Advances in Environmental Biology, 10(7) July 2016, Pages: 90-98

AENSI Journals

Advances in Environmental Biology

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/AEB/

Influence of several substrates on growth parameters and yield of potato minitubers (*Solanum tuberusum*)

[1]Nacera Hennouni, [1]Kaouter Madi, [1]Faiza Taibi, [2]Tahar Kestali, [1]Salim Etsouri, [3]Mokhtar Boudelaa

[1]Laboratory of Research on Biodiversity and Ecosystem Pollution, ChadliBendjedid University, El-Tarf, Algeria
[2]Laboratoryof in vitro, Technical Institute of Industrial Crops and vegetable. El-Tarf, Algeria
[3]Department of Biology, Badji Mokhtar, University, Annaba, Algeria

Address For Correspondence:
Nacera Hennouni, Laboratory of Research on Biodiversity and Ecosystem Pollution, ChadliBendjedid University, El-Tarf, Algeria
E-mail: hennouni_nacera@yahoo.fr

Received 12 June 2016; Accepted 28 July 2016; Available online 25 August 2016

ABSTRACT

Background: Micropropagation product minitubers that are now the basis ofthe breeding programs and the production of potatoes in open fields **Objective:** In order to demonstrate the best substrate for crops and to obtain minituber high quality, potato *vitroplants* of Spunta variety were inoculated in MS medium (Murashige and Scoog), then transplanted into four culture mediaT1 (reference substrate) made of topsoil, peat and grape marc ; the second substrate T2 (consists of 100% peat) ; the third one T3 (made of peat and sandy loam soil) and the fourth one T4 (consists of more pozzolan peat). This first micropropagation generation is intended to obtain a sufficient amount of sound minitubers. Growth parameters (stem length, collar diameter and number of nodes) and outputs (number, diameter and mini-tubers weight) were measured. **Results:** Results were analyzed statistically (ANOVA) to show that at maturity stage, the T2 plants (100% peat) present the best growth performance (greater length of the rod, more nodes, larger collar diameter). In terms of performance parameters, transplanted seedlings into T4 substrate presented a higher number of minitubers compared to the other substrates. Minitubers from the T3 substrate plants presented the best calibre.The ANOVA test allowed us to classify different culture substrates based on individual measurements. **Conclusion:**According to these results, we notice some diversification in the behaviour of our plants following treatment. The results show that the best growing medium is T4, followed by T2 and T1 reference substrate and finally T3.

KEYWORDS: Vitro Propagation, Potato, Spunta, Middle MS, Substrates, Growth parameters, Performance Parameters

INTRODUCTION

Potato culture becomes the fourth food crop in the world after maize and wheat [1]. Food needs in Algeria are fulfilled thanks to local production supplemented by importing additional quantities of agricultural products such as cereals, vegetables, tubers and oils.

Potato production in Algeria meets consumer needs, which make us dependent on foreign imports especially in seed resources, those do not often have the qualifications and their genotype does not always fit in local soil and climate conditions. Similarly, seeds may hold some contaminants since it is well known for its sensitivity to many infections transmitted through generations by the tuber and for which no chemical control is available [2]. Potato diseases considerably lower yields and tuber quality. In vitro cultivation techniques are applied to plant biodiversity collection, propagation and conservation [3]. They allow introducing in vitro techniques, in the domain of explants species with recalcitrant seeds and vegetative propagation. They also enable production, rapid growth and high quality material scale, free from diseases in a short time and on a reduced area, more is independent of weather conditions [4; 5]. The use of in vitro culture is of great interest for

genetic resources conservation of recalcitrant seed and vegetatively propagated species. It allows the rapid production and mass at any time of the year; shortening development cycles; ease of storage and conservation (cold) of millions of plants on very small surfaces in favourable condition and free from contamination; regeneration of a degenerate plant and production of valuable biochemicals for industry .[6; 5]

Tissue culture plantlets in vitro for production of potato plants free of disease, requires expensive and highly qualified personnel technologies. However, with recent advances in biotechnology, countries importing potato plants can expect to reduce their bills. Hope comes from "micro-tubers", tiny potatoes, half a –centimetre diameter which are grown in test tubes [7].

To address these problems, Algeria has been drawn to the application of biotechnology to modernize agricultural research systems. According to [8], development in the recent past, new quality control techniques (ELISA, PCR, isoelectric, RAPD, RFLP and other procedures) combined with in vitro potato micropropagation techniques, enabled a significant increase in overall production quality. Micropropagation has also brought more flexibility and speed in the plant production process [9; 10; 8]

Direct transfer to the field of in vitro cultured plantlets is however not possible. Also, the development of various methods that transfer was necessary. Thus the acclimatization techniques and root-balled vitroplants for a transfer in the field, minituber production techniques, production of those glass-or microtubers were born. Minituber production shelter "insectproof" in organic or inorganic substrates is a promising technical production class tubers and close physiological behavior tuber "seed" traditional [11; 12; 8].

