Improvement of sweet pepino (*Solanum muricatum* Aiton) crop by agri-biotechnological methods in Cotopaxi-Ecuador

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Abstract

This project addresses the interspecific gene flow from *S. caripense* to *S. muricatum*, for the development of improved fruits and certified seeds. The concentration of plant growth regulators (a1c1: 1.0 mg·L^{-1} AIA and 1.0 mg·L^{-1} BAP; a.5c2: 0.5 mg·L^{-1} AIA and 2.0 mg·L^{-1} BAP) was evaluated physiologically *in vitro* (w: with plastic seal; wo: without plastic seal) in NG (triple hybrid) and GP (double hybrid), and the new genotypes were morphologically differentiated. Varieties of tzimbalo FG, GH, and GPGn were used. The treatments were analyzed using quantitative characters, and genotypes were differentiated by analysis of variance. The number of leaves was different (mean ± S.E.), NGa1c1w = 11.38 ± 0.33 (B), GPa1c1w = 8.92 ± 0.59 (A), NGa1c1wo = 7.95 ± 0.44 (A), and GPa0c0w = 7.50 ± 1.44 (A); stem height was different, NGa1c1w = 4.77 cm ± 0.12 (B), GPa1c1w = 4.11 cm ± 0.21 (AB), GP0c0w = 3.90 cm ± 0.52 (AB), and NGa1c1wo = 3.38 cm ± 0.16 (A). In experiment two, the number of leaves was different due to the effect of plastic seal. In the meta-analysis, the number of leaves was different, TM1 = 16.13 ± 1.35 (D), GPa1c1w = 8.92 ± 1.10 (ABC), GPa0c0wo = 7.50 ± 2.71 (AB), and GPa.5c2wo = 7.47 ± 0.64 (A); and for climatization stage plantlet height was different, TE3 = 34.17 cm ± 1.49 (C), GPa1c1w = 12.12 cm ± 1.72 (A), and GPa.5c2wo = 9.37 cm ± 0.99 (A). The impact of this approach is the obtention of fruits with higher commercial weight, FG = 6.04 g/fruit, Sm800 = 27.48 g/fruit, and NG = 22.03 g/fruit; accumulation of anthocyanins genes; and soluble solids content, FG = 10.67 ± 0.27 °Brix (C), Sm800 = 8.00 ± 0.27 °Brix (A), and NG = 9.25 ± 0.19 °Brix (B).

Keywords: plant breeding; interspecific crosses; phytohormones; *in vitro*; flavour; population.

1. Introduction

The tzimbalo fruit has many seeds, its high germination percentage (Prohens et al. 1999; Morales and Vaca 2016), allows discard the presence of primary dormancy and physical lethargy (Bithell et al. 2002), in contrast to seeds from other species of the genus *Solanum* (Ibrahim et al. 2001; Taab 2009); In addition, the fruit contains more sucrose, vitamin C (Prohens et al. 2005), and minerals, compared to modern varieties of sweet pepino and wild species of the series *Caripensia*. Additionally, the phenolic content of the tzimbalo fruit is considerably higher than that of melon (*Cucumis melo*) and cucumber (*Cucumis sativus*) (Prohens et al. 2016); the fruit of these species is utilized as an antiscorbutic due to the high content of vitamin C, which is higher than that of most fruits (Debbarma et al. 2017); some materials are developed for diabetic people due to its low content of calories (Levy et al. 2006).

The specific objectives were: 1) assess the concentration of indole acetic acid (IAA), and 3-benzyl amino purine (BAP) physiologically *in vitro* in NG (triple hybrid) and GP (double hybrid); 2) differentiate morphologically the new genotypes; 3) consolidate *in vitro* technics as a powerful tool for breeding programmes through meta-analysis; 4) test SSC in the new genotypes generated.

