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INFLUENCE OF HORMONAL TREATMENTS AND OF SUCROSE ON THE MICROTUBERIZATION OF THREE POTATO VARIETIES (*Solanum tuberosum* L.) ADAPTED TO AGROCLIMATIC CONDITIONS IN SENEGAL

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ABSTRACT: In the situation of a research-development project on the in vitro production of potato seeds in Senegal, we attempted to improve the microtuberization of some varieties. The production of vitrotubers is one of the most efficient tools for the propagation of basic material, the transport of germplasm and the conservation of potato varieties cultivated after cleansing. It allows, at any period of the year, to obtain healthy seeds ready to be planted and which can be dispatched within long distances without any specific precaution.

In this context, comparative studies have been achieved under darkness conditions and at 20°C, on two types of Murashige & Skoog (MS/2) media of tuberization, with, on the one hand, a sucrose concentration of 80 or 100 g L^{-1}, without hormones and on the other with different hormonal combinations (Kinetin + BAP) or (Kinetin + Coumarin). Aïda variety presents the best aptitude for the microtuberization with a rate of 91%. The sucrose concentration most favorable being 80 g L^{-1} for the Aïda variety and 100 g L^{-1} for the Atlas and Odessa varieties. The Aïda variety (88.5%) gave more microtubers when the hormonal combination Kinetin + BAP is added to the culture medium, compared to the Odessa (74.5%) and Atlas (66.5%) varieties. The replacement of the BAP by Coumarin in the hormonal combination was not beneficial. We noticed, indeed, a reduction of the microtuberization rate for all varieties whatever was the sucrose concentration added to the culture medium. The output in mass of the microtubers depends as well on the caliber and the number of microtubers.

Keywords: *Solanum tuberosum*, cuttings, microtubers, sucrose, cytokinins, coumarin.

INTRODUCTION

The potato (*Solanum tuberosum* L.) is the most important food and economic plant of the *Solanaceae* family. It occupies the 5th world rank of human food. In Senegal, the potato which bulk of cultivation concentrates in the "Niayes" zone and has become an important horticultural speculation, both by its production and its consumption. It also represents 10% of the national production of vegetables and roughly 50% of trade importation. The national production of 2002-2003 season was estimated at 7.029 tons. This production is insufficient to cover the national needs, and in comparison with the estimated importations of 26,769 tons ; making an amount of 3 billions 588 millions F.CFA. Analysis of the potato production cost illustrates that potato seeds represent one third of importation, which heavily influences the trade balance in Senegal. Thus, the necessity to develop a lasting policy of potato seeds production in our country is imperative.

The development of potato cultivation in the sahelian zone is hindered by the difficulty to obtain a rather unpredictable supply in quality seeds often unsuitable with the periods of field cultivation of the year. A reduced range of varieties adapted to our agroclimatic conditions only does exist. Besides, commercial structured processes for supply, distribution, and conservation of seeds are fragile or not well organized. The Potato can be reproduced in a sexual manner. It produces fruits in berries containing kernels. The use of kernels as seeds draws away a very large genetic heterogeneity within progeny plants.

This is why, the tuber, organ of vegetative multiplication is used like seed. Nevertheless, the assertion of a good health state is more complex, particularly in tropical zones.
To deter phytosanitary problems and of heterogeneity derivations in the progeny, Morel [16] and, later, Nozeran [19] have set a method of in vitro vegetative multiplication also called micropropagation. This method of cuttings adequate and fast conducts to obtaining an important number of juvenile plants. It brings more flexibility and quickness in the processes of plant production and it is a better tool to eradicate viruses [25]. Nevertheless, transferring directly towards the full field the in vitro cultivated plantlets is not possible. Hence, developing various techniques allowing this transfer was performed but each of her has advantages and disadvantages. Currently, the use of microtubers proves to be one of the convenient and efficient means to propagate basics material and preserve cultivated varieties of potato after purification [2 ; 11; 12].
The conditions of the environment of culture which characterize the aptitude for in vitro tuberization have been studied and improved by several authors [6; 7; 31; 8; 10; 18; 27]. Microtuberization techniques also take into account local economic constraints. Microtubers can be kept in cold, as such, during at least a year. Besides, they are much less fragile than the existing microplants from in vitro culture, which require, further frequent subcultures, a very delicate period of weaning, and gripping in greenhouse, before the planting in natural conditions, microtubers are directly produced in vitro from cultivated nodes or nodal cuttings. Nothing differentiates them from tubers produced in the field if it is not their size, in general lower than 10 mm, and their weight. They weigh on average less than 1 gram.
From this perspective, considering the absence of local production of potato seeds, which are totally imported from the North, and to free the producers from constraints linked to supply and to availability of potato seeds in quality, we undertook this study to contribute to potato seed production in Senegal. Consequently were examined, the influence of hormonal treatments and of sucrose in the culture media during microtuberization of three potato varieties adapted to the agroclimatic conditions of the Senegal.

