Aquatic Botany 76 (2003) 291–297

Aquatic botany

www.elsevier.com/locate/aquabot

Growth and development of *Posidonia oceanica* seedlings treated with plant growth regulators: possible implications for meadow restoration

Elena Balestri*, S. Bertini

Dipartimento di Scienze dell'Uomo e dell'Ambiente, Biologia Marina ed Ecologia, Via Volta 6, I-56126 Pisa, Italy

Received 22 July 2002; received in revised form 12 December 2002; accepted 8 April 2003

Abstract

To provide more vigorous seedlings of *Posidonia oceanica* for re-seeding programs, we examined under controlled conditions the effects of application of (a) a plant growth stimulant on seed germination and (b) three different growth regulators on root initiation and development of seedlings. No significant differences on final percentages of germination were observed among seeds exposed for 24 h to Sprintene and those nontreated. Application of Naftal (active ingredient: α-naphtaleneacetic acid (NAA)) and indole-3-butyric acid (IBA) for 1 h stimulated the initiation and growth of adventitious roots in seedlings; this resulted in two- to three-fold reduction of the time for root emergence, as well as in two- to three-fold longer seedling roots compared to the nontreated control. No dose-dependent effects were detected. If extrapolated to field conditions, the use of seedlings treated with auxins could result in a high probability of success following transplanting.

Keywords: *Posidonia oceanica*; Rooting; Seedlings; Transplanting

1. Introduction

Populations of *Posidonia oceanica* (L.) Delile, an endemic seagrass to the Mediterranean, are declining in many areas due to natural and human-induced disturbances (Marbà et al., 1996; Marbà and Duarte, 1997). Given the ecological importance and conservation status of *P. oceanica* meadows, collection of plants is rigorously managed (PNUE, 1990) and considerable efforts are being devoted to protect and restore this habitat. Until now, most *P. oceanica* restoration efforts have relied upon planting of vegetative material collected

* Corresponding author. Tel.: +39-050-500018; fax: +39-050-49694.
E-mail address: balestri@discat.unipi.it (E. Balestri).

0304-3770/$ – see front matter
doi:10.1016/S0304-3770(03)00074-3

from existing beds (Meinesz et al., 1993; Molenaar et al., 1993; Piazzi et al., 1998). Less work has been done with seeds and seedlings as donor material (Meinesz et al., 1993; Balestri et al., 1998a), despite the use of sexual material in restoring seagrass meadows could theoretically have some advantages. For example, even at very low rate the recruitment of seedlings following transplantation could preserve the natural genetic diversity in restored populations, whereas there is some evidence that the use of vegetative material reduces the genetic variability among individuals in transplanted populations (Williams and Orth, 1998).

At present, an important problem with planting seedlings of *P. oceanica* that needs to be solved is the high loss of seedlings occurring during the first months after planting (Cooper, 1979; Balestri et al., 1998a). As in other seagrasses (Duarte and Sand-Jensen, 1996; Kuo and Kirkman, 1996), the root system of young seedlings of *P. oceanica* is insufficient to cope successfully with physical disturbance imposed by water movement and sediment instability (e.g. erosion or burial), as well as to exploit resources for sustaining shoot growth.

Numerous studies show that application of several biostimulants and plant growth regulators (PGRs) may increase the germination ability of seeds and seedling vigour in a wide range of terrestrial plants (Russo and Berlyn, 1990; Crunkilton et al., 1994; Swaminathaan and Srinivasan, 1996). Information on the effects of PGRs on seagrasses is still limited (Kelly et al., 1971; Van Breedveld, 1975; Loquès et al., 1990; Koch and Durako, 1991; Terrados, 1995; Woodhead and Bird, 1998), but there is evidence that exposure of seedlings of the seagrass *Pyllospadix torreyi* S. Watson to auxins may influence root development (Reed et al., 1998).

In the present study, we report the outcome of preliminary investigations aimed at maximising seed germination and obtaining seedlings of *P. oceanica* with a well developed root system for large scale planting programs. Specifically, we tested under controlled conditions the effects of exposure to (a) Sprintene, a product marketed to stimulate seed germination in terrestrial plants, on seed germination, and (b) three different commercially available hormones on root development and growth of germinated seeds.