Technological innovations with the potential to scale benefit farmers through cutting-edge insights into plant tissue culture (Cavusoglu and Sulusoglu 2013; Morales and Vaca 2016), gene expression (PCR tests) (Ballou et al. 2007; Payyavula et al. 2015; Morales et al. 2020; Morales et al. 2021), sequencing or bioinformatics (Zhang et al. 2014; Herraiz et al. 2016; Morales and Chiluisa-Utreras 2022), integrated to traditional plant breeding methods (Rodríguez-Burruezo 2003; Camarena et al. 2014). The high nutritional value and exotic fruity aroma of sweet pepino and tzimbalo fruits (Rodríguez-Burruezo et al. 2011), the high commercial value devoted to local and international markets, the possibility and the fact of developing agro-industrial products (García-García 2017; Morales and Andrade 2023), support this project.

The method is based on interspecific and intraspecific crosses, integrated to biotechnological procedures (Blas et al. 2010; Camarena et al. 2014; Morales and Chiluisa-Utreras 2022; Morales and Andrade 2023). The yield of interspecific hybrids with *S. muricatum* x *S. caripense* and *S. muricatum* x *S. tabanoense* (30-40 t/ha) is comparatively higher than that of their wild parents, with intermediate fruit weight (40-60 g) superior to that of its wild progenitors; *S. caripense* (tzímbalo) and *S. tabanoense* have high soluble solids (SSC) content (10-14%) (at least 8 ºBrix to be acceptable) (Rodríguez-Burruezo et al. 2003; 2011). The close phylogenetic relationship between tzímbalo and pepino dulce makes viable the conservation and use of these genetic resources in plant breeding processes (Prohens et al. 2003; Rodríguez-Burruezo et al. 2011; Herraiz et al. 2016). In Ecuador there are projects that support the export of sweet pepino to Bolivia (Fernandez 2013), Germany (Erazo 2014), and Japan (Pacheco 2015). Indeed, sweet pepino traditionally grown in Carchi-Ecuador is exported to USA (Revista Líderes 2021).

2. Materials and Methods

2.1 Plant Material

Selected material of *S. caripense*, varieties FG (Frequency Gene), GH (G Hybrid), and GPGn (G Purple, Generation n), and their progenies NG (New Generation, triple hybrid) and GP (G Purple, double hybrid) were utilized through the company GENNBIO (Quito, Ecuador); a total of 122 plants were grown.

2.2. Growing conditions

The seeds germinated in plastic vessels containing peat, and sealed to maintain the humidity of the internal microenvironment, under laboratory conditions with temperature at 22 ± 2 °C, and photoperiod of 16 hours of light and 8 hours of darkness. In proliferation stage, seedlings were subcultured in plastic pots using the same substrate and adding growth regulators (a1c1: 1.0 $mg \cdot L^{-1}$ AIA and 1.0 $mg \cdot L^{-1}$ BAP; a.5c2: 0.5 $mg \cdot L^{-1}$ IAA and 2.0 $mg \cdot L^{-1}$ BAP), physiologically *in vitro* (w: with plastic seal; wo: without plastic seal) (Ioannidis et al. 2022). Subsequently, indole butyric acid (IBA) solution was applied, plus micronutrients, and the new genotypes were acclimatized at 2719 meters above sea level (m.a.s.l.) in greenhouse in Cotopaxi-Ecuador.

2.3. Data Analysis

Agronomic characters of stem length, number of leaves, and number of nodes in proliferation stage were measured, physiologically *in vitro*. The analytical measurements were randomly repeated for each hybrid studied. Data were arranged under a completely randomized design

(CRD), using the analysis of means within an additive linear statistical model. Statistical packages InfoStat 2016, Minitab 16, and RStudio 4.1.2 were used.

3. Results and Discussion

NG (triple hybrid) is F1 progeny of Sc4-pl.1 - Sc4 x Sm800 (FG x Sm800).
GP (double hybrid) is F1 progeny of G1 - Gennbiotz x GenPurpura – Gennbiotz (GH x GPGn).

