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

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Summary

This project addresses the development of certified seeds of *S. caripense* x *S. muricatum*. Phytohormones (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) were assessed physiologically *in vitro* (w: with plastic seal; wo: without plastic seal) in NG and GP genotypes. Tzimbalo varieties FG, GH, and GPGn were used. Treatments were analyzed quantitatively, and genotypes were differentiated by analysis of variance. First, 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) due to genotypes, and second, due to plastic seal. Third, 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) due to *in vitro* technics; pre-aclimatized plantlet height was different, too. Finally, fruit weight (g/fruit) FG = 6.04, Sm800 = 27.48, and NG = 22.03, accumulation of anthocyanins, and soluble solids content (mean ± S.E. °Brix) FG = 10.67 ± 0.27 (C), Sm800 = 8.00 ± 0.27 (A), and NG = 9.25 ± 0.19 (B) were improved.

Keywords

Interspecific crosses; plant growth regulators; *in vitro*; flavour; population.

1. INTRODUCTION

The tzimbalo fruit has many seeds, its high germination percentage [1, 2], allows discard the presence of primary dormancy and physical lethargy [3], in contrast to seeds from other species of the genus *Solanum* [4, 5]. In addition, the fruit contains more sucrose, vitamin C [6], 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*) [7]; 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 [8]; some materials are developed for diabetic people due to its low content of calories [9].

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 [2, 10], gene expression (PCR tests) [11, 12, 13, 14], sequencing or bioinformatics [15, 16, 17], integrated to traditional plant breeding methods [18, 19]. The high nutritional value and exotic fruity aroma of sweet pepino and tzimbalo fruits [20], the high commercial value devoted to local and international markets, the possibility and the fact of developing agro-industrial products [21, 22], support this project.

The method is based on interspecific and intraspecific crosses, integrated to biotechnological procedures [17, 19, 22, 23]. 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) [18, 20]. The close phylogenetic relationship between tzímbalo and pepino dulce makes viable the conservation and use of these genetic resources in plant breeding processes [16, 20, 24]. In Ecuador there are projects that support the export of sweet pepino to Bolivia [25], Germany [26], and Japan [27]. Indeed, sweet pepino traditionally grown in Carchi-Ecuador is exported to USA [28].

2. METHODOLOGY

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·L^{-1} AIA and 1.0 mg·L^{-1} BAP; a.5c2: 0.5 mg·L^{-1} IAA and 2.0 mg·L^{-1} BAP), physiologically *in vitro* (w: with plastic seal; wo: without plastic seal) [29]. 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

NG (triple hybrid) is F1 progeny of Sc4-pl.1 - Sc4 x Sm800 (FG x *S. muricatum* 800).

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.

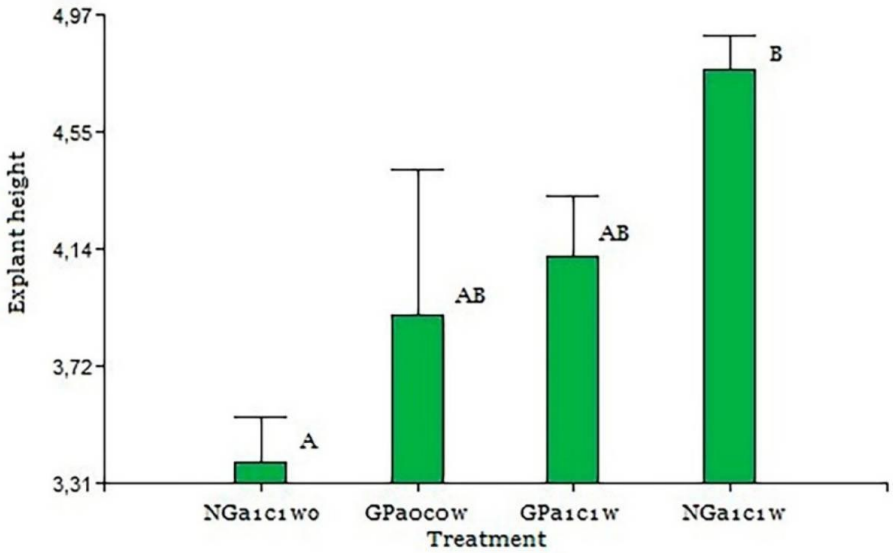

Figure 1: Stem height (mean ± S.E.) in NG (triple hybrid) and GP (double hybrid) treated with different phytohormonal concentrations in proliferation stage. Different letters demonstrate significant differences using Duncan's test (p-value < 0.05).

