminar flow hood. These containers containing the explants were loosely covered and placed in a culture room for 30 days at a temperature of 22 ± 1 °C, 70% relative humidity, a photoperiod of 16 h and a lighting intensity of 1200 lux. the experiments were conducted on the basis of the (Namanda et al. 2015) and (K. Wang 2014). The Namanda et al., (2015) and Wang (2014).

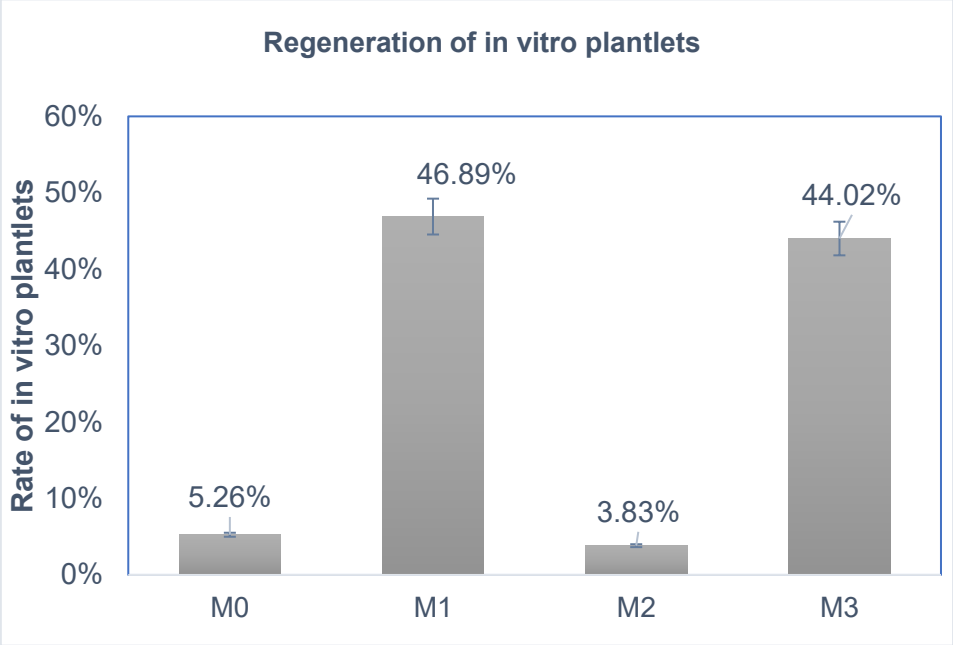
STATISTICAL ANALYSIS

RStudio was used for the various analyses. Boxplots were used to determine the effect of different growing media on seedling growth parameters. The Newman-Keuls test was used to determine the performance of cultivars through the separation of means. Three replicates were performed in the trial and 80 explants were in each replicate. 20 samples in each of the 4 media?

RESULTS

REGENERATION PARAMETERS OF PLANTLETS

Statistical analysis also showed that all the media used favored vegetative regeneration of in vitro plantlets, with the rate varying from one medium to another. As a result, M1 and M3 exhibited the greatest regeneration rates, ranging from 46.89% to 44.02% respectively. However, low regeneration rates were observed in media M0 and M2, at 5.26% and 3.83% respectively (Fig. 1).



in different

Fig.1 Regeneration rate of the in vitro plantlets by culture media

Legend (M0= MS (Murashigue and Skoog 1962) 4.4 g l^{-1} + agar 7 g l-1 ; M1= M0 + 30 g l^{-1} sucrose ; M2= M0 + 0.5 mg l^{-1} auxin 3 indole butyric acid (IBA) et M3= M0+30 g l^{-1} de sucrose + 0.5 mg l^{-1} auxin 3 indole butyric acid IBA)

EFFECT OF MEDIA ON ROOT PROLIFERATION OF IN VITRO GENERATED PLANTLETS

Statistical analysis also showed that all the media used favored root proliferation of in vitro plantlets, with the rate varying from one medium to another. As a result, M1 and M3 exhibited the greatest rooting rates, ranging from 45.04 % to 48.79 % respectively. However, low rooting rates were observed in media M0 and M2, at 0.54 % and 5.63 % respectively (Fig.2).