3.1 Experiment one

It was demonstrated that the stem height (mean ± S.E.) in proliferation stage is statistically different between treatments; the experimental point NGa1c1w = 4.77 cm ± 0.12 (B) was superior, followed by GPa1c1w = 4.11 cm ± 0.21 (AB), GPa0c0w = 3.90 cm ± 0.52 (AB), and NGa1c1wo = 3.38 cm ± 0.16 (A) (Figure 1). Errors have normal distribution with W = 0.97879 and p-value = 0.2642 applying Shapiro-Wilk normality test; and homogeneity of variances exists with p-value = 0.1488 using Bartlett's test.

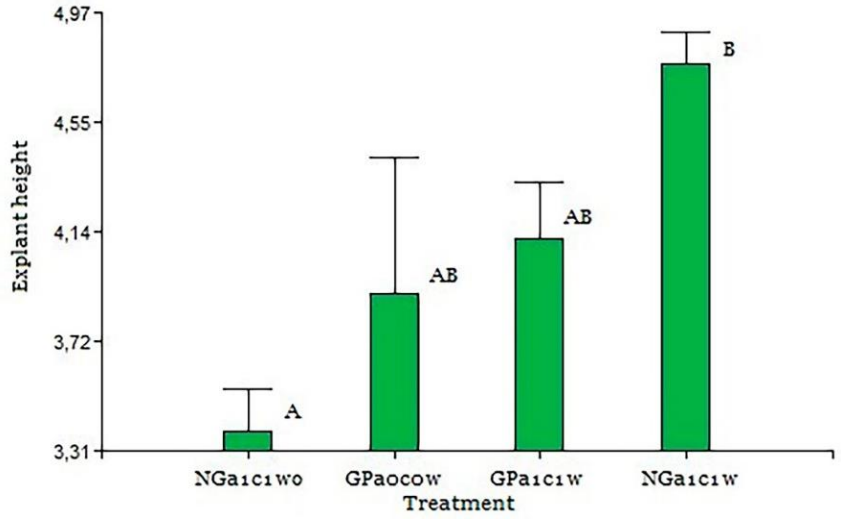

Figure 1. Stem height (mean ± S.E.) in NG (triple hybrid) and GP (double hybrid) treated with different phytohormonal concentrations in proliferation stage. Different letters demonstrate significant differences using Duncan's test (p-value < 0.05).

The effect of treatments is significantly different for stem height, with p-value = 4.38e-08 *** (Table 1); and C.V. = 17.35%. NGa1c1w = 4.77 cm ± 0.12 (B) was superior due to growth regulators and plastic seal (Morales and Vaca 2016), and its genotype which is part of *S. muricatum*, and genes from *S. caripense* corresponding to the largest plants, in contrast to GPa1c1w = 4.11 cm ± 0.21 (AB) that was obtained through intraspecific crosses between intermediate and large plants of *S. caripense* (Morales et al. 2021).

Table 1. ANOVA of steam height in NG (triple hybrid) and GP (double hybrid).

Source of variation	D.F.	M.S.	F-value	p-value
Treatment	3	8.778	16.3	4.38e-08 ***
Residuals	68	0.539		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

The number of leaves (mean ± S.E.) in proliferation stage was statistically different between treatments; the experimental point NGa1c1w = 11.38 ± 0.33 (B) was superior, followed by GPa1c1w = 8.92 ± 0.59 (A), NGa1c1wo = 7.95 ± 0.44 (A), GPa0c0w = 7.50 ± 1.44 (A), (Figure 2). Errors have normal distribution with W = 0.97683, and p-value = 0.2052; and homogeneity of variances exists with p-value = 0.1539.