2. Material and methods

Mature fruits of *P. oceanica* were collected from drift material at Livorno (43°30′N, 10°19′E NW Italy) in late March 2000. Seeds were manually extracted from fruits. In the first set of experiments, seeds were immersed for 24 h (at 18 °C in the dark) in a solution of Sprintene (Aifar Agricola, Italy) at a concentration of 35 ml/l in seawater, following the protocol available for terrestrial plants. Seeds immersed in natural seawater were used as control. There were 20 seeds per treatment and treatments were replicated twice. After treatment, seeds were transferred to 500 ml Erlenmeyer flasks containing 250 ml of seawater through which air was bubbled, and maintained at 18 °C with a 16 h photoperiod and a photon irradiance of 30 μmol m^{-2} s^{-1}. Seeds were checked for germination every 3 days. Germination was deemed to have occurred when the cotyledon was emerged from the seed coat. Final germination percentage was calculated for each treatment. Mean time-to-germinate (MTG) was calculated using the equation

$$\mathrm{MTG} = \sum \frac{nd}{N} \tag{1}$$

where n is the number of seeds germinated between observation intervals, d the incubation period in days after time of observation and N the total number of seeds in the sample that germinated in the treatment.

In the second set of experiments, seeds were germinated in an aquarium (28 cm × 32 cm) containing natural seawater, at the conditions described earlier. Germinated seeds were then randomly assigned to the following treatments: control (no hormone), 5 ml/l Naftal (Aifar Agricola, Italy; active ingredients: α-naphtaleneacetic acid (NAA), 1%), 10 ml/l Naftal, 5 mg/l indole-3-butyric acid (IBA), 10 mg/l IBA and 0.2 ml/l 66 F (L. Gobbi, Italy; active ingredients: NAA and α-naphtaleneacetamide (NAD), 3.1%). Germinated seeds were individually transferred into 250 ml flasks containing 150 ml of seawater supplemented with the rooting agents and incubated for 1 h at 18 °C. There were 10 replicate seedlings per treatment. Following treatment, the seedlings were planted into plastic pots (80 mm in diameter) containing sand supplemented with 0.01 g Osmocote (Bayer, Germany), constant-release (6 months). Pots were kept in 28 cm × 32 cm aquaria (10 pots per aquarium) filled with 10 l of seawater through which air was bubbled and maintained at the conditions described above for 4 months. Seawater was renewed every 2 weeks to prevent excessive microalgae growth. Seedlings died during the course of the experiment were removed from pots to reduce likelihood of infection of healthy plants. Every 3 days during the first month, seedlings were carefully retrieved and checked for the appearance of the first adventitious root; the days until the emergence of the first root were recorded. Subsequently, seedlings were retrieved at monthly intervals and the maximum length of the primary and adventitious roots, the number of leaves per seedling and the maximum length of leaves recorded.

At the end of the experiment, data were analysed by separate one-way analyses of variances (ANOVAs). When significant differences in ANOVAs were found, means were compared a posteriori using the Student–Newman–Kuels (SNK, Underwood, 1997). Before running the analysis, leaf length data were log $(x + 1)$ transformed. In the case of the time of root emergence, variances were shown to be heterogeneous and could not be stabilised. Nevertheless, analysis of variance is considered sufficiently robust to the departures from the assumption, particularly with balanced designs (as in our case). Thus, the untrasformed data were used and results were interpreted with the more conservative probability level of 0.01 (Underwood, 1997).

3. Results

In both treated seeds and controls, the starting of germination occurred after a lag phase of 12 days. There was a tendency for seeds treated with Sprintene to germinate less (42.5% ± 2.5) when compared with control seeds (57.5% ± 2.5), but the ANOVA did not detect significant differences among treatments ($F_{1,2} = 18$; $P > 0.05$).The highest percent of seed germination was reached after 26 days. The MTG (±ES) was 15.9 (±0.1) days for nontreated controls and 16.7 (±0.1) days for treated seeds.

Emergence of the first adventitious root was observed within a few days after planting (Fig. 1a). Results of ANOVA showed that seedlings treated with Naftal and IBA initiated roots faster ($F_{4,25} = 6.27$, $P < 0.01$) than untreated control and seedlings treated with 66F. Since only two seedlings among those exposed to 66F produced adventitious roots within