MATERIALS AND METHODS

The basic material is constituted of Elite tubers belonging to three varieties of potato: Aïda, Atlas and Odessa. Young plants had been imported from GERMICOPA S.A. (*Qimper, France*). These varieties were chosen because of their adaptability to Senegalese agroclimatic conditions. Tubers-young plants are 28/35 mm caliber and are virus free according to the phytosanitary certificates which accompanied them (Norms of certification ISO 9001). Twenty tubers were chosen regarding the Aïda variety, 30 for the Atlas and 28 for Odessa.
The *in vitro* tuberization is acquired by modifying the experimental conditions of growth of the cuttings, which is what leads them to tuberization. This occurred at, in most cases, by passing in short photoperiods and a lowering of incubation temperatures (15 - 20°C) under favorable nutritional conditions.
The existing vitroplants of germs taken from tubers-young plants were used for this study. They were multiplied on Murashige [17] medium, according to the protocol represented by Couillerot [3]. After 4 weeks of culture, the explants constituted by a portion of stem, an axillary bud, and a leaf, *i.e.* mono nodal cutting, are sowed aseptically in a Murashige & Skoog (1962) medium, where macro-elements are reduced by half (MS/2) and in the presence of 80 g L^{-1} or 100 g L^{-1} of sucrose. To test the effect of hormones on the microtuberization process, the medium of culture has been enriched or not with cytokinin (KIN and BAP: 1 – 2.5 mg L^{-1}) and/or with coumarin (0.025 mg L^{-1}). These culture media, which pH was adjusted to 5.9, have been solidified with agar; at the rate of 8 g L^{-1} and autoclaved to 110°C during 20 min. For every treatment, an experimental unit is formed of a jar filled with 50 mL of solid medium MS/2 and contains 20 explants. The adopted experimental implement is a complete randomization of 8 treatments by variety, which makes 24 treatments. The explants of the 3 varieties (Aïda, Atlas and Odessa) were incubated in darkness under low temperature, which is 20°C ± 1°C during 72 days. For each variety and every treatment, the rate of microtuberization and the output have been calculated.

Different performed treatments have been categorized between multiple comparison of averages and rates, after analysis of variance followed by the test of Student, Newman, and Keuls at the probability threshold of 5 % (SPSS software).

RESULTS

On the MS/2 culture media, enriched with sucrose (80 and 100 g L^{-1}) and complemented or not with cytokinin (KIN (kinetin) and BAP at 1 mg L^{-1} or 2.5 mg L^{-1}) and/or with coumarin (0.025 mg L^{-1}), a precocious tuberization was determined, 13 days after incubation of the vitroplants of Aïda variety. This phenomenon of microtuberization started only, respectively on 19 and 20 days for the Odessa and Atlas varieties. In general, the microtubers of Aïda variety (Plate 1) have a much more spherical aspect than those of Atlas and Odessa varieties, which were rather stretched out to oblong. The largest part of them, whatever can be the variety, developed in the armpit of nodes, out of the culture medium and in epigeal position (92 %).

