

Storage of plant species with desiccation-sensitive germplasm in Compact Germplasm Bank.

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Abstract

Plant species sensitive to desiccation are difficult to store and transport in the germplasm bank for space travel. Applying plant tissue culture can help to create a Plant Germplasm Bank for this species. For this purpose, the Compact Germplasm Bank (CGB) was created to store and transport *in vitro* explants, maintaining them for long periods in slow-grown storage. This study aimed to evaluate the CGB efficacy in the storage *in vitro* explant of Taioba (*Xanthosoma sagittifolium*). For this, three CGBs were connected, side by side, with different LED light spectra (CGB1: Red spectrum; CGB2: 50% Red + 50% Blue spectra; CGB3: Blue spectrum), containing each one nine test tubes with taioba explants (one per test tube), and LED lights intensity adjusted for $30 \mu\text{mol m}^{-2}\text{s}^{-1}$. The CGBs were maintained for 120 days in the darkroom, at 25°C temperature and 50-60% humidity. Explant storage in CGB3 showed lower root numbers and root lengths than in CGB1 and CGB2. The Blue spectrum in CGB3 reduced the root oxidation and browning, resulting in 100% live explants. The CGB fulfilled its proposed function of storing the *in vitro* explants.

Key Message

Compact chamber for transporting and storage of *in vitro* plant germplasm.

Introduction

Storage and transport of the Plant Germplasm Bank (PGB) is a challenge to maintaining plant-based bio-regenerative systems for the success of space trips (Wheeler 2010). Extremophile seeds, tolerant to desiccation, can be transported in dry environments through space. In contrast, numerous plant species produce seeds that are partially or fully sensitive to desiccation or propagated clonally and cannot be maintained in the dry state. Important species such as potato, yam, garlic, sweet potato, cassava, sugar cane, and banana are propagated through vegetative tissue. In addition, propagative explants, such as shoot tips, dormant buds, somatic embryos, or undifferentiated calluses, are highly sensitive to desiccation (Visscher et al. 2023).

Therefore, to maintain the PGB during space travel, plant tissue culture techniques are the most suitable as they maintain plant health, reduce the volume transported, and require low inputs (Wheeler 2010). The storage of *in vitro* PGB requires periodic changes in the culture medium depending on the function of the laboratory. This operation involves procedures such as adequate structure, use of culture medium, and qualified labor (Tavazza et al. 2015).

Applying the Slow-Grown Storage (SGS) technique reduces *in vitro* plant growth. In this way, SGS aims to reduce the plant's metabolism and consequently increase the *in vitro* period of the explant without altering the genetic characteristics and quality of the *in vitro* PGB (Kamińska et al. 2016). In *in vitro* growth incubation, the cultivation conditions can be changed, significantly reducing the plant's metabolism. Conditions such as temperature, light intensity, photoperiod, and components of the culture

medium can be adjusted to maintain the plants *in vitro* in SGS (Gopal et al. 2010; Kaur et al. 2012; Carvalho et al. 2014; Thakur et al. 2015). In addition to these factors, the light spectra can be adjusted to reduce the metabolism of the plant and contribute to the SGS technique. The blue spectrum contributed significantly to the reduction of root growth and oxidative stress in the roots during *in vitro* storage of *Heliconia champneiana* for 12 weeks (Rodrigues et al. 2018). These results were corroborated using the same technique for 5 months *in vitro* SGS in banana cv. Prata Catarina (Rodrigues et al. 2022).

To transport and maintain *in vitro* PGB, on Earth or in a Space Lab, the Compact Germplasm Bank (CGB) was created. This device has a small size, reduced weight, light intensity control, light spectra adjustment, and a photoperiodic system. In addition, the CGB hasn't been screwed, it is mobile if powered by a 12V battery, and can be connected side by side to increase the useful area. Thus, the present work demonstrates how the CGB works with *in vitro* taioba explants in different light spectrums for long-term *in vitro* storage.

