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Original Article

Effect of Naphthaleneacetic Acid (NAA) and 6-Benzylamino Purine (BAP) on *In-Vitro* Propagation of "Mashua" (*Tropaeolum tuberosum* Ruíz & Pavón) Morphotypes from Peru

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ABSTRACT

Background: *Tropaeolum tuberosum* Ruız & Pavón "mashua" is a native species of the central Andes and different propagation methods *in vitro* have been evaluated. However, this research has studied the *in vitro* propagation of 15 mashua (*Tropaeolum tuberosum* Ruíz & Pavón) morphotypes from the Ayacucho and Apurímac regions (Peru) **Objective:** To evaluate the effect of the additives naphthaleneacetic acid (NAA) and 6-benzylamino purine (BAP) on the micro-propagation rate of *T. tuberosum.* **Material and Methods:** For *in vitro* establishment, seedlings were used after disinfection with sodium hypochlorite, propagated in Murashige and Skoog (MS) medium. For the evaluation of the effect of NAA and BAP additives, nodes were isolated from *in vitro* seedlings after 30 days of cultivation and transferred to MS medium supplemented with 3% sucrose, pH 5.6 and 7 g/L of agar according to T1 (MS + NAA 1ppm) and T2 (MS + BAP 1ppm) treatments. The samples were cultivated for 28 days at 19 °C ± 2 °C with photoperiods of 16 h/light and 8 h/darkness. **Results:** After evaluating 20 repetitions for each of the three treatments, the addition of NAA or BAP does not improve the micropropagation rate of practically all the morphotypes studied. Furthermore, BAP behaved as an inhibitor of the development of mashua seedlings. **Conclusion**: The best micropropagation medium of *Tropaeolum tuberosum* Ruíz & Pavón (mashua) is the basic Murashige and Skoog (MS) medium without the NAA or BAP additives.

Key words: *Tropaeolum tuberosum*, Mashua, Micropropagation, NAA, BAP.

INTRODUCTION

Tropaeolum tuberosum Ruız & Pavón "mashua" is a native species of the central Andes[1] (See Figure 1) and it is characterized by growing in poor, shallow soils with a pH of 5.3 to 7.5, without the use of fertilizers or pesticides.[2] Mashua is cultivated between 300 and 3800 masl, although Andean farmers generally cultivate it between 2400 and 4300 masl.[3] It is a frost and drought tolerant crop; although the optimum temperature is in the range of 8 to 11°C.[4] It is resistant to insects, nematodes, fungi and other pathogens that attack Andean tuberous roots.[5] Mashua is used by the Andean population for its nutritional and medicinal properties. Recent studies indicate that mashua has a high content of bioactive substances, such as phenolic antioxidants, glucosinolates,[6] which by the action of the enzyme myrosin are converted into isothiocyanates, sulfuranes, nitriles and thiocyanates with antibiotic, insecticide, nematicide, antineurodegenerative, diuretic and anticancer properties.[7] Mashua itself (lyophilized) contributes effectively to the reduction of benign prostatic hyperplasia.[8]

Mashua is propagated by tubers that in many cases are infected by different viruses that affect productivity in the traditional Andean propagation system.[9] Peña *et al.*,[10] have reported the propagation of Andean tuberoses by *in vitro* propagation using a temporary immersion system that allows massive propagation and conservation of diversity. This technique allows genetic manipulation and improvement, production of bioactive compounds, meristem cultivation for virus and other pathogen sanitation, embryo rescue, callus cultivation, somatic embryogenesis, protoplast fusion.[11] The success of micropropagation is associated with factors such as genotype, the physiological state of the donor plants, the type of explant, surface disinfection methods, propagation medium, growth regulators, size of propagation containers, spectral quality, light intensity, photoperiod and temperature.[12] The optimization of the components of the propagation medium and the use of growth regulators, such as NAA and BAP, is another key factor that determines the regeneration and rooting of many plants.[13] In some cases, there is a synergy of growth regulators for the formation of nodes and shoots.[14] The most widely used growth regulators in tissue cultive are the auxins AIB, 2,4-D, AIA, NAA, and the cytokinins BAP and ZEA. The concentration range used varies with the growth regulator, the species and the type of explant to be used; therefore, their combinations and concentration ranges must be optimized for each species, genotype and multiplication stage.[13]

MATERIALS AND METHODS

The research was carried out in the Laboratory of Cellular and Molecular Biology of the Faculty of Biological Sciences of UNSCH (Universidad Nacional de San Cristóbal de Huamanga), Ayacucho (13°08'43''S 74°13'16''W, 2790 masl).