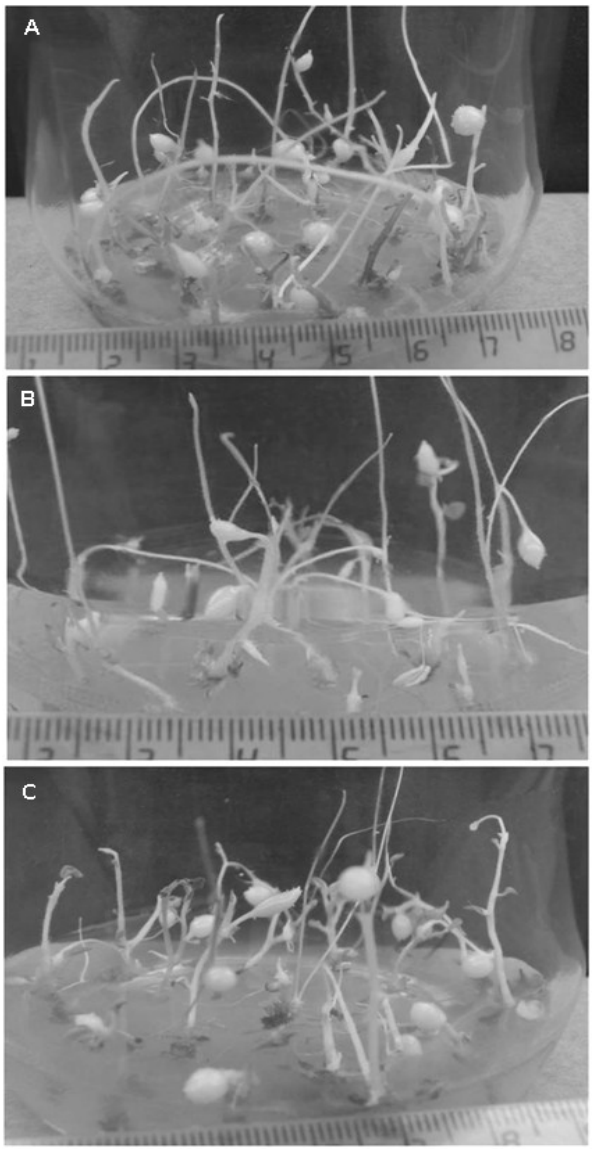

Plate 1: Appearance of microtubers of three potatoes varieties: Aida (A), Atlas (B) and Odessa (C) formed in MS/2 with added sucrose, cytokinins and / or coumarin, after 72 days of incubation into darkness at 20°C.

1. Sucrose Influence on the microtuberization

Microcuttings were planted on MS/2 medium, with sucrose added in different concentrations (80 and 100 g L^{-1}; Figure 1). The rate of tuberization varied according to the concentration in sucrose in the culture medium. Optimal tuberization rate was acquired for Aïda variety (91 %) in the presence of 80 g L^{-1} of sucrose. On the other hand, for Odessa et Atlas varieties, it was respectively 67 % and 28.5 %, in the presence of 100 g L^{-1} of sucrose. Aïda variety presented a better aptitude therefore for the microtuberization (Table 1).

Table 1: Effects of sucrose concentrations and hormonal combinations on the microtuberization of the three potato varieties after 72 days of incubation at 20°C.

Treatments	Rate of microtuberization (%)		
	AÏDA	ATLAS	ODESSA
MS/2 + Sucrose 80 g L^{-1}	91 a	22 j	46 gh
MS/2 + Sucrose 100 g L^{-1}	77,5 bc	28,5 i	67 cde
MS/2 + KIN 1mg L^{-1} + BAP 1mg L^{-1} + Sucrose 80 g L^{-1}	61,5 def	66,5 cde	69 cde
MS/2 + KIN 1mg L^{-1} + BAP 1 mg L^{-1} + Sucrose 100 g L^{-1}	55,5 efg	43,5 gh	66,5 cde
MS/2 + KIN 2.5 mg L^{-1} + BAP 1mg L^{-1} + Sucrose 80 g L^{-1}	67 cde	51,5 fg	71 cd
MS/2 + KIN 2.5 mg L^{-1} + BAP 1mg L^{-1} + Sucrose 100 g L^{-1}	88,5 a	37,5 hi	74,5 cd
MS/2 + KIN 2.5 mg L^{-1} + Coum 0.025 mg L^{-1} + Sucrose 80 g L^{-1}	85,5 ab	43,5 gh	51 fg
MS/2 + KIN 2.5 mg.L^{-1} + Coum 0.025 mg.L^{-1} + Sucrose 100 g L^{-1}	66,5 cde	28 i	31,5 i

In the last column, nuùbers followed by the same letters significantly do not differ at the probability threshold of P <0,05 (Test of Newman-Keuls).

