INFLUENCE OF THE CONDITIONS OF STORAGE ON THE SEED QUALITY OF *Stevia rebaudiana* (Bertoni) Bertoni

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**Abstract**

*Stevia rebaudiana* (Bertoni) Bertoni is a native species of Northeast Paraguay, which has been used for its sweetening properties for many years. In Córdoba Province there is no research work related to different growing conditions nor to seed storage.

The aim of this paper was to determine the influence of two storage conditions over the germinative power, vigour and germinative energy of *S. rebaudiana*. At the beginning of May 1996, the seeds were harvested in Bower, Santa Maria, in Córdoba Province, Argentina. Repetitions of one hundred seeds were sown following a completely randomized design at the Seeds Analysis Laboratory of the Faculty of Agricultural Science, University of Córdoba. These were stored for 11 months at 4°C and at environmental temperature and moisture. The normal seedling number was recorded at six dates and, at the end of this study, the fresh and dry weights were obtained. The number of abnormal seedlings was also evaluated.

For the six dates studied, there were highly significant differences between the two ways of storage, as to the germinative power, showing that the conservation in the refrigerator at 4°C can be recommended for this species.

Differences were observed between the fresh and dry weight of the normal seedlings, showing that the storing condition in the refrigerator, produced higher average values, at the same time presenting more vigorous seeds than those in the other treatment.

Concerning the number of abnormal seedlings, significant differences were not found.

1. Introduction

*Stevia rebaudiana* (Bertoni) Bertoni ("yerba dulce") is a native species from Northeast Paraguay that has been used since the time of the guarani Indians for its sweetening properties. It belongs to the family Compositae, subfamily Asteroidae, tribe Eupatorieae (Cabrera et al., 1996).

The leaf of "yerba dulce" contains glucosides, among which steviosides and A rebaudiosides are outstanding, due to their sweetening power (300 and 450 times greater than that of saccharose, respectively). Other components were found in different parts of *S. rebaudiana*, such as glycosides, B, C, D and E rebaudiosides, A, B, C, D, E, F, G, and H sterebins, A and B dulcosides, etc. (Gupta, 1995)

*S. rebaudiana* also has a hypoglycemic, hypotensive, antifertility, antimicrobial and anticares activities.

Nowadays, *S. rebaudiana* is grown commercially in Japan, Continental China, South Korea, Taiwan, Thailand, Paraguay and Brazil. In Argentina, it is adapted to subtropical and temperate climates, the best results in dry matter yield being obtained in the former environments.
Its reproduction is sexual by seeds, or agamic by partition of bushes, by buds or by "in vitro" micropropagation, starting from the node segments and meristems. The most advantageous way of reproduction is agamic, starting from the improved material and with a good amount of A rebaudiosides, but it is not always possible to obtain it. However, the alternative of propagating it by seeds is also reasonable.

Cabanillas and Díaz, (1996), reported the performance of seeds of this species under different temperature and light conditions, studying factors like storage and time in which the seeds keep their germinative power.

Once the seeds have been harvested and dried, the preservation of the physiological quality depends on the conditions of the storage. Other factors to consider in this respect are seed moisture and temperature, and the environment in which they are stored.

As the seeds are hygroscopic, they absorb or lose moisture to achieve a balance with the environmental air. During the storage, the increase of the temperature, combined with the high moisture leads to an acceleration of the metabolic activities of the seeds and also those of the fungi that accompany them.

Quasem and Christensen (1968), mentioned by Castillo Niño (1984) found that fungi from the storage are possibly the main cause of the loss of viability in stored seeds. Fungi of the genera Aspergillus and Penicillium, develop at temperatures starting at 5°C during storage.

Due to this fact, the Faculty of Agricultural Sciences, University of Córdoba, carried out a test in which the objective was to evaluate the germinative power, the germinative energy and the vigour of S. rebaudiana, under two storage conditions.

2. Materials and methods

2.1. Materials

At the beginning of May 1996, seeds of S. rebaudiana were harvested in Bower, District of Santa María in the province of Córdoba, Argentina.

The initial germination power of the seeds was 63%. Part of the harvested seeds were kept in a refrigerator at 4°C, in closed plastic containers, and another part of these seeds were put into plastic bags and kept moist at room temperature.

2.2. Experimental Design

At the eleventh month after the harvest the Laboratory of Seed Analysis at the Faculty of Agricultural Sciences tested the response of the seeds at the two storage conditions.

Three groups of a hundred seeds each were kept in the fridge (treatment I) and three groups of seeds stored in non-controlled environmental conditions (treatment II) were placed in a 20-30°C temperature-alternating chamber, following a completely random design. The seeds in each group were placed on a double sheet of wet paper on a tray wrapped in a plastic bag (International Seed Testing Association, 1996).

After 4 days the number of normal and abnormal seedlings was recorded along with germination percentages. This record was extended for 5 dates to establish the behaviour profiles of both ways of storage in time.

In order to illustrate its growth the fresh and dry weights of the normal seedlings and the amount of abnormal seedlings were evaluated at the end of the test.
2.3. Data Treatment

Due to the fact that normal seedling is a discrete variable, the use of the normal theory for statistical tests is not allowed. So, a linear generalized model with a Binomial error was fitted, with a logistic link function and linear predictor corresponding to a model of analysis of variance with bifactorial structure for the treatments, with the conditions factor with two levels, and dates factor with six levels.