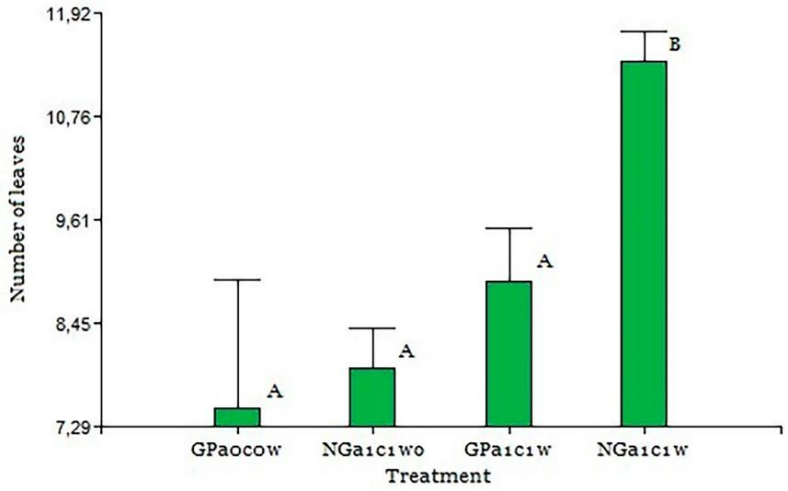

Figure 2. Number of leaves (mean ± S.E.) in NG (triple hybrid) and GP (double hybrid) treated with different phytohormonal concentrations in proliferation stage. Different letters demonstrate significant differences using Duncan's test (p-value < 0.05).

The effect of the treatments demonstrates significant differences in the number of leaves, with p-value = 1.62e-07 *** (Table 2); and C.V. = 20.62%. As above, NGa1c1w = 11.38 ± 0.33 (B) was superior due to growth regulators (rate 1:1), plastic seal, and its genotype, and followed by GPa1c1w = 8.92 ± 0.59 (A) corresponding to intraspecific crosses. In previous studies with *S. caripense*, the higher number of leaves was obtained through 0.5 mg·L^{-1} BAP (TM1), followed by 0.5 mg·L^{-1} AIA and 0.5 mg·L^{-1} BAP (TM4), under controlled conditions (Morales and Vaca 2016).

Table 2. ANOVA of number of leaves in NG (triple hybrid) and GP (double hybrid).

Source of variation	D.F.	M.S.	F-value	p-value
Treatment	3	61.18	14.8	1.62e-07 ***
Residuals	68	4.13		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Finally, the number of nodes (mean ± S.E.) in proliferation stage was statistically different between treatments (Figure 3), with C.V. = 19.96%. Errors seem to have normal distribution with W = 0.96669, and p-value = 0.05331; and no homogeneity of variances exists with p-value = 0.005469.

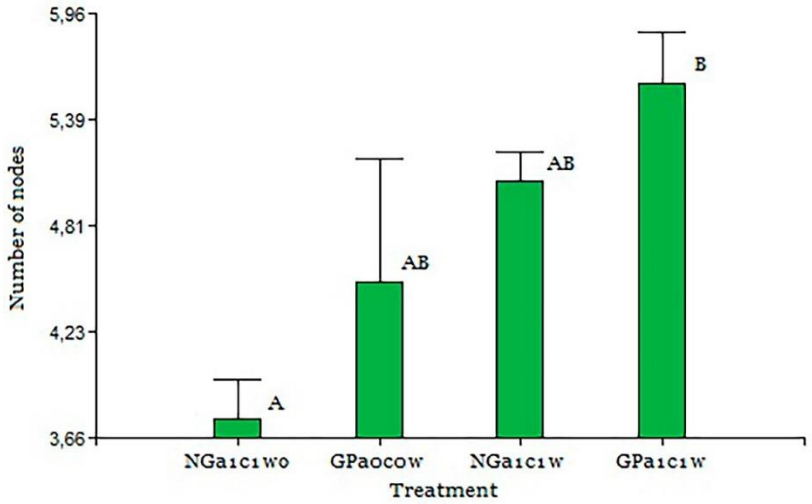

Figure 3. Number of nodes (mean ± S.E.) in NG (triple hybrid) and GP (double hybrid) treated with different phytohormonal concentrations in proliferation stage. Different letters demonstrate significant differences using Tukey's test (p-value < 0.05).