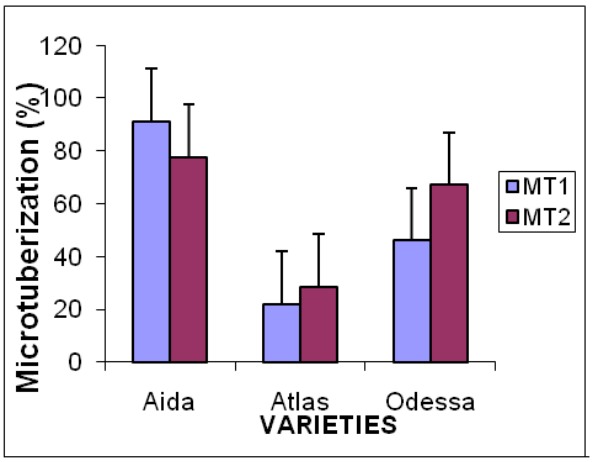

Figure 1: Effect of sucrose on the microtuberization of Aïda, Atlas and Odessa varieties after 72 days of incubation at 20°C.

Legend: MT 1 = MS/2 + Sucrose 80 g L^{-1} MT 2 = MS/2 + Sucrose 100 g L^{-1}

2. Combined Effects of cytokinins (KIN + BAP) and of sucrose.

The combination MS/2 + KIN 2.5 mg L^{-1} + BAP 1 mg L^{-1} + Sucrose 100 g L^{-1} favorized better the tuberization of minicuttings with a rate reaching 88.5 % for the Aïda variety.

The Odessa variety also gave a good rate of tuberization (74.5 %) but inferior to that of Aïda's. For the Atlas variety, the combination MS/2 +KIN 1 mg L^{-1} + BAP 1 mg L^{-1} + 80 g L^{-1} of sucrose, was the most efficient, with a rate of tuberization equivalent to 66.5 %. The Aïda variety better formed tubers during the addition of the hormonal combination KIN + BAP compared with the Odessa variety (74.5 %) and the Atlas variety (66.5 %) (Figure 2 and Table 1).

3. Combined effects of Kinetin and Coumarin in the presence of sucrose on microtuberization.

The rate of microtuberization varied from 28 % to 85.5 %. The combination MS/2 + KIN 2.5 mg L^{-1} + COUM (coumarin) 0.025 mg L^{-1} + Sucrose 80 g L^{-1} revealed itself more advantageous for the microtuberization for all varieties. So, it was noticed a 85. 5 % rate for the Aïda variety, 51 % for the Odessa, and 36 % for the Atlas (Table 1).

The substitution of BAP by the Coumarin in the hormonal combination was not advantageous because it was showed a reduction in the rate of microtuberization for all varieties, no matter what the concentration in sucrose added in the culture medium was.

We have got a better output in microtubers on the medium of culture MS/2 enriched with 80 g L^{-1} of sucrose, with a number equal to 182 for Aïda variety. This output significantly did not differ from that of medium MS/2 + KIN 2.5 mg. L^{-1} + BAP 1 mg L^{-1} + Sucrose 100 g L^{-1} (177) and MS/2 + KIN 2.5 mg L^{-1} + COUM 0.025 mg L^{-1} + Sucrose 80 g L^{-1} (171). We can, therefore, conclude that sucrose and cytokinins both favor induction to the tuberization (Table 2).

Table 2: Number of microtubers formed by variety on different media of *in vitro* tuberization.