Materials and Methods

The plant material was *in vitro* explants of Taioba (*Xanthosoma sagittifolium*), from *in vitro* Germplasm Bank (GB) of Laboratory Tissue Culture Ornamental Plants (LTCOP), University of São Paulo. Taioba is propagated only from rhizomes, raw material for the *in vitro* GB. From this GB, aseptic taioba explants with 2.0 cm length x 0.5 cm Ø were selected and inoculated (one per tube) in the Pirex® test tubes (15.0 cm x 2.5 cm) with a cap, containing 7.0 mL of a semi-solid culture medium MS salts and vitamins (Murashige, T.; Skoog, F. 1962) plus 30 g L⁻¹ sucrose, and 2.0 g L⁻¹ Phytigel® (Sigma-Aldrich) – no Plant Growth Regulator (PGR); the pH of the medium was adjusted to 5.8 before autoclaving for 20 min at 121°C. The explants were introduced and incubated in three different CGBs, each one with a different LED light spectrum, CGB1 = 100% Red (660 nm), CGB2 = 50% Blue + 50% Red spectrum (considered control), and CGB3 = 100% Blue (450 nm), for 120 days in a dark room, maintained at 25°C ± 2°C and 50–60% humidity (similar ISS conditions). The CGB consists of one piece of aluminum cut and bent into a container measuring approximately 32 x 270 x 170 mm (A x B x C), comprising a frame with a lying “U”. In the upper inner portion (Fig. 1a-2) is placed a Photosynthetic Active Radiation (PAR) COB (Chip On Board) LED tape. On the side (Fig. 1a-3), fit the photosynthetic cycle system and the Photosynthetic Photon Flux (PPF) dimmer. The front cover (Fig. 1a-5) and the back cover (Fig. 1a-4) are fixed, wherein the frame and fitting portion have folded flaps and fastening hook and loop fasteners (3M Dual Lock™) disposed on the outer portion of the folded flaps for the firm and removable fit of the front and rear covers. The side fitting portion (Fig. 1a-6) is fitted to the frame through a side opening, locking the CGB (Fig. <link rid="fig1">1</link>a-1). Each CGB weighs 592g (plus front and rear aluminum covers). Full of test tubes (culture media + explant), the CGB weighs 930 g.

The CGBs Photosynthetic Cycle (PC) was adjusted with the mini digital-timer (THT 2401-0) for 12 h day/night, it was supplied by PAR-COB LED tape (CGB1, CGB2, and CGB3), at a photosynthetic photon flux density (PPFD) adjusted to 30 µmol m⁻²s⁻¹ (LI-250A, LI-COR) for all treatments. The treatments (CGBs) were connected side by side with fastening hook and loop fasteners (3M Dual Lock™) but

separated by a black acrylic shell one each other (Fig. 1b). The CGBs were connected to a power supply LSF-CB-12V60W5A (AC100-265V), and a Nobreak (SMS LITE600). The experiment was installed in a completely randomized design, with 9 replicates for each CGB. After 120 days, the CGBs were opened and the plants were taken from the test tubes, washed with tap water (to remove the culture media), and measured plantlet height (cm), number of roots, root length (cm), fresh mass (g), dry mass (g), and plantlet survived (%).

For statistical analyses, initially, descriptive and exploratory analyses of the data were performed. A joint analysis of a group of two experiments was performed, and it was initially found that the variances obtained by the individual analyses were homogeneous. It was found that the data on plant length, root length, number of roots, fresh mass, and dry mass did not meet the assumptions for an analysis of variance (ANOVA). Therefore, it was necessary to apply the logarithmic transformation for the root length data and the number of roots data, and the square root transformation for the fresh mass data and the dry mass data, according to the Box-Cox methodology. In addition, for the fresh mass data, an outlier from the mixed light treatment of the second experiment was removed. For the plant length data, no transformation was found that would make the data meet the assumptions, so the non-parametric Kruskal-Wallis test was applied. After the transformations and removal of the outliers, the data were submitted to ANOVA. Cohen's effect size was also calculated. The effect size was interpreted according to Table 1, according to Cohen (2013). To verify the behavior of the variables studied together, multivariate analysis of variance (MANOVA) was applied, followed by linear discriminant analysis (LDA). For this, the variables that met the assumption of normality of the residuals after the Box-Cox transformation were used (logarithm of root length and number of roots, and square root of fresh and dry mass, removing an outlier from the mixed light treatment). It was verified that the MANOVA assumptions were met. The normality of the residuals within the light color levels was verified by the Shapiro-Wilk test, and the asymmetry and kurtosis by the Mardia tests (Mardia, 1970). Multiple comparisons were made using the Tukey test. All analyses were performed using R software (R Core Team, 2024) at a significant level of 5%.

Table 1
Interpretation for Cohen's f effect size.

