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# Biotechnological plant breeding applied to purple blackberries

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**Abstract:** The current project addresses the great potential of *S. caripense* Dunal (Tzimbalo) for intraspecific breeding and interspecific gene flow towards the related commercial crop *S. muricatum* Aiton (Pepino) to develop fruits with improved antioxidants, flavor, and fruit weight. This study aims to determine the interaction between genotype x altitude and identify significant differences between treatments according to fruit weight. Tzimbalo varieties GenPurpura, Gennbiotz, and GenDulce, were used. Fruit weight was analyzed using a factorial experiment under a completely randomized design (CRD). The interaction Var. x m.a.s.l. was significant (mean ± SE), Gennbiotz:a1 (4.88 g ± 0.44; C) and GenDulce:a2 (4.38 g ± 0.25; BC), followed by GenPurpura:a1 (3.33 g ± 0.36; AB); also the principal effect Var. was significant, Gennbiotz (3.93 g ± 0.23; B) and GenDulce (3.64 g ± 0.25; B), followed by GenPurpura (2.90 g ± 0.19; A). These results demonstrate distinctness, uniformity, and stability (DUS) of at least one tzimbalo variety. Fruit weight and other characteristics are relevant to improve quality and commercial potential. They are used to develop biofortified beer, jam, ice cream, and plant tissue culture media with sucrose and vitamins to strengthen biotechnological production in Cotopaxi-Ecuador.

**Key words:** Factorial experiment, tzimbalo varieties, fruit quality, genotype, agri-biotechnology.



## Introduction

The tzimbalo plant is phylogenetically complex[1], mostly wild, and native to the Andean region[2,3]. This species is the ancestor of cucumber due to chromosomal similarities and the generation of interspecific hybrids[4-6]. The fruit of tzimbalo contains significantly more sucrose, vitamin C[7], and minerals compared to modern varieties of Pepino[8]. Even some developed materials of these fruits are suitable for diabetic people. The great potential of S. caripense for interspecific gene flow to related commercial Solanum crops includes genomic studies of these species[9,10], applied biotechnological tools[11,12] and plant breeding methods[13,14] as discussed in this article, which help to overcome production, marketing and export constraints.

In Ecuador, cucumber fruit is included as one of the sixteen non-traditional products (mango, pineapple, abaca, eddo, dragon fruit, papaya, passion flower, golden berry, cucumber, asparagus, soursop, tree tomato, passion fruit, lemons, avocado and orange), with international demand and added value promoted by an export model[15]. In addition, the National Finance Corporation (CFN) mentions that 269.5 million dollars (USD) were export credits, and 25% of that amount to agribusiness[16], Approximately 67375,000 USD, or 3396.77 ha in sweet cucumber production. The estimated agricultural extension for permanent and transitory crops and cultivated pastures were 4872049.88 ha, 19% of national territory[17]. The high nutritional value and exotic fruity aroma of cucumber and tzimbalo[6] fruits, their high commercial value for local and international markets and the possibility of developing industrial products[18] encourage the improvement of these natural resources. The information about the extension (ha) of the Pepino crop is limited; nevertheless, the opportunity to introduce non-traditional products to the market is feasible. Therefore, some projects support cucumber export to Bolivia[19], Germany[20] and Japan[21]. Cucumber fruit traditionally produced in Carchi is being exported to the United States[22]. Plant breeding with tzimbalo and Pepino is carried out through backcrosses. It contributes to estimating sucrose and ascorbic acid concentrations[23], antioxidants, chlorogenic acid quantification[12], and, subsequently, heritability parameters. Additionally, fruit flavor, seed diameter, corolla color, fruit stripes, fruit length, inner placental area length, and inner placental area breadth contribute more to the variability; they are agronomically essential to increase the commercial potential[12].

