Journal of Applied Botany and Food Quality 92, 94 - 99 (2019), DOI:10.5073/JABFQ.2019.092.013

[1]Laboratory for Plant Breeding and Conservation of Genetic Resources, Centro de Bioplantas, Universidad de Ciego de Ávila, Ciego de Ávila, Cuba
[2]Escuela Superior Politécnica Agropecuaria de Manabí Manuel Félix López (ESPAMMFL), Campus Politécnico El Limón, Carrera de Ingeniería Agrícola, Calceta, Manabí, Ecuador
[3]School of Life Sciences, University of Kwazulu-Natal, Durban, South Africa

Cryopreservation of sorghum seeds modifies germination and seedling growth but not field performance of adult plants

Ariel Villalobos[1], Melissa Arguedas[1], Doris Escalante[1], Julia Martínez[1], Byron E. Zevallos[2], Inaudis Cejas[1], Lourdes Yabor[1], Marcos Edel Martínez-Montero[1], Sershen[3], José Carlos Lorenzo[1]

(Submitted: November 22, 2018; Accepted: February 7, 2019)

Summary

Climate change poses risks to both wild and crop plant biodiversity, which can be mitigated by cryopreservation (usually at -196 °C in liquid nitrogen [LN]) of crop germplasm. Cryopreservation is widely regarded as a reliable method for the *ex situ* conservation of plant genetic resources but its effects on subsequent field performance of popular crop species such as sorghum are largely unknown. This hampers the large-scale implementation (i.e. germplasm banks) of cryostorage for such species. This short communication describes the early stages of germination and field performance of plants derived from cryopreserved sorghum seed. Compared with the control, cryopreservation significantly increased seed electrolyte leakage and from 24 to 120 hours, percentage of germination of the control was ~2.6 folds higher than cryopreserved seeds. At 0 days, chlorophyll *a*/*b* rate was ~1.7 folds higher in the control and at 7 and 14 days, chlorophyll *a* level (~1.5 folds) and chlorophyll *a*/*b* rate (~1.8-1.9 folds) were higher in the control. Contrastingly, at 7 days, seedlings derived from cryopreserved seeds (treatment seedlings) showed ~1.5 folds more superoxide dismutase activity and ~1.9 folds more peroxidase activity. In contrast, treatment and control adult plants were statistically comparable in terms of chlorophylls, proteins, superoxide and peroxidase activities, plant architecture, and yield components. The fact that differences in biochemical indicators observed between control and treatment seedlings did not persist in adult plants validates the use of seed cryopreservation for the conservation of sorghum genetic resources.

Keywords: field performance; germplasm preservation; liquid nitrogen; *Sorghum bicolor* (L.) Moench.

Introduction

Sorghum, an African grass related to sugarcane and maize, is the fifth most important cereal crop globally and is known to tolerate low-nutrient soils and drought. Cultivated varieties are phenotypically diverse (Kayodé et al., 2006; Labeyrie et al., 2016) and are grown for food, feed, fiber and fuel due to the high soluble sugar content of their stems (Breeze, 2018; Manzelli et al., 2006; Mathur et al., 2017; Paterson et al., 2009). In 2016, the world area harvested for sorghum reached 44 771 056 ha and the world production, 63 930 558 t (Food and Agriculture Organization of the United Nations Statistics (FAOSTAT, 2017)).

Access to sorghum seed is crucial for farming and shortfalls are common in many countries. Farmer-farmer exchange is important for providing locally-adapted seed to fill this gap but access varies considerably among households, affecting quantities supplied and terms of exchange (McGuire, 2008; Otieno et al., 2018; Smale et al., 2018). In the context of climate change with risks to lose diversity, cryopreservation of plant materials in liquid nitrogen (LN) has been described as a suitable technology to conserve genetic resources of several species (Panis, 2018; Berjak et al., 2010), such as *Actinidia deliciosa* (Mathew et al., 2018), *Solanum betaceum* Cav. (Graça et al., 2018), *Elaeis guineensis* Jacq. (Beulé et al., 2018) and *Lilium* (Pan et al., 2018). However, the potential effects of cryostsorage of explants and seeds on subsequent plant growth in the field must be established before large-scale implementation in cryobanks (Engelmann and Ramanatha, 2012). In this regard, studies have shown that cryostorage of seed-derived germplasm can compromise the vigor of recovered plants (Berjak et al., 2010). Studies have also shown that recovery after cryopreservation of excised embryonic axes also seldom results in the production of true-to-type plants (Harding, 2004; Mycock, 1999; Steinmacher et al., 2007; Wesley-Smith et al., 2001).

It has been known for some time now that exposure to different types of stress can alter subsequent plant responses (Bruce et al., 2007), but there is little understanding of how stresses imposed at the embryonic stage for example, are translated or manifested during subsequent plant growth. A few reports suggest that there exists within cryopreserved plant materials some 'memory'-based mechanism that senses environmental signals (Forsyth and Staden, 1983; Kvaalen and Johnsen, 2008), which in turn influence adaptive traits in the seedlings they give rise to. With this background, this short communication describes the early stages of germination, seedling growth and field performance of plants produced from cryopreserved sorghum seed.

