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Interactive effect of genotype and medium on microtuberization of potatoes (*Solanum tuberosum* L.) grown *in vitro*

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Abstract: *This work investigates the interactive effect of six culture media and three photoperiods (darkness, 16h/8h, 8h/16h) on the microtuberization of four potato varieties (Spunta, Désirée, Kondor and Bartina). The objective is to determine the best tuberization under these growing conditions. The measured parameters which are related to the suitability of the tuberization characteristics are: the morphological aspects (shape, position) and the biometric ones (number and diameter of tubers). The obtained results permit to valorize the meristems that constitute the starting explant, and their good organogenetic skills to provide the first generation of micropropagation with healthy vitroplants in a sufficient quantity. Thus, the grown vitroplants on the medium (MS/2+BAP+COU) presented the best values which are related to the percentage of tuberization, the number of microtubercles / vitroplants and weight of tubers. Moreover, the Bartina genotype showed a remarkable superiority over its media and under a 16h/8h photoperiod except for the diameter of the tubers, where this genotype had the best diameter over the medium (MS/2+KIN) under an 8h photoperiod.*

Keywords: MS/2 medium, microtuberization, BAP, kinetin, *Solanum tuberosum* L.

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Introduction

Potato (*Solanum tuberosum* L.) is considered as a strategic cultivation, due to the fact that it occupies the world fourth place in production after Wheat, Rice and Maïs [1]. As a matter of fact in Algeria, potato is the leading vegetable crop, both in terms of area and production level [2]. However, the local potato production does not meet the needs of the consumer, which makes it dependent on the large outside importation as well as the expensive seeds imports [3]. To remedy this inconvenience, several countries introduced the micropropagation and microtuberization techniques in seeds production industry [4]. However, these techniques are little and partially used in Algeria. Microtuberization produces microtubers, which can be considered as seeds of the future because of their great usefulness for agriculture: it is their small size that makes them advantageous so that they can be stored for a long time until they are used [5]. They can be transported from one region to another without any difficulty and are produced at any time of the year [6].

On the other hand, the knowledge of the physiology of microtuberization makes it possible to improve the health status of seeds and to obtain microtubers (vitrotubers) of a better size, in a shorter time, and also to improve them qualitatively and quantitatively, thus, introducing them into the certified seed production scheme [7]. This is the focus of our work, which aims to study the compositional influence of the culture medium and the photoperiod on some parameters of *in vitro* microtuberization in four potato varieties (Désirée, Spunta, Kondor and Bartina) in order to determine them with the best tuberization rates. In addition of being the least demanding in the culture medium, i.e. genotypes resulting in the lowest production costs.

Material and Methods

1. Plant material

The explants used in this study are made up of four varieties: Desirée, Spunta, Kondor and Bartina of the species *S. tuberosum*, supplied by "the agro-development company: SAGRODEV". The tubers of these varieties are germinated at room temperature for 20 days. The sprouts obtained measure 5-10 cm in length and are used for the collection of meristem and the regeneration of whole plants. These plants, 4 weeks old and virus-free, were used for the collection of knotted cuttings. Plants from this type of cuttings are used for the production of microtubers *in vitro* (microtuberization).

2. Environmental conditions

Plant growth (micropropagation) is done in a culture chamber under semi-controlled conditions (a photoperiod of 16 hours of light/8 hours of darkness, a temperature of 22±2°C and a light intensity of 24 µmol m^{-2} s^{-1}).

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3. Growing media

The apical ends are grown in glass jars with a capacity of 380 ml, sealed with plastic lids. In contrast, cuttings and vitroplants are grown in Pyrex glass test tubes (200 mm long, 20 mm diameter, 25 ml volume). The culture medium chosen is that of Murashige and Skoog [8], in which the macroelements are halved (MS/2). This medium is recommended by several authors [9, 10, 11, 12] and is autoclaved at 120°C for 20 minutes at a pressure of 1 bar, its pH is adjusted to 5.7 by NaOH and HCl (1N) solutions, then solidified by agar (7g L^{-1}) and supplemented with 30 g of sucrose (case of the medium used during the Micropropagation phase). The volume of the medium is 10 ml/tube and 120 ml/jar.

4. Microtuberization

This phase of the experiment consists in triggering the formation of microtubers by adding the microtuberization culture medium in the tubes resulting from micropropagation (first generation of micropropagation). In our case, the application of the treatment with the two prepared culture media is carried out on vitroplants aged 21 days of incubation in the culture room. This operation is carried out under a laminar flow hood and in conditions of total asepsis. It proceeds by adding 10 ml of liquid microtuberisation culture medium based on growth hormones: BAP (6-benzyl amino purine), Kinetin (6-furfuryl amino purine) and Coumarin (2H-1-benzopurane-2-one) and without hormones and supplemented with a quantity of 80 g of sucrose (this quantity normally corresponds to the same quantity used during the preparation of the tubes for micropropagation). However, the quantity of medium to be added also depends on the length of the vitroplant which must be taken into account in order to avoid that it is totally emulsified in the liquid medium (Figure 1).

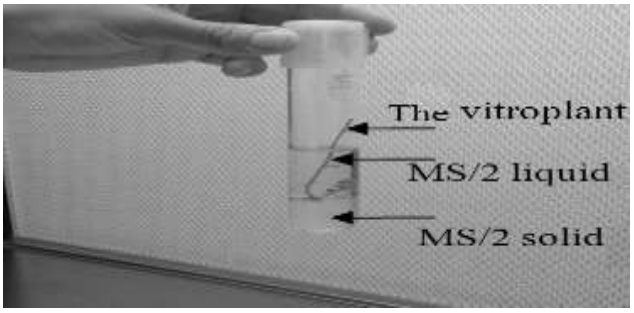

Figure 1. Potato vitroplant grown on solid medium MS/2
and adding the microtuberization medium (liquid medium MS/2)

Treatments with growth hormone, coumarin and sucrose media only concern normally developing vitroplants. Infected and stunted seedlings are eliminated. The BAP (6-benzyl amino purine) hormone dose (2.5 mg/l) is recommended by LÊ and Thomas [7], whereas the dose of kinetin (2.5 mg/l) and coumarin (25

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mg/l) is recommended by Diémé et al. [12]. To facilitate their use, BAP and kinetin are dissolved in a few drops of NaOH, while coumarin is dissolved in 96% ethanol. After this operation, the tubes are incubated under three photoperiod conditions (darkness, 16h/8h, 8h/16h), and at a constant temperature of 22C° for 6 weeks [24] (Table 1).

