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Residual Effects of Sucrose and Hormonal Treatments of the Tuberization Medium on *in Vitro* Germination of Potato (*Solanum tuberosum* L.) Microtubers

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ABSTRACT

The residual effects of sucrose concentrations (80 or 100 g·L^{-1}) and hormonal treatments (BAP + Kinetin or Coumarin) of tuberization medium on *in vitro* microtubers germination of three potato varieties (*Solanum tuberosum* L.) so called Aida, Atlas and Odessa, are described. After 3 weeks of incubation at 28˚C ± 1˚C, 70% of Aida microtubers variety, previously formed in the MT2 medium [MS/2 + 80 g·L^{-1} Sucrose], germinated. The best germination rate for varieties Atlas (100%) and Odessa (66.66%) was obtained on microtubers previously formed in the medium MT2 [MS/2 + 100 g·L^{-1} Sucrose]. The addition of hormones in the tuberization medium allowed optimizing the microtubers germination of the Aida variety unlike the other varieties. Indeed, for the Aida variety, the combination M5 [Kin 2.5 mg·L^{-1} + Coum 0.025 mg·L^{-1} + Sucrose 80 g·L^{-1}] increased the germination rate from 70% up to 93.33%. The best germination rate (90%), noticed with microtubers of Atlas variety, initially formed in M2 medium [Kin 1 mg·L^{-1} + BAP 1 mg·L^{-1} + Sucrose 100 g·L^{-1}], was lower than that one (100%) obtained on medium without hormones. For Odessa variety, the maximum germination rate (53.33%) of microtubers, from the medium M4 [Kin 2.5 mg·L^{-1} + BAP 1 mg·L^{-1} + Sucrose 100 g·L^{-1}], was also lower than that one (66.66%) observed in the medium without hormones. Aida and Atlas varieties thus offer a better germination rate than Odessa after their cold storage.

Keywords: *Solanum tuberosum*; Microcuttings; Microtubers; *In Vitro* Germination; Sucrose; Cytokinins; Coumarin

1. Introduction

The potato is the fourth largest food crop production in the world, after the three major cereals such as wheat, rice and maize. Potato culture is a strategic sector in Senegal. This production occurs from November to March in the Niayes zone, during which the North Atlantic fringe of the country benefits from winds relenting temperatures and thus allow the tuberization of this speculation. The potato culture represents 10% of the national production of vegetables and roughly 50% of trade importation. Analysis of the national potato production cost illustrates that potato seeds represent one third of importation, which heavily influences the trade balance in Senegal. Indeed, conventional seed production systems are currently completely disorganized so that the needs of quality seeds are important. Local certified pest-free seeds (5170 tons in 2010) do not cover all the national requirements, consequently, a massive import of seeds is practiced (24,000 tons per year).

Most varieties of potatoes grown in the world are varieties with tetraploid true-seeds giving heterogeneous progeny. Thus, the use of the potato tuber as basic seed is a current practice and can easily meet the requirement of genetic conformity [1,2]. But the conventional production of potato plants is a speculation that requires significant technical expertise as produced tubers must include a minimum rate of plant pathogenic infections and any viral amount respecting the original genetic traits of the variety. The techniques developed have always strived to bring in cropping systems plant material with a minimum of infective load. For this, the traditional culture techniques obey a clonal selection model that includes a number of measures favorable to the preservation of good health status over multiplication and thus the intrin-

sic qualities of varieties. However, this method of mass selection has severe limitations especially for latent viral infections that may be transmitted to the next generations. The advent of *in vitro* culture techniques enabled us through micropropagation to accelerate the number of seedlings produced and improve their health status while providing virus-free seeds with the development of serological detection methods such as meristem culture coupled with thermotherapy. The discovery of new chemical molecules also contributed to improve the possibilities of fight against the vectors of viral diseases such as aphids and their transmission to plants. The techniques of vegetative propagation permit getting four types of seeds: plantlets, microtubers, minitubers and tubers. Indeed, the acclimatization step of plants can be replaced by *in vitro* microtuberization *i.e*. vitrotubers production which is one of the practical ways, the most effective for the propagation of basic material, the transport of germplasm and the conservation of potatoes varieties grown after sanitation [3,4].