Materials and methods

Plant material

Harvested sorghum seeds (cv. CIAP2) were air dried at room temperature from 15% to 6% moisture content and then stored for 4 months at 4 °C in the dark in hermetically sealed containers. Seeds with 6% moisture content (fresh weight basis) (ISTA, 2005) were used in subsequent experiments.

Seed cryopreservation and recovery

One batch of the seeds was placed in cryo-vials (volume: 2 ml; 5 seeds per cryo-vial) and directly plunged in LN for 24 h (referred to hereafter as treated/cryopreserved seeds). Recovery of seeds from LN was performed according to Stanwood and Bass (1981). Another batch of seeds was stored at 4 °C until further use (referred to hereafter as control seeds).

Seed electrolyte leakage, germination and seedling growth from 0 to 144 hours

Electrolyte leakage from seeds was measured as recommended by Martínez-Montero (2002) immediately after seed recovery from LN. Percentage of seed germination (0-144 hours) and seedling weight (0-72 hours) were also evaluated by incubating seeds on filter papers in Petri dishes (Ø: 10 cm; 15 mL distilled water; five replicates of 10 seeds/dish). These parameters were also measured for control seeds.

Studies of seed and seedlings from 0 to 14 days

A second group of control and treated seeds was set out to germinate as described above (five replicates of 10 seeds/dish) and evaluated at 0, 7 and 14 days for chlorophyll pigments (Porra, 2002), total proteins (Bradford, 1976), and activities of superoxide dismutase (Kumutha et al., 2009) and peroxide oxidase (Sagiv and Bar-Akiva, 1972) were evaluated in the seeds (0 day) or the primary leaves (7 and 14 days). Hypocotyl length, primary leaf length, radicle length, total plantlet fresh weight, and total plantlet dry weight were also recorded.

Growth of adult plants in a plant bed until harvest at 110 days

Ninety treated and control seeds were randomly selected and sown in a plant bed as described in Fig. 1. Technical instructions provided by the Cuban Ministry for Agriculture to cultivate sorghum were applied. Border plants, which had more space to grow, were not considered. Levels of chlorophyll pigments (Porra, 2002), total proteins (Bradford, 1976), and activities of superoxide dismutase (Kumutha et al., 2009) and peroxide oxidase (Sagiv and Bar-Akiva, 1972) were evaluated in middle-aged leaves at 62 days after planting on soil (anthesis). Additionally, the following agricultural traits were evaluated according to Peacock (1990) at 62 days of growth: plant height, number of leaves per plant, middle-aged leaf length and width, number of stems per plant, and fresh and dry weight of plants. All plants were harvested after 110 days of growth and the following traits were recorded: panicula length and width, number of branches per panicula, number of grain per panicula branch, number of grains per panicula, fresh weight of 1000 grains and dry weight of 1000 grains.

Statistical analysis

SPSS (Version 17.0 for Windows, SPSS Inc.) was used to compare growth and physiological parameters between control and treated plants/seeds using a Students t-test ($p \leq 0.05$).

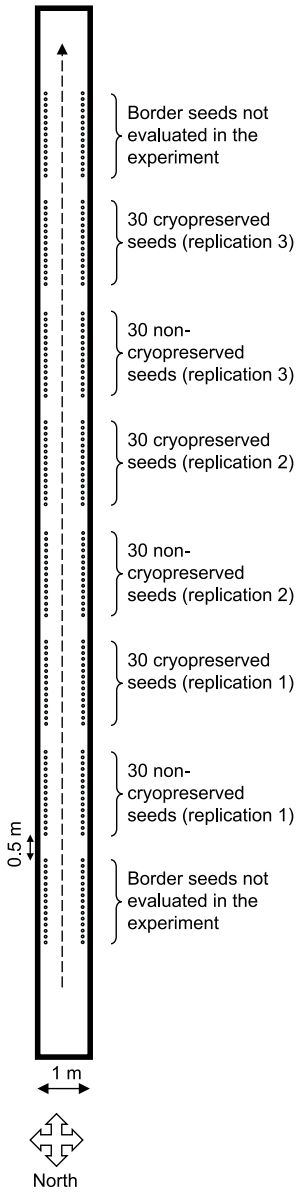

Fig. 1: Superior view of the useful area of the plant bed, made of Ferralytic-red soil and filter-cake-sugarcane ashes (1:1, v:v). Dots symbolize seeds (70 cm × 25 cm apart). The broken arrow in the middle of the plant bed represents the microject irrigation system, which watered the plants for 5 min every 8 h. The plant bed was 50 cm high and its bottom contained a 10 cm-high stones layer to facilitate drainage.

Results

Seed electrolyte leakage, germination and seedling growth from 0 to 144 hours

Compared with the control, cryopreservation significantly increased electrolyte leakage from seeds (~2 folds: 19.1% / 9.4%, Fig. 2A) immediately after recovery from LN. Although percentage of germination was similar at 144 hours, from 24 to 120 hours, percentage of germination of the control was ~2.6 folds higher (average, Fig. 2B). Seedling weight of control group was also higher (average ~1.2 folds, Fig. 2C).

