



RESEARCH PAPER

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Evaluation of the effectiveness of post-harvest conservation methods for seedlings of *Solenostemon rotundifolius* (Poir. J. K. Morton)

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Abstract

Solenostemon rotundifolius is a tuberous plant with great food and economic potential in Burkina Faso. One of the major problems in its production is the loss of seedlings during storage, resulting in a shortage of raw materials at planting time. The aim of this study was to assess the effectiveness of methods for preserving *S. rotundifolius* seedlings. A randomized block design with three (03) replicates was used. Twelve (12) preservation methods were tested. Measurements were made on the rate of budded seedlings, budding time, bud length and seedling loss rate. The results showed that six (6) conservation methods produced seedlings with a budding rate of over 80% and low seedling loss rates, ranging from 2.38% to 6.19%. These are: conservation in "Bitatoré" with millet husks as additive (BITA+G) with a seedling budding rate of $89.05 \pm 2.27\%$, canaries with sand as additive (CAN+S) with a budding rate of $87.62 \pm 2.17\%$, "Bitatoré" without additive (BITA) with a budding rate of $86.19 \pm 2.33\%$, *Storage in Sand and Sprouting* (Tri S) with a budding rate of $85.71 \pm 2.72\%$, canaries with wood shavings as additive (CAN+CB) with a budding rate of $85.24 \pm 2.35\%$ and canaries without additive (CAN) with a budding rate of $85.24 \pm 2.54\%$. In addition, the seedlings produced by these methods had respective seed loss rates of 2.86%, 2.86%, 6.19%, 2.38%, 3.81% and 5.71%. The results also showed that seedling budding time varied from 51 ± 4 to 70 ± 3 days, depending on the storage method.

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Introduction

Solenostemon rotundifolius (Poir.) J. K. Morton, native to tropical Africa (Tindall, 1983), is an annual herbaceous member of the Labiaceae family (Schippers, 2002). It is cultivated in several African countries, notably in West Africa (Burkina Faso, Ghana, Mali, Nigeria, Togo), in Central Africa (Cameroon, Chad) and in parts of South and East Africa.

In Burkina Faso, *S. rotundifolius* is mainly grown for its edible tubers. Indeed, *S. rotundifolius* tubers are used as a staple food in rural areas and as a dietary supplement in urban areas (Nanema, 2010). *S. rotundifolius* tubers contain protein, carbohydrates, fiber, lipids and are rich in minerals such as calcium, magnesium, iron, potassium, sodium, phosphorus, manganese, copper, zinc and chromium (Gouado *et al.*, 2003; Prematilake, 2005, Enyiukwu *et al.* 2014, Sethuraman *et al.*, 2020; Kwazo *et al.*, 2021). In addition to these nutritional values, *S. rotundifolius* is of great medicinal importance. Due to the intermediate glycemic index content of its tubers, *S. rotundifolius* is recommended as a meal for people with type 2 diabetes mellitus (Eleazu *et al.*, 2017). Tubers reduce blood cholesterol levels (Abraham *et al.*, 2005) and possess strong antioxidant activity (Sandhya *et al.*, 2000, Kwarteng *et al.*, 2018). Also, the leaves and tubers are used in the treatment of several illnesses such as coughs, angina, dysentery, sore eyes (Ouédraogo *et al.*, 2007) and fungal and viral infections in humans (Kwarteng *et al.*, 2018). In addition, the marketing of tubers is a source of income for producers. Thus, a survey conducted in Ouagadougou, Burkina Faso, revealed that the price of one kilogram of *S. rotundifolius* tubers was 1.2 to 3 USD (Nanéma *et al.*, 2017). Grubben (2004) also reported trade in *S. rotundifolius* tubers between northern Ghana and Burkina Faso. Despite the plant's many potential uses, it remains under-exploited for a variety of reasons. In Burkina Faso, *S. rotundifolius* is generally grown by elderly people on small areas (Ouédraogo *et al.*, 2007). In addition, one of the major problems is the difficulty of preserving the seedlings that are the agricultural raw material,

particularly their loss during storage (Tindall, 1983). Studies have shown that the lack of appropriate methods for the post-harvest conservation of tubers is the cause of huge losses that can reach 20 to 40% of production (Sugri *et al.*, 2013). Indeed, more rotting occurs during seed conservation. Also, pre-harvest and post-harvest operations damage the tuber integuments, making them more susceptible to attack by micro-organisms (Mohammed, 2013).

In rural areas, growers have developed endogenous methods for preserving *S. rotundifolius* seedlings. These methods involve keeping the tubers in cool, dry conditions, away from light, cooking salt and fats (Bognounou, 1970, Gouado *et al.*, 2003, Ouédraogo *et al.*, 2007). Seedlings are generally mixed with crop residues (millet husks) and stored in containers such as granaries and canaries (Ouédraogo *et al.*, 2007). However, the problem of preservation remains a major constraint, hampering production. The aim of the present study is to identify the best methods for conserving *S. rotundifolius* seedlings. Specifically, it aims to: (i) assess the effect of conservation methods on seedlings, (ii) identify conservation methods that promote better seedling budding.

Materials and methods

Experimental site

To obtain seedlings, tubers were multiplied in the garden of the Life and Earth Sciences Training and Research Unit (UFR-SVT) of Joseph KI-ZERBO University during the 2021/2022 rainy season. Following this multiplication, the conservation experiment was carried out in the laboratory from December 1^{er} 2022 to May 31 2023, i.e. over a conservation period of six (6) months.

Plant material

The plant material used in the present study consists of tubers from seven (07) accessions of *S. rotundifolius* (E120, E186, UW072ID, UW086M, E165, White and UE088). These accessions, three (3) from Burkina Faso and four (4) from Ghana, were selected on the basis of their agronomic performance. The accessions were composed of three morphotypes:

black-skinned (E120, UE088 and UW072ID), red-skinned (UW086M, E186 and E165) and white-yellow (White). Tubers with a diameter of less than 26 mm were selected for the storage test. In this study, small tubers used as seeds are referred to as seedlings.

Storage equipment

The preservation equipment consisted of six (6) canaries, six (6) "Bitatoré", 21 plastic basins with a volume of 17.5 liters, 756 fabric bags 25 cm long and 15 cm wide, and three types of additives: millet husks, fine sand and wood shavings. The "Bitatoré" is a straw basket whose interior is lined with insulating material to reduce heat exchange between the outside and inside of its contents (Fig. 1A.). The canary is a spherical earthenware vessel of variable dimensions, with an opening allowing access to the contents (Fig. 1B.). Millet husks are the protective wrappings of the millet flowers obtained after threshing and winnowing the ears of millet. Wood shavings are fragments of wood obtained by carpenters during the planning process.

Experimental set-up

The experimental design is a completely randomized block with three (03) replicates. Two factors were studied: the primary factor was the type of preservation, with twelve (12) methods tested. Accessions with seven (7) modalities constituted the secondary factor. The experimental unit consisted of ten (10) seedlings. A total of 2,520 seedlings (12 methods x 7 accessions x 10 seedlings x 3 replicates) were used for the experiment. The following conservation methods were considered:

conservation in additive-free canaries (CAN);
 conservation in canaries with millet husks as an additive (CAN+G);
 conservation in canaries with sand additive (CAN+S);
 conservation in canaries with wood chips as additive (CAN+CB);
 conservation in additive-free Bitatoré (BITA);
 conservation in "Bitatoré" with millet husks as an additive (BITA+G);
 conservation in "Bitatoré" with sand additive (BITA+S);

conservation in "Bitatoré" with wood shavings as additive (BITA+CB);

conservation in additive-free pits (F);

conservation in pits with millet husks as additive (F+G);

conservation in pits with sand as additive (FS);

Storage in Sand and Sprouting or triple S (Tri S) system.