The yield of the selected interspecific hybrids *S. muricatum* x *S. caripense* and *S. muricatum* x *S. tabanoense* (30-40 t*ha$^{-1}$) is comparatively higher than their corresponding wild parents. Fruit weight is intermediate (40-60 g) and considerably higher than their connected wild progenitors. The *S. caripense* (tzimbalo) and *S. tabanoense* show high soluble solid content (SSC) (10-14 %; at least 8 % to be acceptable). Export-oriented exploitations of Pepino fruit exist in Ecuador, Peru, Colombia, New Zealand and Australia, and it is mentioned that innovative and entrepreneurial farmers from Brazil, Europe and USA are interested in scaling the production and consumption of this crop. The availability of new cultivars improved for fruit quality is critical for expanding commercial exploitation[6,23]. According to analytical methods, from *S. muricatum* × *S. caripense* and *S. muricatum* × *S. tabanoense* materials, several individuals of the first backcross towards *S. muricatum* (BC1) are selected for high SSC (9.2–11.7 %), yield (23–121 t*ha$^{-1}$), and fruit weight (65–262 g). These BC1 selected individuals are selfed to accumulate favorable alleles from the wild species for

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SSC in homozygosis in the segregating offspring (BC1Ø). Then a preliminary clonal selection is performed in BC1Ø populations; clones are propagated and evaluated. The best BC1Ø clones are selected and utilized for a second backcross toward *S. muricatum* (P1×BC1Ø). Clonal propagation and evaluation are performed, and varieties are chosen for high yield (38–82 $t*ha^{-1}$), commercial fruit weight (200–300 g), and high SSC (8.4–11.2 %)[7].

The expression levels of candidate genes identified and quantified through RT-PCR and RT-qPCR[12] and the selection of genotypes that demonstrate exemplary performance in front of different crop conditions represent a solution for introducing new varieties into agro-markets, focused on the conservation and utilization of Andean resources (Figure 1). In Ecuador, there is a community of traditional producers of S. muricatum (Pepino) in Chimborazo. Each bag of fruit is marketed between Alausí, Riobamba and Cañar; Cucumber production in Ibarra, Checa, Patate, Píllaro, and Vilcabamba. In other growing environments, the monthly yield of cucumber above 2000 m.a.s.l. (14-18 ºC) after five months of sowing is equivalent to 6000 kg[24]. In contrast, in Imbabura, the yield of Pepino (Sweet round) is 72.80 t/ha[25]; the production zone is Pimampiro and part of Chota valley, where the Pepino fruit has a high commercial value at local markets; traditional production also exists in Pichincha and Loja[26].

In Ecuador, the tzimbalo fruit is used to quench thirst and eliminate skin blemishes and freckles[27]. It is also used to treat sore throats, flu and diarrhea in children[28]. The natives make necklaces with the fruits of the tzimbalo for their children to use in healing ceremonies[29] and as a curdling agent to make soft cheese[30]. There are poems related to the tzimbalo[31]. Finally, the Pepino fruit was significant during pre-Columbian times; in its region of origin exists pottery representations and depictions from the Mochica (approximately 500 AD)[32,33], and Nazca cultures in Peru[34], and many references by the first Spanish chroniclers[35]. There was a rediscovery of the Pepino for commercial exploitation in the 1970s–80s, stimulated by the attempts to introduce exotic fruits[36-38], and several cultivars were released at that time[36,39,40].

The biological wealth of third-world countries is 91.1% of germplasm of the International Plant Genetic Resources Bank and 23% from Latin America[41]; this explains the significant input of Andean countries to food and agriculture[30]. The remarkable climatological and orographic configuration of Ecuador originates a wide range of resources in its four natural regions (Coast, Highlands, Amazon, and Galapagos); the environmental conditions generate an impressive diversity of habitats and types of vegetation; flora comprises almost 25000 species of vascular plants, with an endemism of 32.25%[42].

Native edible species are essential for the food security of the Andean countries and the entire world due to their nutritional potential and medicinal and economic values. The genus *Solanum L.* with about 1500 species, is one of the largest among flowering plants[43]. Includes cultivated species such as tomato (*S. Lycopersicum* L.), potato (*S. tuberosum* L.), and others of importance in South America, such as Pepino (*S. muricatum* Aiton) and naranjilla (*S. quitoense* Lam.)[44].

Factorial experiments are treatment orderings to be analyzed into experimental designs such as completely randomized design (CRD), randomized complete block design (RCBD), Latin square design (LSD), and others. Factorial arrangements provide simultaneous studies of two or more factors, with two or more levels for each; they are also used in agricultural, biological and sociological research. Using factorial arrangements makes it possible to obtain information on the factors independently and with interaction[45]. The advantages of applying factorial structures are the more efficient use of the available resources, studied factors are under conditions closer to reality, and these are analyzed under many experimental designs. The number of degrees of freedom for the error is high and contributes to decreasing the experimental error and increasing the accuracy of the experiment.