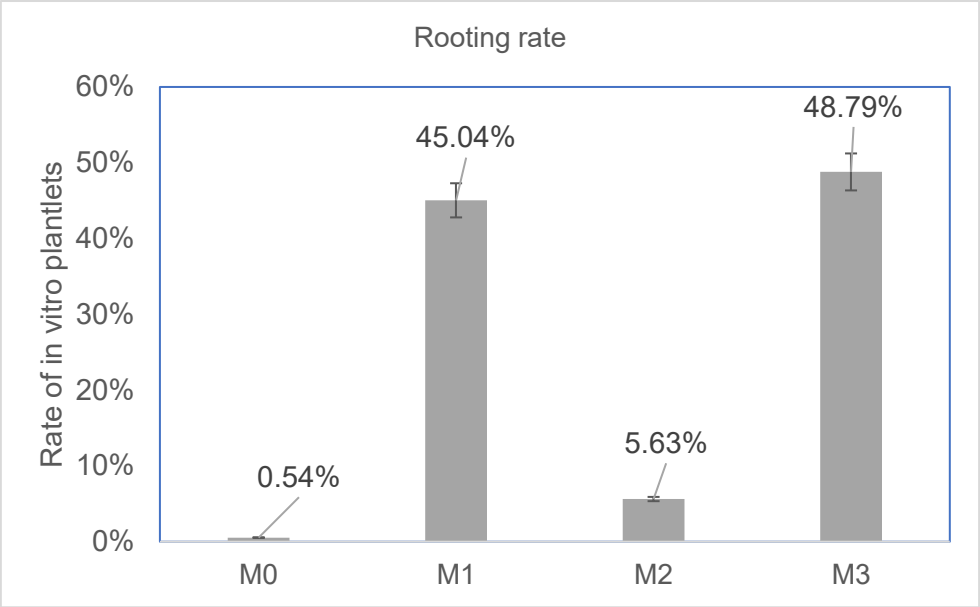


Fig.2: ~~Rooting rate of in vitro plantlets~~. Effect of sucrose and/or IBA on root proliferation

Legend: M0= (Murashigue and Skoog 1962) 4.4 g l-1 + 7 g l^{-1} agar; M1= M0 + 30 g l^{-1} sucrose; M2= M0 + 0.5 mg l^{-1} auxin 3 indole butyric acid (IBA) and M3= M0+30 g l^{-1} sucrose + 0.5 mg l^{-1} auxin IBA)

sucrose and/or IBA in MS media

EFFECT OF MEDIA ON THE GROWTH PARAMETERS OF IN VITRO GENERATED PLANTLETS

The analysis showed that media M1 and M3 had a significant effect on the growth parameters of the in vitro plantlets (Fig.3).✓