Conducting the conservation trial

For each method, ten (10) seedlings, including five (5) small-diameter seedlings ($D \leq 16$ mm) and five (5) medium-diameter seedlings ($16 < D \leq 26$ mm), were selected by accession to form the experimental unit. The seedlings were then packed in cloth bags with or without additives. The quantities of additives used per experimental unit were 50 g for millet glumes, 400 g for fine sand and 50 g for wood shavings (Fig. 2.A., B., C.). The seedlings in the bags were then stored in the "Bitatoré" and canary containers, and the canaries were covered with the bag and its lid (Fig. 1 A., B.).

For preservation using the triple S method, 21 basins with a volume of 17.5 liters and newspaper to absorb moisture were used, following the method of the International Potato Center (2019). A thin layer of fine sand (0.5 kg) was deposited on the newspaper before placing the seedlings (Fig. 1C.) and covered with a layer of fine sand (4.5 kg) approximately 5 cm thick. The basins were stored in the laboratory.

The pits were 20 cm in diameter and 20 cm deep, placed under a tree to take advantage of the shade. The pits were made in a completely randomized design with three (3) replicates (Fig. 3.). The replicates constitute the blocks and were spaced 40 cm apart. Each consisted of 21 pits subdivided into three rows of seven (7) pits each. In all, sixty-three (63) pits were made. Each row corresponded to a conservation method. The distance between two rows was 40 cm, and between two pits 20 cm. Seedlings were placed at the bottom of the pits and covered with soil for the method without additives. For pits with additives, seedlings were laid out with alternating layers of 50 g of millet husk or 400 g of fine sand per

experimental unit.

Data collection and statistical analysis

Temperature, relative humidity and CO₂ concentration were recorded weekly in the storage containers using a Voltcraft CO-100. Averages were calculated monthly.

The budding time and the rate of budded seedlings were evaluated. Seedling budding time corresponds to the number of days between the start of storage and bud emergence. It is calculated by taking the difference between the date on which the seedlings were placed in storage and the date on which the buds began to emerge.

The number of budded seedlings was determined by counting them after visual observation, with reference to bud emergence (Fig. 4.). They were counted at the end of the storage period. This number of budded seedlings was used to calculate the budded seedling rate (BSR) using the following formula:

$$\text{BSR}(\%) = \frac{\text{Number of budded seeds}}{\text{Number of seeds preserved}} \times 100 \quad (1)$$

The number of rotten seedlings was counted at the end of the storage period following visual observations. This number was used to calculate the seedling loss rate (SLR) using the following formula:

$$\text{SLR}(\%) = \frac{\text{Number of rotten seeds}}{\text{Number of seeds preserved}} \times 100 \quad (2)$$

The rate of water loss from the seeds was determined. The initial weight (Pi) of the seedlings, which is the weight before storage, and the weight of the seedlings at the end of each month, considered as the final weight (Pf) of the month, were determined using a precision balance (Fig. 5.). The monthly seedling water loss rate (SWLR) was evaluated using the following formula:

$$\text{SWLR}(\%) = \frac{(P_i - P_f)}{P_i} \times 100 \quad (3)$$

With : Pi = initial weight of seedlings before storage;

Pf = final seedling weight.

Bud length was measured at the end of storage using a wire to follow the curvature of the bud and then placed on a graduated ruler to read the value. Measurements were taken per experimental unit on five (5) budded seedlings randomly selected from among the budded seedlings. Average bud length was calculated according to the following equation:

$$\text{LMB} = \frac{1}{n} \sum_{i=1}^n L_i$$

With: Li = individual bud length; n = number of budded seedlings measured.

The data collected was entered and processed using Microsoft Excel 2019. The same spreadsheet was used for calculations and graphing. The data were then subjected to an analysis of variance (ANOVA) using R software version 4.3.1. Means were compared using the Tukey test with a threshold of 5%. R software version 4.3.1 was also used to produce boxplots.

Results

Temperature and relative humidity in containers

During seedling storage from December to May, the average monthly temperature varied from 28.63°C to 32.35°C in the "Bitatoré" and from 27.43°C to 32.95°C in the canaries. As for monthly relative humidity, it varied on average between 32.4% and 43.5% inside the "Bitatoré" and between 30% and 43.16% inside the canaries. However, analysis of variance showed no significant difference between containers for these two parameters (Table 1).

CO₂ content inside containers

The average monthly CO₂ content varied between 1253.33 ± 62.09 and 2612.5 ± 246.81 ppm inside the "Bitatoré", and between 1150 ± 36 and 2127.5 ± 96.09 ppm inside the canaries. The highest levels were recorded in February inside the "Bitatoré" and inside the canaries, with 2612.5 ± 246.81 and 2127.5 ± 96.09 ppm respectively. On the other hand, low concentrations were recorded in March in the "Bitatoré" and in the canaries, averaging 1253.33 ±

62.09 and 1150 ± 36 ppm respectively. With the exception of April, CO₂ levels were slightly higher in the Bitatoré than in the canaries (Table 2). Analysis of

variance showed a significant difference ($P = 0.03$) between Bitatoré and canaries for this parameter in May.

Table 1. Average temperature and relative humidity inside containers during months of storage.

	Temperature (°C)				
	January	February	March	April	May
Bitatoré	$29.03 \pm 1,53^a$	$32.20 \pm 0,13^a$	$30.08 \pm 0,18^a$	$32.35 \pm 0,20^a$	$28.63 \pm 0,12^a$
Canaries	$27.43 \pm 1,18^a$	$32.95 \pm 0,58^a$	$29.78 \pm 0,15^a$	$31.77 \pm 0,40^a$	$28.89 \pm 0,10^a$
P	0.36 ns	0.28 ns	0.31 ns	0.27 ns	0.06 ns
	Relative humidity (%)				
	January	February	March	April	May
Bitatoré	$41.5 \pm 0,58^a$	$43.5 \pm 0,50^a$	$32.4 \pm 0,1^a$	$32.5 \pm 0,76^a$	$33.17 \pm 2,46^a$
Canaries	$43.3 \pm 0,88^a$	$43.17 \pm 0,67^a$	$31.33 \pm 0,44^a$	$32.82 \pm 0,44^a$	$30 \pm 1,26^a$
P	0.33 ns	0.71 ns	0.77 ns	0.72 ns	0.31 ns

Legend: ns = difference not significant, P = probability.

Table 2. Evolution of CO₂ concentrations inside containers.

	January	February	March	April	May
Bitatoré	$1887.25 \pm 49,18^a$	$2612.5 \pm 246,81^a$	$1253.33 \pm 62,09^a$	$1312.5 \pm 78,10^a$	$1723.33 \pm 11,21^a$
Canaries	$1858.75 \pm 42,24^a$	$2127.5 \pm 96,09^a$	1150 ± 36^a	$1422.5 \pm 47,32^a$	$1607.5 \pm 32,70^b$
P	0.76 ns	0.27ns	0.15 ns	0.2 ns	0,03 *

Legend: * significant difference, ns = non-significant difference, P = probability

Seedling budding time

Budding time varied between 51 ± 4 and 70 ± 3 days depending on the storage method. Seedlings stored in "Bitatoré" pits with sand as an additive had a longer budding time of 70 ± 3 days after storage. In contrast, seedlings stored in pits without additives had a shorter budding time of 51 ± 4 days (Fig.6.).

Table 3. Proportion of budded seedlings (%) by preservation method.