Studies of seed and seedlings from 0 to 14 days

Tab. 1 shows the effects of cryopreservation on early stages of germination (0, 7, 14 days). Except for chlorophyll *a* + *b* on 0 day, statistically significant differences were observed on all sampling days (Tab. 1): At 0 day, chlorophyll *a*/*b* rate was ~1.7 folds higher in the control treatment (1.13 / 0.65); at 7 days the level of chlorophyll *a* was ~1.5 folds higher in the control (21.37 µg g^{-1} fresh weight / 14.02 µg g^{-1} fresh weight); and chlorophyll *a*/*b* rate was ~1.9 folds higher in the control (1.31 / 0.71). Contrastingly, at 7 days, plantlets derived from cryopreserved seeds showed ~1.5 folds (0.42 U mg^{-1} proteins / 0.27 U mg^{-1} proteins) more specific superoxide dismutase activity and ~1.9 folds (0.71 U mg^{-1} proteins / 0.37 U mg^{-1} proteins) more specific peroxidase activity.

At 14 days, plantlet chlorophyll *a* levels was ~1.5 folds higher in the control (25.31 µg g^{-1} fresh weight / 17.13 µg g^{-1} fresh weight) and chlorophyll *a*/*b* rate was ~1.8 folds (1.38 / 0.75) higher in this group (Tab. 1). Contrastingly, the specific peroxidase activity was ~1.7 folds higher in cryopreserved seed-derived seedlings (0.60 U mg^{-1} proteins / 0.36 U mg^{-1} proteins).

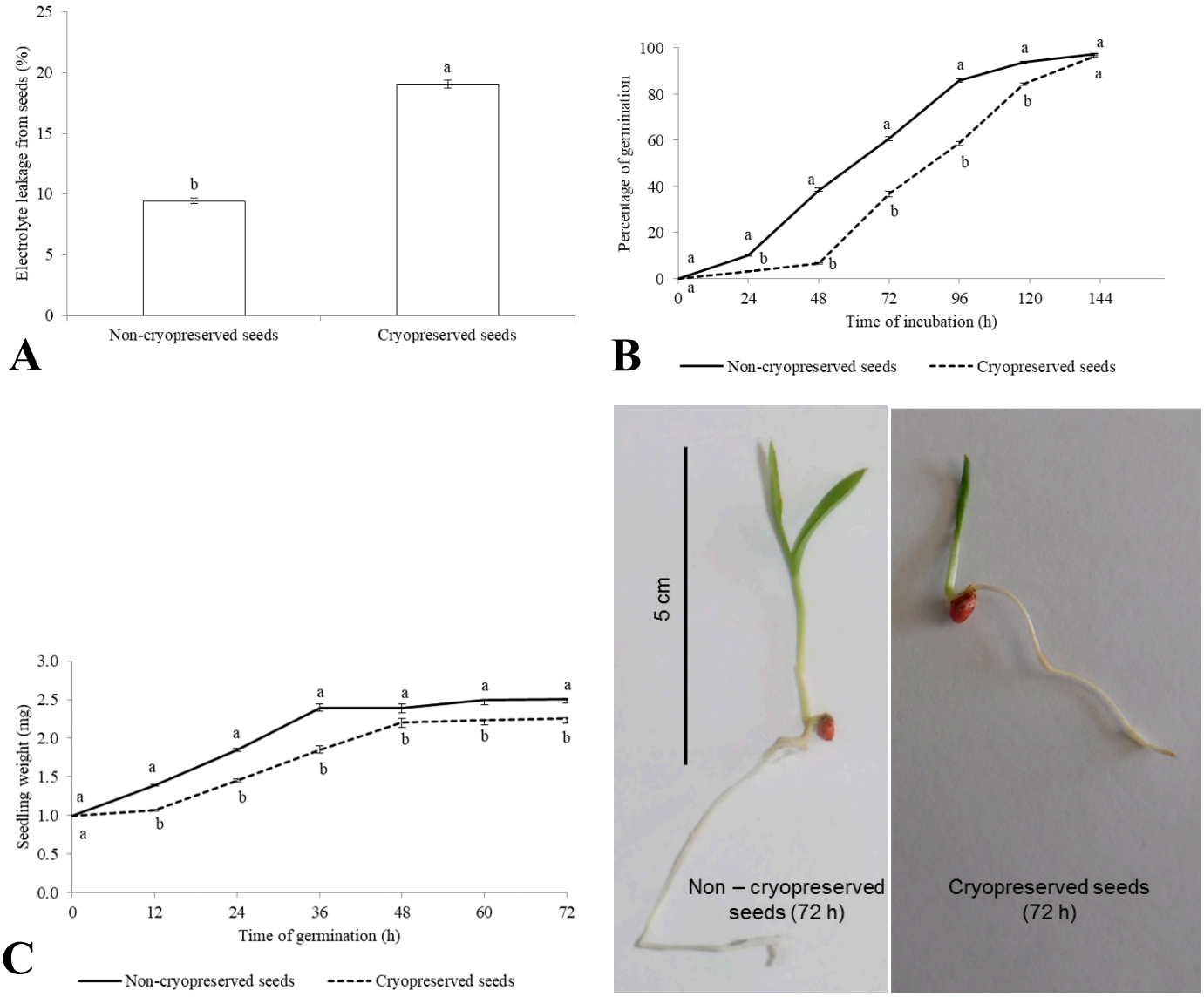

Fig. 2: Effect of seed cryopreservation on seed electrolyte leakage (A), germination (B) and seedling growth (C) from 0 to 144 hours. In each moment of evaluation, results with the same letters are not statistically different (t-test, p>0,05). Vertical bars represent SE.