The effect of treatments demonstrates significant differences in the number of nodes, with p-value = 2.12e-06 *** (Table 3). The treatment GPa1c1w was superior due to the fact that *S. caripense* plants tends to generate more nodes than *S. muricatum.*

Table 3. ANOVA of number of nodes in NG (triple hybrid) and GP (double hybrid).

Source of variation	D.F.	M.S.	F-value	p-value
Treatment	3	10.794	12.01	2.12e-06 ***
Residuals	68	0.899		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

3.2 Experiment two

Simultaneously, the difference in number of leaves was significant between treatments with GP genotype in a second experiment. The experimental point GPa1c1w = 8.92 ± 0.45 (B) was superior, followed by GPa0c0w = 7.50 ± 1.10 (AB), and GPa.5c2wo = 7.47 ± 0.26 (A) (Figure 4), with p-value = 0.0271. Errors have normal distribution with W = 0.97374, and p-value = 0.3263; and homogeneity of variances exists with p-value = 0.1982.

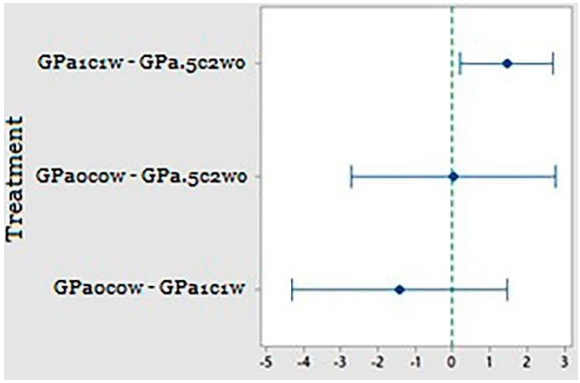

Figure 4. Comparisons using Tukey's test (p-value < 0.05) for number of leaves in GP (double hybrid) in proliferation stage. If an interval does not contain zero, its corresponding means are significantly different.

The effect of treatments demonstrates significant differences in the number of leaves, with p-value = 0.0271 * (Table 4). GPa1c1w = 8.92 ± 0.45 (B) was superior, nevertheless GPa0c0w = 7.50 ± 1.10 (AB) is statistically similar to GPa1c1w, and GPa.5c2wo = 7.47 ± 0.26 (A) is different to GPa1c1w; therefore, the effect of plastic seal is very important for this variable.

Table 4. ANOVA of number of leaves in GP (double hybrid).

Source of variation	D.F.	M.S.	F-value	p-value
Treatment	2	9.496	3.902	0.0271 *
Residuals	47	2.434		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

3.3 Meta-analysis

It was demonstrated for *S. caripense* genotypes that the number of leaves (mean ± S.E.) in proliferation stage is statistically different between treatments among biotechnological (Morales y Vaca 2016), and other methods; the experimental point TM1 = 16.13 ± 1.35 (D) is statistically different from GPa1c1w = 8.92 ± 1.10 (ABC), and from GPa.5c2wo = 7.47 ± 0.64 (A); the higher number of leaves obtained through *in vitro* technics was using 0.5 $mg \cdot L^{-1}$ BAP, through physiology *in vitro* was with 1.0 $mg \cdot L^{-1}$ AIA and 1.0 $mg \cdot L^{-1}$ BAP, and finally without plastic seal (Figure 5), with p-value < 0.0001 (Table 5), and C.V. = 35.20%. Additionally, GPa0c0w (AB) is different from TM0 (ABCD) in two letters. Homogeneity of variances exists in the improved genotypes.