Storage methods	Rate of budded seedlings (%)
FS	$60,95 \pm 4,52^b$
F+G	$63,81 \pm 4,70^b$
F	$68,57 \pm 3,03^b$
BITA	$86,19 \pm 2,33^a$
BITA+CB	$83,81 \pm 3,12^{ab}$
BITA+G	$89,05 \pm 2,27^a$
BITA+S	$83,81 \pm 3,61^{ab}$
CAN	$85,24 \pm 2,54^a$
CAN+G	$82,86 \pm 3,24^{ab}$
CAN+CB	$85,24 \pm 2,35^a$
CAN+S	$87,62 \pm 2,17^a$
Tri S	$85,71 \pm 2,72^a$
P	< 0,0001***

In addition, the budding times of seedlings from the accessions evaluated varied considerably from one method to another, ranging from 23.33 ± 0.66 to 88 ± 3.99 days (Fig. 7.).

Table 4. Proportion of budded seedlings (%) by accessions.

Accessions	Rate of budded seedlings (%)
E 186	$86,38 \pm 1,91^a$
EU 088	$84,44 \pm 2,50^a$
White	$83,05 \pm 3,49^a$
E165	$82,77 \pm 2,6^a$
E 120	$79,72 \pm 2,53^{ab}$
UWo86 M	$79,16 \pm 2,59^{ab}$
UWo72 ID	$66,11 \pm 2,63^b$
P	< 0,0001***

In fact, seedlings from accession UEo88 had the shortest budding times after conservation in the following methods: "Bitatoré" without additives, "Bitatoré" with millet husk as additive, "Bitatoré" with sand as additive, canaries without additives, canaries

with sand as additive, canaries with wood chip as additive, pits with sand as additive, pits with millet husk as additive, pits without additive and in the Storage in Sand and Sprouting or triple S system (Fig. 6.,7.,8. and 9.). Budding times for seedlings from accession UEO88 using the above methods were 35 ± 2.66 days, 31 ± 0.98 days, 45 ± 3.99 days, 38 ± 1.73 days, 50 ± 3.99 days, 42.33 ± 0.87 days, 35.66 ± 8.41 days, 31.33 ± 10.32 days, 23.33 ± 0.66 days and 39.66 ± 8.66 days respectively. Seedlings from accession E186 had the shortest budding times in the "Bitatoré"

with wood shavings as additive, i.e. 37 ± 14.99 days, and those of White in the canary with millet husks as additive, i.e. 51 ± 6.99 days (Fig. 6 and 7).

On the other hand, seedlings of accession E120 budded late in the following methods: "Bitatoré" with millet glumes as additive, "Bitatoré" with wood shavings, canaries without additive and the Storage in Sand and Sprouting or triple S system with budding times of 88 ± 3.99 days, 83 ± 4.4 days, 88 ± 3.99 days and 84 ± 9.01 days respectively.



Fig. 1. Storage system with different containers: A. "Bitatoré"; B. canaries and C. Storage in Sand and Sprouting or triple S system.

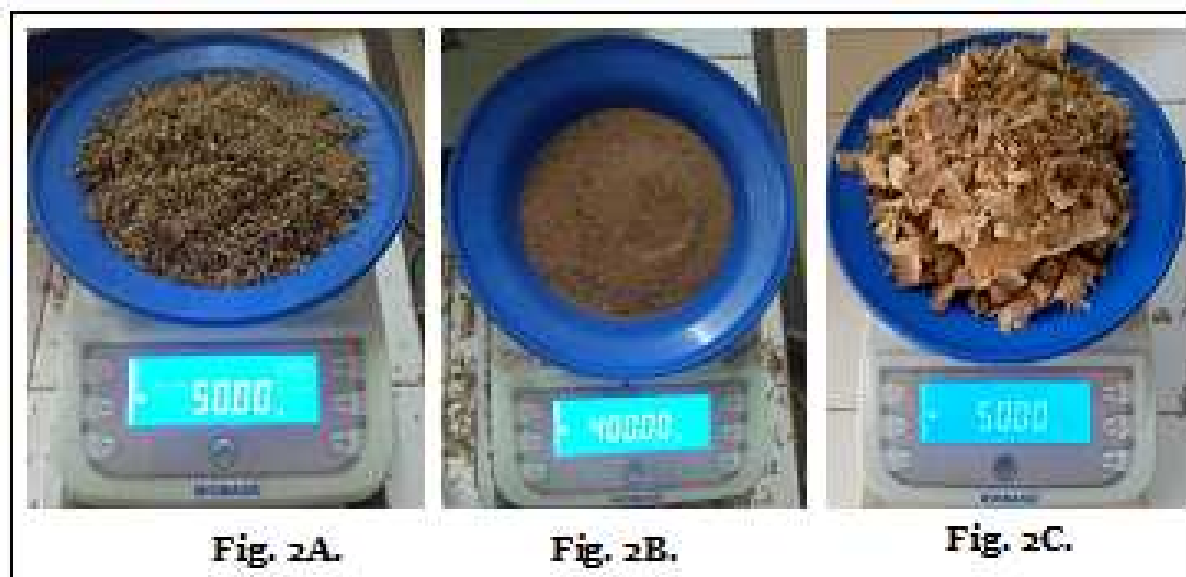


Fig. 2. Additive weighing: A. Millet glumes; B. Fine sand and C. Wood shavings.

In addition, seedlings of accession UWO72ID budded late in the following methods: "Bitatoré" with sand as additive, canaries with wood chip as additive, canaries with sand as additive, canaries with millet glumes as additive, pits without additive and pits with sand as additive with respective budding times of 86 ± 7.5 days, 86.33 ± 5.66 days, 83 ± 8.99 , 83 ± 1.66 days, 61.66 ± 4.66 days and 74 ± 4.93 days. Seedlings from accessions UWO86M and E165 budded late in the "Bitatoré" without additive (80 ± 1.73 days) and in the pits with glumes as additive (68.66 ± 9.02 days) respectively. Furthermore, analysis of variance showed a highly significant difference ($P < 0.0001$) between preservation methods and accessions evaluated (Fig. 6.,7.,8.,9. and 10.).

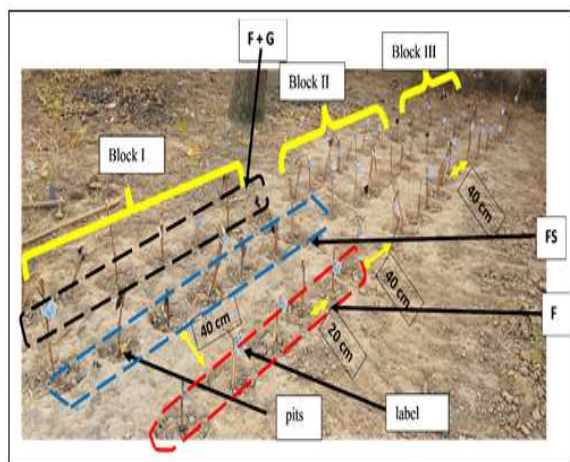


Fig. 3. Conservation device in the pits

Legend: F + G: conservation in pits with millet husks as additive; FS: conservation in pits with sand as additive; F: conservation in pits without additive.

Rate of budded seedlings (%)

The rate of budded seedlings varied between $60.95 \pm 4.52\%$ and $89.05 \pm 2.27\%$, depending on the storage method. The preservation method in "Bitatoré" with millet husks as additive (BITA+G) resulted in a higher rate of budded seedlings, i.e., $89.05 \pm 2.27\%$, whereas the low rate of budded seedlings was noted in seedlings preserved in pits without additives, with an average of $60.95 \pm 4.52\%$. In addition, six (6) preservation methods recorded a budded seedling rate higher than 85%, which are the preservation methods in "Bitatoré" with millet husk as additive (89.05 ± 2.27), "Bitatoré" without additive (BITA) ($86.19 \pm 2.33\%$), canary without additive (CAN)

($85.24 \pm 2.54\%$), canary with wood shavings as additive (CAN+CB) ($85.24 \pm 2.35\%$), canary with sand as additive (CAN+S) ($87.62 \pm 2.17\%$) and the Triple S method (Tri S) ($85.71 \pm 2.72\%$) (Table 3).

