Impact of initial explants on *in vitro* propagation of native potato (*Solanum tuberosum*, Andigena group)

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Abstract

The high nutritional potential of native potatoes makes them an invaluable genetic resource for breeding. However, pathogens have caused both yield and industrial quality losses, and plant tissue culture is a promising alternative to obtain clean plant material. We compared distinct segments excised from apical and lateral sprouts taken from tubers as initial explants for *in vitro* culture of sixteen native potato genotypes. Thus, apical-distal (AD), mid-apical (MA), lateral-distal (LD), and mid-lateral (ML) segments were all grown on Murashige & Skoog (MS) medium. These explants were aseptic, reactive, and viable for all genotypes (with a probability greater than 30%), yielding cumulative proliferation rates of up to 1:10 individualizable segments, and about 86% of *in vitro* plants with 2 to 11 roots of up to 117 mm long. Responses were genotype-dependent during all stages of cultivation, and the best responding genotypes were Maravillosa, Duraznillo and Pepina Rodeo. On the other hand, AD sprouts were the best sprout type and segment for *in vitro* establishment, regardless of genotype. This is the first study of its kind with such a large range of Andean potato genotypes and should contribute to their germplasm conservation and increased multiplication efficiency.

Key Message

Distal segments of apical sprouts gave the best *in vitro* morphogenic responses for sixteen different Andean native potato genotypes.

Introduction

Solanum tuberosum (potato) is one of the most important crops in the world, being widely distributed in five continents: Africa, Asia, Oceania, Europe, and the Americas. This vegetable resource serves a major role in agriculture, economy, food security, and poverty eradication (Bradshaw and Ramsay 2009; Bradeen and Haynes 2011; Calliope et al. 2018). Grown potatoes have a large gene pool and include both native and improved varieties (Machida 2015). However, its production and commercialization are centered on a few genotypes, most of which have been genetically improved, derived from wild and native materials (Fock et al. 2000; Berdugo-Cely et al. 2017).

Native potatoes are the result of a long-term selection and diversification process from their origin in the Lake Titicaca basin on the Peru-Bolivia border (Hardigan et al. 2017). These are the oldest domesticated tubers in the Andes and it have a wide range of sizes, shapes, and colors (Tinjaca Ruíz and Rodríguez 2015). Diversity is conserved in rural communities for subsistence use and as a highly valued heritage. Some of these genotypes are considered superfoods because of their the large quantity and quality of nutrients and functional compounds (Calliope et al. 2018).

These tubers provide a rich, unique and diverse source of genetic variation (Tejeda et al. 2020). They adapt to different environmental conditions and are highly tolerant to drought, frost, subfertile soils, plagues and diseases (Calliope et al. 2018). In the present context of climate change, native genotypes

constitute an innovative alternative in favor of global food security (Lizana et al. 2021), and their study would allow the identification of “elite germplasm” for the development of breeding programs in target varieties (Calliope et al. 2018).

Around 400 native materials have been registered in Colombia and that are cultivated mainly in the departments of Nariño, Cundinamarca and Boyacá (Moreno and Valbuena 2006; Tinjacá and Rodríguez 2015). However, production systems of native potato face a lack of certified seed, technologies, and tools to strengthen its production, as well as changing market demands and high commercial competition. These limitations have influenced the cultural detriment of these potatoes and incur their possible extinction. Therefore, it is necessary to implement methods of propagation and conservation of these resources to foster their use genetic and agro-industrial use in order to increase the income of peasant farmers of the Altiplano (Tapia and Fries 2007).

Plant tissue culture has been used extensively as a successful strategy for the propagation of commercial potato varieties, and its deployment in native genotypes is a promising approach. Thus, micropropagation combined with crop diagnosis and sanitation, contributes to the conservation of native potato cultivars, the production of clean plant material, seed production, and genetic improvement programs (Agramonte 1999; Igarza et al. 2012; Valderrama et al. 2018). However, standardizing of efficient *in vitro* propagation protocols has been complex due to the strong correlation of genotype-dependent responses, physiological and phytosanitary conditions of the mother plant (Naik and Buckseth 2018), as also the selected explant type, the applied surface disinfection treatment, and the physicochemical culture conditions (Mohapatra and Batra 2017).