Growth of adult plants in a plant bed until harvest at 110 days

The data shown in Tab. 2 indicates that seed cryopreservation has no significant effects on adult plants relative to the control. Interestingly, *ex vitro*, 100% of seed germination was recorded in both treatments. Differences in biochemical indicators between treatment and control seedlings reported above (Tab. 1) did not persist in adult plants (Tab. 2), e.g. levels of chlorophylls and proteins, and superoxide dismutase and peroxide oxidase activities.

Discussion

Cryopreservation imposes a series of stresses on plant material both during storage and upon recovery, and this can induce modifications in plants produced from cryopreserved explants/seeds (Benson, 2008a; Benson, 2008b). For example, partial dehydration and cryopreservation reduced the number of *Amaryllis belladonna* excised zygotic embryos that produced seedlings, as well as the subsequent biomass of these seedlings relative to non-cryopreserved embryos (Berjak et al., 2010). Those authors showed that *A. belladonna* seedling produced from cryopreserved explants also exhibited lower CO_2-assimilation rates and stomatal density, abnormal root growth, damage to the photosynthetic apparatus and less efficient adjustment of leaf water potential relative to control seedlings. Other studies have also reported phenotypic variation in *in vitro* recovery times, plant heights and modes of development (Steinmacher et al., 2007) and regeneration in plants recovered from cryopreserved germplasm (Harding, 2010). Similarly, our results for sorghum suggest that cryopreservation increased electrolyte leakage from seeds (Fig. 1A), and delayed germination (Fig. 1B) and seedling growth (Fig. 1C). Cell membranes are one of the main targets of numerous stress events, including cryopreservation (Berjak et al., 2010; Close, 1996, 1997; Harding, 2010; Sangwan et al., 2002; Uemura and Steponkus, 1994).

Previously, our group has studied the effects of LN on the subsequent germination and growth of common bean, tomato, tobacco, maize and *Teramnus labialis* (L.F.) Spreng seeds. In brief, these studies showed that cryopreservation induced some morphological, physiological and biochemical (e.g. chlorophyll, carotenoids, proteins, malondialdehyde, other aldehydes, soluble and cell wall-linked phenolics, and peroxidase and superoxide dismutase activity) changes

Tab. 1: Studies of seed and seedlings from 0 to 14 days. In each moment of evaluation (0, 7, 14 days after exposure to LN), results with the same *letter* are not statistically different (t-test, p>0.05). Intervals represent average ± SE.

	0 days (seeds were evaluated)		7 days (primary leaves were evaluated)		14 days (primary leaves were evaluated)	
	Non-cryopreserved seeds	Cryopreserved seeds	Non-cryopreserved seeds	Cryopreserved seeds	Non-cryopreserved seeds	Cryopreserved seeds
Chlorophyll *a* (μg · g^{-1} fresh weight)	16.85 ± 0.39 a	12.37 ± 0.27 b	21.37 ± 0.28 a	14.02 ± 0.18 b	25.31 ± 0.25 a	17.13 ± 0.30 b
Chlorophyll *b* (μg · g^{-1} fresh weight)	14.97 ± 0.30 b	19.17 ± 0.40 a	16.39 ± 0.31 b	19.95 ± 0.47 a	18.36 ± 0.36 b	22.93 ± 0.57 a
Chlorophyll *a* + *b* (μg · g^{-1} fresh weight)	31.82 ± 0.49 a	31.53 ± 0.43 a	37.76 ± 0.34 a	33.97 ± 0.58 b	43.66 ± 0.40 a	40.06 ± 0.56 b
Chlorophyll *a*/*b*	1.13 ± 0.03 a	0.65 ± 0.02 b	1.31 ± 0.04 a	0.71 ± 0.02 b	1.38 ± 0.03 a	0.75 ± 0.03 b
Protein content (mg proteins · g^{-1} fresh weight)	5.06 ± 0.16 a	4.13 ± 0.17 b	6.76 ± 0.22 a	5.12 ± 0.15 b	7.51 ± 0.17 a	6.23 ± 0.16 b
Superoxide dismutase activity (mg · g^{-1} fresh weight)	1.70 ± 0.04 b	1.88 ± 0.02 a	1.83 ± 0.02 b	2.12 ± 0.02 a	1.88 ± 0.00 b	2.15 ± 0.01 a
Specific superoxide dismutase activity (U mg^{-1} proteins)	0.34 ± 0.02 b	0.46 ± 0.02 a	0.27 ± 0.01 b	0.42 ± 0.01 a	0.25 ± 0.01 b	0.35 ± 0.01 a
Peroxidase activity (mg · g^{-1} fresh weight)	2.43 ± 0.08 b	2.79 ± 0.07 a	2.49 ± 0.07 b	3.60 ± 0.05 a	2.71 ± 0.06 b	3.71 ± 0.03 a
Specific peroxidase activity (U mg^{-1} proteins)	0.48 ± 0.01 b	0.68 ± 0.02 a	0.37 ± 0.01 b	0.71 ± 0.02 a	0.36 ± 0.01 b	0.60 ± 0.01 a
Hypocotyl length (cm)	---	---	2.79 ± 0.01 a	2.46 ± 0.04 b	6.93 ± 0.04 a	6.44 ± 0.04 b
Primary leaf length (cm)	---	---	2.91 ± 0.02 a	2.71 ± 0.03 b	7.26 ± 0.06 a	6.69 ± 0.03 b
Radicle length (cm)	---	---	4.98 ± 0.06 a	4.61 ± 0.12 b	8.00 ± 0.10 b	8.19 ± 0.13 a
Total plantlet fresh weight (mg)	---	---	0.24 ± 0.00 a	0.20 ± 0.00 b	0.51 ± 0.00 a	0.41 ± 0.00 b
Total plantlet dry weight (mg)	---	---	0.08 ± 0.00 a	0.07 ± 0.00 b	0.17 ± 0.00 a	0.13 ± 0.00 b