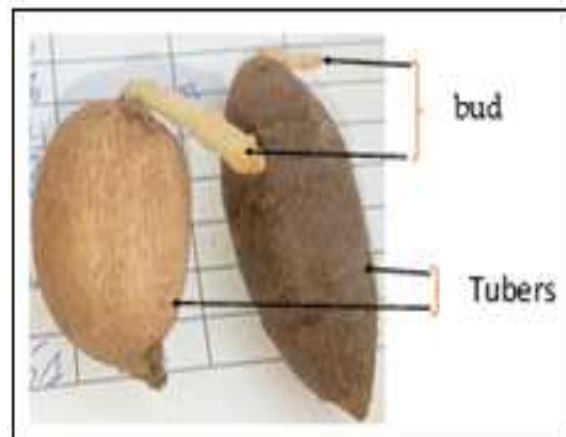


Fig. 4. Seedlings with apical buds.

As for the accessions evaluated, the highest rate of budded seedlings was recorded for accession E186 with $86.38 \pm 1.91\%$, while seedlings from accession UWO72ID had the lowest budding rate at $66.11 \pm 2.63\%$ (Table 4). Analysis of variance of the rate of budded seedlings showed a highly significant difference ($P < 0.0001$) between conservation methods and between accessions.



Fig. 5. Weighing seedlings in April.

Bud length (cm)

Seedling bud lengths ranged from 1.87 ± 0.43 to 5.34 ± 0.46 cm, depending on the storage method (Fig.11.). Seedlings stored in canaries with millet glumes as additive (CAN+G) and those stored in canaries without additive (CAN) had the longest buds, at 5.34

± 0.46 and 4.86 ± 0.46 cm respectively. On the other hand, seedlings kept in pits with millet husks as additive had the shortest buds at 1.87 ± 0.43 cm. Bud lengths of seedlings from the accessions evaluated ranged from 1.8 ± 0.09 to 5.33 ± 0.3 cm with the longest buds observed in seedlings from accession

E165, i.e. 5.33 ± 0.3 cm. On the other hand, the shortest buds were observed in seedlings of accession E120, i.e. 1.8 ± 0.09 (Fig.12.). The results of the analysis of variance also showed highly significant differences ($P < 0.0001$) between conservation methods, and between the accessions tested.

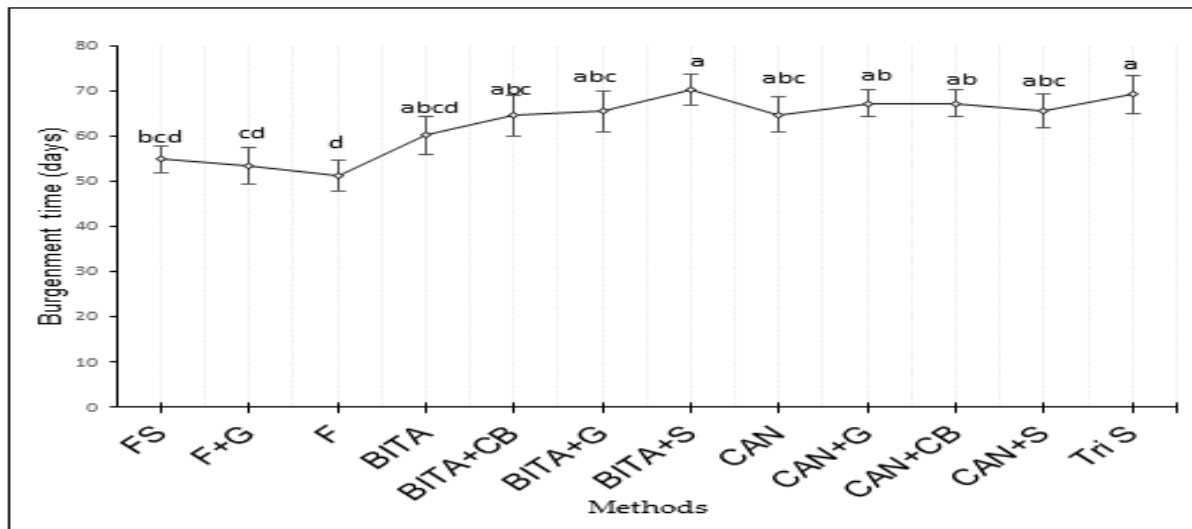


Fig. 6. Variation in budding time depending on conservation methods

Legend : FS = conservation in pits with sand as additive, F+G = conservation in pits with millet glumes as additive, F = conservation in pits without additive, BITA = conservation in "Bitatoré" without additive, BITA+CB = conservation in "Bitatoré" with wood chips as additive, BITA+G = conservation in "Bitatoré" with millet glumes as additive, BITA+S = conservation in "Bitatoré" with sand as additive, CAN = conservation in canaries without additives, CAN+G = conservation in canaries with millet husks as additive, CAN+CB = conservation in canaries with wood shavings as additive, CAN+S = conservation in canaries with sand as additive, Tri S = conservation with the Storage in Sand and Sprouting system or triple S.

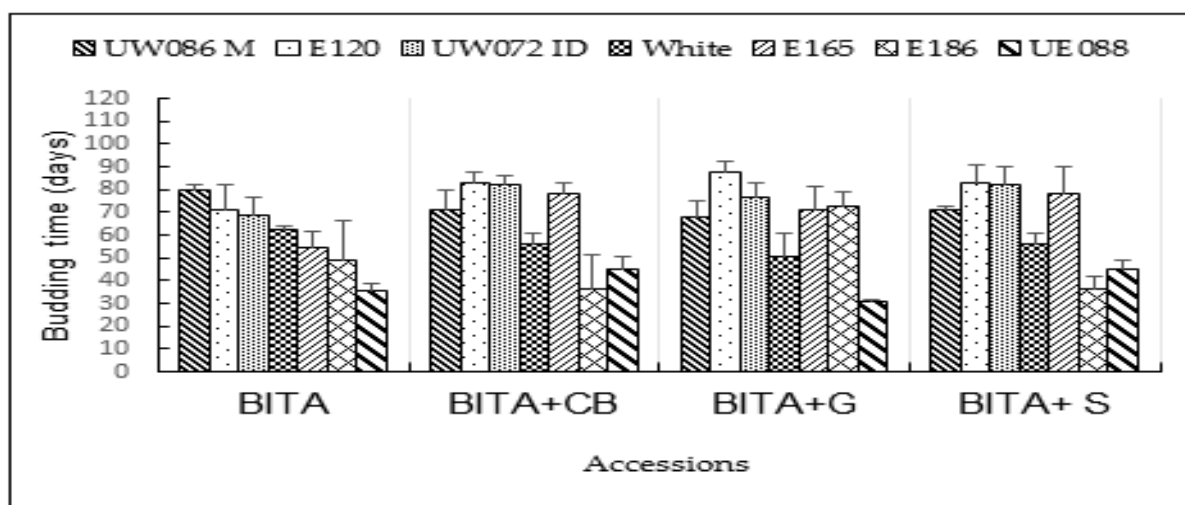


Fig. 7. Budding time in the "Bitatoré"

Legend: BITA = conservation in "Bitatoré" without additives, BITA+CB = conservation in "Bitatoré" with wood chips as additives, BITA+G = conservation in "Bitatoré" with millet husks as additives, BITA+S = conservation in "Bitatoré" with sand as additives.

