American Journal of Plant Sciences, 2013, 4, 2271-2274
Published Online November 2013 (http://www.scirp.org/journal/ajps)
http://dx.doi.org/10.4236/ajps.2013.411280

Scientific Research

Effect of 24-Epibrassinolide on Growth of *in Vitro* Shoot Tips of Different Yam (*Dioscorea* Spp.) Species

Isabelle Engelmann-Sylvestre, Florent Engelmann

IRD, UMR DIADE, Agropolis, Montpellier, France.
Email: florent.engelmann@ird.fr

Received September 26th, 2013; revised October 29th, 2013; accepted November 10th, 2013

ABSTRACT

In this work we compared the effect of the growth regulator content of the culture medium on the growth of *in vitro* shoot tips of five yam accessions belonging to four yam species (one *Dioscorea alata*, one *D. rotundata*, one *D. cayenensis* and two *D. trifida*). Medium S contained 0.6 µM benzyl adenine, 1.07 µM naphthalene acetic acid and 0.23 µM gibberellic acid while medium EBR contained 0.23 µM gibberellic acid and 0.1 µM 24-epibrassinolide. After 2 months of culture, oxidation level was significantly reduced on medium EBR compared to medium S for four of the five accessions tested. By contrast, medium EBR did not have any positive effect on shoot length since length of shoots produced after 2 months of culture on medium S and EBR were similar, except with accession 3-45T, for which shoot length was shorter on medium S compared to medium EBR. These results underline the potential of 24-epibrassinolide to reduce oxidation phenomena during *in vitro* culture and call for its utilization for regeneration of cryopreserved yam shoot tips, which is often impeded by oxidation phenomena.

Keywords: Yam; *Dioscorea* Spp.; 24-Epibrassinolide; Oxidation; Shoot Tip; *In Vitro* Culture; Cryopreservation

1. Introduction

Cryopreservation (liquid nitrogen, −196˚C) currently is the only safe and cost-effective option for long-term conservation of vegetatively propagated plants such as yam [1]. Indeed, at this temperature, all cell divisions stop and metabolism is virtually arrested. Explants can thus be conserved for extended periods (several thousand years) without modification or alteration, sheltered from contamination, in a limited volume and with reduced maintenance.

Cryopreservation protocols have been developed for *Dioscorea alata*, *D. cayenensis*, *D. rotundata* and *D. floribunda* shoot tips using different techniques [2-7]. A droplet-vitrification protocol jointly established by the International Institute of Tropical Agriculture (IITA, Ibadan, Nigeria) and our laboratory has been applied to a total of 42 *D. cayenensis*, *D. rotundata*, *D. alata*, *D. bulbifera* and *D. mangotiana* accessions, with an average recovery of 29% (Gueye *et al.* unpublished results). Cryopreservation experiments performed in IRD Montpellier with American yam (*D. trifida*) shoot tips showed that, even though positive results were obtained, no reproducible protocol was yet available for this species (Engelmann-Sylvestre *et al.* unpublished results). In all these reports, the authors mentioned that if high survival could be consistently achieved, regeneration of whole plantlets from cryopreserved shoot tips was variable, depending on the species and the technique used, and was generally much lower than survival. The occurrence of severe oxidation phenomena was also consistently reported.

In order to optimize the regrowth rate and pattern of *D. trifida* shoot tips, an experiment was performed recently with one *D. trifida* accession (N˚ 278) to compare the effect of the recovery medium used after their cryopreservation, which included 6-benzylaminopurine (BAP), naphthalene acetic acid (NAA) and gibberellic acid (GA_3), with media in which BAP and NAA were replaced by 24-epibrassinolide (EBR), zeatine riboside or meta-topolin [8]. This experiment showed that EBR induced the production of well developed shoots and significantly reduced oxidation compared to the other media tested.