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Table 1: Code data of 15 morphotypes of *Tropaeolum tuberosum* "mashua" from Peru.

Morphotype codes	Region	Province	District	Location	Altitude/m	S.E. Lat.	S.W. Long
MAC 001	Ayacucho	Cangallo	Morochucos	Condorccocha	3 609	586371.04	8513193.58
MAC 006	Ayacucho	Cangallo	Morochucos	Condorccocha	3 609	586371.04	8513193.58
MAC 06B	Ayacucho	Huamanga	Vinchos	Yaruca	3 739	555290.51	8524274.99
MAC 08A	Ayacucho	Cangallo	Morochucos	Condorccocha	3 609	586371.04	8513193.58
MAC 015	Ayacucho	Cangallo	Morochucos	Condorccocha	3 609	586371.04	8513193.58
MAC 021	Ayacucho	Huanta	Uchuraccay	Iquicha	3 802	601807.25	8582772.00
MAC 042	Ayacucho	Huanta	Uchuraccay	Iquicha	3 802	601807.25	8582772.00
MAC 051	Ayacucho	Cangallo	Morochucos	Codorccocha	3 609	586371.04	8513193.58
MAC 057	Ayacucho	Cangallo	Morochucos	Codorccocha	3 609	586371.04	8513193.58
MAC 061	Ayacucho	Cangallo	Morochucos	Codorccocha	3 609	586371.04	8513193.58
MAC 080	Apurimac	Andahuaylas	Huayana	Patahuasi	3 868	657128.19	8451202.10
MAC 083	Apurimac	Andahuaylas	Uripa	Uripa	4 060	646838.51	8500799.01
MAC 091	Ayacucho	Huamanga	Acocro	Pumapuquio	3 680	601865.03	8530546.20
MAC 092	Ayacucho	Huamanga	Acocro	Pumapuquio	3 680	601865.03	8530546.20
MAC 120	Ayacucho	La Mar	Chiquintirca	Osccoccocha	3 669	634555.00	8558368.76

Figure 1: Mashua cultivation fields in Iquicha location, Ayacucho Region – Peru (S.E. Lat. 586371.04; S.W. Long. 8513193.58) at 3 609 masl

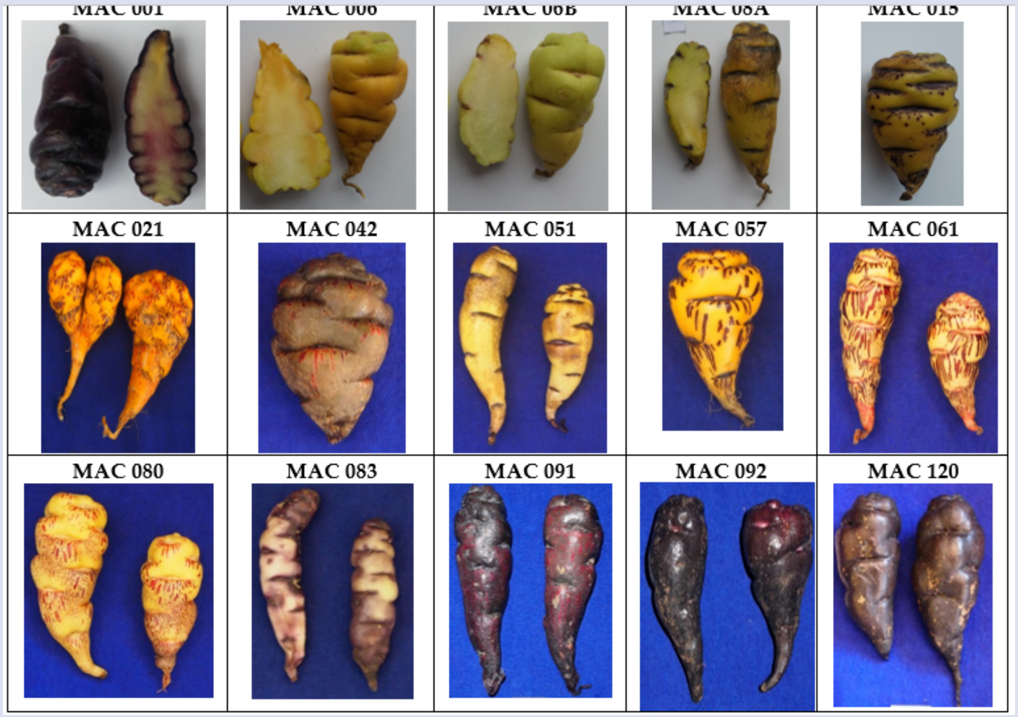

Figure 2: Tubers of 15 mashua morphotypes from the peruvian Ayacucho and Apurímac regions