Seedling loss rate (%)

The loss rate of seedlings varied between 2.38 ± 1.17 and $16.19 \pm 3.75\%$ depending on the preservation method (Fig. 13). Low losses were observed in seedlings preserved using the triple S method, the

"Bitatoré" method with millet glumes as additive, the "Bitatoré" method with sand as additive and the canary method with sand as additive, with respective loss rates of $2.38 \pm 1.17\%$; $2.86 \pm 1.22\%$; $2.86 \pm 1.97\%$; and $2.86 \pm 1.22\%$.

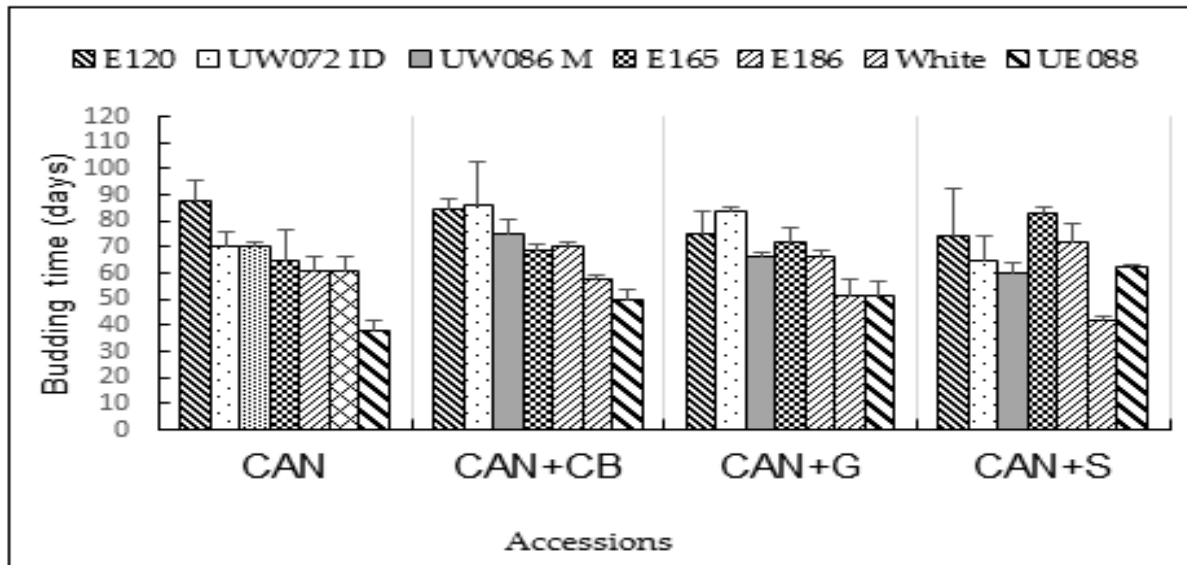


Fig. 8. Budding time in canaries

Legend: CAN = conservation in canaries without additives, CAN+G = conservation in canaries with millet husk as additive, CAN+CB = conservation in canaries with wood shavings as additive, CAN+S = conservation in canaries with sand as additive.

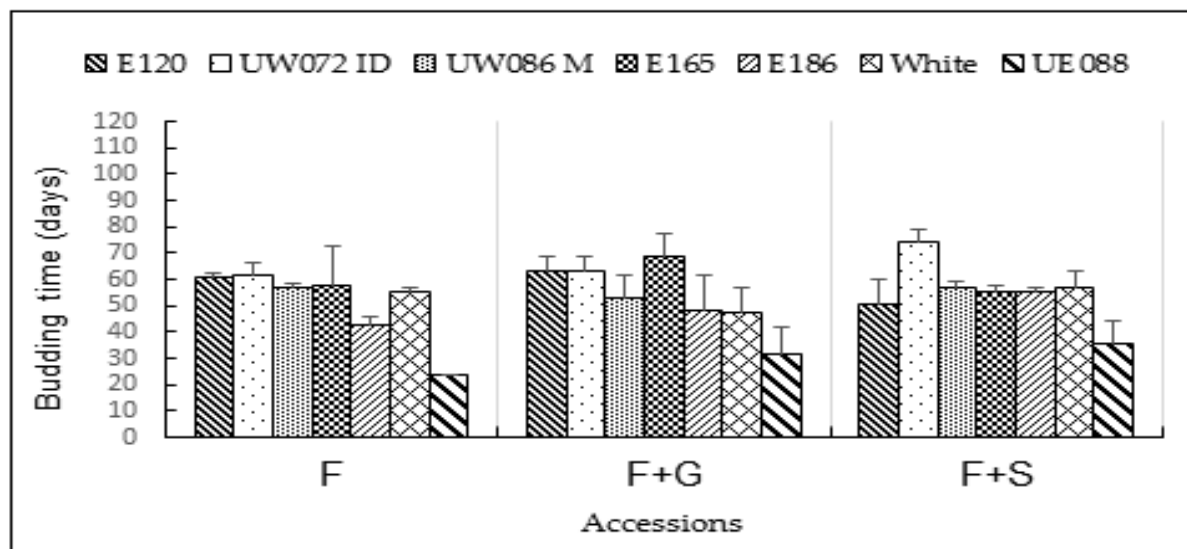


Fig. 9. Budding time in pits

Legend: FS = conservation in pits with sand as additive, F+G = conservation in pits with millet husk as additive, F = conservation in pits without additive.

On the other hand, the highest seedling losses were observed for seedlings kept in pits with sand as additive and pits with millet husks as additive, i.e.,

$16.19 \pm 3.75\%$ and $14.76 \pm 5.05\%$ respectively. Accession UW072ID recorded less seedling loss (2.5%), while accession White recorded the highest

seedling loss (11.66%) compared with the other accessions (Fig. 14.). In addition, analysis of variance of the seedling loss rate revealed a very highly

significant difference between methods ($P < 0.0001$) and highly significant between accessions ($P = 0.002$).

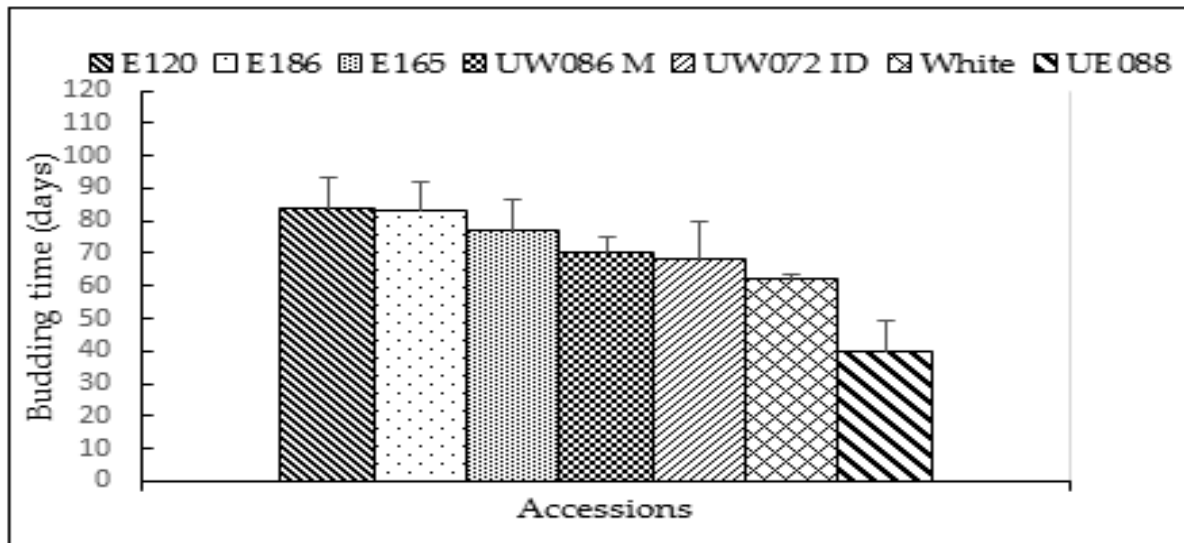


Fig. 10. Budding time in triple S.