MATERIAL AND METHODS

Plant materials used in our experiments were potato vitro-seedlings of Spunta variety. The mother culture medium used for plants tissue culture is Murashige and Skoog (MS) [13]. The latter is mainly characterized by very high mineral contents, in particular potassium and a high nitrogen concentration [14]. The final solution is prepared from the mother one with the required concentrations [15].

To prevent contamination by pathogens and lead our experiment in clean conditions, the environment and culture instruments sterilization is vital [16]. Medium sterilization is performed by autoclaving at a temperature of 120°C and 15 psi pressure for 20 minutes. The *in vitro* culture technique requires such a temperature, to ensure the microbial destruction [2]. All metal instruments (pliers, points, knives…etc.) or glass (Beakers, culture tubes, Petri dishes) are coated with aluminum and paper then placed in an oven at 170°C to 200°C temperature for two hours. During handling, metal instruments are immersed in 70% alcohol and then passed over the Bunsen beak flame to burn alcohol [16]. After autoclaving the culture medium, the latter is distributed as hotas possible in trays, using a dispenser in the laminar flow at a rate of 44ml / tray.

The technique followed by culturing or seeding explants is that of [17]. Transplanting explants on culture media is carried out under the hood in optimal hygienic conditions. Sections were carefully carried out at the nodes between the plantlets having an average 5 pieces/seedling 4 weeks old. The explants are planted out in trays at a rate of 20 units per tray. Finally, all will be placed in the growth chamber where the main environmental factors are present (temperature 20 °C under fluorescent light to 16-hours day / 8 hours night for a used intensity of about 3,000 lux).

Four different culture substrates were tested namely: T1 reference substrate consists de 85% soil + 10% Grape marc Peat + 5%; the second substrate T2 is composed of 100% peat; 3rd Substrate T3 includes 50% peat + 50% soil and finally, the fourth substrate T4 consists of50% Peat + 50% Pozzolan. The latter'spH were7.42; 5.60; 7.10 and 6.92 successively. These substrates were sterilized according to the method of Messiaen&*al.*[18].

An acclimatization process vitroplants (removing agar beyond the base of plants) before planting. The trays are moved greenhouse insect-proof, previously disinfected, with an area of 144m², with 18m length and 8m width, during one week. Transplanting is carried out on the shelves, with 28 seedlings/0.5m^2microplots, with a distance of 10cm between rows and in both senses. The adopted experimental design is randomized complete block design with three replications. Greenhouse, a statement of the average daily temperature was performed throughout the experimental period. All maintenance operations carried out are irrigation, hoeing and earthing up, fertilization, plant protection treatments and replacement of failed plants.

Growth parameters measurements concerned with: The average stem length, collar diameter, number of nodes, number of branches and performance parameters such as: the number, size and weight minitubers.

Variance analysis using ANOVA allowed us to compare and classify the different types of growing media used according to their performance in microtubules.

RESULTS AND DISCUSSION

1. Effect of various substrates on the average length of the rods:

During this experiment, the growth cycle of culture, transplanting to the production of mini-tubers is 103 days; this confirms the work of [19] reports that the cycle of potato lasts 75 to 103 days. At transplanting, all the

explants had an average 07 cm stem length. This corroborates with [20] work. Throughout the growing cycle of the plant, the highest stem length was achieved in vitro plants of T2 and T3 substrates, followed by those of T4 and T1, the substrate where the growth recovery that has begun from the 2nd stage. However, arrived at maturity stage, all plants showed approximately the same length of the shaft, which left us assume that the substrate type had no influence on this parameter. This may be due to the acclimatization problems, already pointed to the potato by [21] and [22]. This phase is very important; it almost alone determines micropropagation success. It is defined as the process of plant adaptation to the environment changes. Certains authors like [23; 24; 25] report that plant growth depends on various environmental and agronomic factors such as water, temperature and light. Otherwise the same parameters were studied on rice cultivation by [26] and on the bulbs beet at different stage of maturity [27], they recorded a higher growth rate in the effect of organic ferlitizer especially during flowering and harvest phases. Also, several researchers revealed that organic manuring increased the vegetative growth and biomass production effectively [26, 28].Statistical analysis showed a highly significant effect between treatments for the first three stages where $p < 0.0001$ and an insignificant effect for the Maturity stage (Table 1, Figure 1).