EBR belongs to brassinosteroids (BRs), a class of

plant steroid hormones, which possess significant growth-controlling activity, and are involved in the promotion of cell elongation, cell division, differentiation, disease resistance, stress tolerance, and senescence throughout the plant life cycle. BRs have been reported to help modulating the plant antioxidant defence system and thus scavenging the free radicals and help the plant protecting itself from oxidative stress [9] and have also been found to have an activity *in vitro*. They were reported to increase the rate of cell division and colony formation of Chinese cabbage mesophyll protoplasts [10] and *Petunia hybrida* protoplasts [11]. BRs are also proved to be essential for the differentiation of isolated *Zinnia* mesophyll cells into tracheary elements [12] and in the morphogenesis of *Arabidopsis* [13].

In this study, we compared the regrowth of shoot tips of a total of five yam accessions including one *D. cayenensis*, *D. rotundata* and *D. alata* accession and two *D. trifida* accessions on medium containing EBR and GA_3, or BAP, NAA and GA_3, which are the plant growth regulators (PGRs) usually added to the regeneration medium after cryopreservation of *yam* shoot tips. Our objective was to observe if the EBR-containing growth medium which had been successfully employed with *D. trifida* accession N˚ 278 [8] was also effective with other *D. trifida* accessions and accessions of the other yam species selected.

2. Materials and Methods

2.1. Plant Material

This study was performed using *in vitro* shoot cultures of three yam accessions provided by the International Institute of Tropical Agriculture (IITA, Ibadan, Nigeria) including accessions N˚ 1454 (*D. alata*), N˚ 2790 (*D. cayenensis*) and N˚ 3675 (*D. rotundata*) and of two accessions provided by the Institut National de la Recherche Agronomique (INRA) Guadeloupe, French West Indies (*D. trifida* accessions N˚ 278 and 3-45T).

2.2. Methods

Mother-plants were subcultured every 3 - 5 months on medium containing Murashige and Skoog [14] basal salts and vitamins, 3% sucrose, 0.2% activated charcoal and 0.7% agar. The pH was adjusted to 5.8 ± 0.1 and the medium was autoclaved for 20 min at 120˚C. Cultures were maintained at 27˚C ± 1˚C under a 12 h light/12 h dark photoperiod and a light intensity of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Single node cultures were transferred to yam multiplication medium consisting of MS basal salts and vitamins [14], 0.476 µM KIN, 0.164 µM L-cysteine, 3% sucrose and 0.7% agar. After 3 weeks, shoot tips (approx. 1 - 2 mm in length) were excised under the binocular microscope and used for experiments.

Half of the shoot tips were cultured on medium S, which was used for regeneration of yam shoot tips after cryopreservation, which consisted of MS mineral salts and vitamins [14], 0.164 µM L-cysteine, 0.22 mM adenine hemisulfate, 0.6 µM BAP, 1.07 µM NAA, 0.23 µM GA_3 (filter-sterilized), 3% sucrose and 0.7% agar.

The other half of the shoot tips was cultured on medium EBR, consisting of MS mineral salts and vitamins [14], 0.164 µM L-cysteine, 0.22 mM adenine hemisulfate, 0.23 µM GA_3 (filter-sterilized), 0.1 µM 24-epibrassinolide (Sigma-Aldrich ref E1641, filter-sterilized), 3% sucrose and 0.7% agar. Shoot tips were kept in the dark for 1 week, and then transferred to the culture conditions employed for mother-plants.

2.3. Observations Performed and Statistical Analysis of Results

The experiment was performed once, with three replicates of 10 shoot tips per experimental condition. After 1 and 2 months of culture on medium S or EBR, the size of shoot tips (mm) was measured and the oxidation level was evaluated using a scale from 0 (no oxidation) to 3 (very high oxidation). One-way ANOVA was performed to compare the growth of shoot tips and oxidation levels on medium S and EBR. Means were statistically differentiated using Duncan test at a significance level of $Po \leq 0.05$.