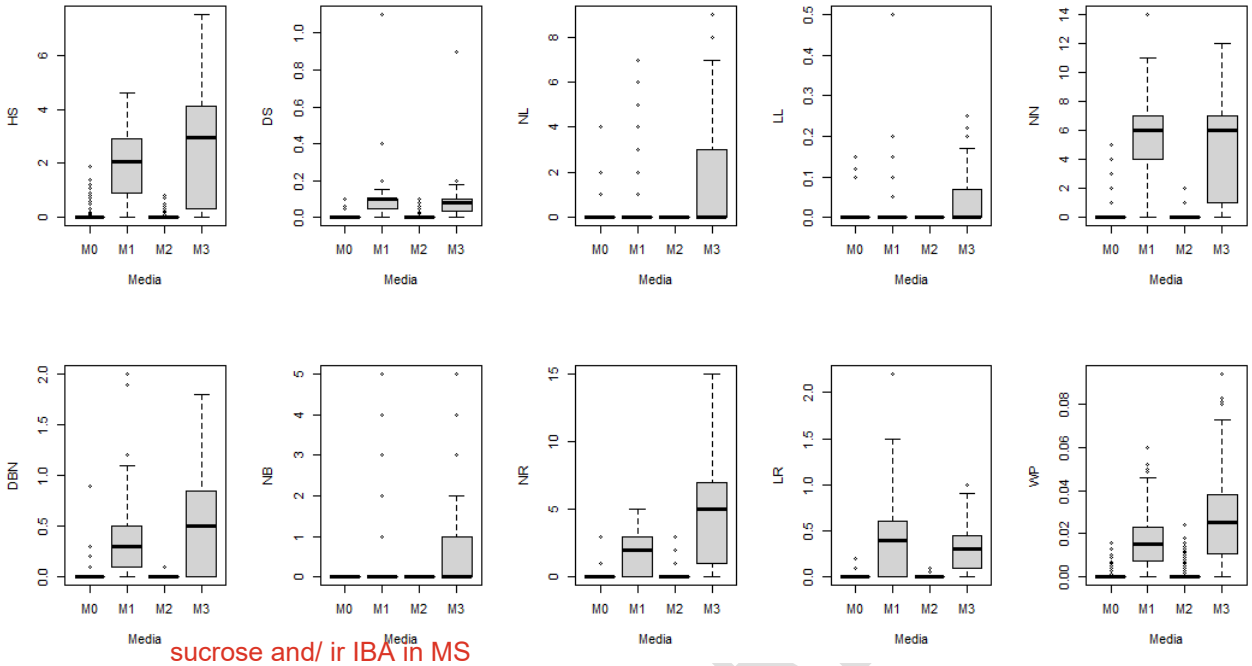
Medium M1 particularly affected stem diameter and root length. In fact, 50% of the in vitro plantlets grown in this medium had a stem diameter of at least 1 mm and a root length of at least 0.4 cm.

Medium M3 was more favourable in terms of stem height, distance between nodes, number of roots and weight of the in vitro plantlets. The median of the parameters shows that 50% of the in vitro plantlets had a stem height of at least 3 cm, a distance between nodes of 0.5 cm, a number of roots equal to or greater than 5 and a weight of at least 0.025 g.

Regarding the number of nodes, environments M1 and M3 seem to have a similar effect, with a median of 6 nodes per plantlet. However, the interquartile range was lower for M1, reflecting the homogeneity of this parameter compared to M3.

✓

On the other hand, M0 and M2 media showed no significant effect on the growth parameters of the in vitro plantlets.



sucrose and/ ir IBA in MS

Fig. 3 Effect of media on the development of in vitro plantlet growth parameters

Legend: M0= (Murashigue and Skoog 1962) 4.4 g l^{-1} + 7 g l-1 agar; M1= M0 + 30 g l^{-1} sucrose; M2= M0 + 0.5 mg l^{-1} auxin 3 indole butyric acid (IBA) and M3= M0+30 g l^{-1} sucrose + 0.5 mg l^{-1} auxin IBA). NSH: number of shoots; HS: height of stem (cm); DS: diameter of stem (mm); NL: number of leaves; LL: length of leaf (cm); NN: number of nodes; DBN: distance between nodes (cm); NB number of branches; NR: number of roots; LR: length of roots (cm); WP: weight of in vitro plants (g); NAR: number of aerial roots.

MEDIA-CULTIVARS INTERACTION

Analysis of the interaction between the two factors showed a highly significant interaction between genotypes for most of the parameters studied (Table 2). All parameters were influenced by variation in the growing medium.