Plant material. Sowing in soil

Tuber sprouts of 15 morphotypes from five Peruvian provinces at the Ayacucho and Apurímac regions were used. The tubers, described in Table 1 and Figure 2 were selected morphologically (shape and color) and planted in plastic containers of 12.5 cm in diameter by 6 cm in height containing a substrate of organic matter (black soil).

In vitro propagations of *T. tuberosum*

Fourteen days after planting the tubers in the soil, shoots of approximately 2 cm in length were removed, superficially disinfected using 70% ethanol for five minutes followed by 1.5% sodium hypochlorite for 15 minutes and then rinsed 3 times with sterile distilled water. Each nodal segment was placed in a 25 x 150 mm tube with 10 mL of MS (Murashige and Skoog,[15] propagation medium supplemented with 3% sucrose, pH 5.7 and previously sterilized the propagation medium for 15 minutes at 121 °C and 15 pounds of pressure. The tubes containing the explants in propagation medium were placed in a controlled environment at 19ºC ± 2ºC and subjected to photoperiods of 16 h/light and 8 h/darkness (Figure 3).

BAP and NAA effects on the *in vitro* mashua propagation

In vitro seedlings of the 15 mashua morphotypes, cultivated for 30 days, were used as explant donors. The explants were seeded in MS medium supplemented with 3% sucrose, pH 5.6 and 7 g/L of agar, under the same *in vitro* propagation conditions described above. NAA and BAP were added to the propagation medium in the following treatments: i/ T0, Control (without NAA and BAP); ii/ T1: MS + NAA (1ppm) and iii/ T2: MS + BAP (1ppm). 20 nodes were sown for each treatment. At 28 days, in all cases, shoot size, number of nodes (cm) and number and size of roots were evaluated.

Data analysis

The data obtained were processed in SPSS version 25.0 software and evaluated with the Kolmogorov Smirnov Test, determining that they do not have a normal distribution, so the non-parametric Kruskall-Wallis test was applied to these transformed data in average for each morphotype and in each of the three treatments (T0, T1 and T2).

RESULTS AND DISCUSSION

Effects of NAA and BAP on seedings development

The growth and development of the seedlings, number of nodes (buds) and root development were evaluated after 28 days of cultivation.

Depending of morphotype, the size of mashua seedlings without additives (T0, Figure 4) reaches values between 2.2 (MAC92) and 7.7 cm (MAC80). Five of the morphotypes (MAC8A, MAC15, MAC21, MAC80 and MAC83) exceed 5cm; highlighting MAC15 and MAC80 that exceed 7cm in size. On the contrary, three of them (MAC51, MAC61 and MAC92) reach sizes below 3cm. The addition of NAA (T1 treatment) does not improve seedling development, except for the MAC51 and MAC91 morphotypes. With this treatment the seedlings can reach values between 0.3 and 5cm; being the MAC91 the one that best responds. On the other hand, treatment T2 (addition of BAP) is worse than T1, except for MAC6B and MAC15, since none of the morphotypes exceeds 1.3cm in size. It is important to mention that MAC80 without treatment (T0) reaches a size almost 6 and 10 times larger than with T1 and T2, respectively.