Cohen's f	Tamanho do efeito
Less than 0.10	Very small
Between 0.10 and 0.25	Small
Between 0.25 and 0.40	Medium
Greater than 0.40	Large
Source: Cohen (2013)	

Results and Discussion

The results of the joint analyses can be observed in Table 2. For all variables, there was no significant effect of the experiment ($p = 0.9833$ for root length, $p = 0.7384$ for the number of roots, $p = 0.8845$ for fresh mass, and $p = 0.9710$ for dry mass) nor of the interaction of the experiment with treatment ($p = 0.9783$ for root length, $p = 0.9535$ for number of roots, $p = 0.8182$ for fresh mass and $p = 0.9966$ for dry mass; Table 2). No significant effects of the light colors were observed on the variables plant length (very small effect), fresh mass (medium effect), and dry mass (medium effect). However, a large and significant effect of light color on root length was observed ($p = 0.0414$; $f = 0.3994$). Furthermore, Table 2 shows that the effect of light color on root number was also large and significant ($p = 0.0013$; $f = 0.6007$). Plants from CGB1 had significantly greater root length and root number than those from CGB3. For this study, the CGB2 treatment was considered the control because it combines the two fundamental spectra required for *in vitro* cultivation.

Table 2

Mean $\pm$ standard error of the variables studied as a function of the lights spectra and results of the joint analyses (p -value of the effect of the color of the lights and effect size f) and results of the MANOVA, including sources of variation, value of the Pillai statistic, value of the F statistic, degrees of freedom (DL) of the denominator and numerator for the F statistic and p -value.

Variable	Treatment (light spectra)			p-value (Treatment)
	CGB1	CGB2	CGB3	
Plant length (cm)	12.53 $\pm$ 0.63 a	10.78 $\pm$ 1.07 a	10.33 $\pm$ 0.70 a	$p = 0.1485$; $f = 0.2026$
Root length (cm)	9.43 $\pm$ 1.09 a	7.78 $\pm$ 0.96 ab	6.08 $\pm$ 0.77 b	$p = 0.0414$; $f = 0.3994$
Number of roots	8.80 $\pm$ 0.45 a	7.12 $\pm$ 0.60 ab	5.56 $\pm$ 0.88 b	$p = 0.0013$; $f = 0.6007$
Fresh weight (g)	3.55 $\pm$ 0.32 a	3.34 $\pm$ 0.45 a	2.80 $\pm$ 0.29 a	$p = 0.2927$; $f = 0.2443$
Dry weight (g)	2.42 $\pm$ 0.17 a	1.96 $\pm$ 0.28 a	1.69 $\pm$ 0.22 a	$p = 0.0732$; $f = 0.3595$
Source of variation	Pillai	F	DF (numerator/denominator)	p-value
Experiment	0.0059	0.0579	4 / 39	0.9935
Treatment	0.4590	2.9788	8 / 80	0.0057
Experiment $\times$ Treatment	0.0296	0.1503	8 / 80	0.9963

Table 2 shows, the MANOVA model obtained a significant Pillai trace for treatment and a large effect size (Pillai = 0.4590; $p = 0.0057$; partial $f = 0.5458$). Tukey's test indicated that plants under CGB3 differed significantly from those under CGB1, but plants under CGB2 did not differ from either of the other two treatments.

By linear discriminant analysis (Fig. 2), a cloud of points formed by observations that received more scatter on CGB3 and a cloud of points that received more concentrated data on CGB1 can be observed. The samples on CGB2 cross both clouds of points. It was observed that 75% of the points classified as CGB3 were correct, as were 50% of the points classified as CGB2 and 60% of those classified as CGB1. Furthermore, 25% of the points classified as CGB2 had received CGB3, and another 25% had received CGB1. None of the points classified as CGB3 had received CGB1, while 15% of those that were classified as CGB1 had received treatment with CGB3. The linear discriminant function 1, which explains 87.4% of the discriminatory variance, indicates that the cloud on CGB3 differs from the others by the difference between fresh mass and dry mass (the CGB3 has a lower dry mass content), by the smaller number of roots, and shorter root length.

These analyses show that the plants under CGB3 had a reduced metabolism. They also showed that the results were consistent, so few plants that received blue spectrum (CGB3) had characteristics similar to those that received red spectrum (CGB1). In addition, plants that received blue spectrum treatment showed characteristics that were not observed in plants under the red spectrum (CGB1).