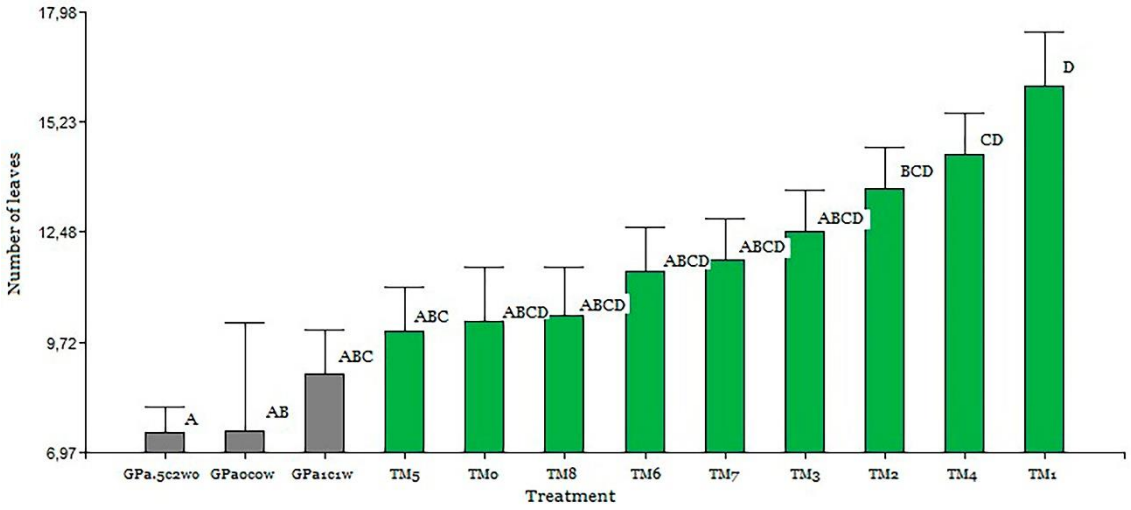

Figure 5. Number of leaves (mean ± S.E.) in GP (double hybrid) and tzímbalo genotypes treated with different phytohormonal concentrations in proliferation stage. Different letters demonstrate significant differences using Tukey's test (p-value < 0.05).

Table 5. ANOVA of steam height in in GP (double hybrid) and tzimbalo genotypes.

Source of variation	D.F.	M.S.	F-value	p-value
Treatment	11	95.69	6.53	< 0.0001
Residuals	144	14.64		

The plantlet height (mean ± S.E.) in climatization stage was statistically different between treatments among biotechnological (Morales y Vaca 2016), and other methods; the experimental point TE3 = 34.17 cm ± 1.49 (C) is statistically different from GPa1c1w = 12.12 cm ± 1.72 (A),

and from GPa.5c2wo = 9.37 cm ± 0.99 (A); the largest plantlets through *ex vitro* rooting were obtained using 2.0 mg·L^{-1} IBA, through physiology *in vitro* with 2.0 mg·L^{-1} IBA, and finally without plastic seal (Figure 6), with p-value < 0.0001 (Table 6), and C.V. = 26.54%. In addition, GPa0c0w (A) is different from TE0 (B).