Here, we evaluated for the first time the effect of four types of initial explants on *in vitro* culture of a wide range of native potato genotypes. The results obtained allow us to suggest the use of distal segments of apical shoots for the massive propagation of plant material with high genetic, morphological, and phytosanitary quality. Our findings will contribute to preserve these genetic resources for future generations, to enhance global food security and to use the promising clones for further breeding programs in the light of changing environmental conditions.

Materials And Methods

The research was carried out at the BIOPLASMA Plant Tissue Culture Laboratory, Universidad Pedagógica y Tecnológica de Colombia (UPTC). The initial plant material consisted of seed tubers from sixteen native potato genotypes (*Solanum tuberosum*, Andigena group) cultivated in the Boyacá department in Colombia. The native genotypes are: *Aguacata* (Ag), *Alcarrosa* (Al), *Amapola* (Am), *Balbanera* (Ba), *Chaucha Botella* (Cb), *Duraznillo* (Du), *Macachona* (Ma), *Manzana* (Mnz), *Maravillosa* (Mar), *Mortiña Azul* (Maz), *Pacha Negra* (Pn), *Pepina Rodeo* (Pr), *Rastrera* (Ra), *Ratona Morada* (Rm), *Tornilla Crema* (Tc) and *Yema de Huevo* (Yh). For each genotype, fifteen tubers of similar sizes were randomly selected and stored until sprouting under dark conditions, with an average temperature of 17 °C and a vapor pressure deficit of 0.7.

***In vitro* establishment.**

Apical and lateral sprouts (2 to 3 cm long) were excised from the tubers and the distal and middle 1-2 cm long segments with at least one developing bud were isolated. For each genotype, 32 explants were isolated and uniformly distributed in four categories according to the sprouting type (Apical or Lateral), and segment position within the sprout (Mid or Distal), into the four categories: Apical-Distal (AD), Mid-Apical (MA), Lateral-Distal (LD), and Mid-Lateral (ML). Thus, we examined whether the type or origin of the cultured explant had any effect on the genotype's response to *in vitro* culture.

After isolation, the explants were (i) washed with tap water for 30 minutes prior to surface disinfection under a laminar flow hood; (ii) rinsed with sterile distilled water (SDW) + Tween® 20 (polysorbate) for 10 minutes; (iii) immersed in EtOH (30% v/v) for 45 seconds; (iv) rinsed again with SDW; (v) immersed in 3% (v/v) sodium hypochlorite (5.25% w/v NaClO) for 10 minutes; and finally (vi) rinsed 5 times with SDW. The disinfected portions were placed in 10 ml glass containers with 3.5 ml of MS (Murashige and Skoog 1962) basal culture medium. The asepsis, reactivity, and viability of explants were evaluated every 5 days until day 25. To evaluate the effect of the explant on the *in vitro* culture response throughout all the stages, the traceability of the initial explant (AD, MA, LD, or ML) origin was maintained.

Sprout multiplication

Apical portions (1-2 cm long) with at least one bud were excised from these established explants. For each category (AD, MA, LD, and ML), between 1 and 9 such portions were cultured in glass containers with 15 ml of MS medium (subculture #1), and subcultured monthly (#1, #2 and #3) for multiplication. At each subculture, the thickness of the main stem (thin: < 1 mm and thick: > 1 mm), the total length of the explant (mm), the number of secondary stems, number of nodal segments (NS), and the cumulative proliferation rate (PR) were recorded.

For each genotype, the number of nodal segments (NS) (shoot of ~1.5 cm with at least one bud) in each subculture was calculated as $NS = S_o$ = obtained nodal segments and S_c = nodal segments initially cultured. Additionally, the relationship between the number of nodal segments (NS) per nodal segment initially cultured (S_c) was determined to know the cumulative proliferation rate (PR).

Sprout rooting

From the plant material obtained in multiplication, 4-6 apical segments (1-2 cm long and with at least one bud) of each category (AD, MA, LD, and ML) were grown on MS medium. The presence of the main root, secondary roots, and adventitious roots was evaluated after 20 days of culture. In addition, the root number and the total root length (mm) per explant were determined.

Growing conditions

The pH for all culture media was adjusted to 5.7 prior to autoclaving at 15 psi and 121 °C for 20 min. The cultured explants were incubated at 24 ± 1 °C with continuous light ($70-80 \mu\text{mol m}^{-2} \text{s}^{-1}$) supplied by 75W

fluorescent lamps.