## Materials and methods

### Plant material

Selected material of *S. caripense* GenPurpura (5.67-10.33 °Brix), Gennbiotz (8.33-10.50 °Brix), and GenDulce (8.83-11.0 °Brix) were used, provided by the company GENNBIO (Quito, Ecuador); a total of sixty-four (n=64) plants of the registered genotypes were biotechnological cultivated at different altitudes (m.a.s.l.).

### Growing conditions

*In vitro* plantlets were acclimatized in Cotopaxi-Ecuador after laboratory propagation at 15-28 °C throughout the


(a) (b) (c)

**Figure 1.** (a) Genetic variability of the Pepino (*Solanum muricatum* Aiton), and varieties of its ancestor tzimbalo *(Solanum caripense* Dunal) with high soluble solid content. (b) The cross-section of Pepino fruit. (c) Derived agri-industrial natural products. Source: GENNBIO

field experiment. Plants were spaced 0.3 m within the row and 1.0 m between rows. Plants were trained with vertical strings. Mechanically irrigation was used, and nutrients were provided through doses of commercial fertilizer plus micronutrients. Due to the significant self-incompatibility of these species, some materials were hand-pollinated to set fruits.

### Data analysis

The agronomic traits, fruit weight (g), and other traits were recorded. Analytical measurements of fruit weight were randomly repeated for each variety of *S. caripense* (1000 fruits). The data were disposed under CRD in a factorial experiment 3 x 2 with three genotypes (Var.) and two altitudes (m.a.s.l.) for analysis with the mean values into a linear additive statistic model[47,48]. Thus, there are three effects of interest without considering decomposition and three null hypotheses raised with their corresponding alternative hypothesis. The level of corruption or detail of the study depends on the number of groups utilized in each factor. Statistic packages InfoStat 2018, Minitab 16, and RStudio 4.1.2. were used.

Where *u* is the general mean; a*i* is the effect of the level *i*=1,2 of factor A; b*j* is the effect of the level *j*=1,2 of factor B; (ab)*ij* represents effects of double interaction on the levels *ij*, respectively; E*ijk* represents the random error in the combination *ijk*, and *k* are replicated.

The mathematical model on the factorial experiments is linked to the experimental design model in which the data is analyzed, except that in these cases, the effect of treatments (Ti) is decomposed in as many effects as factors and interactions are studied in factorial arrangements[45,49].

## Results

GenPurpura is a population of selected clones from a segregating progeny, cross-pollinated with Gennbiotz to set fruits; Gennbiotz is a population of selected clones from $F_1$ progeny after mass selection on the previous generation, cross-pollinated with GenPurpura; and GenDulce is a self-compatible progeny from previous breeding proceedings, too[13,46].

### Interaction genotype x altitude

It was observed that fruit weight is statistically differentiated by genotype (Var.) and altitude (m.a.s.l.); the fruit weight (mean ± SE) was higher in the experimental points corresponding to Gennbiotz:a1 (4.88 g ± 0.44) and GenDulce:a2 (4.38 g ± 0.25), followed by GenPurpura:a1 (3.33 g ± 0.36), Gennbiotz:a2 (2.98 g ± 0.11), GenDulce:a1 (2.91 ± 0.44), and GenPurpura:a2 (2.46 ± 0.13) (Figure 2). The null hypothesis is accepted with p-value = 0.4528 (W = 0.98155). Therefore, the errors have a normal distribution.

The interaction effect Var. x m.a.s.l. demonstrates significant differences in the mean fruit weight (Table 1) between the genotype of tzimbalo varieties and levels of altitude in meters above sea level, p-value < 0.0001. The principal effects of Var. are significant also, suggesting that the effect of genotype contributes more to the differences in fruit weight due to its F-value = 6.72, followed by the principal effects of m.a.s.l. which F-value = 2.75, and no significant p-value = 0.1027. The CV = 20.56 %.