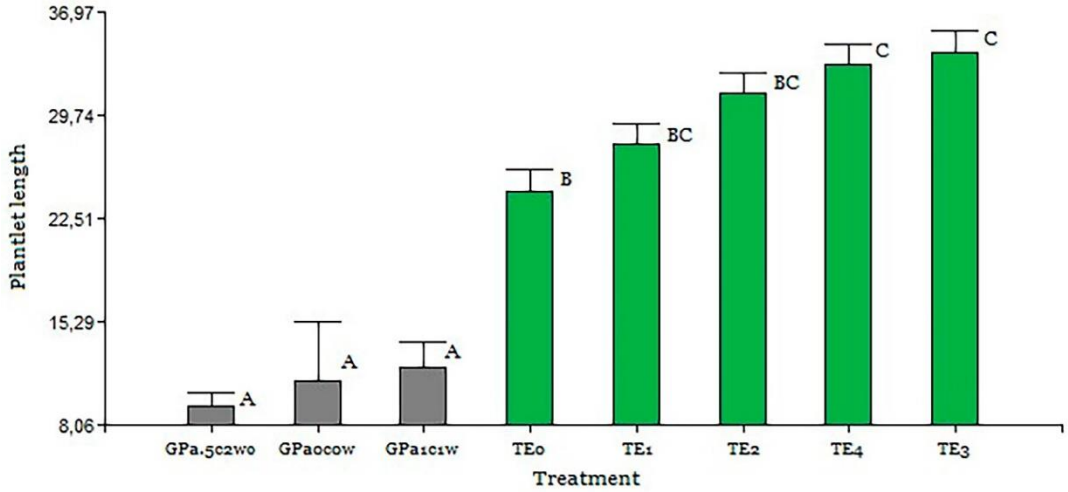

Figure 6. Plantlet height (mean ± S.E.) in GP (double hybrid) and tzimbalo genotypes treated with different phytohormonal concentrations in climatization stage. Different letters demonstrate significant differences using Tukey's test (p-value < 0.05).

Table 6. ANOVA of plantlet height in GP (double hybrid) and tzimbalo genotypes.

Source of variation	D.F.	M.S.	F-value	p-value
Treatment	7	1929.08	54.54	< 0.0001
Residuals	120	35.37		

Finally, through this method based on interspecific and intraspecific crosses, integrated to biotechnological procedures (Blas et al. 2010; Camarena et al. 2014; Morales and Chiluisa-Utreras 2022; Morales and Andrade 2023), the fruit weight of triple hybrid with *S. muricatum* x *S. caripense* (NG = 8.55-53.55 g; > 20 t/ha) is comparatively higher than that of their wild parents (FG = 5.58-6.60 g), as mentioned before with this species and *S. muricatum* x *S. tabanoense* (40-60 g; 30-40 t/ha) (Rodríguez-Burruezo et al. 2003; 2011); and this triple hybrid with *S. muricatum* x *S. caripense* have high soluble solids (SSC) content (6.5-10.0%), at least 8 ºBrix to be acceptable (Prohens et al. 2003; Rodríguez-Burruezo et al. 2003; 2011), this suggest that these varieties are also distinct from other exotic fruits in the Andean region, as example, uchuva (*Physalis peruviana* L.) with fruit weights (± S.D.) of 5.62 g ± 0.92 (heterogeneous collection), 4.8 g (Regional Nariño), 2.77 g ± 0.67 (golden berry), 6.79 g (Colombia), and 6.95 g ± 1.49 (American Southern) (Morales and Andrade 2023).

4. Conclusions

In experiment one, the higher number of leaves was obtained with NGa1c1w = 11.38 ± 0.33 (B), corresponding to the triple hybrid genotype, and GPa1c1w = 8.92 ± 0.59 (A) corresponding to the double hybrid, both using 1.0 mg·L^{-1} AIA and 1.0 mg·L^{-1} BAP by *in vitro* physiology. The greater number of nodes generates more rate of *in vitro* multiplication for both interspecific and intraspecific crosses.

In experiment two, with the double hybrid genotype among *S*. *caripense* varieties, the higher number of leaves was obtained with GPa1c1w = 8.92 ± 0.45 (B) using 1.0 mg·L^{-1} AIA and 1.0 mg·L^{-1} BAP by *in vitro* physiology.

In experiment three by means of meta-analysis, it is proved that biotechnology methods are superior than other methods using growth regulators or without plastic seal; and simultaneously through the breeding process, and standardized technics, the variance within genotypes decreased as well as the time for seed germination (Figure 7; Figure 8; Figure 9).

Figure 7. Biotechnology applied to sweet pepino breeding; fruits of triple hybrid population. Source: GENNBIO

Figure 8. *In vitro* seedling of selected triple hybrid plants. Source: GENNBIO

Figure 9. Standardized protocol for i*n vitro* propagation of sweet pepino; and other crops. Source: GENNBIO

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