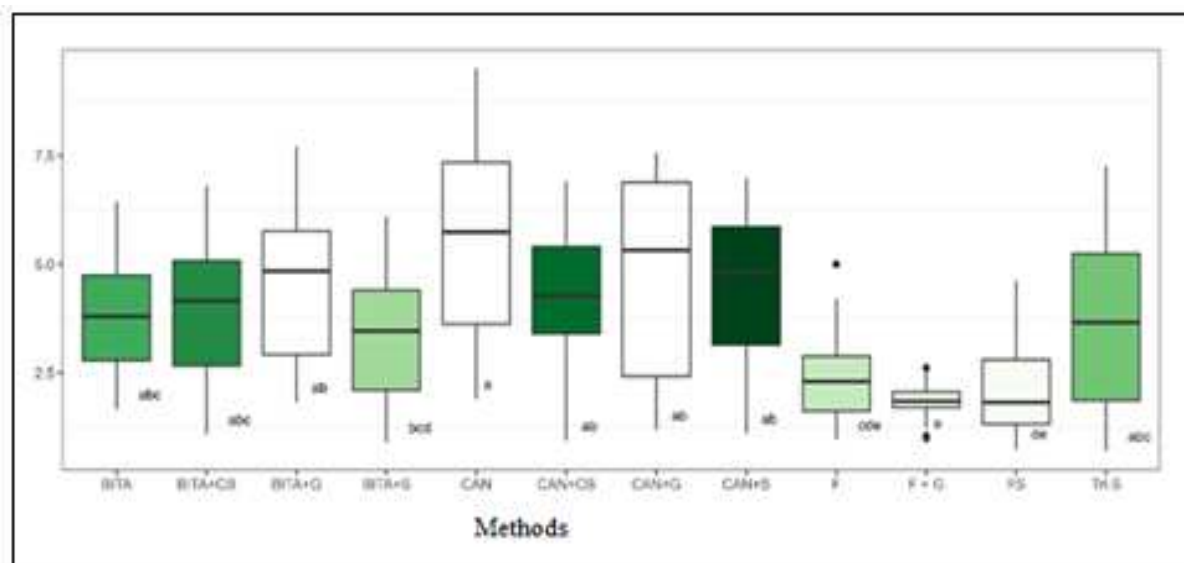


Fig. 11. Comparison of seedling bud length averages according to preservation methods

Legend : FS = conservation in pits with sand as additive, F+G = conservation in pits with millet glumes as additive, F = conservation in pits without additive, BITA = conservation in "Bitatoré" without additive, BITA+CB = conservation in "Bitatoré" with wood chips as additive, BITA+G = conservation in "Bitatoré" with millet glumes as additive, BITA+S = conservation in "Bitatoré" with sand as additive, CAN = conservation in canaries without additives, CAN+G = conservation in canaries with millet glumes as additive, CAN+CB = conservation in canaries with wood shavings as additive, CAN+S = conservation in canaries with sand as additive, Tri S = conservation with the Storage in Sand and Sprouting or triple S system.

Seedling water loss (%)

The seedlings produced by the different methods showed highly variable water losses from one method

to the next, and progressive losses from the beginning to the end of storage (Fig.15.). Water loss rates ranged from 2.32 ± 0.59 to $7.90 \pm 0.82\%$ in January, from

3.71 ± 0.27 to 9.89 ± 0.89% in February, from 8.85 ± 0.71% to 15.1 ± 0.88% in March, from 22.62 ± 0.99 to 35.15 ± 3.02 in April and from 32.64 ± 1.87 to 47.68 ± 2.72 in May (Fig. 15.). However, the greatest water

losses at 47.68 ± 2.72 % were recorded in seedlings kept in pits with sand as an additive. On the other hand, the lowest water loss rates were recorded for seedlings from the triple S method, at 32.64 ± 1.87 %.

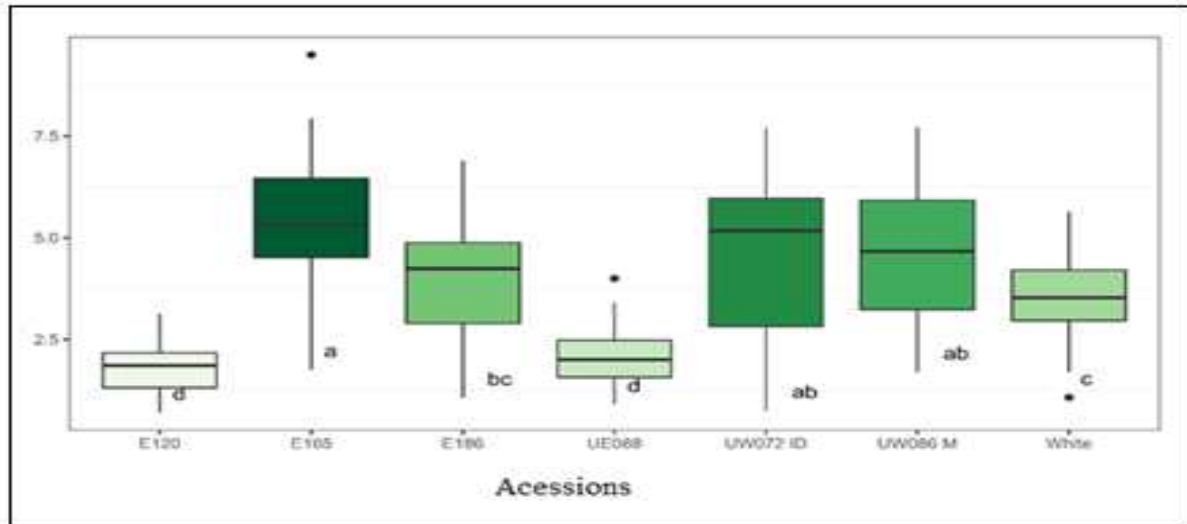


Fig. 12. Comparison of seedling bud length averages by accessions.

