The seeds used in this study were collected through SCUBA diving in a C. nodosa prairie located at Juan Grande in the southeast coast of Gran Canaria (27º 48’ 00” N; 15º 25’ 40” W). Seeds were surface sterilized and then cultivated in Magnifick-407 (Sigma Co.) vessels filled with 40 ml autoclaved sand and 200 ml of liquid culture medium as shown in Figure 1. Enrichment of the medium was achieved by adding nutrients, using the formulation of PES culture medium (69), adjusted as in García-Jiménez et al. (2005) (69). Cultures were placed inside a growth chambers at 24 °C, 12:12 light:darkness photoperiod and 50 ± 5 μmol photons m⁻² s⁻¹ at the level of culture vessels. Viosaplicity shock (5, 11, 18 with 36 psu as control) was used as method for the induction of germination. Then, further development through different 0 to stage 3 (Fig. 1) at different salinity conditions was also monitored to check for the viability of the seeds after their transference to natural salinity.

For acclimation, plantlets (45-60 days after germination in vitro) were transferred to tanks (160 m³) under continuous seawater flow (RT). Leaf and root growth and emission were monitored during 45 days in cultivated plant to determine when plantlets may be ready for transplantation (Fig. 2). A sheltered place was chosen for transplantation to allow for easy monitoring of growth during three month after transplanting, using indexes such as % survival (as % plantlets apparently pigmented and healthy) and leaf and root growth described above. Three methods were evaluated (Fig. 3): seeds scattered all over a nylon mesh which were grounded with woody stakes (1,1), aggregated in a set of 12 biodegradable pots (1,2), and non-acclimated seeds aggregated in the ground (1,3).

RESULTS

An effective methods have been developed that provide with C. nodosa plantlets for seagrass meadows restoration. The entire process consists of GERMINATION of collected seeds under hypoosmotic treatments. In the case of C. nodosa from the Canary Islands, optimal salinity reduction was seen at half of natural salinity (50), though salinity may be down until 11. Several authors have reported that hypoosmotic condition may favour the germination of seeds from seagrass species (Hootsmans et al., 1987 in Zostera marina and Z. noltii (69); Harrison, 1991 in Z. noltii (69); Conacher et al. 1994 in Z. capricornis (69); Ashlock and Shaffer, 2006 in Zostera marina and Zostera nolteia (69); and in C. nodosa since Caya and Meiner (1986, 1990, 69, 70), as they have secondary dormancy which can be broken by physical treatments such as the increase in external water potential and subsequently seeds imbibition. Therefore, the hypoosmotic treatments may be an indirect method for other seagrass species. Particularly in those species, like C. nodosa, in which seeds accumulated are to be encountered in the natural populations, where natural germinations rates are rather low. The induction of germination in the laboratory by physical procedures such as hypoosmotic treatment is a cost effective method to ensure rates up to 70% of the cultivated seeds, as seen when the procedures were scaled up for massive propagation. For propagation purposes, the number of plantlet eventually produced is important. In this regard, it is worth noting that the highest germination rates did not necessarily provide with higher biomass for further propagation. There seems to be a threshold, which was determined at 5 psu in the study with C. nodosa, in which dormancy is broken and germination proceeds with reduced seedling growth and early death. There is no significant document that the threshold may depend on temperature, light, or even chemical treatments should not be discarded as effective methods to break secondary dormancy (69). ACCLIMATIZATION in aquaria or tanks, as big as 100 cubic meters, of hundreds of seedlings germinated in vitro under non-hypoosmotic hypoosmotic treatments. The cultivated seedlings stabilized their growth rate, thus producing healthy plantlets, ready for transplanting to the natural environment as soon as 30 with yields of 100% of cultivated seedlings. TRANSPLANT of acclimated plantlets to the natural populations, with the highest rates of survival and growth when seeds were cultivated very closed in set of biodegradable pots or directly in the ground. In such conditions, the transplanted plantlets grew and produced plagiotropic rhizome, thus revealing the initiation of a new patch formation (17). Although methods must be still improved to face with harsher conditions to be encountered in natural meadows, they may provide with seagrass biomass in form of plantlets coming from seeds force to germinate in vitro, what otherwise would be lost at natural populations, genetically diverse and in quantity enough to allow for multiple assays until maximum adaption will be achieved. To date, we provide with 300-400 seedlings each 4 months for restoration of natural populations in the Canary Islands.

DISCUSSION

An effective methods have been developed that provide with C. nodosa plantlets for seagrass meadows restoration. The entire process consists of GERMINATION of collected seeds under hypoosmotic treatments. In the case of C. nodosa from the Canary Islands, optimal salinity reduction was seen at half of natural salinity (50), though salinity may be down until 11. Several authors have reported that hypoosmotic condition may favour the germination of seeds from seagrass species (Hootsmans et al., 1987 in Zostera marina and Z. noltii (69); Harrison, 1991 in Z. noltii (69); Conacher et al. 1994 in Z. capricornis (69); Ashlock and Shaffer, 2006 in Zostera marina and Zostera nolteia (69); and in C. nodosa since Caya and Meiner (1986, 1990, 69, 70), as they have secondary dormancy which can be broken by physical treatments such as the increase in external water potential and subsequently seeds imbibition. Therefore, the hypoosmotic treatments may be an indirect method for other seagrass species. Particularly in those species, like C. nodosa, in which seeds accumulated are to be encountered in the natural populations, where natural germinations rates are rather low. The induction of germination in the laboratory by physical procedures such as hypoosmotic treatment is a cost effective method to ensure rates up to 70% of the cultivated seeds, as seen when the procedures were scaled up for massive propagation. For propagation purposes, the number of plantlet eventually produced is important. In this regard, it is worth noting that the highest germination rates did not necessarily provide with higher biomass for further propagation. There seems to be a threshold, which was determined at 5 psu in the study with C. nodosa, in which dormancy is broken and germination proceeds with reduced seedling growth and early death. There is no significant document that the threshold may depend on temperature, light, or even chemical treatments should not be discarded as effective methods to break secondary dormancy (69). ACCLIMATIZATION in aquaria or tanks, as big as 100 cubic meters, of hundreds of seedlings germinated in vitro under non-hypoosmotic hypoosmotic treatments. The cultivated seedlings stabilized their growth rate, thus producing healthy plantlets, ready for transplanting to the natural environment as soon as 30 with yields of 100% of cultivated seedlings. TRANSPLANT of acclimated plantlets to the natural populations, with the highest rates of survival and growth when seeds were cultivated very closed in set of biodegradable pots or directly in the ground. In such conditions, the transplanted plantlets grew and produced plagiotropic rhizome, thus revealing the initiation of a new patch formation (17). Although methods must be still improved to face with harsher conditions to be encountered in natural meadows, they may provide with seagrass biomass in form of plantlets coming from seeds force to germinate in vitro, what otherwise would be lost at natural populations, genetically diverse and in quantity enough to allow for multiple assays until maximum adaption will be achieved. To date, we provide with 300-400 seedlings each 4 months for restoration of natural populations in the Canary Islands.