Figure 3a, c, and e show the CGB1, CGB2, and CGB3 with *in vitro* taioba plant test tubes, after 120 days on SGS. The CGBs operated stably and without failures after 120 days of the experiment. In day PC operating, the maximum temperature near the electronic components and COB LEDs (35 W/m) was 26°C ($\pm 1^\circ\text{C}$), and on the external surface of the CGB (aluminum structure) was 27°C ($\pm 1^\circ\text{C}$), for the adjusted PPF of $30 \mu\text{mol m}^{-2}\text{s}^{-1}$. The test tubes' temperature (with culture medium and explant) was 25°C, the same as room temperature. During the night PC operating, the temperature of the entire system and test tubes was constant at 25°C, the same temperature as the dark room. The relative humidity recorded in the dark room varied between 50 and 60%, which did not interfere with the performance of the CGBs or the development of the *in vitro* explants. The *in vitro* relative humidity (inside the test tubes) has a range between 90 and 97% (Chen, 2004). In this way, the test tube caps were suitable for maintaining the environmental conditions within the test tube. The ISS temperature should range from 18–25°C; in the same way, relative humidity ranges from 25–75% (Laurentini et al. 1999). During the experiment, the temperature range in the *in vitro* explant environment was maintained at 25°C ($\pm 1^\circ\text{C}$), with a relative humidity of 50–60%, similar to the ISS environment.

Figure 3b, d, and f show the taioba plantlets after 120 days. In all treatments, there was a lack of uniformity in the final size of the plantlets. Even though the explants were selected at the beginning of the trial, this variation in plantlet size may occur at the end of 120 days, mainly because auxin was not added to the culture medium. Thus, the explants used the natural hormonal balance for elongation and rooting, as observed in the explants of Satoimo taro (*Colocasia esculenta* (L.) (Karyanti et al. 2025).

Although the CGB is isolated from external light, gas exchange is facilitated using Velcro and the corners that are not welded. This configuration helps with the gas exchange of test tubes. Even so, the accumulation of ethylene in the test tubes can be a determining factor in the success of the SGS (Rodrigues et al. 2022). In the CGB1 and CGB2 treatments, greater rooting occurred than in CGB3. This

rooting process induces more ethylene production, oxidation, and consequent browning and plant death. The yellow circle in Fig. 3b and d shows this effect, resulting in 88% survival. On the other hand, in CGB3 all plantlets survived (100%) and showed less yellowing in the leaves and oxidized roots (Fig. 3f). These results were similar to the *in vitro* SGS of *Heliconia champneiana* and Banana cv. Prata Catarina (Rodrigues et al. 2018; Rodrigues et al. 2022). In these works, the authors suggest that the use of the blue spectrum *in vitro* can help reduce oxidation, browning, rooting, and yellowing of leaves.

To date, there are no reports of compact and mobile tools for the storage and transportation of *in vitro* explants for use on Earth or in space. The CGB described in this work may be an option for studies on the storage and transportation of plant germplasm for long periods. The development of this type of equipment helps in the maintenance of food production systems and techniques for astronauts and in the improvement of bioregenerative technologies for life support systems in space. The authors of the present work address the same question as Visscher et al. 2023: How do we transport plant species with desiccation-sensitive germplasm in space?"

The CGB is small, light, easy to operate, and resistant. Inside, it can be adapted that cloud-based sensor networks can continuously measure temperature, light spectra and intensity, growth metrics, and a subset of platforms can even implement AI-based alerts or adaptive controls. Thus, the CGB, applying SGS techniques, is ready to operate on Earth, connected to a clinostat for microgravity studies of *in vitro* plants. Its application on Earth could reduce costs and anticipate results in the storage of *in vitro* plants.

In conclusion, the CGB fulfilled its proposed function of storing the explants *in vitro*, and the blue spectrum kept the taioba explants in the best condition.

Abbreviations

MS - Murashige and Skoog medium, PGB - Plant Germplasm Bank, SGS - Slow-Grown Storage, GB - Germplasm Bank, LS – Light Spectra, ISS - International Space Station, PC - Photosynthetic Cycle

Declarations

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Paulo Hercílio Viegas Rodrigues: Conceptualization, Methodology, Formal analysis and investigation, Writing - original draft preparation, Writing - review and editing, Funding acquisition.