3. Results

When comparing the growth of shoot tips of the five accessions studied on the two culture media tested, it appeared that accessions 278 and 2790 had a lower growth compared to the other three accessions on both S and EBR medium (**Table 1**). Studying the effect of the culture medium on the growth of each individual accession revealed that shoot length was lower after 1 month on medium S compared to medium EBR for three accessions (1454, 278 and 3-45T). By contrast, no differences in shoot length were observed after 2 months of culture on medium S and EBR, except with accession 3-45T, for which shoot length was lower on medium S compared to medium EBR.

When comparing the effect of culture medium on oxidation level, it was observed that accessions reacted differently depending on the culture medium and the culture duration (**Table 2**). After 1 month on medium S, oxidation was significantly lower in accessions 2790 and 3675, compared to the other three accessions. After 2 months on medium S, oxidation remained significantly lower in accession 2790, and was comparably higher in the four other accessions. After 1 and 2 months on medium EBR, oxidation was significantly higher in accession 1454 compared to the other four accessions studied. When

Table 1. Effect of culture medium and culture duration on shoot length (mm) of five yam accessions. The S medium contained 0.6 µM BAP, 1.07 µM NAA, 0.23 µM GA_3 and the EBR medium 0.23 µM GA_3 and 0.1 µM 24-epibrassinolide. In lines, different lower case letters indicate significant differences between accessions (Po ≤ 0.05). In columns, different upper case letters indicate significant differences between treatments (Po ≤ 0.05).

		Shoot length (mm)				
Medium	Culture duration	Accession N°				
		1454	278	2790	3-45 T	3675
S	1 month	5.27 ± 2.83 a/B	2.97 ± 2.31 b/B	1.89 ± 1.15 b/A	1.86 ± 1.22 b/B	4.65 ± 2.56 a/A
	2 months	6.73 ± 2.56 bc/A	11.60 ± 9.77 a/A	3.47 ± 2.55 d/A	3.97 ± 2.81 cd/B	8.15 ± 3.76 b/A
EBR	1 month	6.67 ± 2.22 a/A	6.57 ± 5.10 a/A	1.55 ± 0.69 b/A	3.13 ± 2.90 b/A	4.95 ± 2.72 a/A
	2 months	7.93 ± 3.03 b/A	13.96 ± 4.14 a/A	5.15 ± 3.98 c/A	6.70 ± 3.70 bc/A	7.65 ± 3.56 b/A

Table 2. Effect of culture medium and culture duration on oxidation level (0 to 3) of five yam accessions. The S medium contained 0.6 µM BAP, 1.07 µM NAA, 0.23 µM GA_3 and the EBR medium 0.23 µM GA_3 and 0.1 µM 24-epibrassinolide. In lines, different lower case letters indicate significant differences between accessions (Po ≤ 0.05). In columns, different upper case letters indicate significant differences between treatments (Po ≤ 0.05).

		Oxidation level				
Medium	Culture duration	Accession N°				
		1454	278	2790	3-45 T	3675
S	1 month	2.07 ± 0.94 a/A	2.00 ± 0.87 a/A	0.32 ± 0.48 b/A	2.28 ± 1.07 a/A	0.50 ± 0.89 b/A
	2 months	2.43 ± 0.73 ab/A	1.93 ± 0.83 b/A	1.11 ± 0.94 c/A	2.48 ± 0.83 a/A	2.15 ± 1.18 ab/A
EBR	1 months	0.97 ± 0.67 a/B	0.20 ± 0.41 b/B	0.10 ± 0.31 b/A	0.13 ± 0.35 b/B	0.00 ± 0.00 b/B
	2 months	1.43 ± 0.77 a/B	0.54 ± 0.74 bc/B	0.60 ± 0.60 bc/A	0.73 ± 0.52 b/B	0.25 ± 0.55c/B

studying the effect of the culture medium on the oxidation level measured on each individual accession, it was observed that the EBR medium induced significantly lower oxidation at 1 and 2 months in all accessions studied except in accession 2790, for which no significant differences between experimental conditions were noted. The positive effect of EBR on oxidation level after 2 months of culture on medium EBR compared to medium S is illustrated on **Figure 1** with accessions N° 3675 (*D. rotundata*) and 1454 (*D. alata*).