Table 2 Media-cultivars interaction Interaction of 8 cultivars with the media

Parameters	Cultivars		Media		Cultivars-Media	
	F-value	**P**	**F-value**	**P**	**F-value**	**P**
DBN	**9.13**	**<.001**	**254.68**	**<.001**	**4.32**	**<.001**
DS	**2.67**	**0.005**	**130.59**	**<.001**	**2.44**	**<.001**
HS	**10.48**	**<.001**	**349.73**	**<.001**	**5.24**	**<.001**
LL	**6.20**	**<.001**	**65.13**	**<.001**	**3.53**	**<.001**
LR	**6.81**	**<.001**	**262.67**	**<.001**	**7.16**	**<.001**
NL	**8.47**	**<.001**	**113.83**	**<.001**	**5.02**	**<.001**
NN	**10.23**	**<.001**	**444.20**	**<.001**	**4.45**	**<.001**
NR	**5.52**	**<.001**	**349.03**	**<.001**	**3.89**	**<.001**
NAR	**3.29**	**<.001**	**26.13**	**<.001**	**2.52**	**<.001**
NS	**4.99**	**<.001**	**380.17**	**<.001**	**3.59**	**<.001**
NB	**3.00**	**0.002**	**48.24**	**<.001**	**1.93**	**0.003**
WP	**9.33**	**<.001**	**285.77**	**<.001**	**4.23**	**<.001**

Legend: NSH: number of shoots; HS: height of stem (cm); DS: diameter of stem (mm); NL: number of leaves; LL: length of leaf (cm); NN: number of nodes; DBN: distance between nodes (cm); NB number of branches; NR: number of roots; LR: length of roots (cm); WP: weight of in vitro plants (g); NAR: number of aerial roots.

PERFORMANCE OF CULTIVARS IN CULTURE MEDIA

The analysis of variance showed significant differences between the cultivars at the 5% level for most of the parameters studied (Table 3).

For the M0 medium, CIP 398208.29 showed a superior performance for the parameters (height, stem diameter and weight of in vitro plantlets) compared to the other cultivars. CIP 393079.4 showed an intermediate performance.

In medium M1, all cultivars performed well for all parameters, with the best performance of the cultivars CIP 398208.29 (in vitro plant height), CIP 393079.4 (in vitro plant height, number of nodes, number of roots and in vitro plant weight) and sahel (in vitro plant height, diameter, number and length of roots).

For the M2 medium, the cultivars CIP 398208.29, CIP 393385.39 and Spunta showed superior performance for the parameters in vitro plant height, in vitro plant diameter and number of nodes. The CIP 393385.39 genotype had ~~a higher in vitro plant weight than the other in vitro plants.~~ had the highest in vitro plant weight.

~~In~~ On M3 medium, all cultivars performed well for all parameters. However, CIP 398208.505 and CIP 393079.4 performed best for most of the parameters studied.