Table 1: Effect of various substrates on the average length (cm) of the rods (n=15,m ± s)

	Planting	Growing	Tuber	Maturity
T 1	7,00 ± 0,00	11,07 ± 1,90	16,67± 3,07	29,47 ± 7,00
T 2	7,00 ± 0,00	14,23 ± 1,90	22,57 ± 4,38	32,93 ± 4,25
T 3	7,00 ± 0,00	12,10 ± 3,57	19,90 ± 3,40	33,33 ± 5,75
T 4	7,00 ± 0,00	9,63 ± 2,54	16,63 ± 4,06	31,00 ± 5,19

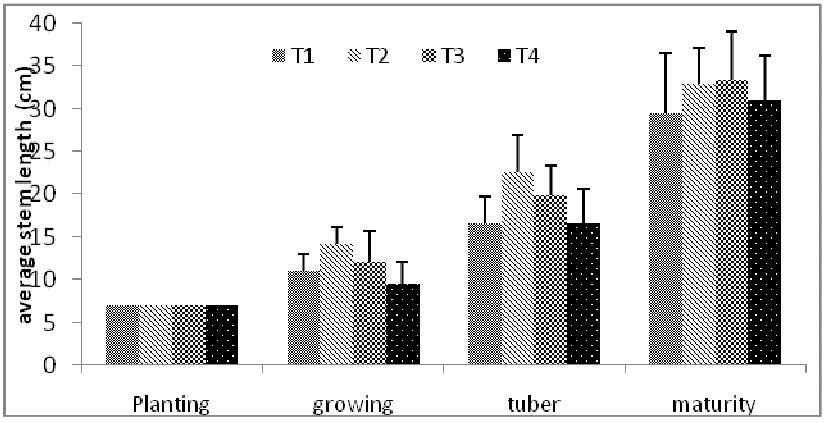

Fig. 1: Effect of various substrates on the average length of stems (cm)

2. Effect of various substrates on the collar diameter (mm):

This parameter is a force indicator. In stage transplanting all plants had the same diameter of the collar. Moreover, tuber growth stages, the diameter of T1 seed substrate is higher than those of other substrates, namely, T2, T3 and T4. However, at the stage maturity, these are the diameters of the T2 plants, T3 and T4 which exceed those planted in the first T1 culture substrate. The analysis of variance shows no significant effect between treatments at different stages (Table 2, Figure 2).

Table 2: Effect of various substrates on the diameter (mm) of the collar (n=15, m±s)

	Planting	Growing	Tuber	Maturity
T 1	1,00 ± 0,00	11,07 ± 1,90	16,67± 3,07	29,47 ± 7,00
T 2	1,00 ± 0,00	14,23 ± 1,90	22,57 ± 4,38	32,93 ± 4,25
T 3	1,00 ± 0,00	12,10 ± 3,57	19,90 ± 3,40	33,33 ± 5,75
T 4	1,00 ± 0,00	9,63 ± 2,54	16,63 ± 4,06	31,00 ± 5,19

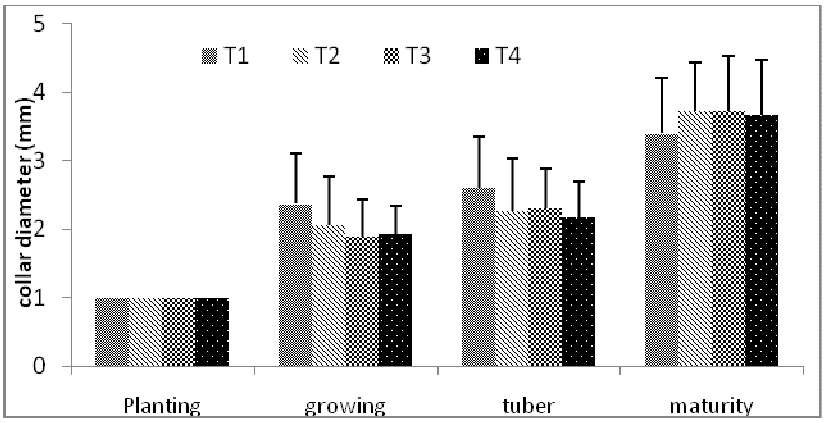

Fig. 2: Effect of various substrates on the collar diameter (mm)

3. Effect of various substrates on the average number of nodes per plant:

The largest number of nodes is presented by the plants of the second T2 substrate (21 internodes) this is proportional to the length of the stem is 33 cm. The more the stem is longer and the number of node increases. This result is confirmed by the work of [29] who reported that a length of 40 cm stem, the plant has a number of 25 internodes (Table 3, Figure 3). The statistical study showed a highly significant effect between treatments in growth stage ($p < 0.001$), a highly significant effect on tuber stage ($p < 0.0001$) and a significant effect Maturity stage ($p = 0.004$).