	Number of microtubers		
Treatments	**AÏDA**	**ATLAS**	**ODESSA**
MS/2 + Sucrose 80 g L^{-1}	**182**	44	92
MS/2 + Sucrose 100 g L^{-1}	155	57	134
MS/2 + KIN 1mg L^{-1} + BAP 1 mg L^{-1} + Sucrose 80 g L^{-1}	123	**133**	138
MS/2 + KIN 1 mg L^{-1} + BAP 1 mg L^{-1} + Sucrose 100 g L^{-1}	111	87	133
MS/2 + KIN 2.5 mg L^{-1} +BAP 1 mg L^{-1} + Sucrose 80 g L^{-1}	134	103	142
MS/2 + KIN 2.5 mg L^{-1} + BAP1 mg L^{-1} + Sucrose 100 g L^{-1}	**177**	75	**149**
MS/2 + KIN 2.5 mg L^{-1} + Coum 0.025 mg L^{-1} + Sucrose 80 g L^{-1}	171	87	102
MS/2 + KIN 2.5 mg L^{-1} + Coum 0.025 mg L^{-1} + Sucrose g L^{-1}	133	56	63

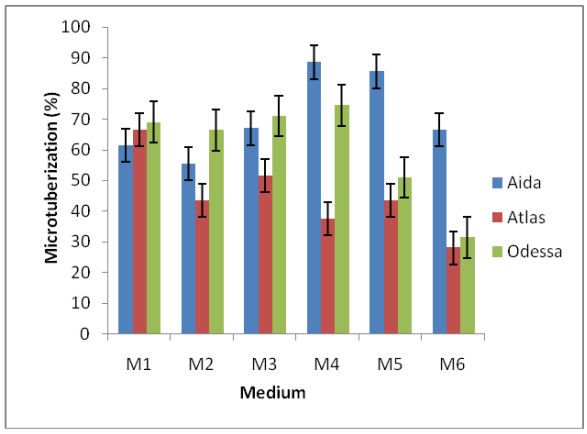

Figure 2: Effect of sucrose and of hormonal combinations on microtuberization of Aida, Atlas and Odessa varieties after 72 days of incubation into darkness at 20°C.

Legend: **M 1** = MS/2 + KIN 1mg L^{-1} + BAP 1mg L^{-1} + Sucrose 80 g L^{-1}
M 2 = MS/2 + KIN 1mg L^{-1} + BAP 1mg L^{-1} + Sucrose 100 g L^{-1}
M 3 = MS/2 + KIN 2.5 mg L^{-1} + BAP 1mg L^{-1} + Sucrose 80 g L^{-1}
M 4 = MS/2 + KIN 2.5 mg L^{-1} + BAP 1mg L^{-1} + Sucrose 100 g L^{-1}
M 5 = MS/2 + Kin 2.5 mg L^{-1} + Coum 0.025 mg L^{-1} + Sucrose 80 g L^{-1}
M 6 = MS/2 + Kin 2.5 mg L^{-1} + Coum 0.025 mg L^{-1} + Sucrose 100 g L^{-1}

The weight of microtubers, formed on the MS/2 medium enriched with 80g L^{-1} of sucrose, was improved, either 10.413 g. Microtubers formed in the medium MS/2 + sucrose 100 g L^{-1}, although they had an output of 155, they weighed 9.045 g which significantly did not differ from 10.413 g. The best output has been obtained for Atlas (133) and Odessa (149) varieties which respectively were acquired on media of tuberization MS/2 + KIN 1 mg L^{-1} + BAP 1 mg L^{-1} + Sucrose 80 g L^{-1} and MS/2+ KIN 2.5 mg L^{-1} +BAP 1 mg L^{-1} + Sucrose 100 g L^{-1}. The hormonal combinations MS/2 + KIN 2.5 mg L^{-1} + BAP 1 mg L^{-1} + Sucrose 100 g L^{-1} and MS/2 + KIN 2.5 mg L + BAP 1 mg L^{-1} + Sucrose 80 g $L^{-1;}$ they gave the most elevated outputs in mass, with respectively 3.45 g for the Atlas variety and 3.78 g for the Odessa variety (Table 3). It seems therefore that the weight of microtubers depends as well on output as on caliber.