that gradually disappeared as the plants grew (Acosta et al., 2018; Arguedas et al., 2018a; Arguedas et al., 2018b; Cejas et al., 2012; Pérez-Rodríguez et al., 2017; Zevallos et al., 2014). This supports our present findings for sorghum where cryopreservation-induced declines in seedling chlorophyll *a* levels and consequently chlorophyll *a*/*b* rate, and enhanced superoxide dismutase and peroxidase activities did not persist in adult plants. Various markers, including electrolyte efflux, peroxide and superoxide oxidations, reflect the structural and functional integrity status of cell membranes after exposure to such stressful events (Dumet and Benson, 2000). Various abiotic stresses decrease the chlorophyll content in plants (Ahmad et al., 2012) and this decline is believed to be due to inhibition of important enzymes, such as δ-aminolevulinic acid dehydratase and protochlorophyllide reductase associated with chlorophyll biosynthesis (Van Assche and Clijsters, 1990). However, a major finding of the present study is that cryopreservation did not appear to affect the phenotype of adult sorghum plants in terms of growth and performance significantly. This report therefore demonstrates the value of seed cryopreservation to conserve sorghum genetic resources, although in large-scale field experiments, the reduced germination speed, seedling weight and the increase of enzyme activities may result in inhomogeneous field establishment and lower yield.

Author contribution: AV, MA, DE, JM, BEZ, IC, LY, MEMM, S and JCL designed the research; AV, MA, DE and JM conducted the experiment; AV, BEZ, IC, LY, MEMM, S and JCL analyzed data; S and JCL wrote the paper; JCL had primary responsibility for the final content. All authors have read and approved the final manuscript.

Acknowledgements
This research was supported by the University of Ciego de Avila (Cuba), the Escuela Superior Politécnica Agropecuaria de Manabí Manuel Félix López (Ecuador), and the University of KwaZulu-Natal (South Africa).

References

Acosta, Y., Hernández, L., Mazorra, C., Quintana, N., Zevallos, B.E., Cejas, I., Sershen, Lorenzo, J.C., Martínez-Montero, M.E., Fontes, D., 2018: Seed cryostorage enhances subsequent plant productivity in the forage species *Teramnus labialis* (L.F.) Spreng. CryoLetters (in press).

Ahmad, P., Kumar, A., Gupta, A., Hu, X., Azooz, M.M., Sharma, S., 2012: Polyamines: role in plants under abiotic stress. Crop Production for Agricultural Improvement. Springer.

Arguedas, M., Gómez, D., Hernández, L., Engelmann, F., Garramone, R., Cejas, I., Yabor, L., Martínez-Montero, M.E., Lorenzo, J.C., 2018a: Maize seed cryo-storage modifies chlorophyll, carotenoid, protein, aldehyde and phenolics levels during early stages of germination. Acta Physiol. Plant. 40, 118. DOI: 10.1007/s11738-018-2695-7

Arguedas, M., Villalobos, A., Gómez, D., Hernández, L., Zevallos, B., Cejas, I., Yabor, L., Martínez-Montero, M.E., Lorenzo, J.C., 2018b: Field performance of cryopreserved seed-derived maize plants. CryoLetters 39 (6), 366-370.

Benson, E., 2008a: Cryopreservation of phytodiversity: a critical appraisal of theory & practice. Critic. Rev. Plant Sci. 27, 141-219. DOI: 10.1080/07352680802202034

Benson, E.E., 2008b: Cryopreservation theory. Plant cryopreservation: A practical guide, 15-32.