Table 3: Effect of various substrates on the average number of nodes per plant (n=15, m±s)

Stade	Planting	Growing	Tuber	Maturity
T 1	4,00 ± 0,00	7,20 ± 1,69	11,00 ± 2,10	19,07 ± 4,20
T 2	4,00 ± 0,00	8.00 ± 1,64	13,00 ± 2,12	21,00 ± 3,06
T 3	4,00 ± 0,00	8,00 ± 1,95	12,40 ± 2,58	21,40 ± 2,69
T 4	4,00 ± 0,00	6,00 ± 1,00	9,00 ± 1,88	17,46 ± 3,11

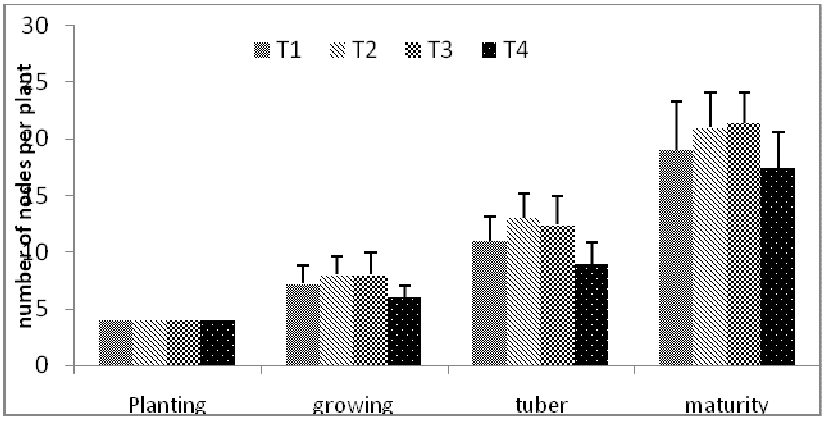

Fig. 3: Substrate variations effect on the average number of nodes per plant.

4. Effect of different substrates on performance parameters:

4.1. Number of mini-tubers per plant:

The plants with the largest number in mini-tubers are those of T4 substrate composed of peat + pozzolan, which gives up to 11 mini-tubers per plant, followed by T1 treatment with a number of 09 mini-tubers per plant. T2 and T3 treatments, by contrast, give the lowest number (07minitubercules per plant).

Our results agree with those of [19], who found that with a planting density of 33 plantlets / m2 yields 112-214 minitubers / m2, equivalent 7 minitubers / plant. In our experiment, the planting density is 56vitroplants / m2 where average production from 236 354 minitubers / m2, the equivalent of 7 to 11 mini-tubers per plant. Other authors like [7] found that the variety Spunta has an average production capacity of between 9-11 microtubers per plant. However, [4], reports that for a density of 200 plants / m2, the multiplier is quite heterogeneous: 2-6 tubers / plant. [30], point out that the number of microtubers by tissue culture plant is 1 to 2, while [31] found that in the variety Spunta, the number of microtubers can be 3 per plant. Regarding this parameter, we can conclude that whatever the physical and chemical state of the environment, plants produce

tubers. This confirms the work of [11] who found that the percentage of tuber plants of the variety Spunta is 58% in the solid medium (Table 4, Figure 4). This parameter is related to the genotype characteristics on one hand; on the other hand it may dependent on external factors [32; 33; 34].

Table 4: Effect of different substrates on the average number of mini-tubers per (n=15, m±s)

Treatments	Number of mini-tubers per plant
T 1	9,47 ± 4,41
T 2	7,13 ± 3,33
T 3	6,80 ± 3,07
T 4	11,00 ± 4,49

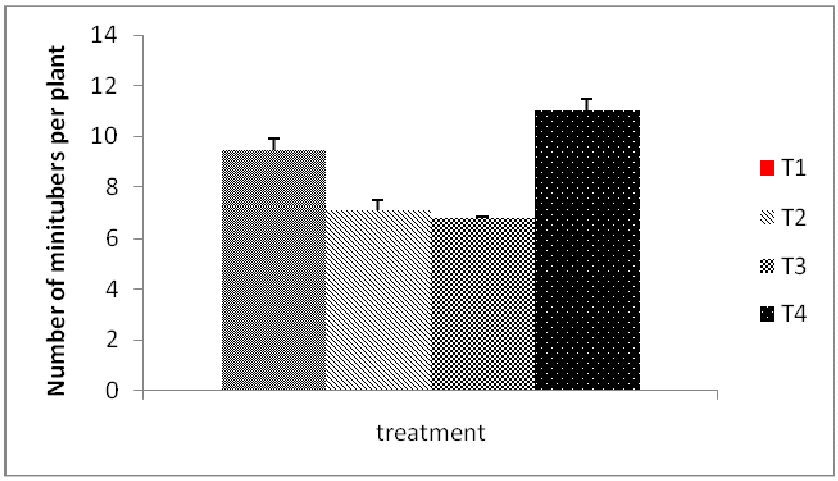

Fig. 4: substrate variations effect on the average number of minitubers per plant.