Table 3: Weight (g) of microtubers produced by variety on different media of *in vitro* tuberization.

Treatments	AÏDA (g)	ATLAS (g)	ODESSA (g)
MS/2 + sucrose 80 g L^{-1}	**10,413**	0,939	0,57
MS/2 + Sucrose 100 g L^{-1}	9,045	1,559	2,2
MS/2 + KIN 1 mg L^{-1} + BAP 1 mg L^{-1} + Sucrose 80 g L^{-1}	3,909	2,07	1,295
MS/2 + KIN 1 mg L^{-1} + BAP 1 mg L^{-1} + Sucrose 100 g L^{-1}	0,352	1,553	1,851
MS/2 + KIN 2 5 mg L^{-1} + BAP 1 mg L^{-1} + Sucrose 80 g L^{-1}	0,44	1,078	**3,787**
MS/2 + KIN 2.5 mg L^{-1} + BAP 1 mg L^{-1} + Sucrose 100 g L^{-1}	4,292	**3,45**	1,772
MS/2 + KIN 2.5 mg L^{-1} + Coum 0.025 mg L^{-1} + Sucrose 80 g L^{-1}	4,532	2,113	2,466
MS/2 + KIN 2.5 mg L^{-1} + Coum 0.025 mg L^{-1} + Sucrose 100 g L^{-1}	7,082	1,178	0,81

DISCUSSION

Studies led on the factors implicated in the *in vitro* tuberization of some potato have been the object of several scientific publications in the sixties [1; 13; 29]. In general, a shortening of the photoperiod (shorter days) and a decrease in temperature, .lead to the tuberization of potato

The medium of culture used for the microtuberization is the nutritious basic medium (MS/2). In fact, a modification of the nutritious conditions of growth of cuttings leads to the tuberization *in vitro,* as well and, is what orientates them to a tuberization on stolons produced to the base. Thus, Dieng [4] dilutes by half the mineral solution (macro-elements) of the medium of culture and, acquires a more precious and important tuberization of the studied varieties. During this experiment of microtuberization, apical and basal explants were eliminated on every vitroplant. In fact, Lê [9] had pointed out that there was a loss of productivity all along numerous subcultures compared with the existent explants of the subjacent levels of insertion.

The study of sucrose influence on the tuberization of the *in vitro* cultivated explants revealed that the optimum potential of tuberization is obtained in the presence of 80 g L^{-1} of sucrose for the Aïda variety, with a rate of microtuberization of 91 %. The Odessa and Atlas varieties have a maximum tuberization with a concentration in sucrose of 100 g L^{-1}. The maximum rate of microtuberization of these two varieties was respectively 67 % and 28.5 %. Similar results were acquired with the same concentration of sucrose by Lê [10] with variety Agria; Dieng [4] with variety Atlantic × LT7 (18) and Ndiaye [18] with variety Bintje. In fact, sucrose acts as carbonaceous substrate in starch biosynthesis. *In vitro* cultivated tissus or explants are broadly heterotrophic concerning the carbon, owing to the absence or insufficiency of chlorophyll assimilation, and this is true specially since incubation is made into darkness. From there, came the idea sustained by Palmer & Smith [20], and then by Lakhoua & Ellouze [8] that the potato stolon ends to tuberize need to be bred in high concentrations in sugar. On a poor environment in sugars, the tuberization is late and weaker.

Adding hormones as cytokinin (KIN and BAP) and the coumarin, in the medium of culture draws a better response of explants to the tuberization of Odessa and of Atlas varieties with respectively a maximum rate of microtuberization of 74.5 % and 66.5 %. These results reinforce those of Dieng [3] on other varieties of potato, which shows that cytokinins play a crucial role in the microtubers initiation. In fact, hormones are at the origin of all the processes which lead to the tuberization, meaning : the activation of cell division [28]; the inhibition of cellular elongation [24]; the stimulation of radial extension of cells [26]; the activation of enzymes [14] and, finally the accumulation of starch in cells [32].