The *in vitro* method of vegetative multiplication allows respecting easily the imperatives of conformity such as permanency and genetic stability for different studied potato varieties [5]. Microtuberization of explants requires an important provision of sucrose and growth regulators. It was more precocious when the culture medium was enriched with cytokinins. Harmey *et al.* [6] stated that adding growth regulators increases the development of microtubers if an amount of 8% sucrose is supplied. Ebadi *et al.* [7] found healthy microtubers with 3 to 4 months of dormancy by cultivating isolated cultures of two to three nodes in bio semi-continuous bioreactors of attained microtubers in concentration above 10 $mg \cdot L^{-1}$ BAP and 8% of sucrose.

The effects of hormones and sucrose on microtuberization were well documented [8] but a few were reported on the influence of these exogenous factors on microtubers germination.

The residual effect of hormones and sucrose can affect the dormancy duration of cold stored microtubers and therefore has an impact on their early germination when put to normal temperature. Indeed, increasing the sucrose concentration from 80 to 140 $g \cdot L^{-1}$ during the tuberization process, it is possible to reduce the six weeks duration of Désirée microtubers dormancy of [9]. Hussey and Stacey [10] obtained immediate sprouting of micro-tubers produced in long days, while those induced by short days in the presence of BAP germinate more slowly and were heterogeneous.

It is important depending on the variety, to know hormonal combinations that are most favorable to the dormancy period reduction of microtubers to obtain a homogeneous and synchronous lifting of germs that can be cultivated in fields during the windy season. Indeed, this speculation is a dry-season crop in Senegal.

This present work describes the residual effect of sucrose and of growth regulators of the tuberization medium on the germination capacity of microtubers after cold storage at 4˚C.

2. Material and Methods

2.1. Characteristics of Varieties

The basic material is constituted of Elite tubers of 28 - 35 mm caliber and belonging to three varieties of potato: Aïda, Atlas and Odessa. They were imported from GERMICOPA S.A. (France). These varieties were chosen because of their adaptability to Senegalese agroclimatic conditions. Tubers-young plants were virus-free according to the phytosanitary certificates (Norms of certification ISO 9001). Twenty tubers were chosen regarding the Aïda variety, 30 for the Atlas and 28 for Odessa.

Atlas is a relatively late variety**,** high yielding and its productivity is stable even in hard conditions. Its tubers are of big caliber, less numerous and less sensitive to desprouting and could support a prolonged storage period. Odessa is an early variety and has a good yield per hectare. Its tubers are oblong with a yellow skin and a yellow flesh and have an ability to support an average conservation period. Aïda is a late variety with an early maturity of tubers and a good yielding. Tubers are oblong and elongated pale-yellow appearance.

2.2. Microtuber Production

Microtubers were obtained by applying the protocol previously described in Dieme *et al*., 2011. Explants consisting of mononodal cuttings were cultured on Murashige & Skoog [11] medium with halved macronutrients (MS/2) and in the presence of 80 $g \cdot L^{-1}$ or 100 $g \cdot L^{-1}$ Sucrose. To test the residual hormonal effect of the culture medium on the germination of microtubers, culture media were previously enriched or not (**Table 1**) with cytokinins (Kin and BAP 1 to 2.5 $mg \cdot L^{-1}$) and/or Coumarin (0.025 $mg \cdot L^{-1}$) for microtuber production. Agar at 8 $g \cdot L^{-1}$ was used to solidify the media; the pH was adjusted to 5.9 before autoclaving at 110˚C for 20 min.

2.3. Storage and Germination of Microtubers

The microtubers of the 3 varieties from different tuberization medium were harvested aseptically and previously stored in a cold room at 4˚C ± 1˚C for 4 months. Experiments on microtubers were conducted to determine their ability to germinate at 28˚C. Three batches of 10

Table 1. Media composition for microtubers production from the microcuttings of the three varieties.

Media	Treatments
MT1	MS/2 + Sucrose 80 g·L^{-1}
MT2	MS/2 + Sucrose 100 g·L^{-1}
M1	MS/2 + Kin 1 mg·L^{-1} + BAP 1 mg·L^{-1}+ Sucrose 80 g·L^{-1}
M2	MS/2 + Kin 1 mg·L^{-1} + BAP 1 mg·L^{-1} + Sucrose 100 g·L^{-1}
M3	MS/2 + Kin 2.5 mg·L^{-1} + BAP 1 mg·L^{-1} + Sucrose 80 g·L^{-1}
M4	MS/2 + Kin 2.5 mg·L^{-1} + BAP 1 mg·L^{-1} + Sucrose 100 g·L^{-1}
M5	MS/2 + Kin 2.5 mg·L^{-1} + Coum 0.025 mg·L^{-1} + Sucrose 80 g·L^{-1}
M6	MS/2 + Kin 2.5 mg·L^{-1} + Coum 0.025 mg·L^{-1} + Sucrose 100 g·L^{-1}

microtubers for each variety, with caliber between 5 and 7 mm, and obtained from different hormonal combinations were used. The microtubers were deposited aseptically on MS (0) medium [11], then incubated in a dark room at 28˚C ± 1˚C. A completely randomized block was used for the experiments. The number of germinated microtubers was counted after 1, 2 and 3 weeks. For each factor studied, three repetitions were performed.