4.2. Number of tubers per treatment:

The results of this parameter indicate that the T4 treatment plants give the best number of mini-tubers with a value reaching (531 minitubers per treatment). The plants of treatment T2, T1, T4 give a much smaller number. It is respectively 390-381-355 minitubers. T4 substrate seems to have a great influence on the yield of minitubers. This is probably due to the physicochemical properties of peat mixed with pozzolan. Indeed [35], reports the use of this mixture is intended to facilitate aeration of soil, allow high water retention, improve drainage and, consequently, affects the behaviour of the plant by improving its production performance. We can say that the number of tubers produced per unit area (m2) is primarily depending on the variety and the type of substrate adopted as the physical and chemical support for the plants. Statistical analysis (ANOVA) showed a highly significant effect between treatments or $p < 0.01$ (Table 5, Figure 5).

Table 5: Effect of various substrates on number of minitubers(n=15, m±s)

Treatments	Number minituber treatment
T 1	381,00 ± 0,57
T 2	390,00 ± 2,00
T 3	354,00 ± 2,52
T 4	531,67 ± 0,57

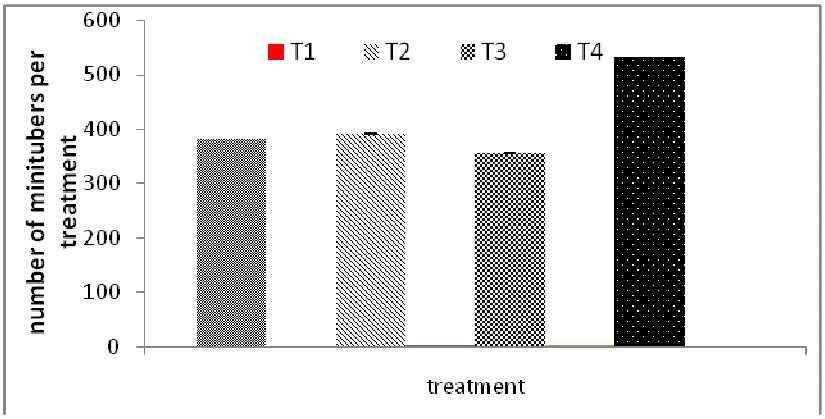

Fig. 5: Substrate variations effect on the number of minitubers per treatment.

4.3. Effect of different substrates on the average size of minitubers per treatment:

The diameter of the microtubers is related to the variety, the environment and photoperiod [31]. The different sizes obtained in our experiment are between 6 and 29 mm. These results confirm those of [19] reports that the vitro plants manage to give mini-tubers with a diameter of between 4 and 29 mm. As ranked by [36], the large caliber should be greater than 25mm; the mean size is between 10 and 25 mm, while the small size is less than 10mm. In our experiment, the large calibre is obtained on minitubers planted on the culture substrate T3 with a diameter of 29 mm. However, on the fourth substrate T4 culture, two types of gauges are observed, the average size with a diameter of 17mm and the small bore with a diameter of 6 mm. As for percentages of 03 calibres obtained, it is noted that the T2 gives approximately the same percentage of 03 types of calibre from 33 to 35%. The results achieved with the T3 indicate a high percentage (39%) in minituber medium size, followed by 38% minituber small arms and finally 23% of large calibre. The results of [7] show that transplanting plantlets at a density of 160 plants / m2 in a bed consisting of a mixture of peat and soil allows the production of 300 to 500 calibre mini-tubers ≥ 15 mm. However, the substrate T4 gives the greatest percentage (58%) in mini tubers but small caliber, 12% of high calibre and 30% of medium calibre. Regarding T1, we record a percentage of 42% of small caliber minitubers, 32% of medium size and 18% of large calibre. In practice, large size microtubers are preferred because they are easy to handle and produce vigorous plants [37; 38]. They are also less prone to drying out in storage and they have a short period of dormancy and a high survival rate when a direct transfer into the soil (Table 6).