Guilherme Bovi Ambrosano: Methodology, Formal analysis and investigation, Writing - original draft preparation, Writing - review and editing.

The authors agree to the availability of the manuscript data.

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References

1. Carvalho V, dos Santos DS, Nievola CC (2014) In vitro storage under slow growth and ex vitro acclimatization of the ornamental bromeliad *Acanthostachys strobilacea*. *S Afr J Bot* 92:39–43. <https://doi.org/10.1016/j.sajb.2014.01.011>
2. Chen C (2004) Humidity in plant tissue culture vessels. *Biosyst Eng* 88:231–241. <http://doi.org/10.1016/j.biosystemseng.2004.02.007>
3. Cohen J (2013) Statistical power analysis for the behavioral sciences. p. 567. New York, NY
4. Routledge
5. Gopal J, Chauhan NS (2010) Slow growth in vitro conservation of potato germplasm at low temperature. *Potato Res* 53:141–149. <https://doi.org/10.1007/s11540-010-9158-x>
6. Kamińska M, Skrzypek E, Wilmowicz E et al (2016) Effect of light conditions and ABA on cold storage and post-storage propagation of *Taraxacum pieninicum*. *Plant Cell Tiss Organ Cult* 127:25–34. <https://doi.org/10.1007/s11240-016-1026-1>
7. Khairiyah H, Sukarnih T, Rudyana Y, Ramadhani RM (2025) Optimization of shoot multiplication in satoimo (*Colocasia esculenta* L.) using 6-benzylaminopurine (BAP) and thidiazuron (TDZ) *in vitro*. In *IOP Conference Series: Earth and Environmental* 1476, No. 1, 012056. <https://doi.org/10.1088/1755-1315/1476/1/012056>
8. Kaur D, Ogra RK, Bhattacharya A et al (2012) Changes in sugar levels during slow growth of *Dendrocalamus hamiltonii* somatic embryos due to liquid paraffin overlay. *Vitro Cell Dev Biol -Plant* 48:120–126. <https://doi.org/10.1007/s11627-011-9392-1>
9. Laurini D, Thirkettle A, Bockstahler K (1999) Environmental Control & Life Support for the Multi-Purpose Logistics Module. *ESA Bull Number* 98
10. Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15:473–497
11. R CORE TEAM (2024) R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Available at: <https://www.R-project.org/>
12. Rodrigues PHV, Arruda F, Forti VA (2018) Slow-grown *in vitro* conservation of *Heliconia champneiana* cv. Splash under different light spectra. *Sci Agric* 75:163–166.

13. Rodrigues PHV, Oliveira EL, Demetrio CA, Ambrosano GB, Piedade SMS (2022) Effects of different light spectra on the slow-grown *in vitro* storage and quality of banana plantlets cv. Prata Catarina (AAB). Plant Cell Tiss Organ Cult 150:479–485. <https://doi.org/10.1007/s11240-022-02280-x>
14. Tavazza R, Rey AN, Papacchioli V, Pagnotta MA (2015) A validated slow-growth in vitro conservation protocol for globe artichoke germplasm: a cost-effective tool to preserve from wild to elite genotypes. Sci Hort 197:135–143. <https://doi.org/10.1016/j.scienta.2015.09.024>
15. Visscher AM, Pritchard HW, Neri G, Ballesteros D (2023) How do we transport plant species with desiccation-sensitive germplasm in space? Life Sciences in Space Research. 36:135–137. <https://doi.org/10.1016/j.lssr.2022.09.008>
16. Wheeler RM (2010) Plants for human life support in space: from Myers to Mars Gravitational and Space Biology. Gravitational Space Biology 23:25–36