Table 1. Different treatments used during the experiment

Treatments of Media	Sucrose (g/L)	BAP (mg/L)	Kinetin (mg/L)	Coumarin (mg/L)
Medium 1	80	-	-	-
Medium 2	80	2.5	-	-
Medium 3	80	2.5	-	25
Medium 4	80	-	2	-
Medium 5	80	-	2	25
Medium 6	80	2.5	2	25

Medium 1: Medium for control, (-): without dose of the substance

5. Statistical analysis

The device adopted is random with 3 factors studied: four potato genotypes (Bartina, Désirée, Kondor and Spunta); each genotype was subjected to 5 treatments (M1, M2, M3, M4, M5) and a control (untreated). The treatments were subjected to three photoperiods (four replicates). An analysis of variance with three classification criteria was carried out on the different parameters using the SPSS.20 (Statistical Package for Social Sciences.20) software and a correlation matrix obtained on the 4 varieties and calculated with the Excel Stat (2014) software. The mean values of the different parameters measured were used for a principal component analysis (PCA) to determine the best performing genotype.

Results and Discussion

According to the table 2 of the mean squares, the genotype effect is significant to very highly significant for all the studied parameters. The conditioning effect is non-significant on the RL, DRW, SL and FWS parameters and significant to very highly significant for the rest of the measured variables; whereas the condition effect is non-significant on RL (root length), FRW (fresh root weight), DRW (Dry root weight), SL (stem length) and FWS (Fresh weight stem) and significant to very highly significant for the rest of the parameters: NM (number of microtubers), FWM (fresh weight of microtubers), DWM (dry weight of microtubers) and DM (diameter of microtubers) (Table 2).

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Table 2. ANOVA's measured parameters

Source of variation	ddl	RL	FRW	DRW	SL	FWS	NM	FWM	DWM	DM
Genotype	3	11.5	290.23*	20.56*	25.73*	4311.64*	1.20*	11120.67	419.17*	0.59*
Media (M)	5	0.97	37.55*	12.13^{n}	2.22^{ns}	342.49^{ns}	0.74*	2867.92**	101.31*	0.52*
Condition	2	0.08	19.28^{ns}	16.85^{n}	1.07^{ns}	178.58^{ns}	6.54*	16907.81	475.82*	1.39*
G x M	15	0.48	14.15^{ns}	13.56*	1.10^{ns}	257.35^{ns}	0.95*	1697.50**	100.42*	0.17*
G x C	6	0.75	30.74^{ns}	12.54^{n}	1.37^{ns}	598.26*	0.90*	1612.88*	37.64^{ns}	0.13*
G x M x C	30	0.64	15.49^{ns}	10.21^{n}	0.85^{ns}	136.32^{ns}	0.36*	1010.76*	42.88^{ns}	0.07*

***: highly significant **: very significant *: significant ns: not significant (R=5%)

Callus pick-up and formation

During the follow-up of the microtuberisation experiment on the six culture media, callus formation on all parts of the vitroplants was noticed (basal part, caulinary part and even on the tubers); this formation is not similar in all genotypes subjected in the different culture media and variable photoperiod conditions (Figure 2). The same results were obtained by Cysty et al. [13] who reported that the induction of the callogenesis is favoured on cytokinin-rich media. The vitroplants also regenerated and tuberised in the presence of the concentration of 80 g L-1 of sucrose on the used liquid medium. This amount of sucrose facilitated the hydrolysis, synthesis and accumulation of starch and patatin via the cytoplasmic enzymes involved in sucrose metabolism (sucrose phosphate synthase (SPS), sucrose synthase (SuSy) and invertase) [14, 15], however, the yield of microtubers depends on the physical state of the culture medium. For example, the use of a liquid medium has facilitated microtuberization, as agar-agar under certain medium conditions may hinder tuberization. Indeed, alginic acid and D-mannitol, contained in agar-agar, chelate the mineral elements and prevent their absorption, hence, avoiding the accumulation of starch and consequently the formation of the tuber [16, 17]. Our results corroborate those of Mani et al. [11], who obtained a percentage of 70% of plants tuberized at the same concentration of 80 g L-1 of sucrose on the same physical state of the medium (MS/2 liquid). The use of low doses of nitrogen also prompt tuber formation by increasing the carbon/nitrogen ratio [18], as the low concentrations of nitrogen and ammonium nitrate are used to improve induction and growth (ammonium nitrate is one of the major components of the MS medium) [19, 20].

The length of roots and stems

The results in Tables 3 and 4 demonstrate the Spunta genotype has the best root and stem lengths. This highly developed growth in the Spunta variety compared to the other genotypes can be explained by its varietal effect, this genotype has a well-developed root and stem lengths in the field (Potato Varieties Bulletin, 2010). Furthermore, the results were in agreement with those of Margara [34] which shows that across all cultivated species, organogenesis rates are greatly influenced by the intrinsic characteristics of the explants.