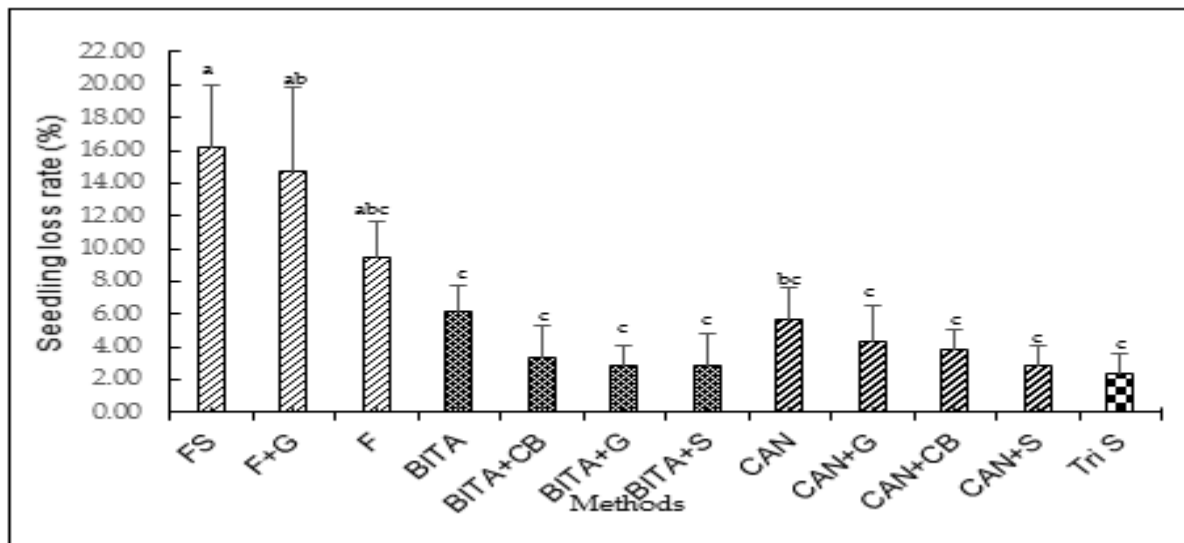


Fig. 13. Comparison of average seed rates according to preservation methods

Legend : FS = conservation in pits with sand as additive, F+G = conservation in pits with millet glumes as additive, F = conservation in pits without additive, BITA = conservation in "Bitatoré" without additive, BITA+CB = conservation in "Bitatoré" with wood chips as additive, BITA+G = conservation in "Bitatoré" with millet glumes as additive, BITA+S = conservation in "Bitatoré" with sand as additive, CAN = conservation in canaries without additives, CAN+G = conservation in canaries with millet glumes as additive, CAN+CB = conservation in canaries with wood shavings as additive, CAN+S = conservation in canaries with sand as additive, Tri S = conservation with the Storage in Sand and Sprouting or triple S system.

The seedlings of the accessions evaluated also showed highly variable and progressive water losses from the beginning to the end of storage (Fig. 16.). In fact,

seedlings from accession UE088 lost a great deal of water in the first three (3) months of storage compared with the other accessions, with water loss

rates of 7.62 ± 0.82 % in January, 10.78 ± 0.9 % in February and 17.12 ± 0.97 % in March. As for the last two months of conservation (April and May), it was the seedlings of the White and UW086M accessions that lost a lot of water compared with the others, with water loss rates of 33.31 ± 2.17 % in April for the White accession and 45.97 ± 1.29 % in May for the UW086M accession. In contrast, seedlings from

accession E165 lost less water in January (4.05 ± 0.32 %), February (5.96 ± 0.4 %) and March (9.88 ± 0.4 %). On the other hand, seedlings from accession UW072ID lost less water in April (19.62 ± 1.26 %) and those from E120 in May (30.30 ± 1.55 %) (Fig. 16.). Analysis of variance revealed a highly significant difference ($P < 0.0001$) between accessions over the 6 months of storage.

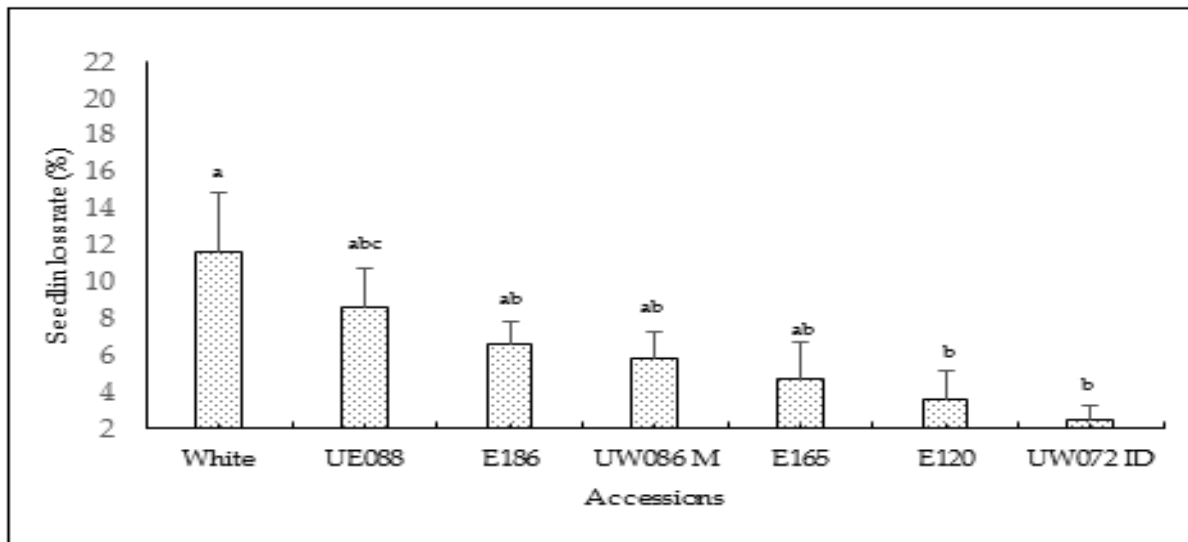


Fig. 14. Comparison of seedling rate averages between evaluated accessions.

The analysis of variance also revealed a very highly significant difference ($P < 0.0001$) between storage methods in the months of January, April and May, and a significant difference ($P = 0.01$) in the month of February. However, there was no significant difference between preservation methods in March.

Discussion

The short budding time of seedlings stored in pits without additives, pits with millet husks as additive and pits with sand as additive have shown that the duration of seedling dormancy depends on the storage method. Indeed, several authors have reported that the duration of seedling dormancy depends strongly on tuber preservation techniques (Aksenova, 2013; Mani *et al.*, 2014; Nanbol *et al.*, 2020). Significant differences were observed between the accessions evaluated, with budding times ranging from 23.33 ± 0.66 to 88 ± 3.99 days after storage. In addition, the budding time of seedlings from the evaluated accessions varied differently from one

accession to another. The variation in seedling budding time observed between accessions is thought to be of genetic origin (Bischoff *et al.*, 2006). Temperatures varied between 27.43°C and 32.95°C inside the "Bitatoré" and the canaries, with relative humidities between 30% and 43.5%. The highest temperatures during storage were observed in February: $32.20 \pm 0.13^{\circ}\text{C}$ inside the "Bitatoré" and $32.95 \pm 0.58^{\circ}\text{C}$ inside the canaries. These high temperatures therefore favored seedling budding. Indeed, according to Law *et al* (2004), tuber storage in thermal conditions below 3°C or above 30°C favors tuber budding. CO_2 levels, ranging from 1150 ± 36 to 2612.5 ± 246.81 ppm, testify to the presence of respiratory activity in the tubers, resulting in the transformation of carbohydrates (starch) into carbon dioxide and water, with the production of heat and consumption of oxygen (Robert, 2011). In this way, starch is mobilized for the first stages of vegetative construction, resulting in the emergence of buds (Richard *et al.*, 2010).