This model was estimated in GLIM (Generalized Linear Interactive Model) (Payne, 1994) and it was chosen because it had the advantage of dealing with the real nature of the observations (counting of seedlings), therefore avoiding the implementation of some transformations in order to use of statistical standard techniques for continuous variables.

The analysis of the abnormal seedlings was similar, though considering only one factor, the storage conditions.

For fresh and dry weight of the seedlings, a normal analysis of variance model was used, and the standard errors were estimated in order to describe these variables.

3. Results

In general terms, a germination period was observed from the fourth day after the sowing date up to the fourteenth day. Table 1 shows the accumulative percentages of the germination of the six observation dates, at 4, 6, 8, 11, 13 and 14 days. Table 1 also shows the dead seedling percentages in brackets, for the last observation date (14 days).

For the six dates studied, there were differences (P<0.01) between the two ways of storage for the power of germination, showing that the conservation in the refrigerator at 4°C produced higher average values. This behaviour was observed for all the dates without interaction with the treatments.

Table 2 presents the average values for fresh and dry weight in grams. Differences were observed (P<0.05) between the treatments, showing that the storage condition in the refrigerator had the higher average values.

The medium average of fresh and dry weight for each seedling also had best values in treatment I (refrigerator) as shown in Table 3.

According to Popinigis (1985), the weight of the dry material is used as a vigour pointer to compare different lots of seeds. The seeds that produce seedlings with more dry material are stronger. Therefore, the values found show that treatment I presents more vigorous seeds than those in the treatment II, since the seedlings have more dry material.

As to the number of abnormal seedlings, significant differences were not found (P>0.05) reporting 5% in treatment I and 6% in treatment II.

4. Discussion

Treatment I has a superior germinative power, energy and vigour for a period of eleven months of storage, which makes it highly recommended for S. rebaudiana seeds. This may be due to the fact that the storage at low temperatures and low moisture reduces the respiration of the seeds, reducing the attack of fungi and insects and maintaining viability of the seed (Hartmann et al., 1994).

In the contrary to Concha Marcavillaca’s (1985) opinion that “the seeds have a short period of germinative power”, the seeds of this species can be kept in refrigeration for as long as eleven months without risking the loss of their germination capacities.
The conditions of storage have no influence on the abnormal seedlings. In both treatments, the main causes of abnormality were found at the level of the primary root. Studies carried out for the genera Beta, Brassica, Daucus, Dianthus, Helianthus, Lactuca, Trifolium and Zinnia (Bekendom et al., 1979) showed that if the primary root is defective, the seedling is classified as abnormal, even though secondary roots have developed.

Although references of abnormality for this species were not found, the values obtained are low. Later works on abnormal S. rebaudiana seedlings would establish the bases to compare the different lots of seeds.

The work done by Alvarez et al. (1994) shows that in case that is impossible to do the sowing of the seeds immediately after the harvest, they should be kept in tightly sealed containers and kept at the refrigerator at 4°C, because the seeds rapidly lose their viability when left at room temperature.

This work also reports that in temperate areas, including the Province of Córdoba, the low-temperature harvests of fall and winter are a cheap and easy way to store seed under healthy storage conditions. Nevertheless, at the beginning of summer, if the seeds are to be kept longer, it is necessary to apply environmentally controlled conditions of temperature and moisture, as a preventive measure to reduce the storage losses and costs.

The conditions of this test make it necessary to carry out new assays, in order to obtain precisions about the period of time the seed preserves its germinative power.

The storage under non-controlled environmental conditions presents a great number of dead seeds. Although for this species there is no evidence in that respect, it is necessary to carry out further studies to determine exactly the elements that cause the losses during storage.

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Ing. Graciela Milano. Computer Laboratory . Faculty of Agricultural Sciences. U.N.C.

6. References


Table 1 - Average values of germination accumulative percentages for treatments and dates and, for the last date, the dead seedling percentages in brackets

<table>
<thead>
<tr>
<th>Dates (days)</th>
<th>Treatment I (refrigerator) (%) germination</th>
<th>Treatment II (room temperature) (%) germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>46</td>
<td>38</td>
</tr>
<tr>
<td>11</td>
<td>56</td>
<td>47</td>
</tr>
<tr>
<td>13</td>
<td>59</td>
<td>50</td>
</tr>
<tr>
<td>14</td>
<td>61 (34)</td>
<td>51 (43)</td>
</tr>
</tbody>
</table>

Table 2 - Average values (grams) and standard errors for fresh and dry weight of seedlings by repetition

<table>
<thead>
<tr>
<th>Weight</th>
<th>Treatment I (refrigerator)</th>
<th>Treatment II (room temperature)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>0.1257 ± 0.0541</td>
<td>0.0991 ± 0.0031</td>
</tr>
<tr>
<td>Dry</td>
<td>0.0167 ± 0.0043</td>
<td>0.0110 ± 0.0017</td>
</tr>
</tbody>
</table>

Table 3 - Average values (grams) for fresh and dry weight for each seedling and their standard deviations in brackets

<table>
<thead>
<tr>
<th>Weight</th>
<th>Treatment I (refrigerator)</th>
<th>Treatment II (room temperature)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>0.00206 (0.0000692)</td>
<td>0.00193 (0.000055)</td>
</tr>
<tr>
<td>Dry</td>
<td>0.000274 (0.0000213)</td>
<td>0.000215 (0.000086)</td>
</tr>
</tbody>
</table>

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