2.4. Statistical Analyses

Multiple comparisons of averages and rates have been done, after analysis of variance, using the test of Student, Newman, and Keuls at the probability threshold of 5% (SPSS software).

3. Results

3.1. Influence of the Sucrose in the Tuberization Medium

The different varieties of microtubers obtained with sucrose concentrations of 80 g·L^{-1} and 100 g·L^{-1} are kept in a cold room and then put in 28˚C ± 1˚C for germination and the germination rate of microtubers determined during 3 weeks (**Figures 1** and **2**).

The effect of high concentrations of sucrose is expressed mainly by reducing the duration of dormancy. Indeed, to obtain 70% germination after 3 weeks at 28˚C, 80 g·L^{-1} of Sucrose in the medium of tuberization is proved sufficient for Aïda variety. Concentration of 100 g·L^{-1} sucrose lowers the germination rate to 53.33%. In addition, the germination process begins earlier, in the presence of Sucrose at 80 g·L^{-1}. However, for the varieties Atlas and Odessa, the best germination rate is obtained in the presence of 100 g·L^{-1} of Sucrose in the initial *in vitro* tuberization medium with 100% and 66.66% of microtubers germinated.

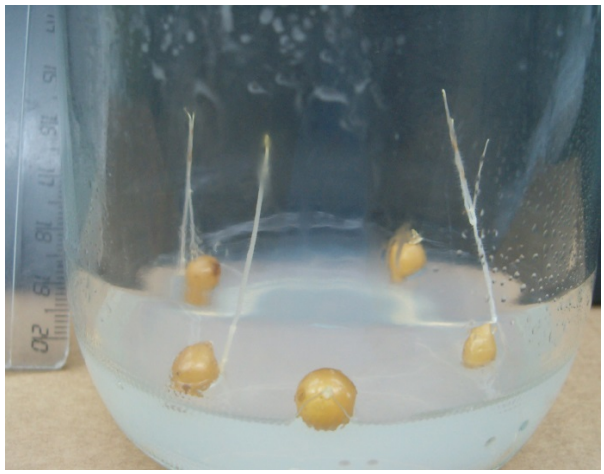

Figure 1. *In vitro* germination of potato microtubers after 21 days of incubation in MT1 medium at 28˚C in darkness.

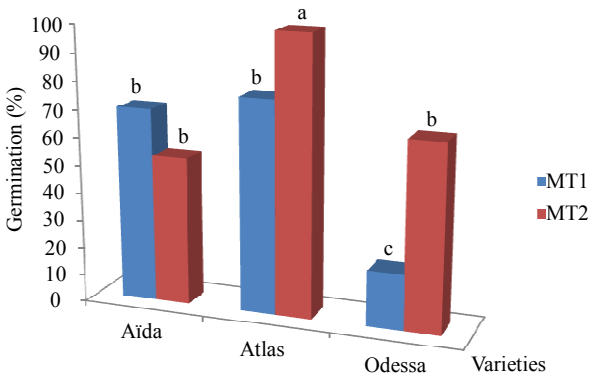

Figure 2. Effect of sucrose of the tuberization medium on the microtuber germination of 3 potato varieties (Aida, Atlas and Odesa), after 3 weeks of incubation at 28˚C. MT1 = MS/2 + Sucrose 80 g·L^{-1}; MT2 = MS/2 + Sucrose 100 g·L^{-1}. Treatments followed by the same letter are not significantly different at probability level of P <0.05 by Student-Newman-Keuls (SNK).

3.2. Combined Effect of Kinetin and BAP and Sucrose in the Tuberization Medium

The combined effect of kinetin and BAP on Aida variety was studied in the presence of two concentrations of Sucrose (80 g·L^{-1} and 100 g·L^{-1}). The highest rate of microtubers germination (86.66%) was obtained with the medium M3 [Kin 2.5 mg·L^{-1} + BAP 1 mg·L^{-1} + Sucrose 80 g·L^{-1}] while the combination M1 [Kin 1 mg·L^{-1} + BAP 1 mg·L^{-1} + 80 g·L^{-1} Sucrose] allows a significantly lower germination rate, of 83.33%. Increasing the concentration of Kinetin from 1 to 2.5 mg·L^{-1} helped to raise slightly the germination rate (**Figure 3**).