Table 6: Effect of various substrates on the mean size (mm) minitubers per treatment (n=15, m±s)

Treatments	Large caliber	Medium caliber	Small caliber
T 1	26,00 ± 1,02	14,00 ± 0,99	8,00 ± 1,00
T 2	25,00 ± 0,95	14,00 ± 1,10	6,00 ± 0,20
T 3	29,00 ± 0,90	15,00 ± 0,89	7,00 ± 0,10
T 4	25,00 ± 0,20	17,00 ± 0,99	7,00 ± 0,10

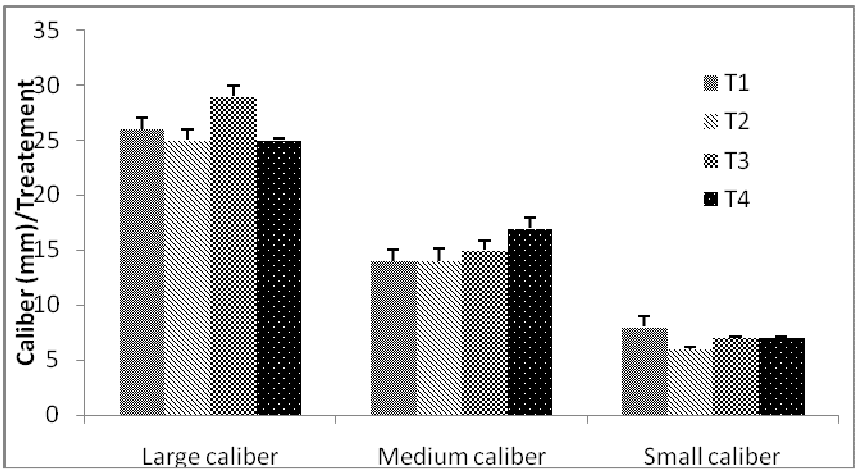

Fig. 6: Effect of different substrates on the caliber (mm) of minitubers

4.4. Effect of different substrates on the total weight of mini-tubers:

In our work, the highest total weight of mini tubers (6532 gr.) was observed in T2 substrate at a rate of 40 grams per minituber. This is explained by the high number of large calibre mini-tubers. In contrast, mini tubers T4 reach a weight of 5200 gr. despite the reduced number of search. [38], reports that the size of the tuber products not only depends on growing conditions, but also the environment is changing every runner. However, both T1 and T3 treatments have weight of around 3000 and 4000 g. The weight of their large caliber minituber is between 26 g and 32 g respectively. For medium caliber, best weight is obtained with T4 plants with a value of up to 10g per 07g minituber and those under other treatments as T1, T2, T3. As for the small size, the weight by minituber is almost identical in all treatments; it is between 1.37 and 2 g minituber. According [39], the low production of microtubers obtained may be due to the reduced amount of nutrients available to the initial explants (Table 7, Figure 6).

Table 7: Substrate variations effect on the total weight (g) minitubers

Treatments	Total weight of minitubers / treatment
T 1	2990
T 2	6532
T 3	3976
T 4	5200

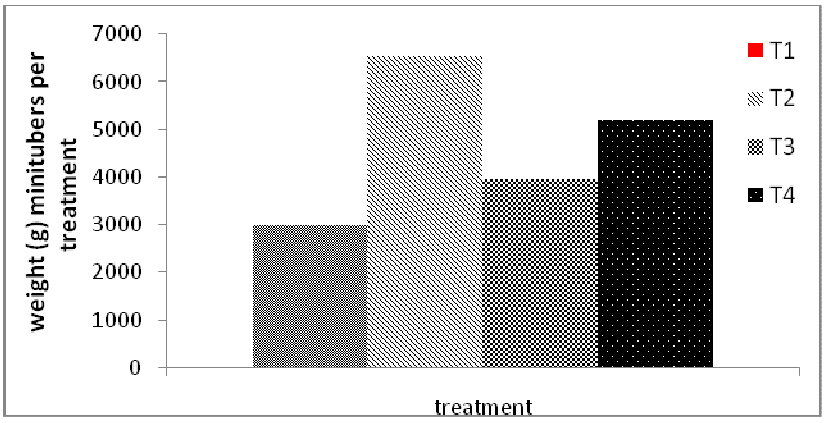

Fig. 7: Effect of Substrats variations on the total weight (g) minitubers

Conclusion:

According to these results, we notice some diversification in the behaviour of our plants following treatment. Concerning growth parameters T2 (100% peat), shows the best growth performance (greater length rod, best number of nodes, the largest collar diameter), but gives a relatively low number minituber compared to other treatments. These minitubers have a greater size and a weight than those of other substrates. T4 (peat and pozzolana), presents the most interesting performance parameters (high number of main stems, many major mini-tubers), but with a size and a lower weight. Nevertheless, we can say that these results are confirmed in standards cited in the literature. This experiment allows us to predict the exclusive use of peat and pozzolana mixture that works best for mini-tubers production but small arms provided to lift their dormancy. However, the substrate called 100% peat is the one giving the most satisfactory number of high calibreminitubers.