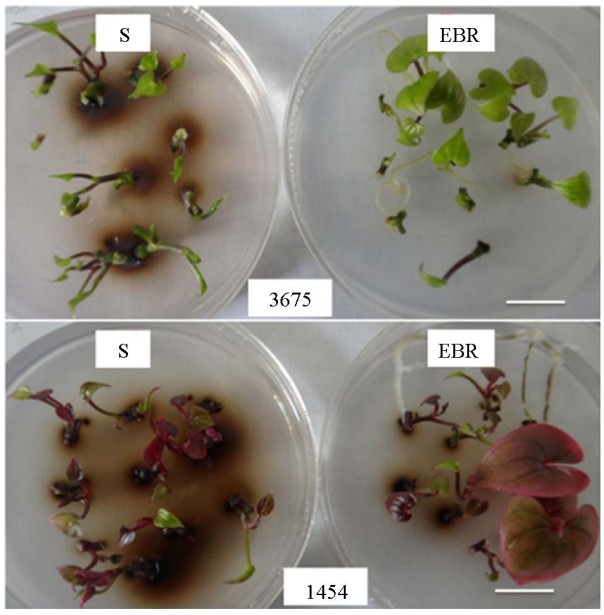

Figure 1. Effect culture for 2 months of shoot tips of yam accessions N° 3675 and 1454 on S medium (0.6 µM BAP, 1.07 µM NAA, 0.23 µM GA_3) or EBR medium (0.23 µM GA_3 and 0.1 µM 24-epibrassinolide) on oxidation level (0 - 3). Bars represent 10 mm.

4. Discussion

Our experiments showed that culture for 2 months of shoot tips of five yam accessions, representing four different yam species, on medium EBR, which contained 24-epibrassinolide, had a positive effect on oxidation level compared to culture on medium S, which contained BAP and NAA. Indeed, oxidation level was significantly reduced on medium EBR for four of the five accessions tested. This result confirms our previous observations performed on *D. trifida* accessions N° 278 [8] and underlines the potential of 24-epibrassinolide to reduce oxidation phenomena during *in vitro* culture.

By contrast, even though medium EBR induced higher shoot growth after 1 month compared to medium S, this positive effect was no more visible after 2 months of culture since shoots grown on medium S and EBR had similar lengths, except with accession 3-45T, for which shoot length was shorter on medium S compared to medium EBR.

It has been demonstrated in numerous species that modifying the PGR composition of the recovery medium can have a dramatic effect on regrowth of cryopreserved plant material [1].

Notably, alteration of post-cryopreservation culture media with PGRs and their application at various stages of growth recovery was crucial for regeneration of shoot tips and formation of *in vitro* plantlets in *D. alata* and *D. bulbifera* [5,7]. A recent report showed that the addition of vitamin E, vitamin C or both vitamins together, which have antioxidant properties, at different steps of the protocol (pre- and/or post-cryopreservation) had a positive effect on regrowth of cryopreserved blackberry shoot tips [15].

In view of the potential of the PGR 24-epibrassinolide in reducing oxidation, which has been shown in this work, experiments will be performed to test its effect on regrowth of cryopreserved yam shoot tips using the five accessions employed in this study.

5. Acknowledgements

This work has received financial support from ARCAD, a flagship programme of Agropolis Fondation (Montpellier, France) (I. Engelmann-Sylvestre). The assistance of Giuseppe Barraco for performing the statistical analysis of results is acknowledged.

REFERENCES

[1] F. Engelmann, "Plant Cryopreservation: Progress and Prospects," *In Vitro Cellular & Developmental Biology—Plant*, Vol. 40, No. 5, 2004, pp. 427-433.

[2] B. B. Mandal, K. P. S. Chandel and S. Dwivedi, "Cryopreservation of Yam (*Dioscorea* spp.) Shoot Apices by Encapsulation-Dehydration," *Cryo Letters*, Vol. 17, No. 3, 1996, pp. 165-174.