However, increasing the sucrose concentration of 80 g·L^{-1} up to 100 g·L^{-1} in the best medium combination has reduced the germination rate to 66.66%. The decrease in hormonal concentration of Kinetin 2.5 mg·L^{-1}

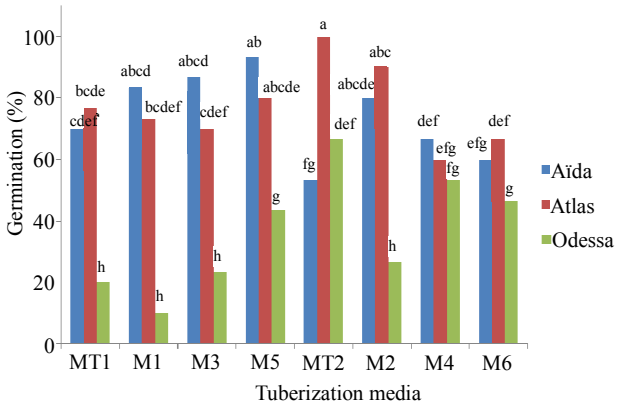

Figure 3. Hormonal effects of the tuberization medium on microtubers germination of potato varieties (Aida, Atlas and Odessa) at 28˚C. MT1 = MS/2 + Sucrose 80 g·L^{-1}; M1 = MS/2 + Kin 1 mg·L^{-1} + BAP 1 mg·Lw^{-1} + Sucrose 80 g·L^{-1}; M3 = MS/2 + Kin 2.5 mg·L^{-1} + BAP 1 mg·L^{-1} + Sucrose 80 g·L^{-1}; M5 = MS/2 + Kin 2.5 mg·L^{-1} + Coum 0.025 mg·L^{-1} + Sucrose 80 g·L^{-1}; MT2 = MS/2 + Sucrose 100 g·L^{-1}; M2 = MS/2 + Kin 1 mg·L^{-1} + BAP 1 mg·L^{-1} + Sucrose 100 g·L^{-1}; M4 = MS/2 + Kin 2.5 mg·L^{-1} + BAP 1 mg·L^{-1} + Sucrose 100 g·L^{-1}; M6 = MS/2 + Kin 2.5 mg·L^{-1} + Coum 0.025 m g·L^{-1} + Sucrose 100 g·L^{-1}; Treatments followed by the same letter are not significantly different at probability level of P < 0.05 by Student-Newman-Keuls (SNK).

to 1 mg·L^{-1} in the presence of BAP 1 mg·L^{-1} gave a higher rate (83.33%) than the M1 medium [Kin 1 mg·L^{-1} BAP + 1 mg·L^{-1} + 100 g·L^{-1} sucrose] (80%).

With Atlas variety, the sucrose concentration in the culture medium most likely to give better germination rate of microtubers was 100 g·L^{-1} with 100% of microtubers germinated after three weeks of incubation. The effect of hormones on the germination of microtubers was also appreciated in the presence of 80 g·L^{-1} and 100 g·L^{-1} Sucrose (**Figure 3**). The experiment revealed a germination rate of 90% of microtubers obtained in the M2 tuberization medium [Kin 1 mg·L^{-1} + BAP 1 mg·L^{-1} + Sucrose 100 g·L^{-1}]. By increasing the concentration of Kinetin from 1 to 2.5 mg·L^{-1} in the hormonal combination, we noticed a decrease in microtubers germination with a maximum rate of 60%. The reduction in sucrose concentration also causes a decrease in the germination rate (73.33%) but it does not seem significantly different from 90% (**Table 1**).

The experiment, in Odessa variety, was performed under the same experimental conditions as those used for Aida variety. For Odessa variety, the M4 combination [Kin 2.5 mg·L^{-1} + BAP 1 mg·L^{-1} + Sucrose 100 g·L^{-1}] gave a germination rate of 53.33% after three weeks of incubation at 28˚C ± 1˚C in darkness (**Figure 3**).

The highest germination rate (23.33%) in the presence of sucrose 80 g·L^{-1} is obtained with the combination Kin 2.5 mg·L^{-1} + BAP 1 mg·L^{-1}. So, this concentration of sucrose is not favorable for the germination of microtubers of this variety in comparison to Aida variety.