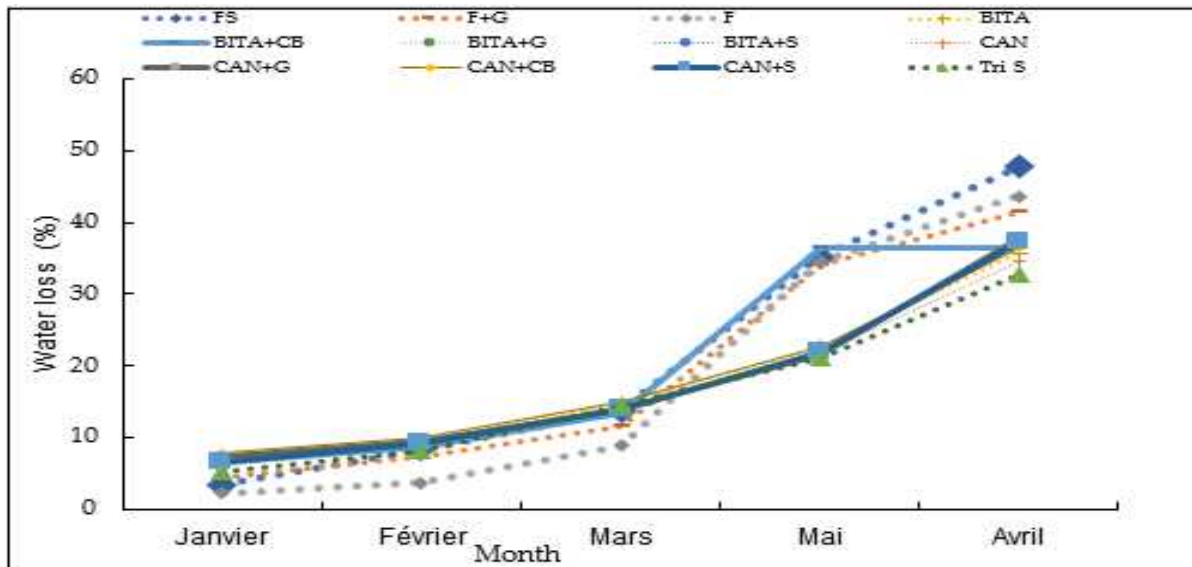


Fig. 15. Evolution of water losses during the month of January to May according to conservation methods
 Legend : FS = conservation in pits with sand as additive, F+G = conservation in pits with millet glumes as additive, F = conservation in pits without additive, BITA = conservation in "Bitatoré" without additive, BITA+CB = conservation in "Bitatoré" with wood chips as additive, BITA+G = conservation in "Bitatoré" with millet glumes as additive, BITA+S = conservation in "Bitatoré" with sand as additive, CAN = conservation in canaries without additives, CAN+G = conservation in canaries with millet glumes as additive, CAN+CB = conservation in canaries with wood shavings as additive, CAN+S = conservation in canaries with sand as additive, Tri S = conservation with the Storage in Sand and Sprouting or triple S system.

These CO₂ concentrations observed inside the containers were conducive to the conservation of *S. rotundifolius* seedlings, as seedlings from containers obtained the highest budding rates. Seedling bud lengths also varied from accession to accession,

ranging from 1.8 ± 0.09 to 5.33 ± 0.3 cm. Indeed, our results on bud lengths are close to those obtained by Nanbol *et al.* (2020) on seedlings from six (6) accessions of *S. rotundifolius* stored in plastic baskets in ambient air.

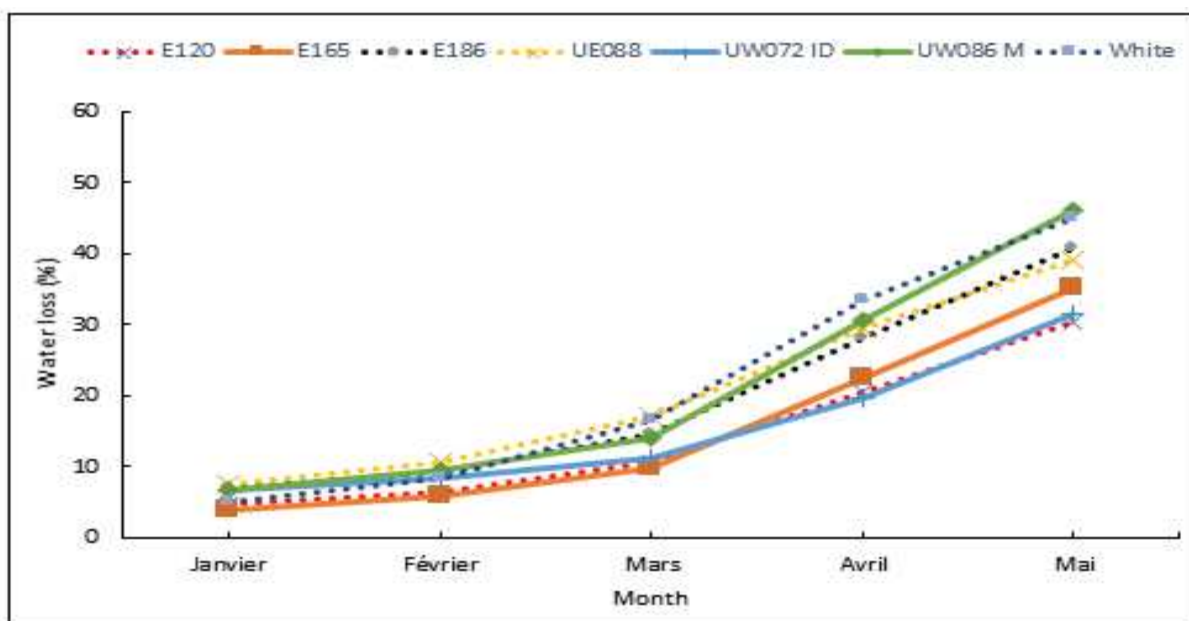


Fig. 16. Evolution of water losses during the month of January to May according to the accessions evaluated.

Seedlings from accessions grown in pits (with or without additives) showed the greatest losses, ranging from $16.19 \pm 3.75\%$ to $14.76 \pm 5.05\%$. Indeed, Dandago *et al* (2011) showed that sweet potato samples stored in shaded pits with alternating layers of fresh river sand rotted after 4 months. In contrast, the "Bitatoré" and canary methods with additives such as sand, millet husk and wood chip all recorded low seedling loss rates (2.86 ± 1.22 to $2.38 \pm 1.18\%$) and long dormancy periods (65 ± 4 to 70 ± 3 days), as did the triple S method. The additives acted as an inhibitor of the seedlings' physiological processes, prolonging dormancy while limiting seedling losses. On the other hand, seedlings produced by these methods lost quite a lot of water (36.50 ± 1.63 to $37.70 \pm 1.46\%$), in contrast to those produced by "Bitatoré" without additives and canaries without additives (34.68 ± 1.77 to $35.70 \pm 1.51\%$). These additives, made up of millet husks, wood shavings and sand, would have played a role in water absorption during seedling storage. Results also showed that seedlings produced using the triple S method had the lowest rates of seedling loss ($2.38 \pm 1.77\%$) and water loss (32.64 ± 1.87). Indeed, studies have shown that the Triple S method resulted in a higher survival rate (81-95%) of sweet potato seed tubers during the dry season compared with local preservation methods (7-57%) (Hundayehu *et al.*,2022). The variations in water loss observed in seedlings during storage are roughly similar to those found by Surgi *et al.* (2021), where water loss in *S. rotundifolius* seedlings stored in different varied between 11.5% and 56.2%.

Conclusions

The results of this study showed that seedlings from the accessions evaluated varied differently from one storage method to another in terms of budding, water loss and seedling loss. From the results obtained from this study, six (6) conservation methods favored better budding, with budding rates higher than 80%. These methods were Bitatoré with millet husk additives (BITA+G), Canary with sand additives (CAN+S) and Bitatoré without additives (BITA), in the triple S system (Storage in Sand and Sprouting)

(Tri S), in canaries with wood chips as additives (CAN+CB) and in canaries without additives (CAN) with budding rates ranging from $85.24 \pm 2.35\%$ to $89.05 \pm 2.27\%$. In addition, these methods recorded low seedling loss rates ranging from $2.38 \pm 1.17\%$ to $5.71 \pm 1.89\%$. Among these methods, seedlings from the "Bitatoré" method with millet husk as additive recorded the best budding rate at $89.05 \pm 2.27\%$ and a low seedling loss rate of $2.86 \pm 1.22\%$. However, seedlings from the six (6) best methods mentioned above budded late, between 60.14 ± 4.19 days and 70.33 ± 3.42 days, in contrast to the conservation methods of pits without additives, pits with sand as additive and pits with millet husk as additive, which shortened seedling dormancy to 51 ± 4 , 53 ± 4 and 55 ± 3 days after conservation.

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Conflicts of Interest

The authors declare no conflicts of interest.

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