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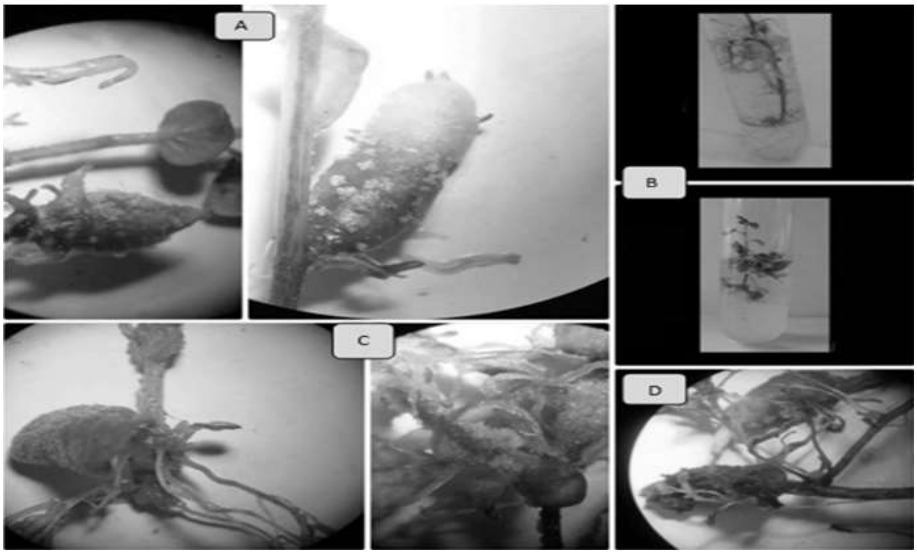

Figure 2. Callus formation (A: on all parts of the vitroplant, B: on the microtubers, C: on the stem, D: on the roots)

The differences in stem elongation between the four varieties grown on the six media were observed; which are explained by the regeneration capacity of each variety. These results are consistent with those of [21-7], which showed that Desirée has an average ability to multiply with the reduced size and contradict those of [22] for the Spunta variety, which still shows low values.(According to 3photoperiods: P0-Darkness, P8-8hours lightness /16 hours obscurity, P16-16hours lightness /8 hours obscurity).

Table 3. Root length (cm)

		Spunta	Bartina	Kondor	Desirée
	P16	2.475	1.800	2.050	1.400
M1	P8	3.225	1.300	1.450	1.700
	P0	1.725	1.825	1.300	1.425
	P16	1.725	1.450	1.275	1.025
M2	P8	2.350	1.100	1.800	2.000
	P0	3.050	1.725	1.352	0.925
	P16	2.270	2.050	1.375	2.200
M3	P8	2.850	1.350	0.875	1.100
	P0	1.920	1.325	1.175	0.650
	P16	2.020	1.400	0.900	1.500
M4	P8	1.650	1.825	1.400	1.250
	P0	2.025	2.025	1.575	1.425
	P16	1.700	1.900	1.025	1.700
M5	P8	1.300	1.700	1.350	1.225
	P0	2.700	1.550	1.675	0.950

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	P16	1.950	1.875	1.050	0.900
M6	P8	1.950	1.475	1.275	1.525
	P0	1.770	1.475	0.600	0.525

Table 4. Stem length (cm)

		Spunta	Bartina	Kondor	Desirée
	P16	3.925	3.400	2.400	3.150
M1	P8	4.575	3.225	2.750	2.425
	P0	3.425	3.000	2.850	2.400
	P16	4.000	3.750	1.750	2.850
M2	P8	3.875	3.600	2.175	2.775
	P0	2.900	2.875	2.125	2.000
	P16	3.975	2.600	2.525	3.100
M3	P8	3.600	3.375	1.850	2.525
	P0	2.800	3.050	3.625	2.200
	P16	3.025	3.550	2.275	2.825
M4	P8	3.875	3.525	3.450	2.375
	P0	3.125	3.225	2.800	2.275
	P16	4.600	2.950	2.675	2.975
M5	P8	3.325	2.600	2.750	2.200
	P0	5.075	2.900	2.375	2.725
	P16	3.375	2.200	1.700	2.100
M6	P8	3.925	3.800	1.950	1.625
	P0	3.175	2.575	1.475	2.550

The fresh and dry weight of the roots

The highest fresh and dry root weights were observed in the Spunta genotype for the control (Table 5), Bartina genotypes grown on BAP medium and Coumarin in the dark (Table 6). Indeed, Pelacho and Mingo-Castel [23] explained that the increased level of rhizogenesis could disrupt or delay microtuberization and that low root weight favours microtuberization. This was observed in our study so that the control medium (MS/2) had the highest root rate and the lowest percentage and speed of microtuberization; thus, it could be explained by the fact that BAP gave the best microtuberization rate and this is because of its inhibitory effect on root development that will induce microtuberization. Therefore, when root growth stops, microtuberization begins.

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Table 5. Fresh root weight (mg)

		Spunta	Bartina	Kondor	Desirée
M1	P16	11.65	10.375	3.000	2.625
	P8	9.950	6.700	3.300	1.870
	P0	4.300	8.325	1.400	5.275
M2	P16	2.175	3.075	1.900	2.125
	P8	2.100	3.075	3.025	2.300
	P0	7.275	3.075	2.725	2.250
M3	P16	1.950	8.750	2.825	3.625
	P8	3.975	8.425	1.975	2.750
	P0	3.425	3.525	1.950	3.200
M4	P16	2.975	6.325	0.900	1.375
	P8	6.375	4.275	1.250	1.275
	P0	2.800	7.700	1.350	8.425
M5	P16	0.725	11.125	1.100	1.950
	P8	10.525	7.625	2.125	2.225
	P0	3.50	3.575	1.925	1.275
M6	P16	2.775	4.625	2.150	1.400
	P8	5.400	5.300	2.050	1.650
	P0	5.025	6.175	2.775	2.475

Another observation determines that the control medium showed better root and stem weight compared to the supplemented medium with the growth regulators BAP and Kinetin, only the latter showed better growth compared to BAP. According to Cysty et al. [13], BAP has two functions *in vitro* potato culture: a stimulating effect on microtuberization and an inhibitory effect on root growth.

The percentage of microtuberization

According to Figure 3, the highest percentage of microtuberization is founded in the Bartina variety grown on the M3 medium (BAP +COU) in the conditions (16h/8h: P1) with a value of 200%, followed by 175% in total darkness (P3). The number of microtubers per vitroplant varies between 1 and 2 and no significant effect and that is observed between the three factors: variety, medium and photoperiod. The results of Cysty et al. [13], confirm the previous one, there was no significant effect between varieties and growth regulators on the number of microtubers per vitroplant.