different

Table 3: Performance of cultivars in culture media

Medium M0											
Cultivars	CIP.13	CIP.22	CIP.29	CIP.39	CIP.4	CIP.505	CIP.58	CIP.704	SAHEL	SPUNTA	Pr(>F)
HS	0.01 b	0.01 b	0.27 a	0.04 b	0.16 ab	0.00 b	0.04 b	0.00 b	0.04 b	0.03 b	0.0006 ***
DS	0.00 b	0.00 b	0.03 a	0.01 b	0.02 ab	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.0003 ***
NN	0.04 b	0.04 b	0.63 a	0.08 b	0.46 ab	0.00 b	0.17 b	0.04 b	0.04 b	0.08 b	0.001 **
NR	0.00 a	0.00 a	0.13 a	0.04 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.497
LR	0.00 a	0.00 a	0.01 a	0.01 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.294
WP	0.0004 b	0.0004 b	0.002 a	0.0006 b	0.002 ab	0.00 b	0.0005 b	0.0003 b	0.00004 b	0.0004 b	0.009 **
Medium M1											
HS	1.57 bc	1.35 bc	2.73 a	1.03 c	2.52 a	1.90 abc	1.59 bc	2.21 ab	2.72 a	1.43 bc	3.17e-08 ***
DS	0.06 ab	0.05 b	0.07 ab	0.05 b	0.09 ab	0.08 ab	0.08 ab	0.09 ab	0.13 a	0.06 ab	0.0374 *
NN	4.79 abc	3.50 c	5.75 ab	3.25 c	6.79 a	6.21 ab	5.71 ab	6.13 ab	4.42 bc	3.46 c	7.89e-07 ***
NR	1.67 abc	0.71 c	2.13 ab	0.79 c	2.50 a	1.71 abc	1.96 ab	2.29 ab	2.75 a	1.33 bc	6.39e-09 ***
LR	0.27 cde	0.17 de	0.40 bcd	0.12 e	0.55 b	0.25 cde	0.47 bc	0.46 bc	0.75 a	0.26 cde	2.67e-12 ***
WP	0.01 bc	0.01 c	0.02 abc	0.01 bc	0.03 a	0.02 abc	0.01 bc	0.03 abc	0.02 ab	0.01 bc	0.000148 ***
Medium M2											
HS	0.027 a	0.000 a	0.079 a	0.050 a	0.038 a	0.000 a	0.000 a	0.000 a	0.000 a	0.058 a	0.0654 *
DS	0.004 a	0.000 a	0.008 a	0.009 a	0.003 a	0.000 a	0.000 a	0.000 a	0.000 a	0.009 a	0.0531 *
NN	0.083 a	0.000 a	0.208 a	0.125 a	0.083 a	0.000 a	0.000 a	0.000 a	0.000 a	0.167 a	0.022 *
NR	0.083 a	0.000 a	0.125 a	0.208 a	0.042 a	0.083 a	0.167 a	0.167 a	0.000 a	0.292 a	0.265
LR	0.000 a	0.000 a	0.002 a	0.000 a	0.004 a	0.000 a	0.000 a	0.000 a	0.000 a	0.006 a	0.311
WP	0.000 b	0.000 b	0.003 ab	0.004 a	0.003 ab	0.001 b	0.000 b	0.000 b	0.000 b	0.001 b	2.6e-05***
Medium M3											
HS	2.03 cde	1.56 de	3.70 ab	2.62 bcde	3.12 abc	4.24 a	1.79 cde	3.01 abcd	2.41 bcde	1.35 e	1.91e-08 ***
DS	0.05 b	0.05 b	0.06 b	0.07 b	0.09 ab	0.13 a	0.05 b	0.06 b	0.06 b	0.08 b	0.00161 **
NN	3.46 c	3.21 c	5.08 bc	5.50 bc	6.71 ab	7.79 a	4.46 bc	5.29 bc	3.63 c	3.33 c	4.69e-08 ***
NR	4.50 abc	3.25 bc	4.62 abc	3.63 bc	5.79 ab	6.37 a	2.75 c	5.46 ab	3.63 bc	3.21 bc	0.0001***
LR	0.29 ab	0.26 ab	0.26 ab	0.31 ab	0.43 a	0.38 ab	0.21 b	0.32 ab	0.26 ab	0.32 ab	0.0387 *
WP	0.02 bc	0.02 c	0.03 abc	0.03 abc	0.04 ab	0.04 a	0.02 c	0.04 ab	0.02 abc	0.01 c	5.91e-08 ***

Legend : CIP381381.13 (CIP.13), CIP 392797.22 (CIP.22), CIP 398208.29 (CIP.29), CIP 393385.39 (CIP.39), CIP 393079.4 (CIP.4), CIP 398208.505 (CIP.505), CIP 393371.58 (CIP.58), CIP 398208.704 (CIP.704). HS: height of stem (cm); DS: diameter of stem (mm); NN: number of nodes; NR: number of roots; LR: length of roots (cm) and WP: weight of in vitro plants (g)