Berjak, P., Bartels, P., Benson, E., Harding, K., Mycock, D., Pammenter, N., Sershen, W., 2010: Cryoconservation of South African plant genetic diversity. *In Vitro* Cell. Dev. Biol.- Plant 47, 65-81. DOI: 10.1007/s11627-010-9317-4

Beulé, T., Ilbert, P., Adeoti, K., Durand-Gasselin, T., Dumet, D., Engelmann, F., Morcillo, F., 2018: Recovery of oil palm (*Elaeis*

Tab. 2: Growth of adult plants in a plant bed until harvest at 110 days. Statistically significant differences were not recorded (t-test, $p>0.05$). Intervals represent average ± SE.

Days after plantings and traits measured	Non-cryopreserved seeds	Cryopreserved seeds
Traits evaluated in middle-aged leaves at 62 days after planting on soil (anthesis).		
Chlorophyll *a* (μg · g^{-1} fresh weight)	85.17 ± 0.37	85.63 ± 0.44
Chlorophyll *b* (μg · g^{-1} fresh weight)	56.10 ± 0.31	55.63 ± 0.44
Chlorophyll *a* + *b* (μg · g^{-1} fresh weight)	141.27 ± 0.41	141.26 ± 0.88
Chlorophyll *a*/*b*	1.52 ± 0.01	1.54 ± 0.00
Protein content (mg proteins · g^{-1} fresh weight)	8.97 ± 0.03	9.05 ± 0.03
Superoxide dismutase activity (mg · g^{-1} fresh weight)	3.36 ± 0.07	3.39 ± 0.07
Specific superoxide dismutase activity (U mg^{-1} proteins)	0.38 ± 0.01	0.37 ± 0.01
Peroxidase activity (mg · g^{-1} fresh weight)	15.03 ± 0.19	15.11 ± 0.18
Specific peroxidase activity (U mg^{-1} proteins)	1.68 ± 0.02	1.67 ± 0.02
Agricultural traits evaluated at 62 days after planting on soil (anthesis).		
Plant height (cm)	168.75 ± 0.53	169.15 ± 0.49
Number of leaves per plant	10.38 ± 0.15	10.25 ± 0.15
Middle-aged leaf length (cm)	97.99 ± 0.24	97.79 ± 0.18
Middle-aged leaf width (cm)	7.72 ± 0.03	7.70 ± 0.01
Number of stems per plant	1.00 ± 0.00	1.00 ± 0.00
Stem diameter (cm)	5.89 ± 0.02	5.88 ± 0.02
Fresh weight of plants (g)	1.10 ± 0.02	1.09 ± 0.03
Dry weight of plants (g)	0.27 ± 0.01	0.28 ± 0.01
Agricultural traits evaluated at at 110 days after planting on soil (harvest).		
Panicula length (cm)	17.91 ± 0.03	17.88 ± 0.02
Panicula width (cm)	6.07 ± 0.02	6.06 ± 0.01
Number of branches per panicula	29.80 ± 0.15	29.83 ± 0.09
Number of grains per panicula branch	37.43 ± 0.77	37.93 ± 0.67
Number of grains per panicula	1119.15 ± 27.93	1131.58 ± 20.96
Fresh weight of 1000 grains (g)	27.98 ± 0.70	28.29 ± 0.52
Dry weight of 1000 grains (g)	5.13 ± 0.07	5.15 ± 0.07

guineensis Jacq.) somatic embryos cryostored for 20 years. CryoLetters 39, 60-66.

Berjak, P., Pammenter, N.W., 2010: Effects of cryopreservation of recalcitrant Amaryllis belladonna zygotic embryos on vigor of recovered seedlings: a case of stress 'hangover'? Physiol. Plant. 139, 205-219. DOI: 10.1111/j.1399-3054.2010.01358.x

Bradford, M., 1976: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Analytical Biochemical 72, 248-254. DOI: 10.1016/0003-2697(76)90527-3

Breeze, E., 2018: Sweet and juicy: identification and origins of the dry alleles in sorghum. The Plant Cell. DOI: 10.1105/tpc.18.00748

Bruce, T.J., Matthes, M.C., Napier, J.A., Pickett, J.A., 2007: Stressful "memories" of plants: evidence and possible mechanisms. Plant Science 173, 603-608. DOI: 10.1016/j.plantsci.2007.09.002

Cejas, I., Vives, K., Laudat, T., González-Olmedo, J., Engelmann, F., Martínez-Montero, M.E., Lorenzo, J.C., 2012: Effects of cryopreservation of *Phaseolus vulgaris* L. seeds on early stages of germination. Plant Cell Rep. 31, 2065-2073. DOI: 10.1007/s00299-012-1317-x

Close, T.J., 1996: Dehydrins: emergence of a biochemical role of a family of plant dehydration proteins. Physiol. Plant. 97, 795-803. DOI: 10.1111/j.1399-3054.1996.tb00546.x

Close, T.J., 1997: Dehydrins: a commonalty in the response of plants to dehydration and low temperature. Physiol. Plant. 100, 291-296. DOI: 10.1111/j.1399-3054.1997.tb04785.x

Dumet, D., Benson, E.E., 2000: The use of physical and biochemical studies to elucidate and reduce cryopreservation induced damage in hydrated/desiccated plant germplasm. In: Engelmann, F., Takagi, H. (eds.), Cryopreservation of Tropical Plant Germplasm: Current Research Progress and Application. JIRCAS/IPGRI, Tsukuba/Rome.