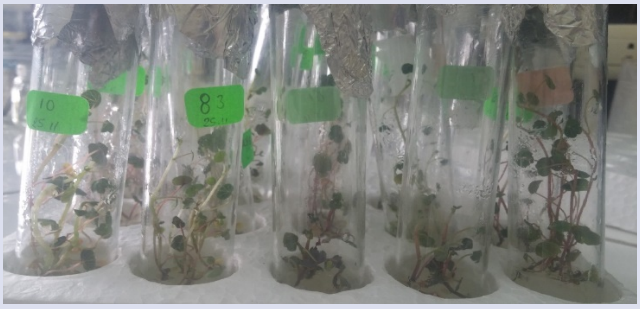

Figure 3: *In vitro* mashua-seedlings

The treatment with additives, such as NAA and BAP, for the development of shoots of tuberoses and other plants has diverse effects. Thus, Ponce *et al.*,[16] and Jiménez *et al.*,[17] reported that cytokinins do not promote the induction and growth of new shoots of, respectively, *Mutisia spinosa* and *Cissus tiliacea*. Armin *et al.*,[18] reported that the best medium to root and propagate *Solanum tuberosum* seedlings *in vitro* is MS without NAA and BAP, since the application of these additives decreases the growth and rooting of seedlings, highlighting the inhibitory character of NAA. Espinoza *et al.*,[19] found that the increase in BAP concentration does not facilitate the growth of *Curcuma longa* seedlings; also, without this additive Ruffo *et al.*,[20] reported a high multiplicative coefficient of nodal segments of *Ruta graveolens*. However, Hoyerová *et al.*,[21] reported that auxins regulate the development of apical meristem of plants and Kumlay[22] found that a small concentration of auxin (0.01 ppm) combined with gibberellic acid improves the multiplication of potato meristems. Basera *et al.*,[14] found the beneficial effect of NAA on the development of nodes and shoots of this tuber. Likewise, Chand *et al.*[23] reported a higher frequency of regeneration of *Psoralea corylifolia* seedlings with NAA and BAP supplements; and in this same line, Mejia *et al.*,[24] reported that both additives improved the propagation of *Oxalis tuberosa* Mol (oca) although the absence of NAA increased the number of shoots per explant. Dhital *et al.*,[25] reported the beneficial effects of the additives zeatin, NAA and gibberellic acid on shoot regeneration, number of shoots and roots per potato explant. In oca cultivation, Indacochea *et al.*,[26] finds differentiated behaviors using BAP. The beneficial effects of BAP were reported by Jena *et al.*[27] in the multiplication and regeneration of *Curcuma zeodoria* buds; Hajare *et al.*,[28] in potato regeneration; and Khan *et al.*,[29] in the micropropagation of *Leucaena leucopcephala* and *Tropaelum majus*, respectively.

Effects of NAA and BAP on knot formation

The number of nodes formed in the mashua seedlings developed without additives (T0, Figure 5), like in the development of its shoots, depends on the morphotype. It reaches values between 4 (MAC61) and 10 (MAC80) knots. Five of the morphotypes exceed 7 nodes (MAC21, MAC42, MAC57, MAC80 and MAC91); highlighting MAC21 and MAC80 that exceed 8 knots. On the contrary, only two of them (MAC1 and MAC61) reach values below 5 knots. The addition of NAA (T1) does not improve the formation of the number of nodes, except for the MAC91, which forms 1 more node than without treatment (T0) and is the one with the best response. In a similar way to the development of shoots, MAC80 without treatment (T0) is the morphotype that manages to form more nodes (almost three times) than with treatments T1 and T2.

Formation and growth of roots. Effects of NAA and BAP

As in the shoots and nodes development, the formation and growth of roots per mashua seedling propagated without additives (T0) depend on the morphotype (Figure 6 and 7). The average number of roots formed per seedling (Figure 6); reaches values between 1 (MAC15 and MAC92) and 3 (MAC80 and MAC91). The T1 treatment does not improve the formation of the number of roots, except for the MAC120 and MAC8A, the latter manages to form almost one more root than with T0 and it is the one that has the best response compared to the rest of the morphotypes. It is noteworthy that both MAC80 and MAC91 without treatment (T0) are morphotypes that manage to form more