DISCUSSION

The very high and highly significant interaction between genotypes for in vitro plantlets growth parameters would mean that in vitro plant growth is influenced by the change in culture medium. The low regeneration of the in vitro plantlets in media M0 and M2 could be due to a lack of sugar in both media. Sugars were missing in these media compared to those in media M1 and M3, which showed good regeneration (more than 5%). In this sense (Cardinal et al. 2000) showed that sugar is an essential element for both the vegetative development of cuttings and microtuberization. According to these authors, the presence of sugar in a growth medium is favorable for cauline and root growth. Our results showed that sugar in the M1 medium had a positive effect on growth parameters. The stem diameter and root length were the most influenced by the effect of sugar. A 30 g l-1 sucrose dose of M1 medium produced in vitro plantlet with a diameter of at least 1 mm and a root length of at least 0.4 cm. The better performance of the CIP4 and Sahel genotypes is due to their adaptation to this environment. Therefore, sucrose at this dose favors the stem and root development of in vitro plantlets. According to (Demo et al. 2008), sucrose improves potato micropropagation. Sugar is a source of energy used by explants for their growth and development. The same applies to exogenous sucrose, which is thought to be directly involved in regulating cell proliferation, photosynthesis and defense mechanisms against reactive oxygen derivatives (Dubuc,2010). According to (Fadaladeen and al. 2022) sucrose is the best source of carbon in Ipomoea batatas L, followed by fructose and glucose. Furthermore, (Rahman et al. 2010), showed that sucrose, glucose and maltose have favorable effects on various growth parameters in potato plants. Therefore, in vitro multiplication of vegetative explants requires a carbon source such as sugar.

The root proliferation of in vitro generated plantlets in the media is thought to be due to the effect of formulation of media into sugar and auxin. Auxin is thought to be produced in the apical buds of plants and transported from stems to roots via a polar transport system (Besnier 2007). It accumulates in the root tips and stimulates rhizogenesis by favoring the initiation and development of lateral roots (Besnier 2007). According to (Belaizi et al. 1989); (Kbiach et al. 2002), IBA positively improves the rooting phase in in vitro culture in cork oak and apple plants. (Fadaladeen and al. 2022) showed that the auxin IBA had better rooting parameters than did NAA for root formation in Ipomoea batatas L. Auxin is known to regulate many aspects of plant growth and development, such as vascular tissue differentiation, embryonic development, root formation and primary and secondary stem formation (Gilroy and Trewavas 2001). Explant regeneration was promoted by the effect of sugar. This regeneration is essential for the production and assimilation of auxin in vitro plants. As a result, the combination of sugar and auxin will accentuate the action of auxin through high root production and will allow good development of the in vitro plantlets observed in the M3 medium compared to those in other media. This combination is thought to be behind the performance of genotype CIP 398208.505, which was better for most of the traits studied. Indeed, (Wotavová-Novotná and al. 2007; Ilczuk and al. 2013) reported that the application of glucose, sucrose and fructose stimulated the development of Dactylorhiza species and Physocarpus opulifolius (L.) plants and that the growth rate and length of the roots increased in the presence of IBA and α-naphthalene-acetic acid. Thus, the combined effect of sugar and auxin in combination allows the regeneration of explants and the growth of their roots. The in vitro plantlets in M3 medium were characterized by an average height, a greater number of roots, a greater number of leaves and a greater weight of plantlets compared to those in the media. These characteristics are very important for good acclimatization of the plantlets. Indeed (Fadaladeen and al. showed that the better rooting rate observed with IBA favored 100% success at the acclimatization stage in sweet potato (Ipomoea batatas L).

CONCLUSION

This study revealed that the four media used favor the regeneration of in vitro plantlets, but M1 and M3 were observed as the best media for good vegetative regeneration. Sugar had a positive effect on growth parameters by favoring the development of stem diameter, root length, number of nodes and fuller leaves. The media M1 and M3 have a positive effect on the root proliferation of in vitro regenerated plants. The combination of sugar and auxin improved the development of growth parameters such as stem height, number of roots, number of expanded leaves and weight of in vitro plantlets in M3 medium compared with those in the other media. The cultivars have performed well in the M1 and M3 media. Thus, the production of plantlets that exhibit good vegetative growth would allow good acclimatization and an improvement in the production of potato seeds.

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