The 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), (Supplementary material: 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.

Finally, the number of nodes (mean ± S.E.) in proliferation stage was statistically different between treatments (Supplementary material: 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.

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) (Supplementary material: 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.

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·L^{-1} BAP, through physiology *in vitro* was with 1.0 mg·L^{-1} AIA and 1.0 mg·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.

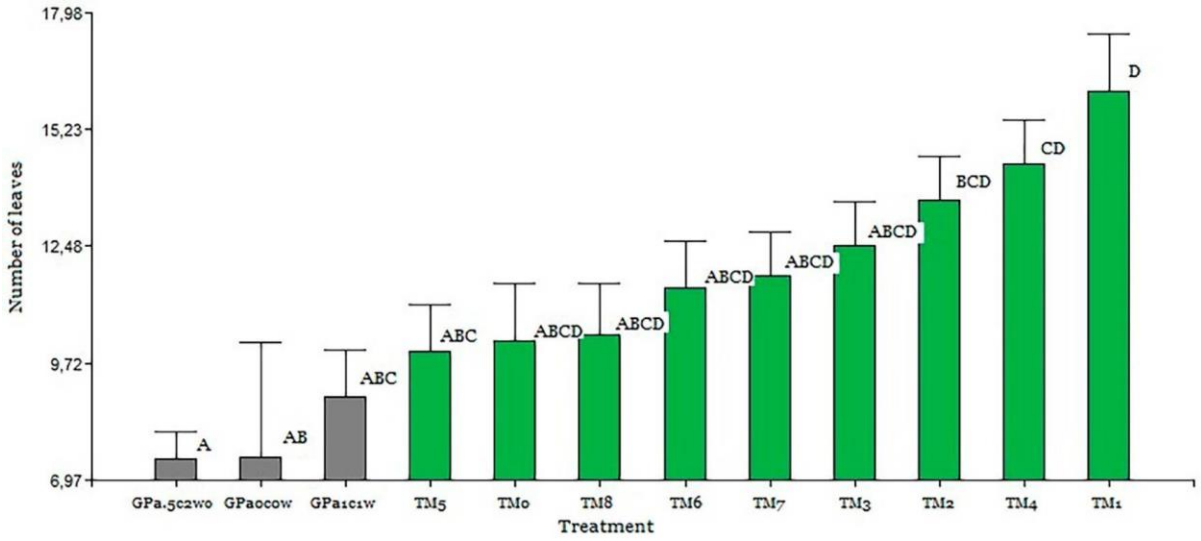

Figure 5: Number of leaves (mean ± S.E.) in GP (double hybrid) and tzímbalo genotypes treated with different phytohormonal concentrations in proliferation stage. Different letters demonstrate significant differences using Tukey's test (p-value < 0.05).

Table 5: *ANOVA of steam height in 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).