For the Aïda variety, the addition of hormones did not give a better response of tuberization because we noticed a 88.5 % rate contrary to the control medium without hormone, which gave 91 %. This result reinforces observations made by Palmer [21] who maintained that it was not obvious that there is a specific stimulation of the tuberization following an application of exogenous growth regulators.

Concerning the Aïda and Odessa varieties, we noticed that explants put into culture in the medium containing 100 g L^{-1} of sucrose, enriched with kinetin at 2.5 mg L^{-1} and BAP 1 mg L^{-1}, showed a more precocious and more important tuberization. As for the Atlas variety, the combination KIN 1 mg L^{-1} + BAP 1 mg L^{-1} + Sucrose 80 g L^{-1} turned out to be the most efficient then. These results reinforce those of Ndiaye [18] who noticed a very important tuberization when they combine KIN 2.5 mg L^{-1} + BAP 1 mg L^{-1} + Sucrose 100 g L^{-1}; but works done by Dieng [4] show that the combination KIN 1 mg L^{-1} + BAP 1 mg L^{-1} + Sucrose 80 g L^{-1} is the most efficient for Atlantic variety × LT7 (18).

The hormonal combination kinetin and coumarin and sucrose at 80 g L^{-1} and at 100 g L^{-1} gives a maximum rate of tuberization but lower than to the one reached during the addition of cytokinin only for all varieties. In fact, Ewing [5] showed that among the numerous activators of growth, cytokinin plays a major role in tuberization induction to *Solanum tuberosum* L. The same would be expected from a number of phenolic compounds as coumarin, which is a very powerful tuberization activator of young excised potato plants [23]. In addition to all that, Stallknecht [30] demonstrated that the tuberization accomplished with a coumarin concentration of 25-50 mg L^{-1} only needs 15 - 20 days to reach 100 % of tuberized vitroplants and that tuberization process was optimum. He noticed as well that microtubers were of bigger sizes. Weston [33] showed that a good tuberization of the vitroplants in the presence of coumarin needs a minimum temperature of 20°C, and a minimal concentration of sucrose of 4 % and a maximum sucrose of 6 to 8 % concentration. A concentration of 12 % leads to an inhibition of the coumarin action on tuberization. These results have been confirmed during our study because the rate of tuberization of the cultivated vitroplants in the medium enriched with 80 g L^{-1} of sucrose, was superior to that acquired in the medium supplemented with 100 g L^{-1} of sucrose.

The weight of the microtubers of the Aïda variety is superior to that of the two other varieties according to the culture medium studied. These results reinforce those of Lakhoua [8] which translated a correlation between the weights of vitrotubers and the strengh of the cultivated young plants. In fact, the most vigorous plants give more homogeneous microtubers and of a higher caliber superior to 5 mm.

The best output in microtubers is obtained with the Aïda variety (182) in medium MS/2 + Sucrose 80 g L^{-1} with an average weight of 10.814 g of the formed microtubers. These results are in adequacy with those of Sidikou [27] which showed that the output (as a whole) of microtubers is proportional to their maturity and to the percentage of sucrose in medium. Moreover, for the Aïda variety, the coefficient of multiplication by tuberized explants is the most high (1 - 3) whatever the tuberization medium used. This coefficient is only in the range of 2 for Atlas and Odessa varieties with an overall output of microtubers which are respectively of 133 and of 149.

CONCLUSION

Coming to the end of this study, a certain number of points need to be underlined. The *in vitro* method of vegetative multiplication used, allows us to respect easily, the imperatives of conformity (permanency and genetic stability) for different potato varieties. Microtuberization of explants requires an important provision of sucrose and of growth regulators.

It is more precocious when the culture medium is enriched with cytokinins. The weak mass of microtubers harvested with Atlas variety is to be correlated to its status of late variety; which did not completely achieve its period of dormancy and to non optimal hormonal combinations into the tuberization media.

These results, in their globality, allow therefore to consider the possibility of a large multiplication of microtubersin a large scale, whose germinative capacity is not only important, but whose plants would have a synchronous development and growth. By the fact, they could be used as seeds to boost the agricultural production in our country, the Senegal.

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