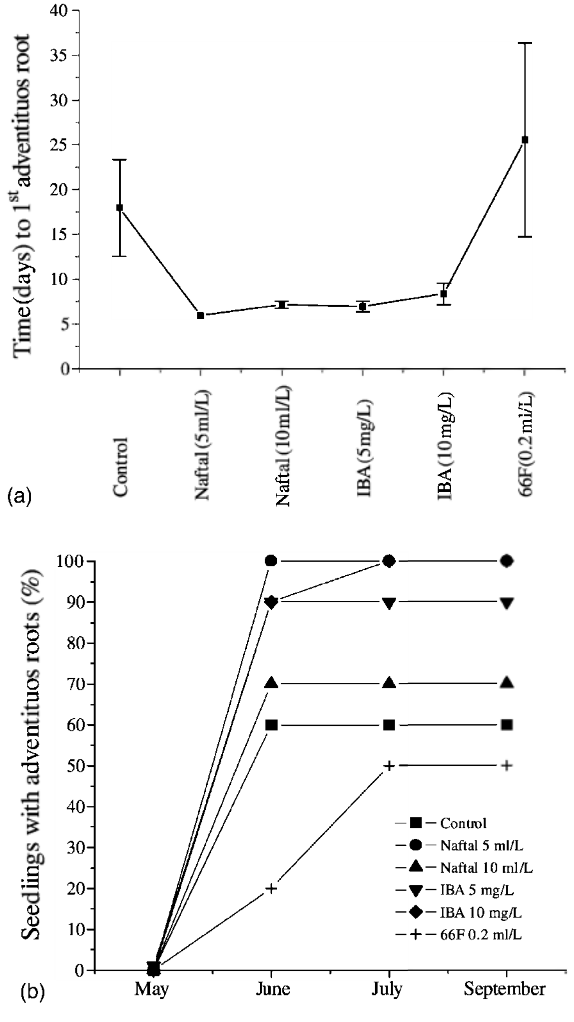

Fig. 1. Mean number of days until emergence of the first adventitious root (a) and percentage of seedlings produced at least one adventitious root (b). Bars indicate standard error.

the first month, this treatment was excluded from the analysis relative to the time for root emergence. Exposure of seedlings to 5 ml/l Naftl and 10 mg/l IBA tended to result in a higher percentage of seedlings producing at least one adventitious root (90–100%) compared with the other treatments (50–70%, Fig. 1b). After 5 months, the roots of seedlings exposed to Naftal and IBA were significantly longer ($F_{5,18} = 11.36$; $P < 0.01$) compared to the 66F treatment and controls (Fig. 2a). Significant differences were observed in the number of roots produced per seedling ($F_{5,42} = 2.50$, $P < 0.05$), although the SNK test did not identify an alternative to the null hypothesis. By contrast, the length of primary root (Fig. 2c, $F_{5,23} = 2.01$, $P > 0.05$), the number of standing leaves (Fig. 2d; $F_{5,18} = 1.71$; $P > 0.05$)