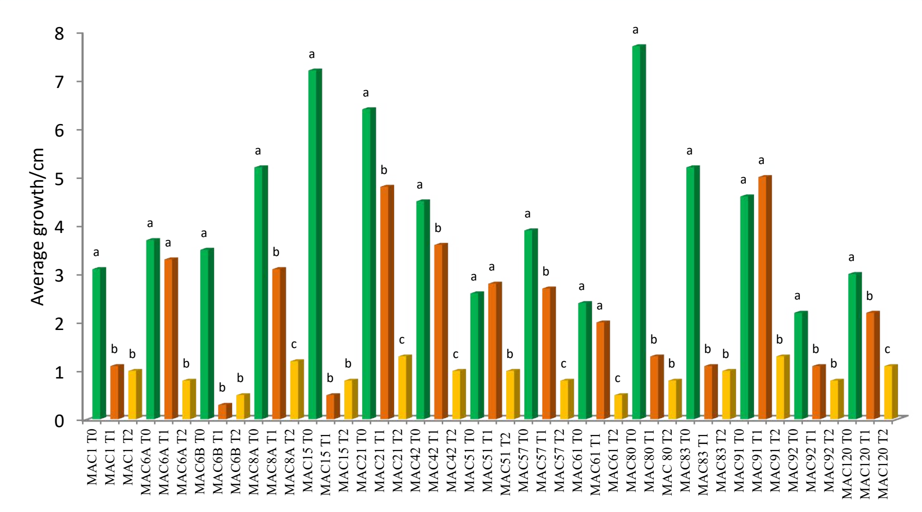

Figure 4: Comparison of average growth (in size) of 15 morphotypes of *Tropaeolum tuberosum* Ruíz & Pavón "mashua" seedlings. NAA and BAP effects: T0 (Green, no supplement), T1 (brown, +NAA) and T2 (yellow, +BAP)

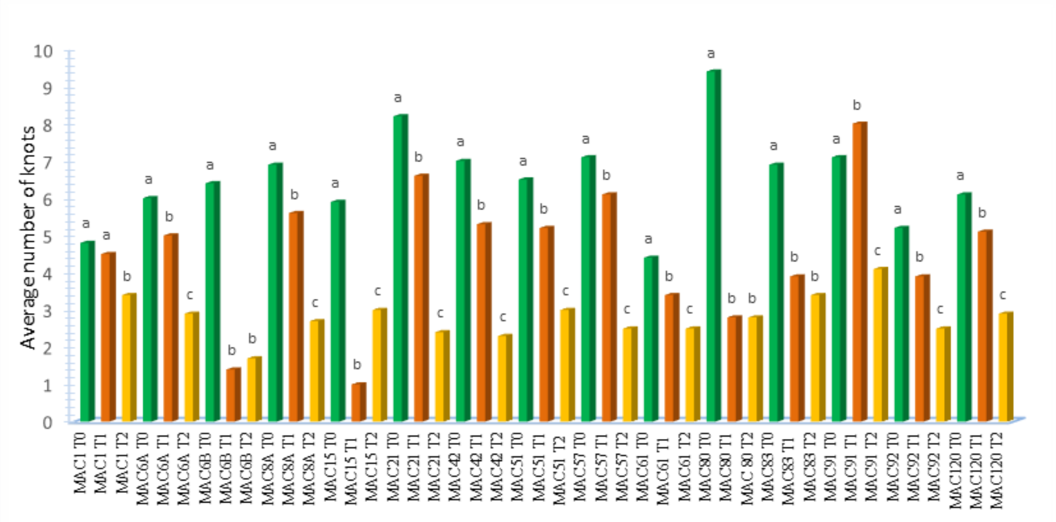

Figure 5: Comparison of the knots average number in the development of 15 morphotypes of *Tropaeolum tuberosum* Ruíz & Pavón "mashua" seedlings. NAA and BAP effects: T0 (Green, no supplement), T1 (brown, +NAA) and T2 (yellow, +BAP)