The study of the influence of genotype, medium and photoperiod on the tuberization of explants grown *in vitro* revealed that the optimal potential tuberization is obtained in the Bartina variety grown on M3 medium (MS/2+BAP+COU) under a photoperiod (16h/8h: P1). So, it is in perfect agreement with those of Sarkar et al. [25], who consider, that BAP has a greater potential microtuberization than kinetin and has an

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effect on the reduction of sugars that produced a sufficient number of microtubers per vitroplant during the exposure of the vitroplants to an 8-hour photoperiod during microtuberization (P2).

Table 6. Dry root weights (mg)

		Spunta	Bartina	Kondor	Desirée
M1	P16	6.175	1.950	1.100	1.200
	P8	5.950	1.225	0.925	0.850
	P0	1.325	1.850	0.125	2.350
M2	P16	0.550	0.675	0.350	0.850
	P8	0.575	0.775	0.675	1.025
	P0	2.900	1.825	0.900	0.925
M3	P16	0.375	1.450	0.925	1.500
	P8	0.850	0.550	0.525	1.075
	P0	0.375	11.675	0.400	0.950
M4	P16	0.475	0.325	0.150	0.325
	P8	0.525	1.100	0.200	0.350
	P0	0.550	2.400	0.225	5.775
M5	P16	0.100	1.400	0.175	1.025
	P8	2.450	0.275	0.725	0.850
	P0	0.850	0.425	0.550	0.325
M6	P16	0.500	1.250	0.775	0.375
	P8	1.000	1.825	0.675	0.475
	P0	1.750	1.675	1.175	1.200

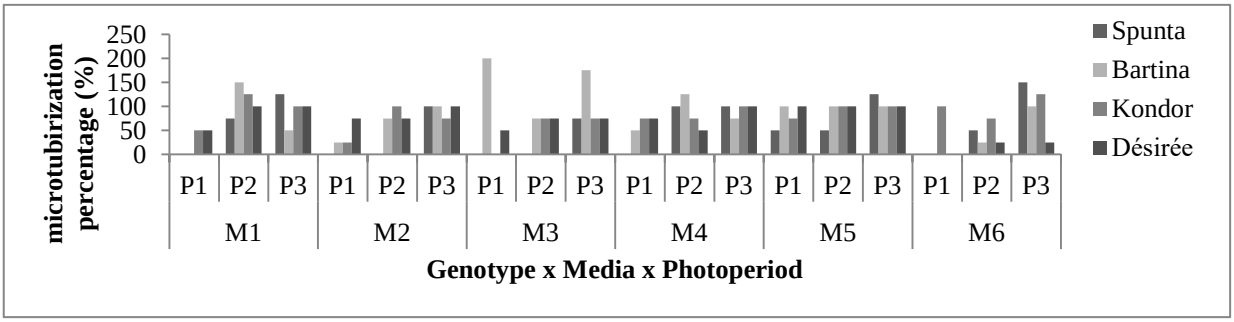

Figure 3. Microtuberization's percentage
(P1= P16: 16/8h - P2 = P8: 8/16h – P3 = P0: Total darkness)

Position and shape of the microtubers

During Microtuberization, the microtubers are positioned in three modes at the budding level: basal, axillary and apical (Figure 4) and take on the oval, round and elongated shapes (Figure 5).

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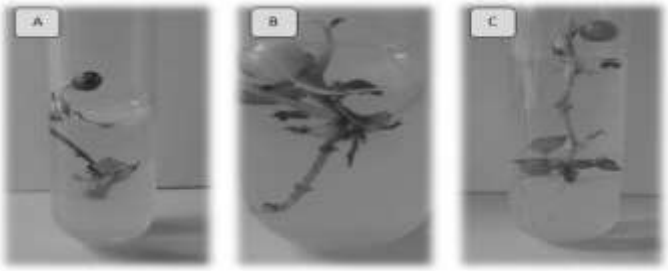

Figure 4. Position of the microtubers, A: apical, B: basal, C: axillary

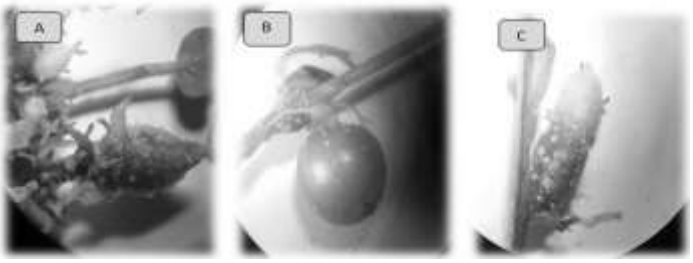

Figure 5. Different shapes of microtubers, A: oval, B: round, C: elongated

The fresh and dry weight of the microtubers

According to Tables 7 and 8, the Bartina variety grown on M2 medium (MS/2+BAP) in total darkness that exhibits the best fresh and dry weight of microtubers with values of 135.4 mg and 22,650 mg respectively. The lowest dry weight was observed in the same genotype grown on the same medium under 16h/8h photoperiodic conditions, while the lowest fresh weight was located in Kondor grown on M2 (MS/2+BAP) medium under (16h/8h) conditions.

Table 7. Fresh weight of microtubers (mg)

		Spunta	Bartina	Kondor	Desirée
	P16	0.000	0.000	1.100	2.775
M1	P8	10.65	5.812	5.450	8.625
	P0	9.550	3.975	3.875	8.000
	P16	0.000	0.650	0.350	2.925
M2	P8	0.000	4.825	5.275	5.350
	P0	5.675	22.65	3.225	8.075
	P16	0.000	9.100	0.000	3.100
M3	P8	0.000	11.075	4.450	2.525
	P0	3.375	12.75	2.375	6.875
	P16	0.000	6.100	1.325	2.450
M4	P8	12.45	19.425	2.000	3.650
	P0	11.65	15.475	4.275	5.950

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	P16	4.500	14.15	3.300	2.025
M5	P8	2.050	11.40	5.025	6.950
	P0	7.450	8.600	6.350	4.025
	P16	0.000	0.000	11.375	0.000
M6	P8	0.825	4.350	1.050	0.550
	P0	3.100	7.000	5.700	0.525

The best microtuber weight results acquired in the dark; whereas Seabrook et al. [26] found the same results but under photoperiodic conditions (8h/16h). Genotypic diversity is observed for fresh and dry microtuber weights (highly significant genotypic effect between varieties, medium and photoperiod on microtuber weight). The interaction effect between varieties, medium and photoperiod on microtuber weight was also significant, which is consistent with those of Pruski et al. [28] (According to 3photoperiods: P0-Darkness, P8-8hours lightness /16 hours obscurity, P16-16 hours lightness /8 hours obscurity).