If one refers to the traditional agricultural field method, the mini-tubers production method from vitro-plants has the advantages:

- Obtaining mini-tubers of better health qualities
- Obtaining a satisfactory amount of mini-tubers with adequate calibre for planting in open fields.

ACKNOWLEDGEMENTS

We thank all those who helped the realization of the practical work and the writing and editing of this article. We thank agricultural farm that hosted experimentation. We thank Dr. Mahieddine BOUMENDJEL for orientations and corrections of the paper.

REFERENCES

[1] Beaulieu, C., 2008. International Year of the Potato, InfoSÈVE, 12: 10.
[2] Himour, S., 2008. Comparative study of regeneration plants vegetatively in vitro culture. Magisterium of memory. UniversityMentouri Constantine: p: 107.
[3] FAO, 2010. Matériel de micropropagation et minitubercules de pommes de terre (Solanumspp.) exempts d'organismes nuisibles et destinés au commerce international Normes internationales pour les mesures phytosanitaires NIMP 33. p: 21.
[4] Fouarge, G., 1994. Evaluation and potentiality of potato mini-tubers produced by in vitro. What next for the production of plants? Ed. AUPELF- UREF. John LibbeyEurotext. Paris, pp: 205-209.
[5] Niyonzima, P., A. Bararyenya, M. Inamahoro, E. Vyizigiro, V. Hakizimana, A. Nizigiyimana and D. Simenya, 2014. The Role of Culture in vitro Laboratory ISABU in Gisozi availing of healthy start planting material for vegetatively propagated crops. Agricultural Research: Upstream of Agriculture and Livestock in Burundi. Quarterly Bulletin, 3: 2-5.
[6] Dieme, A. and MI MO, Sagna, 2011. Influence of hormonal treatments and of sucrose on the microtuberization of three potato varieties (Solanum tuberosum L.) adapté to agroclimatic terms in Senegal. International Journal of Plant, Animal and Environmental Science, 1(3): 69-77.
[7] Barrot, P., 1990. Africa: Winning big with tiny potatoes. International Syfia 2012.
[8] Rolot, J.L., H, Seutin and D. Michelante, 2002. Production of potato mini-tubers by hydroponics: evaluation of a system combining technical "NFT" and "Gravel Culture" for two types of nutrient solutions. Biotechnol. Agron. Soc. About., 6(3): 155-161.
[9] Rossel, G., F.G., De Bertholdi, R. Tizio, 1987. In vitro mass tuberization as a contribution to potato micropropagation. Potato Res., 30: 111-116.