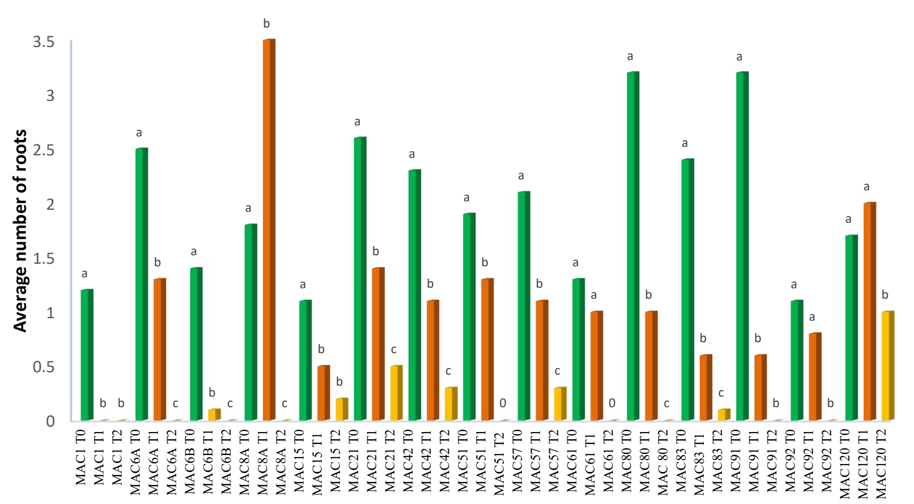

Figure 6: Comparison of the roots average number in the development of 15 morphotypes of *Tropaeolum tuberosum* Ruíz & Pavón "mashua" seedlings. NAA and BAP effects: T0 (Green, no supplement), T1 (brown, +NAA) and T2 (yellow, +BAP)

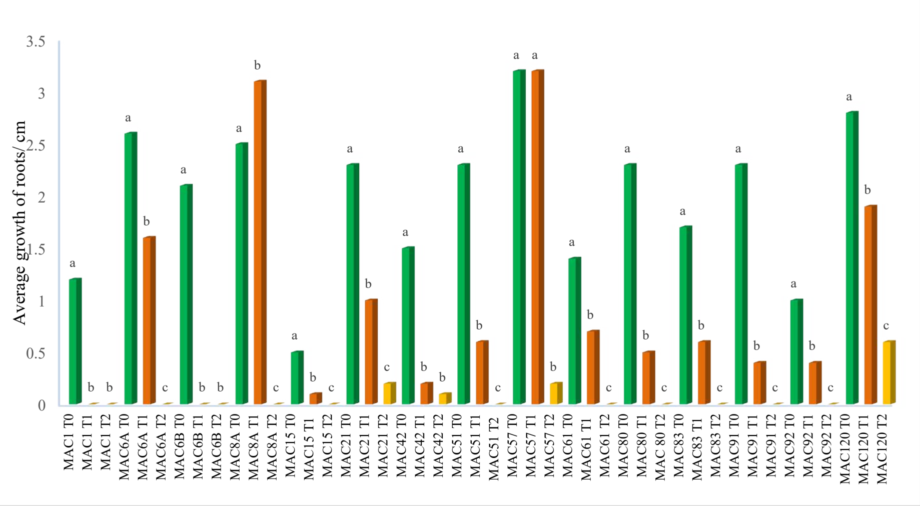

Figure 7: Comparison of the average growth (in cm) of roots of 15 morphotypes of *Tropaeolum tuberosum* Ruíz & Pavón "mashua" seedings. NAA and BAP effects: T0 (Green, no supplement), T1 (brown, +NAA) and T2 (yellow, +BAP)

than three times as many roots as with treatments T1 and T2. For almost all morphotypes, BAP is practically an inhibitor of root formation. The behavior of root growth (Figure 6) is similar to the formation of the number of roots. Without treatment (T0) it reaches values between 0.5 (MAC15) and 3.2 cm (MAC57). Except for MAC8A, the T1 treatment does not improve the growth of the roots of the rest of the morphotypes and, in all cases, T2 practically inhibits their growth.

Regarding the *in vitro* formation and growth of seedling roots, Saidi *et al.*, [30] reported that growth regulators do not improve the formation and development of potato roots. In other reported that the irrelevance of NAA and BAP in the formation of roots and stems of *Solanum tuberosum.*[28] However, several authors reported beneficial effects of additives, such as NAA and auxins, on the root formation of *Ciccus tiliacea,*[17] *Eucryphia glutinosa;*[31] and *Dioscoreas sp.*[32]

CONCLUSIONS

From the analysis of our results, we can conclude that the best micropropagation medium of *Tropaeolum tuberosum* Ruíz & Pavón (mashua) is the basic Murashige and Skoog (MS) medium without the NAA (T1) or BAP (T2) additives; being the last additive rather an inhibitor. Under T0 conditions, better development and growth of shoots, nodes and roots were obtained in practically all morphotypes. We highlight MAC80 (Patahuasi-Apurímac) as the most suitable morphotype for the studied micropropagation. On the other hand, it is important to mention that MAC91 (Pumapuquio-Ayacucho) responded somewhat better with T1 treatment, respect to T0, to the development of shoots and nodes; and the MAC8A (Condorccocha-Ayacucho) to the formation and growth of roots.

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DATA AVAILABILITY STATEMENT

Data available on request.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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