Table 8. Dry weights of microtubers (mg)

		Spunta	**Bartina**	**Kondor**	**Desirée**
	P16	0.000	0.000	4.600	6.425
M1	P8	21.35	32.64	31.30	46.70
	P0	47.125	33.575	22.250	38.525
	P16	0.000	1.900	1.250	14.75
M2	P8	0.0000	27.200	30.925	14.725
	P0	30.050	135.45	0.0000	38.550
	P16	0.0000	32.712	23.750	15.100
M3	P8	0.000	50.70	14.50	12.25
	P0	14.52	54.47	8.875	28.95
	P16	0.000	36.80	15.57	8.050
M4	P8	49.30	75.05	22.55	19.32
	P0	62.40	78.42	19.30	36.85
	P16	17.65	58.95	32.95	11.75
M5	P8	7.550	63.85	30.20	41.725
	P0	45.362	57.625	53.650	25.000
	P16	0.000	0.000	5.700	0.000
M6	P8	8.175	21.67	28.80	2.175
	P0	21.10	36.02	6.425	2.175

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The diameter of the microtubers

Table 9 shows that the largest microtuber diameter is founded in the Bartina genotype (0.82 mm), grown on M4 medium (MS/2+KIN) under photoperiod conditions (8h/16h). The smallest one of 0.05 mm is observed in the varieties, Bartina on medium M2 (MS/2+BAP), Kondor has grown under photoperiod conditions (16h/8h) and Desiree grown on medium M6 in total darkness. The diameter of the microtubers depends on the composition of the culture medium used so that the best ones were obtained on MS/2 a kinetin medium.

The studied results are similar to those of Du Jardin et al. [14] who confirmed the role of cytokinins on the weight and the diameter of microtubers. The effect of kinetin on microtuber diameter was studied by [25-29], who found that this hormone stimulates starch accumulation by reducing total sugars. In this case, the reached results contradict with those found by [30-31-3], for these researchers Kinetin does not produce any significant change on the diameter of the microtubers. The latter is strongly influenced by the cultivar for all microtuberization photoperiods. In this study, the best microtuber diameter was found in Bartina followed by Spunta. Particular characteristics related to the genotype could be at the origin of this variability of tuberization in the controlled culture medium. Moreover, the microtuberization potential of potatoes is a genotype-dependent trait that is strongly influenced by environmental factors [32, 33] (According to 3photoperiods: P0-Darkness, P8-8hours lightness /16 hours obscurity, P16-16hours lightness /8 hours obscurity).

Table 9. Diameter of microtubers (mm)

		Spunta	Bartina	Kondor	Desirée
	P16	0.000	0.000	0.125	0.150
M1	P8	0.500	0.625	0.475	0.750
	P0	0.475	0.325	0.350	0.375
	P16	0.000	0.050	0.050	0.225
M2	P8	0.000	0.275	0.450	0.350
	P0	0.375	0.625	0.200	0.375
	P16	0.000	0.550	0.000	0.250
M3	P8	0.000	0.525	0.225	0.175
	P0	0.250	0.725	0.175	0.375
	P16	0.000	0.450	0.250	0.250
M4	P8	0.775	0.825	0.350	0.375
	P0	0.600	0.450	0.400	0.325
	P16	0.325	0.775	0.275	0.300
M5	P8	0.275	0.725	0.425	0.575
	P0	0.625	0.650	0.350	0.325
	P16	0.000	0.000	0.475	0.000
M6	P8	0.150	0.200	0.125	0.075

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P0	0.425	0.525	0.400	0.050

Principal Component Analysis (PCA)

Principal component analysis (PCA) was applied to the matrix of correlations obtained from the 10 reduced centred variables measured on the 4 potato varieties (genotypes: Spunta, Bartina, Kondor and Désirée) and subjected to three photoperiods (P1: 16/24h, P2: 8/24h and P3: darkness) taken in pairs. This method was carried out, separately, for each of the 6 media studied (M1: MS/2 + 80 g sucrose, M2: MS/2 + 80g sucrose + 2.5mg BAP, M3: MS/2 + 80g sucrose + 2.5mg BAP + 25 mg coumarin, M4 : MS/2 + 80g sucrose + 2 mg Kinetin, M5 : MS/2 + 80 g sucrose + 2 mg Kinetin + 25 mg coumarin and M6 : MS/2 + 80g sucrose + 2.5mg BAP + 2 mg Kinetin + 25 mg coumarin). It is clear that the first axis alone explains 39.46% of the total variation of the initial variables, the first two axes together explain 60.26%. So these two synthetic axes best summarize the information provided by the 10 initial variables.

The correlation circles sometimes make it possible to give a physical interpretation to certain main components. Thus, the examination of figure 6 shows that the variables Callogenesis, FWS, DWShave short vectors indicating low correlations and consequently a poor consideration of these variables by the two axes. The other variables RL (root length), SL (stem length), FRW (fresh root weight), and NM (number of microtubers) are close to the circle of correlation and are therefore better represented. On the other hand, DWM (dry weight of microtubers), FWM (fresh weight of microtubers), and DM (diameter of microtubers) have long vectors close to each other indicating a strong correlation between these two variables.