The mean of fruit weight in the level *i*=2 (Var.=Gennbiotz) of factor A and level *j*=1 of factor B (m.a.s.l. = a1) is significantly different from the mean in the level I =3 (Var. = GenPurpura) of the factor A and level *j*=1 of the factor B (m.a.s.l. = a1), with alpha = 0.05; the null hypothesis ($H_0$: $u_{21} = u_{31}$) is rejected and the alternative hypothesis ($H_1$: $u_{21} \neq u_{31}$) is accepted. This means that the mean fruit weight in Gennbiotz at the first altitude (a1) is significantly different from that of GenPurpura at the first altitude (a1).

On the other hand, the mean of fruit weight in the level *i*=1 (Var.=GenDulce) of factor A and level *j*=2 of factor B (m.a.s.l. = a2), is significantly different from the mean in level i=3 (Var. = GenPurpura) of the factor A and level *j*=2 of factor B (m.a.s.l. = a2), with alpha = 0.05; the null hypothesis ($H_0$: $u_{12} = u_{32}$) is rejected and the alternative hypothesis ($H_1$: $u_{12} \neq u_{32}$) is accepted. This means that the mean fruit weight in GenDulce at the second altitude (a2) is significantly different from that of GenPurpura at the second altitude (a2) (Figure 3).

### Principal effects

The mean of principal effects on genotype, according to the Tukey test with alpha = 0.05 (Table 2), is statistically similar for both Gennbiotz (3.93 g) and GenDulce (3.64



$\mathbf{H_0}$: $(a)_i = 0$ $\quad i=1,2,3$
All levels of factor A have the same effect on the fruit weight.
$\mathbf{H_1}$: $(a)_i \neq 0$ $\quad i=1,2,3$
Not all levels of factor A have the same effect on the fruit weight.

$\mathbf{H_0}$: $(b)_j = 0$ $\quad j=1,2$
All levels of factor B have the same effect on the fruit weight.
$\mathbf{H_1}$: $(b)_j \neq 0$ $\quad j=1,2$
Not all levels of factor B have the same effect on the fruit weight.

$\mathbf{H_0}$: $(ab)_{ij} = 0$ $\quad i=1,2,3; j=1,2$
Interaction between factor A and factor B exists.
$\mathbf{H_1}$: $(ab)_{ij} \neq 0$ $\quad i=1,2,3; j=1,2$
No interaction between factor A and factor B exists.
In factorial arrangements axb, the response variable (Y) is described through the model of effects given by:

$$Y_{ij} = u + a_i + b_j + ab_{ij} + E_{ijk} \qquad (1)$$

$$i=1,2..., a; j=1,2..., b; k=1,2..., n$$

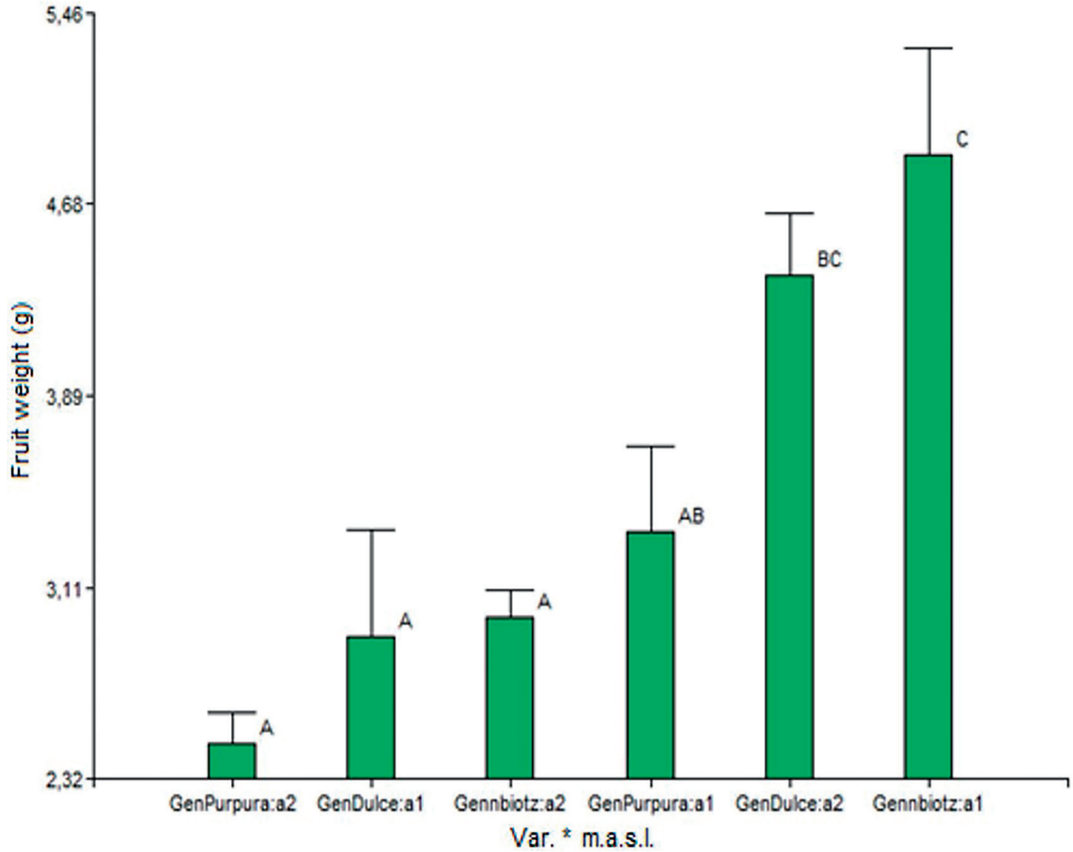


**Figure 2.** Fruit weight (mean ± S. E.) of tzimbalo varieties GenPurpura, Gennbiotz, and GenDulce at different altitudes in meters above sea level. Factorial experiment 3 x 2. Other letters demonstrate significant differences according to the Tukey test (p-value < 0.05).

| Source of variation | D. F. | M. S. | F | P-value |
|---|---|---|---|---|
| Model | 5 | 5.05 | 13.21 | < 0.0001 |
| Var. | 2 | 2.57 | 6.72 | 0.0024 |
| m.a.s.l. | 1 | 1.05 | 2.75 | 0.1027 |
| Var. x m.a.s.l. | 2 | 4.91 | 12.86 | < 0.001 |
| Error | 58 | 0.38 | | |
| Total | 63 | | | |

**Table 1.** ANOVA of fruit weight in tzimbalo varieties GenPurpura, Gennbiotz, and GenDulce.

g), whereas, GenPurpura (2.90 g; A) is statistically different from Gennbiotz and GenDulce. As the mean of principal effects on altitude, a1 and a2 are both statistically similar, and no significant differences were found (Table 3).

## Discussion

Gennbiotz generated the higher fruit weight:a1 (4.88 g; C) and GenDulce:a2 (4.38 g; BC); in previous studies, the fruit weight for tzimbalo EC-40 is 8.8 g[7], ranging from 1.9-9.7 g[50]. Nevertheless, the $F_1$ from Gennbiotz x GenPurpura, and GenPurpura x Gennbiotz cultivated at altitude a1 in our breeding program have higher yield than EC-40 (66 g/plant) due to gene flow of yield and stability traits. It is mentioned that when a variety shows uniform, it can also be considered stable[51]. In contrast, these results demonstrate the stability of GenPurpura at different altitudes.

In the Andean region, it is mentioned that for exotic fruits such as uchuva (*Physalis peruviana* L.), the fruit weight (± SD) of a heterogeneous collection is 5.62 g ± 0.92[52], and for the genotype Regional Nariño the fruit weight is 4.8 g[53]. In other studies, the fruit weight of golden berry is 2.77 g ± 0.67[54]; for the ecotype, Colombia is 6.79 g[55]; and for the American Southern variety, the fruit weight is 6.95 g ± 1.49[56]. This suggests that the fruit weight (± SE) of the tzimbalo variety Gennbiotz:a1 (4.88 g ± 0.44) is similar to that of Regional Nariño, with high SSC.