Engelmann, F., Ramanatha, R., 2012: Major research challenges and directions for future research. In: Normah, M.N.,Chin, H.F., Reed, B.M. (eds.), Conservation of Tropical Plant Species. Springer Verlag, Berlin.

FAOSTAT, 2017: FAO Cereal Supply and Demand Brief. Retrieved from

Forsyth, C., Staden, J.V., 1983: Germination of *Tagetes minuta* LI Temperature effects. Ann. Bot. 52, 659-666. DOI: 10.1093/oxfordjournals.aob.a086622

Graça, D., Correia, S., Ozudogru, E., Lambardi, M., Canhoto, J., 2018: Cryopreservation of tamarillo (*Solanum betaceum* Cav.) embryogenic cultures. In: Jain, S., Gupta, P. (eds.), Step Wise Protocols for Somatic Embryogenesis of Important Woody Plants. Forestry Sciences. Springer.

Harding, K., 2004: Genetic integrity of cryopreserved plant cells: a review. CryoLetters 25, 3-22.

Harding, K., 2010: Plant and algal cryopreservation: issues in genetic integrity, concepts in cryobionomics and current applications in cryobiology. J. Mol. Biol. Biotech. 18, 151-154.

ISTA, 2005: International Rules for Seed Testing. International Seed Testing Association, Bassersdorf, Switzerland.

Kayodé, A.P., Linnemann, A.R., Nout, M.R., Hounhouigan, J.D., Stomph, T.J., Smulders, M.J., 2006: Diversity and food quality properties of farmers' varieties of sorghum from Bénin. J. Sci. Food Agric. 86, 1032-1039. DOI: 10.1002/jsfa.2451

Kumutha, D., Ezhilmathi, K., Sairam, R., Srivastava, G., Deshmukh, P.,

Meena, R., 2009: Waterlogging induced oxidative stress and antioxidant activity in pigeonpea genotypes. Biologia Plantarum 53, 75-84. DOI: 10.1007/s10535-009-0011-5

Kvaalen, H., Johnsen, Ø., 2008: Timing of bud set in *Picea abies* is regulated by a memory of temperature during zygotic and somatic embryogenesis. New Phytol. 177, 49-59. DOI: 10.1111/j.1469-8137.2007.02222.x

Labeyrie, V., Deu, M., Dussert, Y., Rono, B., Lamy, F., Marangu, C., Kiambi, D., Calatayud, C., Coppens d'Eeckenbrugge, G., Robert, T., 2016: Past and present dynamics of sorghum and pearl millet diversity in Mount Kenya region. Evol. Appl. 9, 1241-1257. DOI: 10.1111/eva.12405

Manzelli, M., Benedettelli, S., Vecchio, V., 2006: Agro-biodiversity in subsistence farming systems of South Somalia-Collection and agronomic assessment of Somali sorghum (*Sorghum bicolor* (L.) Moench) germplasm. Tropicultura 24, 213.

Martínez-Montero, M.E., Mora, N., Quiñones, J., González-Arnao, M.T., Engelmann, F., Lorenzo, J.C., 2002: Effect of cryopreservation on the structural and functional integrity of cell membranes of sugarcane (*Saccharum sp.*) embryogenic calluses. Cryoletters 23, 237-244.

Mathew, L., McLachlan, A., Jibran, R., Burritt, D.J., Pathirana, R., 2018: Cold, antioxidant and osmotic pre-treatments maintain the structural integrity of meristematic cells and improve plant regeneration in cryopreserved kiwifruit shoot tips. Protoplasma 255, 1065-1077. DOI: 10.1007/s00709-018-1215-3

Mathur, S., Umakanth, A., Tonapi, V., Sharma, R., Sharma, M.K., 2017: Sweet sorghum as biofuel feedstock: recent advances and available resources. Biotechnol. Biof. 10, 146. DOI: 10.1186/s13068-017-0834-9

McGuire, S.J., 2008: Securing access to seed: Social relations and sorghum seed exchange in eastern Ethiopia. Hum. Ecol. 36, 217-229. DOI: 10.1007/s10745-007-9143-4

Mycock, D., 1999: Addition of calcium and magnesium to a glycerol and sucrose cryoprotectant solution improves the quality of plant embryo recovery from cryostorage. CryoLetters 20(2), 77-82.

Otieno, G., Lacasse, H., Adokorach, J., Mulumba, J.W., Recha, J.W., Reynolds, T.W., Fadda, C., 2018: Social seed networks for climate change adaptation in Uganda: Strategies to improve access to genetic diversity and information. Results from a study to better understand farmers' primary sources of seed and information in the Hoima Climate-Smart Villages. CGIAR: https://hdl.handle.net/10568/93207.