Indeed, four homogeneous groups were obtained by the PCA. The first group (G1) is located on the positive side of axis 1 and has affinities with the Spunta genotype grown in the medium (in the medium M1 in the dark; in the medium M4 in the dark and for a photoperiod of 8/24h and in the medium M5 and M6 for the three previous photoperiod conditions); with the Bartina genotype grown in the medium (M1 in two photoperiod conditions of 16 and 8/24h respectively; in the media (3, 4, 5) under the three photoperiod conditions considered in our experiment and in media 2 and 6 in the dark); with the Kondor genotype cultivated in the media (1, 2 and 5) subjected to the same photoperiod of 8/24h and in medium 6 for the photoperiods of darkness and 16/24h and finally the Desirée genotype planted in media 1 and 4 in darkness and a photoperiod of 8/24h and grouping the measured parameters (FWS: fresh weight of the stems, NM: number of microtubers, FWM: fresh weight of the microtubers, DWM: dry weight of the microtubers, MD: diameters of the microtubers, and Callogenesis). The second group is always located on the same axis of the circle of correlations on the negative end and is characterized by the Bartina variety; the Kondor variety cultivated in media 2 and 4 for a 16/24h photoperiod; and in media (3, 4, 6) and subjected to an 8/24h photoperiod; the Desirée variety in media 2 and 6 for the three periods of luminosity; in medium 3 for an

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8/24h photoperiod and in media 4 and 5 for an 8/24h photoperiod. The third group is characterized by the Spunta variety in medium 1 with two periods of luminosity which are respectively 16 and 8/24h; in media (2, 3) under the different light conditions applied during the experiment and in medium 4 for a 16/24h photoperiod; by the Bartina variety planted in the dark (M1) and at a 16/24h photoperiod (M2) and in two light conditions of 16 and 8/24h respectively, and by the last two varieties Kondor and Desirée which are cultivated in media 1 and 3 and exposed to a 16/24h photoperiod of it groups the parameters (RL: root length, FRW: fresh root weight, DRW: dry root weight and SL: stem length).

Group four is located on the negative side of axis 2 and includes the Bartina variety grown in medium 2 subject to a 16/24h photoperiod; the Kondor varieties of medium 1, 2, 4 and the Desirée variety of medium 3, 5 subject to darkness in the medium; Kondor of medium 3 and Desirée of medium 4 subject to an 8/24h photoperiod and Kondor of medium 5 exposed to a 16/24h photoperiod. (Figure 6).

The results obtained statistically by PCA also show that the first homogeneous group (G1) located on the positive side of axis 1 (F1+), shows that the media M1, M5 and the photoperiods P1(16/8), P2 (8/16) and P3(darkness), as well as the Bartina and Spunta genotypes, show more affinity with the parameters (FWS: Fresh weight of stems, NM: Number of microtubers, FWM: Fresh weight of microtubers, DM: Microtuber diameter and callogenesis). The study of the second homogeneous group (G2) located on the negative side of axis 1 (F1-), shows that the Bartina, Kondor and Desirée genotypes are negatively correlated with axis F1, therefore they have no affinity with the parameters (FSW: Fresh stem weight, NM: Number of microtubers, FWM: Fresh microtuber weight, MD: Microtuber diameter, callogenesis, LR: Root length, FRW: Fresh root weight, DRW: Dry root weight, SL: Stem length) and that the media M2 (MS/2+BAP), M4 (MS/2+KIN) and M3 (MS/2+BAP+COU), M4 (MS/2+KIN) and M6 (MS/2+BAP+KIN+COU) are unfavourable conditions for Bartina and Kondor subjected to (16h/8h) and (8h/16h) photoperiod, and that the media M2 and M6 under the three photoperiodic conditions are unfavourable conditions for Desirée and that the media M3 (MS/2+BAP+COU), M4 (MS/2+KIN) are unfavourable conditions for the development of the Desirée genotype subjected to a photoperiod (8h/16h).

The study of the third group G3 located on the positive side of axis 2 (F2) brings out the following remarks: All the varieties (Spunta, Bartina, Désirée and Kondor) belonging to this group favouring the M1 medium under the condition of a 16h/8h photoperiod. These results reflect the existence of a certain affinity of the varieties studied under the previous conditions with the parameters RL (root length), FRW(fresh root weight), DRW (dry root weight), SL (stem length), etc. On the M3 medium (MS/2+BAP+COU) and at a light period (16h/8h) the Desirée and Kondor genotypes together present medium conditions with a favourable affinity with the above-mentioned parameters. Spunta and Bartina together favour medium 2 in all photoperiodic conditions except Bartina for darkness. The group G2 located on the negative side of axis one

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(F1) and the group G4 located on the negative side of axis two (F2) are thus negatively correlated with respect to the two axes F1 and F2 respectively, therefore no affinity is found between the genotypes and the measured parameters.