3.3. Combined Effects of Kinetin and Coumarin and Sucrose in the Tuberization Medium

For Aida variety, microtubers obtained from the M5 combination [Kin 2.5 mg·L^{-1} + Coum 0.025 mg·L^{-1} + 80 mg·L^{-1} Sucrose], offer a germination rate of microtubers of 93.33% after a three weeks incubation time. The increase in sucrose concentration at this hormonal combination (M6 medium) gave a low rate of 60% (**Figure 3** and **Table 2**). Compared to other hormonal combinations, it can be seen that the M5 combination, [Kin 2.5 mg·L^{-1}+ Coum 0.025 mg·L^{-1} + 80 g·L^{-1} Sucrose], gave more germinated microtubers than the combinations M1 [Kin 1 mg·L^{-1} + BAP 1 mg·L^{-1}] and M3 [Kin 2.5 mg·L^{-1} + BAP 1 mg·L^{-1}].

For Atlas variety, the substitution of the BAP by Coumarin, in the combination Kin 2.5 mg·L^{-1} + BAP 1 mg·L^{-1} + 80 or 100 g·L^{-1} Sucrose, can increase the germination rate three weeks after incubation, with a maximum of 80% when microtubers originated from the M5 hormonal combination [Kin 2.5 mg·L^{-1} + Coum 0.025 mg·L^{-1} + sucrose 80 g·L^{-1}]. However, for the Atlas variety, the combination M2 [Kin 1 mg·L^{-1} + BAP 1 mg·L^{-1} + Sucrose 100 g·L^{-1}] gave the best germination rate (90%).

Regarding the Odessa variety, the replacement of the BAP by Coumarin in the combination [Kin 2.5 mg·L^{-1} + BAP 1 mg·L^{-1} + Sucrose 100 g·L^{-1}] was not beneficial for this variety because there is a fall of the germination rate from 53.33% to 46.66%. For a best germination rate of Odessa microtubers after cold storage (**Figure 3** and **Table 2**), microtuberization must be realized in a medium containing 100 g·L^{-1} Sucrose and supplemented with Kin 2.5 mg·L^{-1} + BAP 1 mg·L^{-1}. The Odessa variety gave a maximum rate of germinated microtubers equal to 53.33% with the medium M4 [Kin 2.5 mg·L^{-1} + BAP 1 mg·L^{-1} + 100 g·L^{-1} Sucrose] against 66.66% for the microtubers formed in the medium MT2 without hormones but supplemented with 100 g·L^{-1} Sucrose.

4. Discussion

4.1. Effects of Sucrose Concentration of the Tuberization Medium on the Microtubers Germination

The sucrose concentration giving the best germination rate varies depending on the variety. Indeed, 80 g·L^{-1} Sucrose gave the best germination rate for the variety Aida while those of the varieties Odessa and Atlas are obtained in the presence of 100 g·L^{-1} Sucrose in the initial *in vitro* tuberization medium. The Atlas variety provides

Table 2. Residual effects of Sucrose and hormonal combinations on the microtubers germination of different varieties, after 3 weeks of incubation at 28˚C.

Media	Treatments	Germination (%)		
		Aïda	Atlas	Odessa
MT1	MS/2 + Sucrose 80 g·L^{-1}	**70.00 cdef**	76.66 bcde	20.00 h
MT2	MS/2 + Sucrose 100 g·L^{-1}	53.33 fg	**100.00 a**	**66.66 def**
M1	MS/2 + Kin 1 mg·L^{-1}+ BAP 1 mg·L^{-1} + Sucrose 80 g·L^{-1}	83.33 abcd	73.33 bcdef	10.00 h
M2	MS/2 + Kin 1 mg·L^{-1} + BAP 1 mg·L^{-1} + Sucrose 100 g·L^{-1}	80.00 abcde	90.00 abc	26.66 h
M3	MS/2 + Kin 2.5 mg·L^{-1} +BAP 1 mg·L^{-1} + Sucrose 80 g·L^{-1}	86.66 abcd	70.00 cdef	23.33 h
M4	MS/2 + Kin 2.5 mg·L^{-1}+ BAP 1 mg·L^{-1} + Sucrose 100 g·L^{-1}	66.66 def	60.00 efg	53.33 fg
M5	MS/2 + Kin 2.5 mg·L^{-1} + Coum 0.025 mg·L^{-1} + Sucrose 80 g·L^{-1}	93.33 ab	80.00 abcde	43.33 g
M6	MS/2 + Kin 2.5 mg·L^{-1} + Coum 0.025 mg·L^{-1} + Sucrose100 g·L^{-1}	60.00 efg	66.66 def	46.66 g

Student-Newman-Keuls (SNK) analysis of difference in rates: in columns, treatments followed by the same letter are not significantly different at a probability level of 0.05.

a better ability to germinate than the other varieties.