Pan, C., Liu, J., Bi, W.-L., Chen, H., Engelmann, F., Wang, Q.-C., 2018: Cryopreservation of small leaf squares-bearing adventitious buds of Lilium Oriental hybrid 'Siberia' by vitrification. Plant Cell Tiss. Org. Cult. 133, 159-164. DOI: 10.1007/s11240-017-1363-8

Panis, B., 2018: 60 years of plant cryopreservation: from freezing hardy mulberry twigs to establishing reference crop collections for future generations. 2018-01; The Third International Symposium on Plant Cryopreservation, Bangkok, Thailand.

Paterson, A.H., Bowers, J.E., Bruggmann, R., Dubchak, I., Grimwood, J., Gundlach, H., Haberer, G., Hellsten, U., Mitros, T., Poliakov, A., Schmutz, J., Spannagl, M., Tang, H., Wang, X., Wicker, T., Bharti, A.K., Chapman, J., Feltus, F.A., Gowik, U., Grigoriev, I.V., Lyons, E., Maher, C.A., Martis, M., Narechania, A., Otillar, R.P., Penning, B.W., Salamov, A.A., Wang, Y., Zhang, L., Carpita, N.C., Freeling, M., Gingle, A.R., Hash, C.T., Keller, B., Klein, P., Kresovich, S., McCann, M.C., Ming, R., Peterson, D.G., Mehboob ur, R., Ware, D., Westhoff, P., Mayer, K.F.X., Messing, J., Rokhsar, D.S., 2009: The *Sorghum bicolor* genome and the diversification of grasses. Nature 457, 551. DOI: 10.1038/nature07723

Peacock, J., 1990: Investigación del ICRISAT sobre Sorgo en los trópicos semiáridos. ICRISAT 17.

Pérez-Rodríguez, J.L., Escriba, R.C.R., González, G.Y.L., Olmedo, J.L.G., Martínez-Montero, M.E., 2017: Effect of desiccation on physiological and biochemical indicators associated with the germination and vigor of cryopreserved seeds of *Nicotiana tabacum* L. cv. Sancti Spíritus 96. *In Vitro* Cell. Dev. Biol.-Plant, 1-9. DOI: 10.1007/s11627-017-9857-y.

Porra, R., 2002: The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b. Photosynth. Res. 73, 149-156. DOI: 10.1023/A:1020470224740

Sagiv, J., Bar-Akiva, A., 1972: Visual demonstration of differences in peroxidase activity in iron and manganese deficient citrus leaves. Experientia 28, 645-646. DOI: 10.1007/BF01944952

Sangwan, V., Örvar, B.L., Dhindsa, R.S., 2002: Early events during low temperature signaling. Plant Cold Hardiness. Springer.

Smale, M., Assima, A., Kergna, A., Thériault, V., Weltzien, E., 2018: Farm family effects of adopting improved and hybrid sorghum seed in the Sudan Savanna of West Africa. Food Pol. 74, 162-171. DOI: 10.1016/j.foodpol.2018.01.001

Stanwood, P., Bass, L., 1981: Seed germplasm preservation using liquid nitrogen. Seed Sci. Technol. 9, 423.

Steinmacher, D.A., Saldanha, C.W., Clement, C.R., Guerra, M.P., 2007: Cryopreservation of peach palm zygotic embryos. CryoLetters 28, 13-22.

Uemura, M., Steponkus, P.L., 1994: A contrast of the plasma membrane lipid composition of oat and rye leaves in relation to freezing tolerance. Plant Physiol. 104, 479-496.

Van Assche, F., Clijsters, H., 1990: Effects of metals on enzyme activity in plants. Plant Cell Environ. 13, 195-206. DOI: 10.1111/j.1365-3040.1990.tb01304.x

Wesley-Smith, J., Walters, C., Pammenter, N., Berjak, P., 2001: Interactions among water content, rapid (nonequilibrium) cooling to -196 °C, and survival of embryonic axes of Aesculus hippocastanum L. seeds. Cryobiology 42, 196-206. DOI: 10.1006/cryo.2001.2323

Zevallos, B., Cejas, I., Engelmann, F., Carputo, D., Aversano, R., Scarano, M., Yanes, E., Martínez-Montero, M., Lorenzo, J.C., 2014: Phenotypic and molecular characterization of plants regenerated from non-cryopreserved and cryopreserved wild *Solanum lycopersicum* Mill. seeds. CryoLetters 35, 216-225.

Address of the authors:
Ariel Villalobos; Melissa Arguedas; Doris Escalante; Julia Martínez; Inaudis Cejas; Lourdes Yabor; Marcos Edel Martínez-Montero; José Carlos Lorenzo, Laboratory for Plant Breeding and Conservation of Genetic Resources, Centro de Bioplantas, Universidad de Ciego de Ávila, Ciego de Ávila 69450, Cuba
E-mail: jclorenzo@bioplantas.cu
Byron E. Zevallos, Escuela Superior Politécnica Agropecuaria de Manabí Manuel Félix López (ESPAMMFL), Campus Politécnico El Limón, Carrera de Ingeniería Agrícola, Calceta, Manabí, Ecuador
Sershen, School of Life Sciences, University of Kwazulu-Natal, Durban, 4001, South Africa