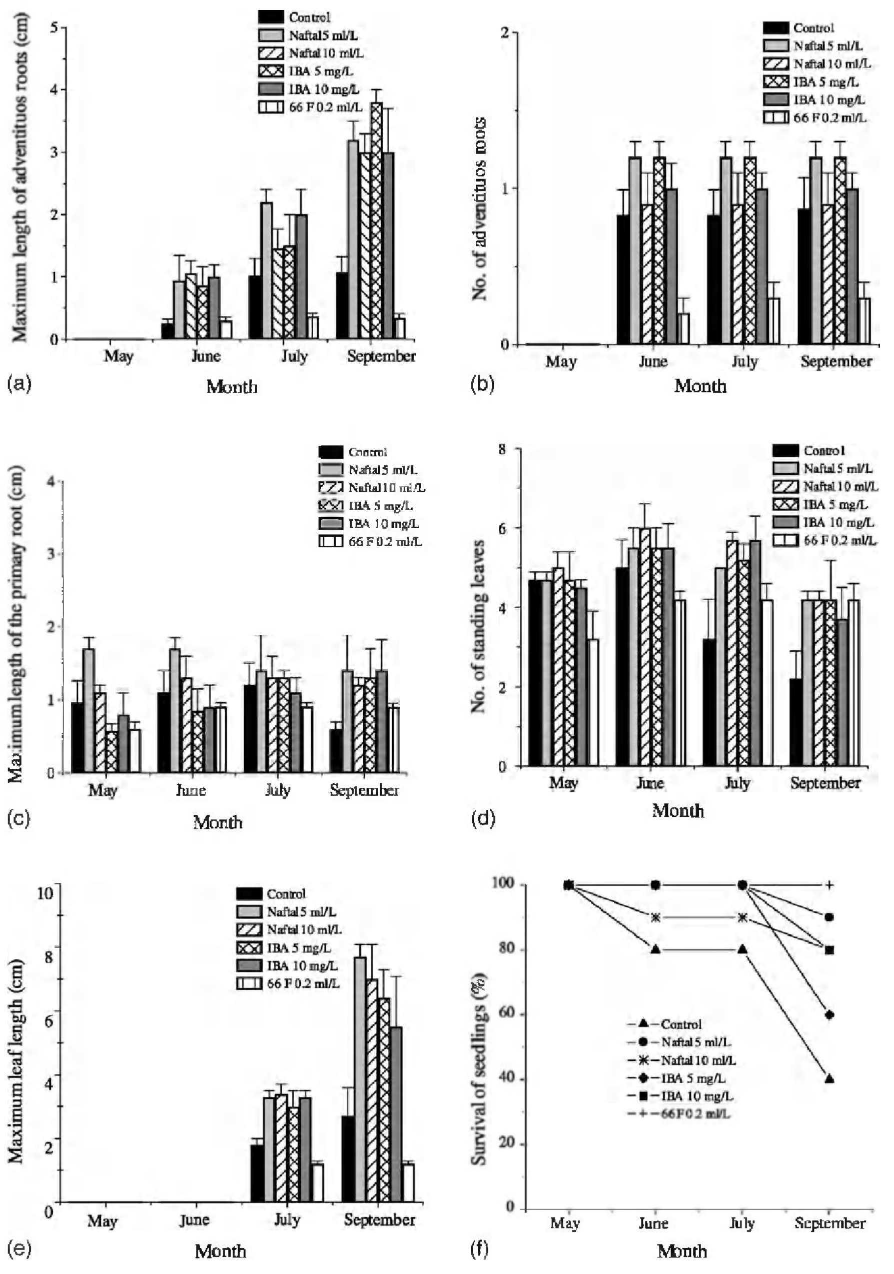

Fig. 2. Effects of the different plant growth substances on (a) maximum length of adventitious roots, (b) number of adventitious root produced per seedling, (c) length of the primary root, (d) number of standing leaves, (e) maximum leaf length and (f) survival of seedlings. Bars indicate standard error.

and the maximum length of leaves of seedlings did not differ significantly among treatments (Fig. 2e; $F_{5,18} = 1.14$, $P > 0.05$). Overall, the percentage of survival tended to be higher in treated seedlings compared to controls (Fig. 2f).

4. Discussion and conclusion

In this study, final percentages of germination appeared within the range reported for this species (30–90%) under laboratory and natural conditions (Cooper, 1979; Caye and Meinesz, 1989; Balestri et al., 1998b; Buia and Mazzella, 1991). No benefit in the use of Sprintene to promote germination was observed. Further experimentation is needed to investigate the effects of other factors that are likely to influence seed germination (such as substrate and oxygen potential). Contrary to previous studies involving the effects of PGRs on the development of *P. oceanica* seedlings (Balestri et al., 1998b), this study showed that application of auxins accelerates root emergence and enhances root growth in germinated seeds. Importantly, such application does not adversely affect shoot growth (in terms of number and length of leaves). These results are consistent with Reed et al. (1998), where, a single application of a commercial-available product containing both NAA and IBA increased root development in the surfgrass *Phyllospadix torrey* S. Watson. Similarly, Kelly et al. (1971) found that NAA increased root production on transplanting cuttings of *Thalassia testudinum* Banks ex König.

In seagrasses only few seedlings, those germinating in richer and stable microenvironments, are likely to establish (Duarte and Sand-Jensen, 1996; Kuo and Kirkman, 1996). The possible value of the application of rooting substances is linked to the quicker establishment seedlings for restoration purposes, together with the relatively low cost. Since seedlings of *P. oceanica* rooted with hormones may develop three- to ten-fold longer roots, theoretically such seedlings have a better chance of establishment than seedling grown conventionally, thereby, increasing the long-term transplantation success. Here, the limited number of seeds available precluded us from assessing the effects of different hormone concentrations. Clearly, further experimentation is needed to identify the optimal concentration and time of exposure of seeds to the rooting agents used. Further studies are also needed to examine the performance and post-planting survival of seedlings artificially rooted in the field, as well as to determine the effective contribution of seedling recruitment following transplantation in restoration of *P. oceanica* meadows.

Acknowledgements

The study was financially supported by the Livorno Provincie and the Industry Solvay and Co, i.e. of Rosignano Solvay (Italy).

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