The effect of high concentrations of sucrose is mainly expressed on reducing the duration of microtuber dormancy. Indeed, the more important is the concentration, the earlier the microtubers germinate. Thus, with Odessa and Atlas varieties, microtubers obtained in the culture medium containing 100 g·L^{-1} Sucrose gave a higher germination percentage than those obtained with 80 g·L^{-1}. These results are consistent with those of Désiré *et al.* [9,12] who found that, by increasing the sucrose concentration from 80 to 140 g·L^{-1} in the phase of tuberization, it is possible to reduce the duration of dormancy of Désiré variety microtubers to 6 weeks. High concentrations of sucrose (140 g·L^{-1}) and cold conditions can shorten the dormancy of microtubers. Storage at 4˚C before transfer to 19˚C is also favorable for the germination of microtubers whatever the time of tuberization. In all cases, the longer is the storage duration at 4˚C, the more rapid and homogeneous is the germination [12].

The influence of carbohydrate nutrition on the duration of dormancy has not been highly documented. Van Ittersum [13] showed a decrease in the duration of the dormancy of 5 to 8 days for tubers obtained from plants grown in the field, when the nitrogen is added during the culture.

For Aida variety, increasing the sucrose concentration in the culture medium from 80 to 100 g·L^{-1} did not affect the germination of microtubers because we had a germination percentage at 100 g·L^{-1} less than that of microtubers obtained at 80 g·L^{-1}. These results contrast to those found in varieties of Odessa and Atlas, and those of Désiré *et al.* [11]. We can therefore argue that the effect of high concentrations of sucrose on reducing the duration of dormancy depends on varieties.

4.2. Effects of Hormone Concentrations and Sucrose in the Tuberization Medium on the Germination of Microtubers

In vitro tuberization of potato is cumulatively controlled by the carbon source (sucrose) and growth regulators. The influence of hormones on the germination of microtubers has not been much reported in the literature. Chinchilla [14] showed that GA_3 reduced the period of dormancy and promoted the formation of very elongated sprouts on classic tubers. Dieng [15] and Desire *et al.* [9] confirmed in their work that GA_3 does not allow the lifting of the dormancy in the classic tubers [16-18] but only stimulates the germination at the end of dormancy cycle [19,20].

For Aida variety, the combination of BAP + kinetin + 80 g·L^{-1} Sucrose in the *in vitro* tuberization medium has allowed an earlier germination of microtubers with a maximum germination rate of 86.66% compared to that of microtubers obtained in MS (0) medium supplemented with 8% sucrose. The increase in sucrose concentration at these combinations reduces the germination rate to 80%.

For Odessa and Atlas varieties, the addition of BAP + kinetin was not beneficial for the germination of microtubers because we noticed a decrease in the rate of germination. The addition of growth regulators was not necessary to obtain a better germination rate. The contribution of hormones on the germination of microtubers varies according to varieties. As for Aida, the hormonal combination, Kin 2.5 m·L^{-1} + Coum 0.025 mg·L^{-1} gave the best germination rate of microtubers compared to that

one obtained in tuberization media without hormones.

Regarding Atlas variety, the contribution of growth regulators is not required; no hormonal combination has given a germination rate higher than that of tuberization control media (MT1 and MT2). According to Sidikou *et al.* [18], *in vitro* tuberization of the Atlas variety is rather optimal in the presence of BAP alone at a high concentration of 5 mg/L. Cytokinins are known to have a significant impact on the size and weight of microtubers formed [21,22]. Hussey and Stacey [10] previously obtained an immediate germination of microtubers produced in long days period, whereas those induced by short days, in the presence of BAP, germinated more slowly and heterogeneously. However, different results were obtained by studying the same factors [23].