Figures

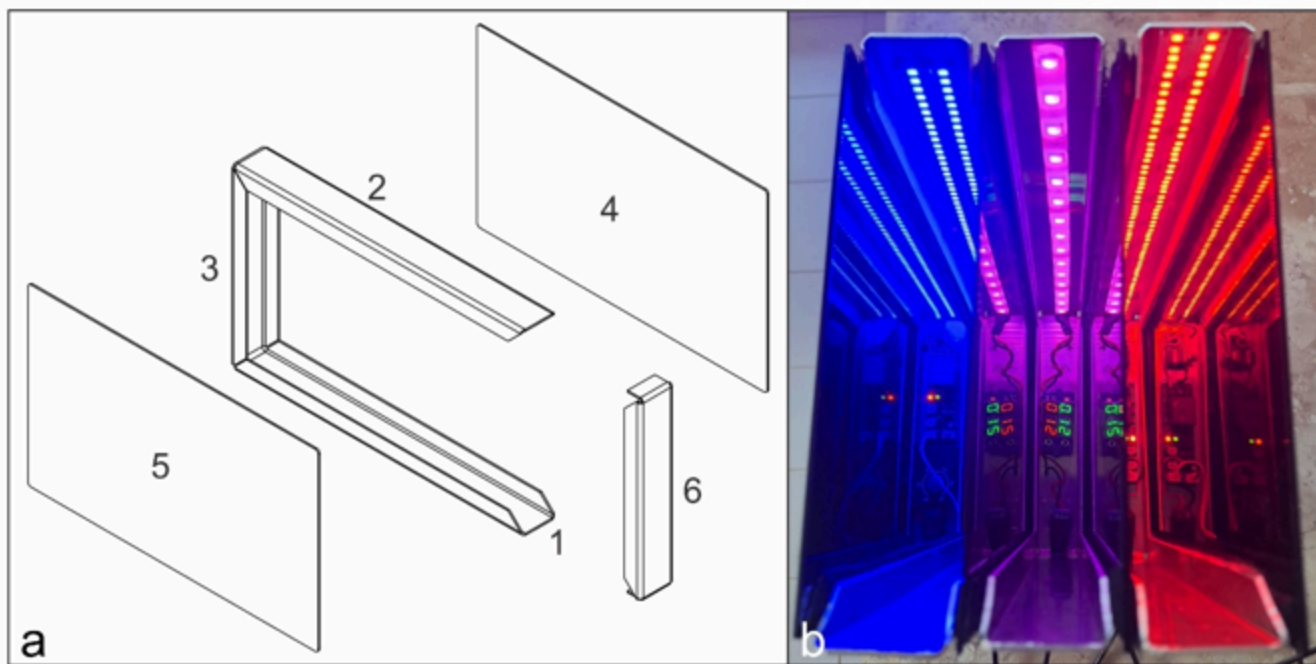


Figure 1

aSchematic drawing of CGB. **b**CGB3, CGB2, and CGB1 are connected side by side.