Statistical analysis

Asepsis, reactivity, viability, stem thickness, main root formation, secondary roots, and adventitious roots values were analyzed by logistic regression using PROC LOGISTIC. A variance analysis of total explant length and root length was performed using PROC GML. The number of secondary stems, the number of nodal segments (NS), and the number of roots was analyzed with a generalized linear model using a Poisson distribution as a link function using PROC GENMOD. For ANOVA and generalized linear model, once significance was tested, Tukey's adjusted least-squares mean comparisons were performed. Analyses were performed using SAS University Edition Software (SAS Institute Inc. 2019) with a confidence level of 95 %.

Results

Genotype, sprout, and sprout segment determined the asepsis, reactivity, and viability of explants.

We evaluated the effects of four different initial explants (AD, MA, LD, and ML) on the *in vitro* establishment of native potato genotypes during 25 days (Figs. S1-3). The distal segments provided the highest number of aseptic explants (probability greater than 50%) in 75% of the genotypes studied (Fig. 1). All types of explants evaluated permitted to establish aseptic cultures *in vitro*. However, the differential response was influenced by time of culture the genotype and sprout segment ($p > 0.001$) (Table S1). The highest rates of reactivity (R: $p > 0.0001$) and viability (V: $p > 0.0070$) were observed with AD segments, where 70% of genotypes gave values higher than 60% (Fig. 1, Table S1). AD segments from *Mnz*, *Pn*, *Du*, *Tc*, *Yh*, *Pr* and *Rm* genotypes yielded the greatest number of aseptic *in vitro* plants (Fig. 1, 2). Additionally, it was interesting to observe that the use of 30% ethanol favored a better response for *in vitro* culture (Table S2).

Sprout multiplication was influenced by genotype.

In order to determine the effect of the four different types of initial explant on sprout multiplication we recorded the thickness of the main stem, the explant length, and the number of secondary stems and of nodal segments for all the native potato genotypes (Tables S3-5). For all genotypes it was possible to multiply the material by clonal culture of nodal segments, with between 2 and 6 segments individualized per subculture and genotype (Fig. 3). In terms of the number of nodal segments, the genotype was statistically significant ($p < 0.0001$) (Table S6). In fact, the genotype was the only factor that influenced the response of each native potato studied. Additionally, to define the cumulative proliferation rates for each genotype we evaluated the number of the nodal segments during 90 days. We found that the cumulative proliferation rates were from 1:5 to 1:10 segments per explant, and that *Pr*, *Mar* and *Rm* were the genotypes with the highest cumulative proliferation rates (Fig. 4).

AD and ML were the best explants in terms of rhizogenic response.

We evaluated the rhizogenic response of the different explant types through the presence of main, secondary, and adventitious roots and number and total length of roots after 20 days of culture (Table S7-9). About 86% of shoots rooted for all initial explants tested. However, the highest roots number (2 to 11) and the longest roots (up to 117 mm) were obtained from AD and ML explants (Fig. 5). The genotype ($p<0.0001$) and sprout segment ($p=0.0138$) were the only factors that influenced rhizogenic responses (Table S8-9). *Du*, *Ma*, *Mar* and *Mnz* were the genotypes with the best rooting responses using AD and ML initial explants.

During the different stages of *in vitro* culture, a better morphophysiological response was observed with the AD explant in genotypes *Mar*, *Pn*, *Pr* and *Rm*, which also showed the best regenerative capacity and establishment of aseptic *in vitro* plants with high proliferation rates (Table S10 and Fig. S4).

Discussion

The ontogenic characteristics of the plant, and the physiological state and position of the shoots with respect to the apex can significantly influence the morphophysiological response of explants *in vitro* (Aguilar et al. 1992; Chanatásig 2004). Therefore, it was relevant to know the impact of the initial explant such as AD, MA, LD, and ML on the *in vitro* response of this native potato genotypes, not yet studied.

Our work allows to fill the knowledge gap regarding the *in vitro* response of native genotypes during all phases of micropropagation, since most of the studies mainly report results for commercial varieties in some stages of the process. The results highlight the need for an adequate selection of the initial explant type, as this was a determining factor both to obtain a high percentage of aseptic, reactive, and viable cultures, as well as for the regeneration and growth of sprouts and roots throughout the *in vitro* culture stages evaluated.

