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EFFECTS OF MELATONIN AGAINST CRYOPRESERVATION-INDUCED OXIDATIVE STRESS IN ZEBRAFISH OVARIAN TISSUE

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Ovarian tissue cryopreservation is an important technique to preserve germplasm and genetic diversity to improve fish stocks for scientific and aquaculture purposes. However, high reactive oxygen species (ROS) production during cryopreservation can lead to an impaired cell function. Melatonin is a potent free radical scavenger and antioxidant, whose effects on different cells have been widely reported. The aim of this study was to investigate the antioxidant effects of melatonin on oxidative stress of zebrafish ovarian tissue cryopreserved with three different melatonin concentrations (10^{-11}; 10^{-9}; 10^{-7}M). The experiment was approved by the Ethics Committee of our University. Ten zebrafish females were euthanized with a lethal dose of tricaine methanesulfonate (0.6 mg/mL, pH 7.4), followed by decapitation. Ovarian tissue fragments (n=20) were distributed among four experimental groups: untreated vitrified ovarian tissue, vitrified ovarian tissue treated with melatonin 10^{-11} M, 10^{-9} M or 10^{-7} M. Vitrified ovarian tissue were exposed to the equilibrium solution (1.5 M methanol and 2.75 M dimethylsulfoxide [Me_2SO]) for 15 minutes. Next, the ovarian tissues were transferred to the vitrification solution (1.5 M methanol, 5.5 M Me_2SO, and 0.5 M sucrose) for 90 seconds, and then plunged into liquid nitrogen. After warming, melatonin antioxidant capability was assessed by measuring the ferric reducing antioxidant potential (FRAP), levels of reactive species (RS) and presence of thiobarbituric acid reactive species (TBARS). The data were submitted to ANOVA, and the means were compared by the Tukey test. There was no significant difference between RS and TBARS levels among the groups. However, inclusion of 10^{-7} M melatonin in the cryoprotective solution showed higher FRAP levels (29.69±4.99) compared to the treatment with inclusion of 10^{-11} M melatonin (20.67±5.84, p= 0.0215). This result suggests that the supplementation of 10^{-7} M of antioxidant melatonin could scavenge excessive ROS improving the developmental potential of vitrified-warmed zebrafish ovarian tissue.

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WITHDRAWN

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EX SITU CONSERVATION OF COCONUT (COCOS NUCIFERA L.) USING EMBRYO CRYOPRESERVATION

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Coconut (*Cocos nucifera* L.) is one of the important palm species, cultivated in tropical and subtropical regions of the world and plays a crucial role in the socioeconomic life of smallholder farmers in these regions. However, despite its great economic importance and multitude of uses, genetic erosion of coconut continues due to pest and disease attack, natural disasters and change in land use patterns. Therefore, there is a need for an applicable and successful conservation protocol to create long-term germplasm collections of this species. Conventional conservation strategies applied to coconut face many problems since the coconut seed is recalcitrant and short-lived, and seed gardens are financially demanding and subject to environmental threats. Thus, cryopreservation is a promising solution for the long-term conservation of coconut germplasm. The aim of this study was to develop an efficient protocol for the cryopreservation of coconut zygotic embryo using rapid physical dehydration. For this purpose, after isolation and surface sterilisation of embryos, they were precultured in a Y3 medium supplemented with sucrose at four concentrations (0.2, 0.3, 0.4 and 0.5 M) for 3, 5 and 7 days. The embryos then were physically dehydrated for 1 to 6 hours using a drying apparatus and placed individually into 2 mL cryovials and plunged directly into liquid nitrogen for 2 hours. To recover the cryopreserved embryos, the frozen vials were removed, warmed in a water bath at 40 ± 1°C for 3 minutes and then placed into a recovery medium. The results showed that sucrose pre-culture is beneficial for post cryopreservation survival of embryos. In the control treatment, survival of embryos was 20% after 6 hours of drying. Whereas, pretreatment of embryos with sucrose for 5 days at concentrations of 0.4 M produced the highest survival rate of 60% after 4 hours of dehydration (moisture content of 20%).

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FIELD PERFORMANCE OF CRYOPRESERVED SEED-DERIVED CARROT, TOMATO AND ASPARAGUS PLANTS

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The potential effects of liquid nitrogen in the subsequent plant growth in the field have not been studied. The research aim was to describe the field performance of cryopreserved seed–derived carrot, cultivar Nants'ka Kharkivs'ka, tomato, cultivars Seven, Potiron Ecarlate, Druzhba and asparagus, cultivars Atlas-15, WB 210-15 plants. The seeds(8 - 10 % humidity) were cryopreserved in 1.8 ml cryovials by a direct plunging into liquid nitrogen. Control seeds were stored at 4°C. The increase in total and marketable yield of carrot roots was by 24 and 17% respectively. The indices of total sugars, ascorbic acid, carotene, nitrates did not change statistically. In respect of tomato, for the Seven cultivar the increase in total and marketable yields was 351 and 268 % respectively. The productivity for each plant had increased almost five times, because the mass of one fruit and the number of them was bigger. For Potiron Ecarlate cultivar the trading productivity increased in 220%, the weight of one fruit was significantly lower, the number of fruits per plant was significantly higher. It was shown that for the Druzhba cultivar the data of total and trading yield increased in 27.8 and 71.9% respectively, number of fruits per plant was significantly higher. The total number of diseased plants decreased by 33% for the Seven variety, for Potiron Ecarlate it did by 6.7%, for the Druzhba cultivar the total percentage of sick and healthy plants did not differ. The use of low-temperature treatments did not affect the germination energy and germination of asparagus. Yield study after 120 days of growing Atlas-15 seedlings showed a higher number of shoots (9.1), their weight (13.3 g) and roots (32.5 g) compared with the control (7.4 units, 10.5 and 29.5 g, respectively), for WB 210-15 cultivar all investigated indices did not differ statistically from the control values.

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