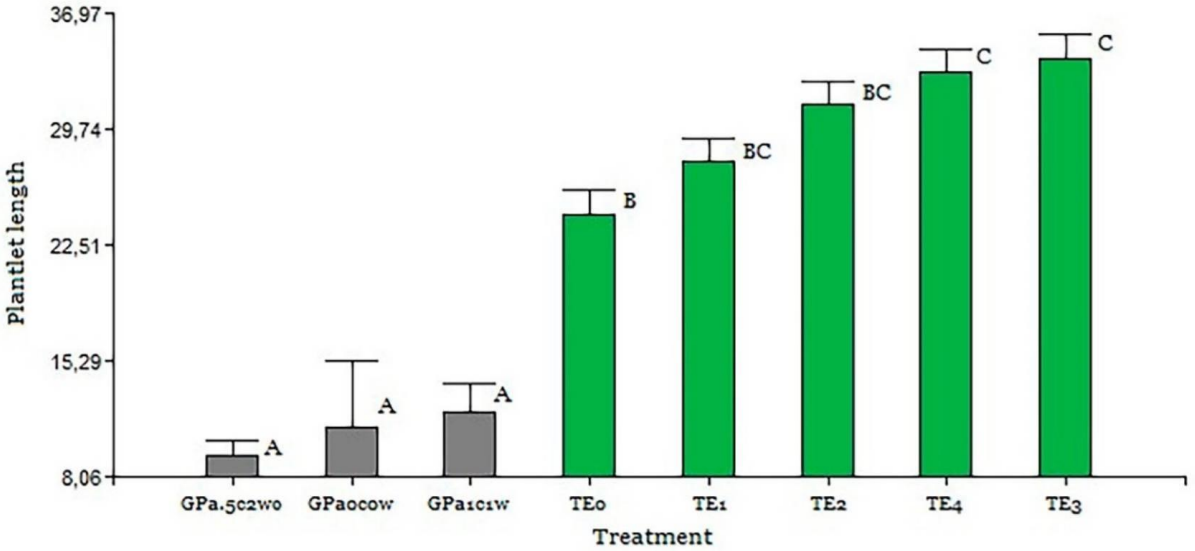

Figure 6: Plantlet height (mean ± S.E.) in GP (double hybrid) and tzimbalo genotypes treated with different phytohormonal concentrations in climatization stage. Different letters demonstrate significant differences using Tukey's test (p-value < 0.05).

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		

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

4. DISCUSSION

4.1. Experiment one

The effect of treatments is significantly different for stem height, with p-value = 4.38e-08 *** (Supplementary material: Table 1); and C.V. = 17.35%. NGa1c1w = 4.77 cm ± 0.12 (B) was superior due to growth regulators and plastic seal [2], 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* [14].

The effect of the treatments demonstrates significant differences in the number of leaves, with p-value = 1.62e-07 *** (Supplementary material: 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 [2].

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

4.2. Experiment two

The effect of treatments demonstrates significant differences in the number of leaves, with p-value = 0.0271 * (Supplementary material: 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 [29].

4.3. Meta-analysis

In experiment three by means of meta-analysis, it is proved that complete biotechnology methods are superior than other methods using growth regulators or without plastic seal.

Finally, through this method based on interspecific and intraspecific crosses, integrated to biotechnological procedures [17, 19, 22, 23], 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) [18, 20]; 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 [18, 20, 24], 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) [22].

5. 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.

Simultaneously through the breeding process (Supplementary material: Figure 7), and standardized technics, the variance within genotypes decreased as well as the time for seed germination.

6. ACKNOWLEDGMENTS

To Universidad Politécnica Salesiana, Universidad Técnica de Cotopaxi, and engineering candidate Andrés Simba for their collaboration in field and greenhouse in order to execute this research work.