In the establishment stage, the AD segments allowed the recovery of a greater quantity of plantlets, with a viability even higher than reported by Badoni and Chauhan (2010), Fawzia et al. (2015) and Shahriyar et al. (2015). The results of these investigations are comparable with ours because they evaluated the *in vitro* establishment of potato, however they did not study the impact of the initial explant in neither of the micropropagation stages. The response observed in our study can be attributed to the presence in the AD explant of the shoot apical meristem (SAM), whose morphogenic activity allows continuous and rapid cell division, and leads to the fast formation and growth of *in vitro* plants (Rzepka-Plevnes et al. 2009; Huamán et al. 2012). Furthermore, the gibberellins content in the SAM would favor cell differentiation, longitudinal growth and cytoskeletal rearrangement (Gao et al. 2017), while auxins would allow the initiation of lateral organs and roots (Ha et al. 2010).

Likewise, the high percentages of asepsis observed in this study were also influenced by the presence of the SAM. The high metabolic activity in these tissue influences the non-uniform distribution of microorganisms in plants, with a progressive reduction from the base towards the apex of the stem (Hernández and González 2010). Although high asepsis was obtained, it must be noted that the number of aseptic explants decreased with time in culture. This could be attributed to the occurrence of

endogenous microbial contamination and/or to the production and accumulation of phytotoxins, which can cause tissue necrosis and a consequent decrease in reactivity and viability of the explants (Slavov 2005; Tekielska et al. 2019).

On the other hand, we observed that for all genotypes, a high proliferation rate and a large number of vigorous plants were obtained. Furthermore, the evaluated genotypes showed development characteristics superior to those reported for other potato cultivars and varieties (García-Águila et al. 2015; Tacoronte et al. 2017; Araque Barrera et al. 2018; Valderrama et al. 2018; Xhulaj and Gixhari 2018), which could be attributed to a genotype-dependent response.

In terms of rooting, this research confirmed that the endogenous hormone concentrations (mainly auxins in the SAM) of native genotypes ensure root growth and development without the addition of external inducers (Ha et al. 2010). In our study, 100% of the evaluated *in vitro* plants showed some rhizogenic response, highlighting a high number of roots in explants derived from AD segments. The formation of the *in vitro* root system influences anchorage to the substrate and water absorption when plants are established *ex vitro*, and therefore, it can improve their survival in the acclimatization stage (Arellano et al. 2010; Mukul and Ginzberg 2020).

A differential *in vitro* response between genotypes was apparent in this study, as also previously reported by Mroginski et al. (2010), Naik and Buckseth (2018) and Singh (2018). Some genotypes such as *Mar*, *Pn*, *Pr* and *Rm* showed a differential response capacity during the propagation stage similar or even superior to commercial varieties, which would allow the identification of “elite germplasm” to be used as parents in future breeding programs (Calliope et al. 2018). Likewise, the genetic improvement of plants together with new technologies (e.g., haploid and double haploid) would allow obtaining crops with high productive potential and desired agronomic traits with enormous benefits to the local producer and consumer (Núñez-Zarantes 2020).

This research reports, for the first time, the impact of the initial explant on the efficient propagation of native potato genotypes. Our results show the relevance of the adequate selection of the initial explant (AD) from the beginning of the process to guarantee a large number of plantlets, with high multiplication rates and rhizogenic capacity. This work constitutes a useful tool for the establishment of *in vitro* germplasm banks and micropropagation systems extrapolatable to other cultivars and/or potato varieties with food relevance. Thus, our results provide a reliable method to obtain plants of high genetic, morphological, and phytosanitary quality at any time of the year, thereby decreasing operating and production costs.

It is expected that this research will promote studies towards the industrial use and conservation of native potatoes cultivated in Colombia. In this way, the use of these forgotten genetic resources will be able to contribute to the food security under the current changing climatic conditions. As well as with the preservation of traditional cuisine, the creation of new gastronomic trends and their cultural and traditional value will be highlighted. Additionally, this study underlines the need to deepen the

multidisciplinary research of local genotypes, with a view to granting and dignifying the potential of these Andean tubers, which are part of the cultural and agronomic heritage of South America.

Declarations

Conflict of interest

The authors have no conflict of interest.

Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals

Not applicable.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available online in “Link Supporting data - Correa et al. 2021”.

Author contributions

LC, DG, MB EA, JU, DA and ZO contributed to the study conception and design; JU analyzed the data; LC, DG, MB, and EA interpreted the data and wrote the manuscript with contributions of all authors. All authors read and approved the final manuscript.