With Odessa variety, if the concentration of kinetin is increased from 1 to 2.5 $mg \cdot L^{-1}$ with 8% Sucrose, a slight increase in germination was noticed whereas with 10% Sucrose, a reduction of this rate was obtained. However, for Atlas the increasing of the concentration of kinetin to 2.5 $mg \cdot L^{-1}$ causes a significant reduction in the speed and rate of germination (**Figure 3**). In a previous study [5], the *in vitro* method of vegetative multiplication used, allows respecting easily, the imperatives of conformity such as permanency and genetic stability for different potato varieties. Microtuberization of explants requires an important provision of sucrose and of growth regulators. It was more precocious when the culture medium was enriched with cytokinins. These results were in accordance with those of [24,25]. The weak mass of microtubers harvested with Atlas variety was to be correlated to its status of late variety, which did not completely achieve its period of dormancy and non optimal hormonal combinations into the tuberization media. In previous works, it was demonstrated that the more the potatoes tuber are physiologically old, the more rapid is their germination [9,26]. When microtubers are physiologically young, they are either dormant or in a slight phase of germination. Then, with aging, their germination vigor increases and therefore germination is accelerated. The effect of the size of microtubers on germination was studied by Désiré *et al.* [11]. Indeed when the microtuber diameter increases, the germination capacity is important.

The combined effect of Kinetin and Coumarin was very successful for Aida variety because the speed of germination was important and the rate is optimal (93.33%). This combination was not very effective for Odessa and Atlas varieties because the maximum germination rates are lower than those obtained in the presence of cytokinins. Couillerot [19] showed that gibberellic acid is incorporated in plant metabolism and therefore, it is not excluded that it modifies some physiological phenomena, namely the germination of microtubers. Similarly, it can be suggested that Cytokinins and Coumarin can modify some physiological phenomena such as germination of microtubers.

With regard to the emergence and homogeneous growth of shoots stemming from Aida and Atlas microtubers, we can correlate them to a consequence of a similar physiological age inherent to the method of *in vitro* culture that synchronizes and harmonizes their development. The results allow therefore considering the possibility of a large multiplication of microtubers in a large scale, in which germinative capacity is not only important, but in which plants would have a synchronous development and growth.

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REFERENCES

[1] L. Lakhoua and O. Ellouze, "Induction de la Microtubérisation Chez *Solanum tuberosum* Variété: Spunta," *Cinquantenaire de la Culture in Vitro Versailles* (*France*), Paris, 24-25 October 1990.

[2] FAO, "Production Year Book," Vol. 53, 1999, pp. 170-199.

[3] P. J. Wang and C. Y. Hu, "*In Vitro* Mass Tuberization and Virus Free Seed Potato Production in Taiwan," *American Potato Journal*, Vol. 59, No. 1, 1982, pp. 33-37. doi:10.1007/BF02854881

[4] C. L. Lê and G. F. Collet, "Conservation *in Vitro* de L'Assortiment Suisse des Variétés de Pomme de Terre," *Revue Suisse d'Agric*culture, Vol. 20 No. 5, 1988, pp. 277-281.

[5] A. Dieme, M. Sagna and M. O. Sy, "Influence of Hormonal Treatments and of Sucrose on the Microtuberization of Three Potato Varieties (*Solanum tuberosum* L.) Adapted to Agroclimatic Conditions in Senegal," *International Journal of Plant, Animal and Environmental Sciences*, Vol. 1, No. 3, 2011, pp. 69-77.

[6] M. A. Harmey, M. P. Crowley and P. E. M. Clinch, "The Effect of Growth Regulator on Tuberisation of Cultured Stems of *Solanum tuberosum*," *European Potato Journal*, Vol. 9, 1966, pp. 146-151. doi:10.1007/BF02364301

[7] M. Ebadi, A. R. Iranbakhsh and G. R. Bakhshi Khaniki, "The Study of Shoot Formation and Microtuberization in Continuous and Semicontinuous Bioreactor," *Pakistan Journal of Biological Sciences*, Vol. 10, No. 6, 2007, pp. 861-867.

[8] M. Ebadi and A. Iranbakhsh, "The Induction and Growth of Potato (*Solanum tuberosum* L.) Microtubers (Sante Cultivar) in Response to the Different Concentrations of 6-Benzylaminopurine and Sucrose," *African Journal of*

Biotechnology, Vol. 10, No. 52, 2011, pp. 10626-10635.

[9] S. Désiré, J. P. Couillerot, J. Hilbert and L. J. Vasseur, "Protein Exchange in *Solanum tuberosum* L. during Storage and Dormancy Breaking of *in Vitro* Microtubers," *Plant Physiology and Biochemistry*, Vol. 33, No. 4, 1995, pp. 479-487.

[10] G. Hussey and N. J. Stacey, "*In Vitro* Propagation of Potato (*Solanum tuberosum* L.)," *Annals of Botany*, Vol. 48, No. 6, 1981, pp. 787-796.