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Supplementary material

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

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

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

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

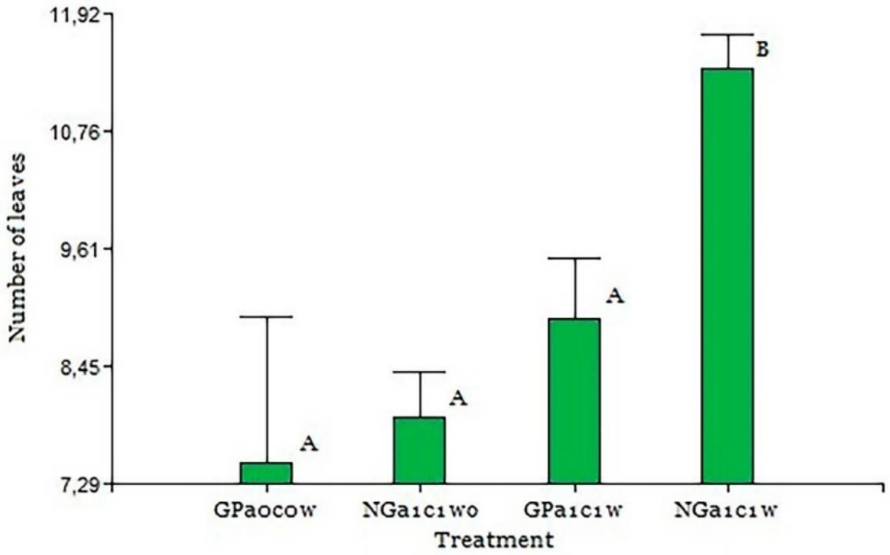

Figure 2: Number of leaves (mean ± S.E.) in NG (triple hybrid) and GP (double hybrid) treated with different phytohormonal concentrations in proliferation stage. Different letters demonstrate significant differences using Duncan's test (p-value < 0.05).

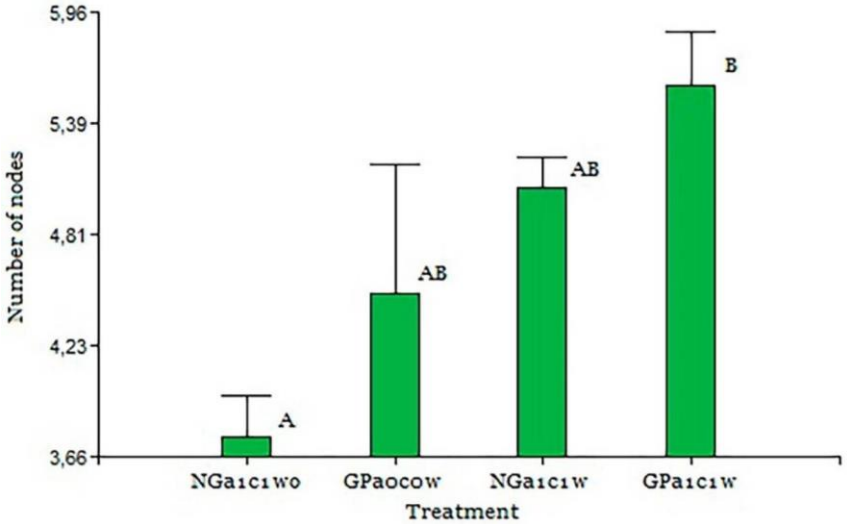

Figure 3: Number of nodes (mean ± S.E.) in NG (triple hybrid) and GP (double hybrid) treated with different phytohormonal concentrations in proliferation stage. Different letters demonstrate significant differences using Tukey's test (p-value < 0.05).

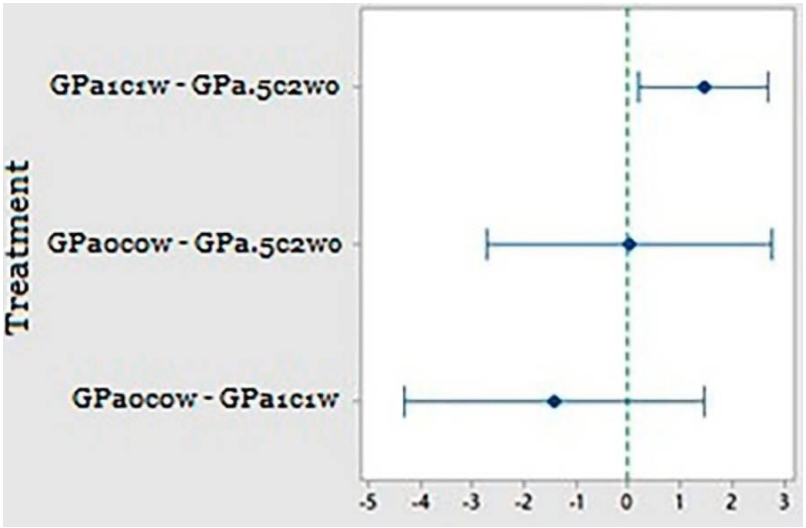

Figure 4: Comparisons using Tukey's test (p-value < 0.05) for number of leaves in GP (double hybrid) in proliferation stage. If an interval does not contain zero, its corresponding means are significantly different.

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