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Figures

Figure 1

Response of native potato genotypes during the *in vitro* establishment stage. Aseptic (a), reactive (b), and viable (c) explants at 20 days of culture (predicted probability) according to initial explant type: Apical-Distal (AD), Mid-Apical (MA), Lateral-Distal (LD), and Mid-Lateral (ML). **Ag:** *Aguacata*, **Al:** *Alcarrosa*, **Am:** *Amapola*, **Ba:** *Balbanera*, **Cb:** *Chaucha Botella*, **Du:** *Duraznillo*, **Ma:** *Macachona*, **Mnz:** *Manzana*, **Mar:** *Maravillosa*, **Maz:** *Mortaña Azul*, **Pn:** *Pacha Negra*, **Pr:** *Pepina Rodeo*, **Ra:** *Rastrera*, **Rm:** *Ratona Morada*, **Tc:** *Tornilla Crema* and **Yh:** *Yema de Huevo*

Figure 2

Page 12/14

Explant source plant material (tuber and sprouting tuber), and *in vitro* plants established from AD explants, corresponding to sixteen native potato genotypes. **Ag:** *Aguacata*, **Al:** *Alcarrosa*, **Am:** *Amapola*, **Ba:** *Balbanera*, **Cb:** *Chaucha Botella*, **Du:** *Duraznillo*, **Ma:** *Macachona*, **Mnz:** *Manzana*, **Mar:** *Maravillosa*, **Maz:** *Mortaña Azul*, **Pn:** *Pacha Negra*, **Pr:** *Pepina Rodeo*, **Ra:** *Rastrera*, **Rm:** *Ratona Morada*, **Tc:** *Tornilla Crema* and **Yh:** *Yema de Huevo*

Figure 3

Identifiable Nodal segments in three subcultures per genotype and initial explant type: Apical-Distal (AD), Mid-Apical (MA), Lateral-Distal (LD), and Mid-Lateral (ML). Different letters between genotypes indicate statistically significant differences, according to the least significant difference (LSD) multiple comparison test ($p \leq 0.05$). **Ag:** *Aguacata*, **Al:** *Alcarrosa*, **Am:** *Amapola*, **Cb:** *Chaucha Botella*, **Du:** *Duraznillo*, **Ma:** *Macachona*, **Mnz:** *Manzana*, **Mar:** *Maravillosa*, **Maz:** *Mortaña Azul*, **Pn:** *Pacha Negra*, **Pr:** *Pepina Rodeo*, **Ra:** *Rastrera*, **Rm:** *Ratona Morada*, **Tc:** *Tornilla Crema* and **Yh:** *Yema de Huevo*

Figure 4

In vitro response of native potato genotypes during the multiplication stage. **A.** Cumulative number of culturable shoots per genotype and initial explant: Apical-Distal (AD), Mid-Apical (MA), Lateral-Distal (LD), and Mid-Lateral (ML). **B.** Proliferation rate and mean shoot length; cumulative values after three subcultures (90 days). **Ag:** *Aguacata*, **Al:** *Alcarrosa*, **Am:** *Amapola*, **Cb:** *Chaucha Botella*, **Du:** *Duraznillo*, **Ma:** *Macachona*, **Mnz:** *Manzana*, **Mar:** *Maravillosa*, **Maz:** *Mortaña Azul*, **Pn:** *Pacha Negra*, **Pr:** *Pepina Rodeo*, **Ra:** *Rastrera*, **Rm:** *Ratona Morada*, **Tc:** *Tornilla Crema* and **Yh:** *Yema de Huevo*

Figure 5

Root development in potato explants for each genotype and per type of initial explant: Apical-Distal (AD), Mid-Apical (MA), Lateral-Distal (LD), and Mid-Lateral (ML). **Ag:** *Aguacata*, **Al:** *Alcarrosa*, **Am:** *Amapola*, **Cb:** *Chaucha Botella*, **Du:** *Duraznillo*, **Ma:** *Macachona*, **Mnz:** *Manzana*, **Mar:** *Maravillosa*, **Maz:** *Mortiña Azul*, **Pn:** *Pacha Negra*, **Pr:** *Pepina Rodeo*, **Ra:** *Rastrera*, **Rm:** *Ratona Morada*, **Tc:** *Tornilla Crema* and **Yh:** *Yema de Huevo*. Initial explants that are not represented for some of the genotypes were not grown at the rooting stage.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SMF2.docx](#)
- [SMT1.docx](#)