[11] T. Murashige and F. Skoog, "A Revised Medium for Rapid Growth and Bioassays, with Tobacco Tissue Culture," *Physiologia Plantarum*, Vol. 15, No. 3, 1962, pp. 473-497. doi:10.1111/j.1399-3054.1962.tb08052.x

[12] S. Désiré, J. P. Couillerot, J. Hilbert and L. J. Vasseur, "Dormancy and Germination of Microtubers of Potato (*Solanum tuberosum* L.) Produced *in Vitro*: Effects of Sucrose Concentration in the Medium of Tuberization, the Shelf Life at 4˚C and Treatment with Gibberellic Acid," *Acta Botanica Gallica*, Vol. 142, No. 4, 1995, pp. 371-378. doi:10.1080/12538078.1995.10515257

[13] M. K. Van Ittersum, "Relation between Growth Conditions and Dormancy of Seed Potatoes: Effects of Nitrogen," *Potato Research*, Vol. 35, No. 4, 1992, pp. 355-364. doi:10.1007/BF02357592

[14] C. M. Chinchilla, "Effecto Temperature Almacenamiento y Algunas Sustancia Quimica in the Interrupcion del Papa Reposo TB (*Solanum tuberosum* L.). Cv. Atzimba," *Agronomy Costarr*, Vol. 9, No. 2, 1985, pp. 187-191.

[15] A. Dieng, "Microtuberization and Micropropagation of Two Varieties of Potato (*Solanum tuberosum* L.) Adapted to the Agro-Climatic Conditions in Senegal," Mémoire de DEA, Université Cheikh Anta Diop de Dakar, FST/BV, 1993.

[16] C. G. N. Turnbull and D. E. Hanke, "The Control of Bud Dormancy in Potato *Solanum tuberosum* Cultivar Majestic Tubers: Evidence for the Primary Role of Cytokinins and a Seasonal Pattern of Changing Sensibility to Cytokinins," *Planta*, Vol. 165, 1985, pp. 359-365. doi:10.1007/BF00392233

[17] H. Mioduszewska and M. Biclinska-Czarnecka, "Influence of GA_3 Treatment on Acid Phosphatase Activity in Potato Tubers towards the End of Growth, in Dormancy and Sprouting," *Acta Physiologiae Plantarum*, Vol. 6, No. 21, 1984, pp. 75-81.

[18] R. D. S. Sidikou, D. Sihachakr, D. Lavergne, A. Nato, D. Ellissèche, B. Jouan and G. Ducreux, "Study of Microtuberisation of Potato (*Solanum tuberosum* L.) in the Sahel," *Revue Agriculture* (*Montrouge*), Vol. 12, No. 1, 2003, pp. 7-14.

[19] J. P. Couillerot, "Effects and Distribution of 14C-Gibberellic Acid Application to Seed after Potato Microtubers," *Phyton*, Vol. 54, 1993, pp. 67-73.

[20] A. Dieme, "Influence de Traitements Hormonaux et du Saccharose sur la Capacité Germinative de Microtubercules de Pomme de Terre Après Conservation au Froid," Mémoire de DEA, Université Cheikh Anta Diop de Dakar, FST/BV, 2006.

[21] Y. Du Jardin, C. Hdider and J. de Rick, "Carbon Nutrition *in Vitro* Regulation and Manipulation of Carbon Assimilation in Micropropagated System. Automaten and Environmental Control in Plant Tissue Culture," Kluwer Academic Publishers, Dordrecht, 1995, pp. 411-471.

[22] T. Roitch and R. Ehness, "Regulation of Sucrose/Sink Relations by Cytokinins," *Plant Growth Regulation Journal*, Vol. 32, 2000, pp. 359-367.

[23] A. J. Abbot and A. R. Belcher, "Potato Tuber Formation *in Vitro*. Plant Tissue Culture and Its Agricultural Application," *Butterworths*, Vol. 11, 1986, pp. 113-122.

[24] C. L. Lê and D. Thomas, "Production de Microtubercules de Pomme de Terre *in Vitro*: Effet de la Durée de Culture," *Recherche Agronomique Suisse*, Vol. 1, No. 11-12, 2010, pp. 404-409.

[25] F. Mani, M. Mhamdi, T. Ettaieb and C. Annachi, "Effet du Saccharose sur la Tubérisation *in Vitro* de la Pomme de Terre (*Solanum tuberosum* L.)," *Revue Nature & Technologie*, Vol. 7, 2012, pp. 38-43.

[26] N. J. Vakis, "Influence of Physiological Ageing of Seed Potatoes on Yield and Earliness," *Potato Research*, Vol. 29, No. 3, 1986, pp. 417-425. doi:10.1007/BF02359973