This current work through intraspecific gene flow between tzimbalo varieties leads to the development of hybrids by introducing relevant genes, which are utilized to improve stability, uniformity, and nutritional values, to generate simultaneously original varieties of Pepino. A difference between the two varieties is clear depending on many factors, considering the type of expression of the measurements analysed[12,13]. Visual measurements refer to sensory

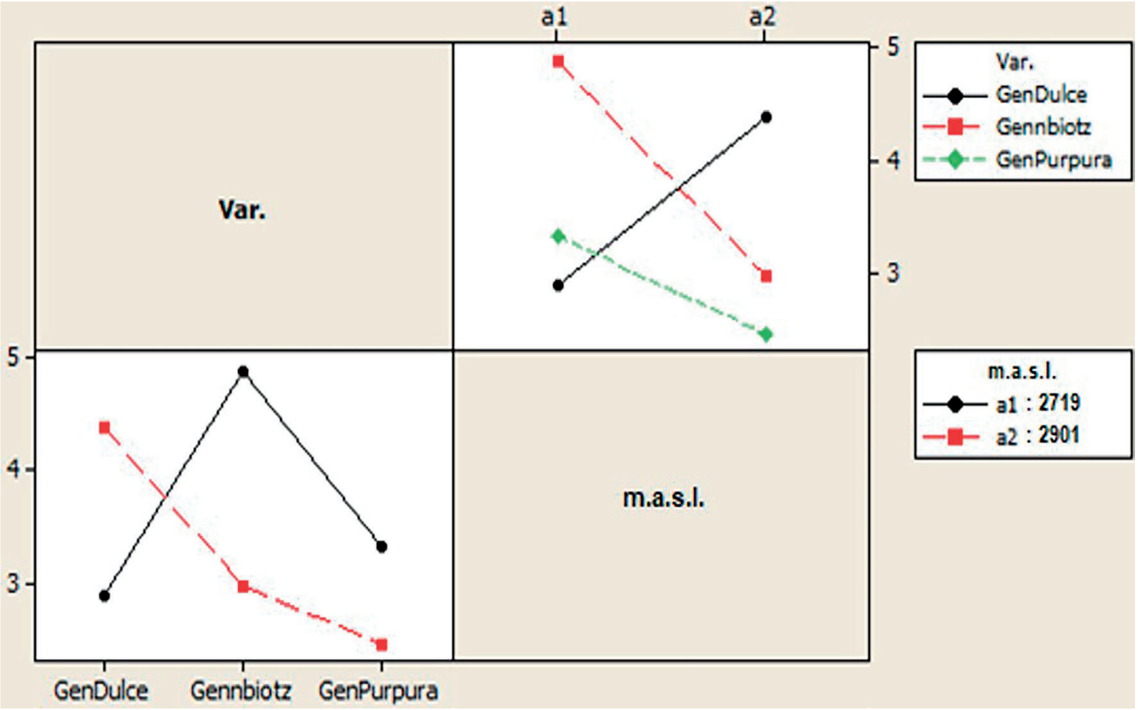


**Figure 3.** Interaction for fruit weight in tzimbalo varieties GenPurpura, Gennbiotz, and GenDulce at different altitudes in meters above sea level.

| Var. | Mean | N | SE. | |
|---|---|---|---|---|
| GenPurpura | 2.90 | 25 | 0.19 | A |
| GenDulce | 3.64 | 8 | 0.25 | B |
| Gennbiotz | 3.93 | 31 | 0.23 | B |

**Table 2.** Fruit weight (mean ± S. E.) of tzimbalo varieties GenPurpura, Gennbiotz, and GenDulce.

| m.a.s.l. | Mean | SE. | |
|---|---|---|---|
| a2 | 3.28 | 0.10 | A |
| a1 | 3.71 | 0.24 | A |

**Table 3.** Fruit weight (mean ± S. E.) of tzimbalo varieties at different altitudes.

observations of experts, including smell, taste, and touch, as well as statements with reference points as diagrams, examples of varieties, and others[51].

## Conclusions

Gennbiotz generated the higher fruit weight:a1 (4.88 g ± 0.44) and GenDulce:a2 (4.38 g ± 0.25), which increase over generations through compatible crosses between tzimbalo varieties. The tzimbalo variety GenPurpura (2.90 g ± 0.19) was stable at different altitudes; it demonstrates these species' stability through biotechnological plant breeding. Our distinct varieties of tzimbalo are the base for improving the Pepino crop to increase the nutritional quality and healthy food in the highlands region of Cotopaxi-Ecuador.

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### Conflicts of Interest

The authors declare no conflict of interest.

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