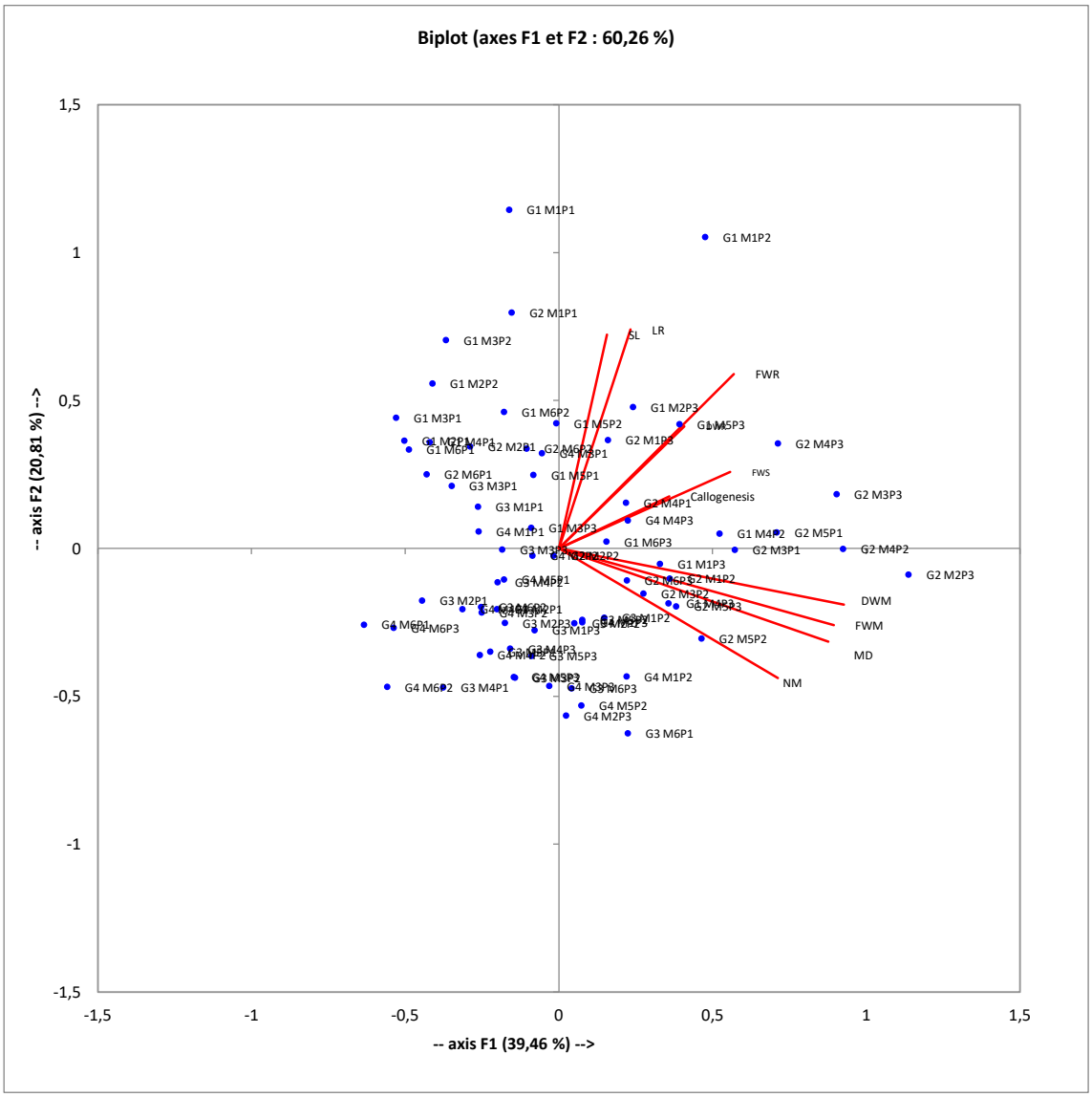

Figure 6. Circle of Correlations

Conclusion

At present, the use of *in vitro* culture techniques for seed production in potatoes is a crucial alternative procedure that is constantly evolving to replace the traditional techniques that cause problems of various kinds. Its generation is mainly justified by its low budget and its effectiveness in producing plants of better

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phytosanitary quality in a much-reduced period of time. The present work on four potato varieties (Spunta, Desirée, Kondor and Bartina), whose objective is to obtain microtubers on six different growing media, and to target the best combination of growth hormones and favourable photoperiodic conditions, leads to the improvement of certain parameters of the micropropagation and microtuberization of four varieties, such as callogenesis rate, percentage of microtuberization, average lengths of roots and stems, and dry as well as fresh weights of roots and stems, percentage of tuberization, number of tubers/vitroplant, and best microtuber characteristics (weight and diameter).

The results for the different parameters studied showed the following: The rate of microtuberisation callogenesis of the four varieties studied (Spunta, Desirée, Kondor and Bartina) is optimal on the media (MS/2 + BAP + COU) and this under photoperiods (8h/16h and 16h/8h) with the presence of genotypic effect for the Bartina genotype; the latter presented in majority the best scores for all the parameters and under all the conditions of the medium and the photoperiod. In potatoes, cytokinins are stimulators of microtuberization and that the combination (MS/2+ BAP+COU) seems to be better to generate high values for all the studied parameters. Darkness and (8h/16h) photoperiod also seems to be involved in giving high values for all the different parameters.

References

[1]. FAO.; FaostatDataBase Results, Roma FAO, **2007.**

[2]. Badaoui, M, I.; Berkani, A.; Lotmani, B., Les entomopathogènes autochtones, Nouvel espoir dans le contrôle biologique de *Tuta absoluta Meyrick* 1917 (*Lepidoptera Gelechiidae*) en Algérie, *Faunistic Entomology*, **2011**, *63*, *3*, 165-169.

[3]. Khelifi, S, A, D.; Pavlista, M, M.; Meagher, P,E, Read., Invertase activity as affected by cytokinin-like compounds during potato tuberization *in vitro*, Am. J. *Potato Res*, **2000**, *77*, 57-61.

[4]. Momena, K.; Adeeba, R.; Rahman, L., *In vitro* Microtuberization of Potato (*Solanum tuberosum* L.) Cultivar through Sucrose and Growth Regulator, *Journal of Bioscience and Agriculture Research*, **2014,** *Vol. 02*, 02, 76-82.

[5]. Agud, E.; Laslo, V., Tratament with low temperatures applied to the bulbs of *Narcissus poeticus* L. for the stimulation of the *in vitro* regeneration and bulbification, *Natural resources and sustainable development*, **2018,** *Vol. 8*, No 1, 49-58.

[6]. Fouarge, G., Quel avenir pour l'amélioration des plantes? *Ed. Aupelf-Uref. Paris*, **1994,** 205-209.

[7]. LÊ, C.; L, Thomas, D., Production de microtubercules de pomme de Terre *in vitro:* effet de la durée de culture, *Revue Suisse Agric*, **2010,** *1*, 11-12, 404-409.

[8]. Murashige, T.; Skoog, F., A revised medium for rapid growth and bioassays with tobacco tissue culture,

Corresponding author: kadizahia6@yahoo.fr Full Paper
DOI: 10.2478/asn-2020-0003

Plant. Physiol, **1962,** *Vol. 15*, 473-497.

[9]. Dieng, A., Micropropagation et microtubérisation de deux variétés de pomme de Terre (*Solanum tuberosum* L.) adaptées aux conditions agroclimatiques du sénégal Mémoire de DEA, *FST/BV/UCAD*, **1993,** 84.

[10]. Sidikou, R, D, S.; Sihachakr, D.; Lavergne, A, N.; Ellisséche, D.; Jouan,B.; Ducreux, G., Etude de la micotubérisation de la pomme de Terre (*Solanum tuberosum* L.) au sahel, *Revue Agricultures (Montrouge)*, **2003,** *12*, 1, 7-14.