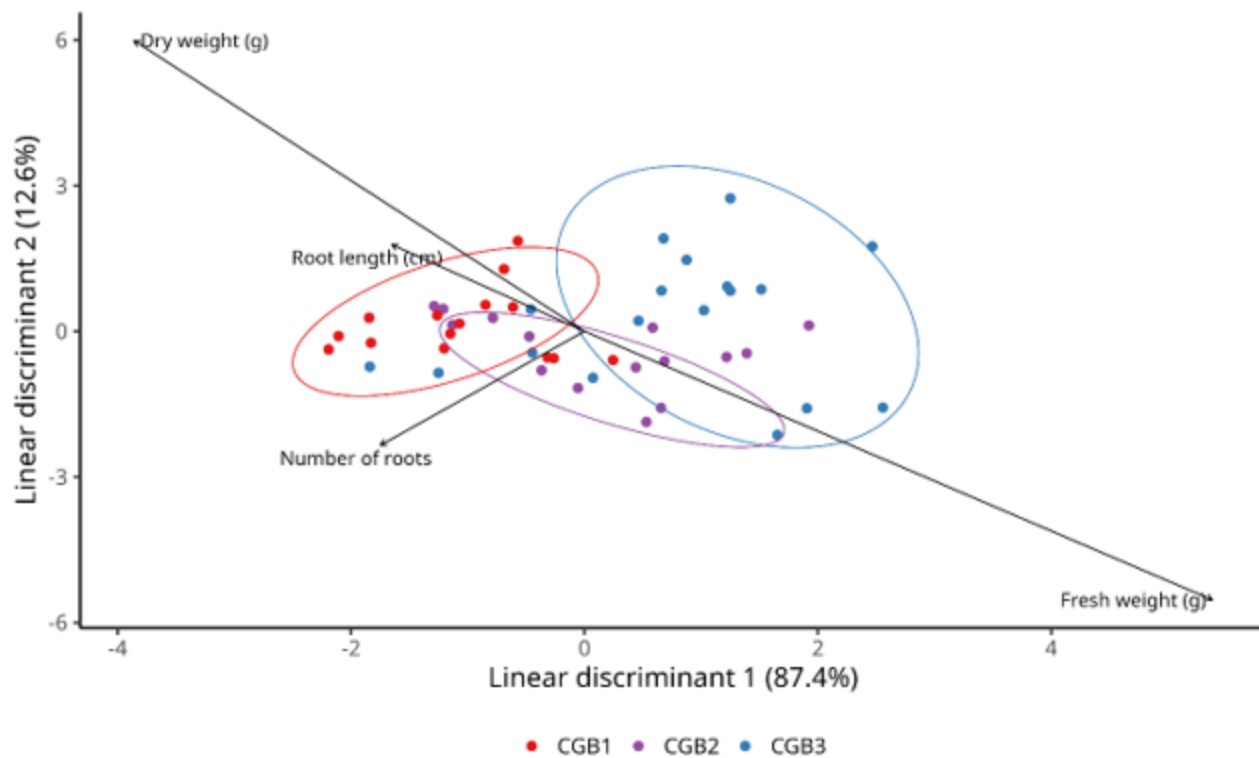


Figure 2

Projection of observations from treatments CGB1, CGB2, and CGB3 onto the first two linear discriminant functions. Arrows represent the standardized discriminant coefficients for the dry mass (g), root length (cm), number of roots, and fresh mass (g).

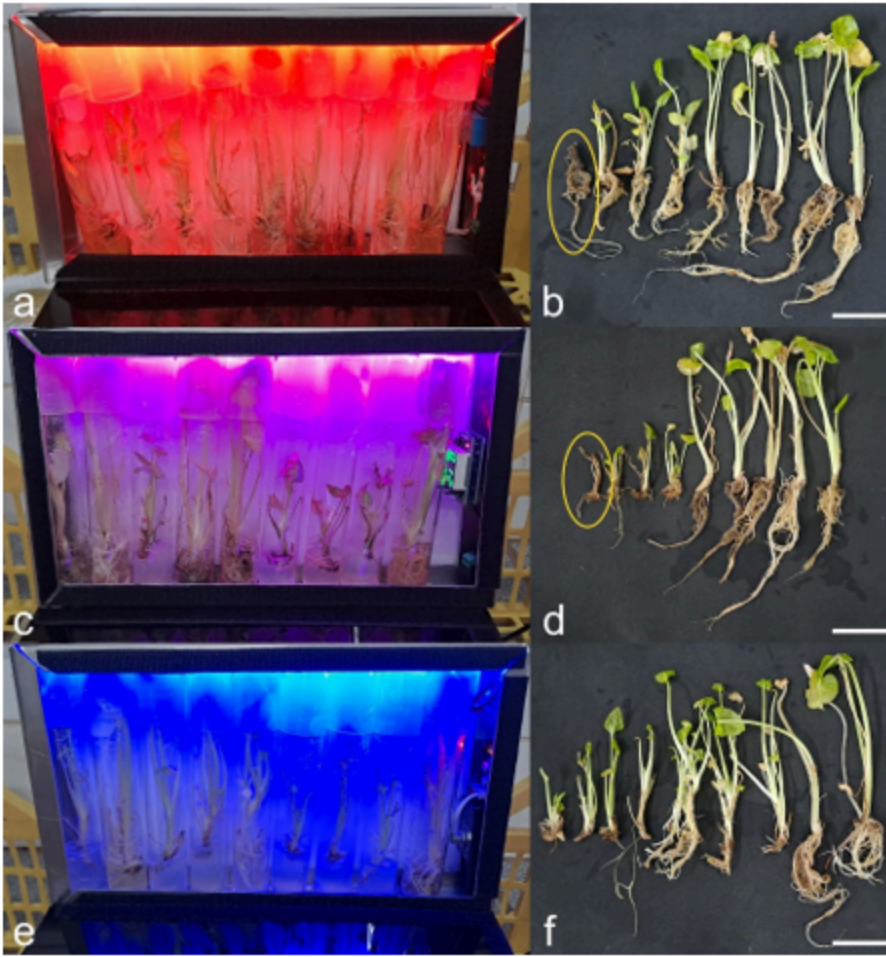


Figure 3

a, c, and e CGBs working test in different LS. **b** In vitro Taioba plants from CGB1 (yellow circle shows plant died). **d** In vitro Taioba plants from CGB2 (yellow circle shows plant died). **f** In vitro Taioba plants from CGB3 (100% of plants live). Barr: 3.0 cm.