[3] B. Malaurie, M. F. Trouslot, F. Engelmann and N. Chabrillange, "Effect of Pretreatment Conditions on the Cryopreservation of *in Vitro* Cultured Yam (*Dioscorea alata* and *D. bulbifera*) Shoot Apices by Encapsulation-Dehydration," *Cryo Letters*, Vol. 19, No. 1, 1998, pp. 15-26.

[4] S. Leunufna and E. R. J. Keller, "Cryopreservation of Yam Using Vitrification Modified by Including Droplet Method: Effects of Cold Acclimation and Sucrose," *Cryo Letters*, Vol. 26, No. 2, 2005, pp. 93-102.

[5] B. B. Mandal and S. Ahuja-Ghosh, "Regeneration of *Dioscorea floribunda* Plants from Cryopreserved Encapsulated Shoot Tips: Effect of Plant Growth Regulators," *Cryo Letters*, Vol. 28, No. 5, 2007, pp. 329-336.

[6] B. B. Mandal, S. Ahuja-Ghosh and P. S. Srivastava, "Cryopreservation of *Dioscorea rotundata* Poir.: A Comparative Study with Two Cryogenic Procedures and Assessment of True-to-Type of Regenerants by RAPD Analysis," *Cryo Letters*, Vol. 29, No. 5, 2008, pp. 399-408.

[7] P. Mukherjee, B. B. Mandal, K. V. Bhat and A. K. Biswas, "Cryopreservation of Asian Yam *Dioscorea bulbifera* and *D. alata* by Vitrification: Importance of Plant Growth Regulators," *Cryo Letters*, Vol. 30, No. 2, 2009, pp. 100-111.

[8] Engelmann-Sylvestre and F. Engelmann, "Effect of Various Growth Regulators on Growth of Yam (*Dioscorea trifida* L.) *in Vitro* Shoot Tips," *African Journal of Biotechnology*, submitted.

[9] A. Verma, C. P. Malik and V. K. Gupta, "*In Vitro* Effects of Brassinosteroids on the Growth and Antioxidant Enzyme Activities in Groundnut," *ISRN Agronomy*, Vol. 2012, 2012, Article ID: 356485. http://dx.doi.org/10.5402/2012/356485

[10] N. Nakajima, A. Shida and S. Toyama, "Effects of Brassinosteroid on Cell Division and Colony Formation of Chinese Cabbage Mesophyll Protoplasts," *Japanese Journal of Crop Science*, Vol. 65, No. 1, 1996, pp. 114-118. http://dx.doi.org/10.1626/jcs.65.114

[11] M. H. Oh and S. D. Clouse, "Brassinolide Affects the Rate of Cell Division in Isolated Leaf Protoplasts of *Petunia hybrida*," *Plant Cell Reports*, Vol. 17, No. 12, 1998, pp. 921-924. http://dx.doi.org/10.1007/s002990050510

[12] T. Iwasaki and H. Shibaoka, "Brassinosteroids Act as Regulators of Tracheary-Element Differentiation in Isolated *Zinnia* Mesophyll Cells," *Plant Cell Physiology*, Vol. 32, No. 7, 1991, pp. 1007-1014.

[13] J. Li, P. Nagpal, V. Vitart, T. C. McMorris and J. Chory, "A Role for Brassinosteroids in Light-Dependent Development of *Arabidopsis*," *Science*, Vol. 272, No. 5260, 1996, pp. 398-401. http://dx.doi.org/10.1126/science.272.5260.398

[14] T. Murashige and F. Skoog, "A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Cultures," *Physiologia Plantarum*, Vol. 15, No. 3, 1962, pp. 473-497. http://dx.doi.org/10.1111/j.1399-3054.1962.tb08052.x

[15] E. E. Uchendu, M. Muminova, S. Gupta and B. M. Reed, "Antioxidant and Anti-Stress Copmounds Improve Regrowth of Cryopreserved *Rubus* Shoot Tips," *In Vitro Cellular & Developmental Biology—Plant*, Vol. 46, No. 4, 2010, pp. 386-393. http://dx.doi.org/10.1007/s11627-010-9292-9