[10] Jones, E.D., 1991. Progress in seed production technology. Am. Potato J. 68: 247-248.
[11] Mani, F., M. Mhamedi, T. Bettaieb and C. Hannachi, 2012. Effect of sucrose on the in vitro tuber of the potato (Solanum tuberosum L.). Nature & Technology, pp: 38-43.
[12] Rolot, J.L., 2012. The in vitro micropropagation of potato: essential and sufficient technical for the development of a sector 'plant' International Exhibition of Meknes. CRA-W.
[13] Evans, D.A., W.R., Sharp, E.C. Flien, 1981. Grouth and behavior of cell cultures embryogenesis and organogenesis in plant tissue culture methods and agriculture application. Throupe T.A Ed Academic Press, pp: 45-113.
[14] Del Vesco, L.L., P.M. Guerra, 2001. The effectiveness of nitrogen sources in somatic embryogenesis Feijoa. Plant Cell Tissue and organ Culture: p: 64.
[15] Volsef, A.K. And W. Preil, 2005. Liquid Culture systems for in vitro plant propagation. Sringer., p: 588.
[16] Anonymous., 2012. Die Potato, ITCMI Bulletin 6
[17] Espinoza, N., R. Lizarraga, S.C. Siguna, J. Brayn and J.H. Dodds, 1992. Tissue culture micropropagation, conservation and export of potato germplasm. CIP Research Ghide, edition. CIP: p: 19.
[18] Messiaen, C.M., D. Blancard and F. Rouxel, 1991. Diseases truck-plants. INRA; Paris.. ISBN; 2-7380-0286-2. : p: 568.
[19] Ahloowalia, B.S., 1994. Production and performance of mini-tubers potato. Kinsealy Research Center, Agriculture & Food Development Authority. FAO; Ireland: p: 10.
[20] Vittorelli, C., O. Page, N. Sonnino, R. Pallais, H. Sawyer, J. Mendoza, S. Dodds, M. Peloquin, P. Jones, L. Van Der Zaag and E. Sikka, 1984. Innovatives methods for propagation potatoes, p: 283.
[21] Schwärzel, H.J., J.T. Lindstrom, J.A. Fiola, 1999. The use of tissue culture propagation of strawberry in the United States. - In Boxus (P.), Larvor (P.) In Vitro Culture of Strawberry Plants. - Comm. Eur. Communities, Rep. EUR 10871. EN-ER: pp: 79-100.
[22] Duong, T.N., H. Nguyen, D. Thi, 2006 A novel in vitro hydroponic culture system for potato (Solanum tuberosum L.) microtuber production . D.T. Nhut et al. / Scientia Horticulturae, 110: 230Ŕ234.
[23] Li, Q., C. Xie, Z. Feng, 2004. An analysis on the comparative advantages and export competitive capability of potatoes in China. Chin Potato J pp: 129-32.
[24] Yadav, S.H., 2008. Potato production processing and marketing, New Delhi, India.
[25] Najm, A.A., M.T. Hadi, MRHS, Darzi, F. Fazeli, 2013. Influence of nitrogen fertilizer and cattle manure on the vegetative growth and tuber production of potato. International Journal of Agriculture and Crop Sciences, 5(2): 147-154.
[26] Alagappan, S., and R, Venkitaswamy., 2016. Physiological parameters, leaf nitrogen content, nitrogen use efficiencies and grain yield of rice (oryza sativa l.) As affected by different sources organic manures in comparison with RDF and INM under sitespecific organic farming condition. American-Eurasian Journal of Sustainable Agriculture, 10(1): 38-46.
[27] Farouk, G., and H, Sharawy, Effect of different fertilizers on growth and bioconstituents of red beet bulbs during three maturity stages. Advances in Environmental Biology, 10(1): 33-41
[28] Alagappan, S., and R, Venkitaswamy., 2016. Impact of different sources of organic manures in comparison with TRRI practice, RDF and INM on growth, yield and soil enzymatic activities of rice- greengram cropping system under site-specific organic farming situation. American-Eurasian Journal of Sustainable Agriculture, 10(2): 1-8.
29] Rozier, C., 1971. Some aspects of the influence of the seed tuber and number of stems on the plant growth Potato. Items Potato Research Volume 14, Issue 3: 219.-231.
[30] Garner, N., J, Blake., 1989. The induction and development of potato microtubers in vitro on media free of growth regulating substances. Annals of Botany 63, 663-674.
[31] Kechrid M, 2005. Physiology and Biotechnology microtuberization of Potato Solanum tuberosum. L. Magisterium of Memory in Plant Biotechnology 158 p. Mentouri University of Constantine (Algeria).
[32] Seabrook JEA., S, Coleman., D, Levy., (1993). Effect of photoperiod on in vitro tuberization of potato (Solanum tuberosum L.). Plant Cell Tiss. Org. Cult. 34:43-51.
[33] Belleti, P., S, Lanter., F, LotitoSaracco. 1994. Production of potato micro-tubers through in vitro culture. ActaHorticulturae 362 Seed Research in Horticulture: 141-149
[34] SidikouDjermakoyeSeyni, R., D, Sihachakr., D, Lavergne., A, Nato., D, Ellisseche., B, Jouan., and G, Ducreux., 2003. Contribution of plant biotechnology in the adaptation of the apple potato (Solanum tuberosum L.) in the Sahel: Study microtuberization among ten cultivars. Agr notebook 12: 7-14.
[35] White D, 1987. The off-ground crops. INRA; Paris. 2ed. Pp
[36] Leclerc Y., DJ, Donnelly and J.E.A, Seabrook.,1994. Microtuberizationoflayered shoots and nodal cuttings of potato: the influence of growth regulators and incubation periods. Plant Cell Tissue & Organ Culture 37 : 113 – 120.
[37] Cong-Linh L., T, Daniel. 2010. Production of potato microtubers in vitro: effect of culture duration. AgrarforschungSchweiz 1 (11-12): 404-409.

[38] Struik, P. C., 2007. Above-ground and below-ground plant development. In: Potato Biology and Biotechnology-Advances and Perspectives. Ed Dick Vreugdenhil, Elsevier, ISBN-13:
[39] Kämäräinen-Karppinen, T., E, Virtanen., V.M, Rokka., and A.M, Pirttilä., 2010. Novel bioreactor technology for mass propagation of potato microtubers. Plant Cell Tiss. and Org. Cult. 101: 245-249.