[11]. Mani, F.; Mhamdi, M.; Bettaieb, T.; Hannachi, C., Effet du saccharose Sur la tubérisation *in vitro* de la pomme de terre (*Solanum tuberosum* L.), *Revue, Nature & Technologie*, **2012,** *07/Juin, 38-43.*

[12]. Diémé, A.; Sambe, M, A, N.; Agbangba, E, C.; Ouréye, Sy, M., Residual effects of sucrose and hormonal treatments of the tuberization medium on *in vitro* germination of potato (*Solanum tuberosum* L.) microtubers, *American Journal of Plant Sciences*, **2013,** *4*, 1872-1878.

[13]. Cysty, Borda Yapez C.; Toledo, J.; Golmirzaie, A.; Roca, W., Effecto de inductors de tuberización y fotoperiodo sobre la microtuberización de *Solanum tuberosum* L. *in vitro*, *Centro International de la Papa* (CIP), **2001**.

[14]. Du Jardin, Y.; Hdider, C.; de Rick, J., 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,** 411-471.

[15]. Roitch, T.; Ehness, R., Regulation of sucrose/sink relations by cytokinins, *Plant Growth Regul*, **2000,** *32*, 359-367.

[16]. Mercier, M.; Laffite, C.; Borderies, G.; Fournier, G., The alagal polysaccharide carrageenans can act as an elicitor for plant defence, *New Phytologist*, **2001,** *Vol. 149*, 43-51.

[17]. Kamarainen-Karppinen, T.; Virtanen, E.; Rokka, V, M.; Pirttilä, A, M., Novel bioreactor technology for mass propagation of potato microtubers, *Plant Cell Tiss. Org. Cult*, **2010,** *101*, 245-249.

[18]. Sattelmacher, B.; Marschner, H., Relation between nitrogen nutrition, cytokinin activity and tuberization in *Solanum tuberosum* L**.,** *Physiologia Plantarum*, **1978,** *44*, 65-68.

[19]. Zarrabeitia A., Lejarcegui X., Veramendi J., Mingo-Castel A. M., Influence of nitrogen supply on micropropagation and subsequent microtuberization of four potato cultivars, *Am Potato J*, **1997,** *370*, 369-378.

[20]. Sarkar, D.; Naik, P, S., Effect of inorganic nitrogen nutrition on cytokinininduced potato microtuber production *in vitro*, *Potato Res*, **1998,** *41*, 211-217.

[21]. Jarret, R, L; Hasegawa, P, M.; Erickson, H,T., Factors affecting shoot initiation from tuber discs of potato (*Solanum tuberosum.* L.), *Article in Physiologia Plantarum*, **1980,** *49*, *2*, 177-184.

Corresponding author: kadizahia6@yahoo.fr Full Paper
DOI: 10.2478/asn-2020-0003

[22]. Belguendouz, A., Essai de substitution des milieux de culture en micropropagation et la Physiologie de la microtubérisation de la pomme de terre terre (*Solanum tuberosum* L.), Memoir de fin d'étude en vue de l'obtention de magistèr en sciences agronomiques, université de Tlemcen, Algerie, **2012**.

[23]. Pelacho, A, M.; Mingo-Castel, A, M., Jasmonic acid-induced tuberization of potato stolons cultured *in vitro*, *Plant Physiology*, **1991,** *97*, 1253-1255.

[24]. SAGRODEV, Société Agro-développement Sétif , Algérie, **2019**.

[25]. Sarkar D.; Pandey, S, K and Sharma S., Cytokinins antagonize the jasmonates action on the regulation of potato tuber formation *in vitro. Plant Cell, Tissue and Organ Culture*, **2006,** *87*, 285-295.

[26]. Seabrook, J, E, A.; Coleman, S.; Levy, D., Effect of photoperiod on *in vitro* tuberization of potato (*Solanum tuberosum* L.), *Plant Cell. Tissue and Organ Culture*, **1993,** *34*, 43-51.

[27]. AL-Hussaini, Z, A.; Yousif, SH, A.; AL-Ajeely, S, A., The role of sucrose and light duration on *in vitro* tuberization for two cultivars of Potato (*Solanum tuberosum* L.), Int. J. *Curr. Microbiol. App. Sci*, **2015,** *4, 2*, 277-283.

[28]. Pruski, K.; Astatkie, T.; Nowak, J., Jasmonate effects on *in vitro* tuberization and tuber bulking in two potato cultivars (*Solanum tuberosum* L.) under different media and photoperiod conditions, Society for *in vitro* Biology. *in vitro Cell. Dev. Biol.-Plant*, **2002,** *38*, 203-209.

[29]. Aryakia, E.; Hamidoghli, Y., Comparison of Kinetin and 6-Benzyl Amino Purine Effect on *in vitro* Microtuberization of Two Cultivars of Potato (*Solanum tuberosum* L.), *American-Eurasian J. Agric. & Environ. Sci*, **2010**, *8, 6*, 710-714.

[30].Anjum, M, A.; Villiers, T, A., Induction of microtubers *in vitro* from stem segments of *Solanum tuberosum* L., *S. compersion* Due and *S. acaule* Bitt., *Scientia Horticulturae*, **1997,** *70*, 231-235.

[31].Amina, Kanwal.; A, A, Shoaib, K., *In vitro* microtuberization of potato (*Solanum tuberosum* L.) cultivar Kuroda-a new variety in Pakistan, *International J. Agriculture and Biol.*, **2006,** *8*, 337-340.

[32]. Ranalli, P.; Bizarri, M.; Borghi, L.; Mari, M., Genotypic influence on *in vitro* induction, dormancy length, advancing age and agronomical performance of potato microtubers (*Solanum tuberosum* L.), *Ann. Appl. Biol.*, **1994,** *125*, 161-172.

[33]. Hossein, M, J., *In vitro* microtuberisation in potato obtained from diverse sources, *Plant Tissue Cult. & Biotech*, **2005,** *15, 2*, 157-166.

[34]. Margara, J., Base de la multiplication végétatives. Les méristèmes et l'organogenèse, *Institut National de la recherche Agronomique (INRA)*, **1984.**

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