Chapter 8

Seed Germination Technologies for Helophyte Production Used in Wastewater Treatment

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Additional information is available at the end of the chapter

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Abstract

Constructed green wetlands with horizontal surface for wastewater treatment are gaining acceptance. Many countries have published innovative experiences with this technology. A great variety of wastewaters from industries have been treated. Different plant species have been tested. Seed technology development provides interesting tools to produce these species in nurseries. It is a sustainable new business. But studies on seed germination of aquatic and lacustrine plants are very few. That is why we have made the following bibliographic review. We have summarised and analysed the state of the art of this innovative topic, concluding that seed technology for multiplication of helophytes needs further experimental work. But there is enough information to produce right now, tens of different species. Significant efforts have been done. Even though it is a challenge to produce from now on, experimental results are ready to be transferred to those who are trading with this type of plants. Helophytes have a promising future as sustainable elements of the upcoming sewage equipment. Improvements on the biotechnology of these species are a worthwhile researching line. To this aim, the following revision is an essential compilation with which to begin.

Keywords: germination protocol, green filter, helophyte, wastewater, production

1. Introduction

Constructed green wetlands with horizontal surface for wastewater treatment are getting social recognition. They usually have plants as an essential component of the design. Many countries have published innovative experiences with this technology: the USA, Canada, the UK,

Germany, France, the Netherlands, Switzerland, Norway, Poland, Slovenia, Lithuania, Italy, Spain, Portugal, Australia, Japan, China, India, Taiwan, South Africa, Turkey, Kenya, Uganda and México [1, 2]. A great variety of wastewaters from industries have been treated: chemical, petrochemical, textile, pulp and paper, tannery, abattoir, food processing, distillery and winery factories. Effluents from pig farms, fish farms, shrimp culture, cobalt recovery, mining or coke plants have also been managed this way. Run-offs from airports, highways, hospitals, agricultural activities or storm water have been tested as well. Finally, landfill leachate or polluted rivers have been experimentally decontaminated using constructed wetlands with vascular plants as the principal component [1, 3]. Uptake of rare earth elements (REEs) from obsolete equipment of modern technologies has also been reported [4].

Different species have been used to date, all over the world. They belong to different genera and families. Frequently they are helophytes, which means plants growing in marsh partly submerged in water, so that they regrow from buds below the water surface. The helophytic plants used to build wastewater green filters in constructed wetlands have in common with these significant characteristics: (a) a rich below-ground organ, root or rhizome, as to provide substrate for attached bacteria and oxygenation (as much as possible) of areas adjacent to the radicular apparatus; (b) a high-tolerance nutrient and organic loadings; and (c) the production of a high amount of above-ground biomass for winter insulation in cold and temperate regions, as well as nutrient removal via harvesting [1].

From a botanical point of view, a great list of species could be used in each country, because plant biodiversity is high. Ad hoc species for each biogeographical region could be found, attending to the above-mentioned characteristics. However, in practical terms, a limited number of species have been tested by the industry. Vymazal [1] summarised the most important, and we have taken its publication as a framework. Later references [4–12] have also been consulted, and **Table 1** shows the helophytes that we have considered most relevant.

For the production and multiplication of plants in nurseries to the global market, it is necessary to standardise the operations aimed at getting living plants that can be installed in the constructed sewages. Plant reproduction can be made by vegetative way (cuttings) or by seeds (sexual way). Nowadays international trade is essential, so those systems better adapted to international transport and management are more competitive. For this reason seed multiplication systems are very interesting. Seeds are more resistant and better prepared than cuttings, to adverse environmental conditions (light, humidity, temperature), that can occur during international transport. Thus, seed technology development provides interesting tools to produce in nurseries good quality plants. It is a sustainable new business.

Various authors have highlighted that studies on seed germination of aquatic and lacustrine plants are very few [13]. This topic is a much less studied subject that other aspects of seed biology, physiology or ecology. However it has a great importance from an applied point of view. That is why we have made the following bibliographic review. It will let us summarise and analyse the state of the art of this interesting topic.

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2. Seed germination conditions for the main helophyte groups for wastewater treatment

A prospective analysis of the publications issued in specialised databases allows us to know the main techniques proposed for seed germination, fitting us to the scope of (**Table 1**) helophyte species. We set out below the most relevant conditions for a successful germination of the genera and species internationally used, excluding palms and aquatic ornamentals.

Species	Family
Acorus calamus L.	Acoraceae
Arundo donax L.	Poaceae
Asclepias incarnata L.	Apocynaceae
Baumea articulata Gaudich.	Cyperaceae
Brachiaria mutica (Forssk.) Stapf	Poaceae
Canna glauca L.	Cannaceae
Canna indica L.	Cannaceae
Canna x generalis L. H. Bailey	Cannaceae
Carex acutiformis Ehrh.	Cyperaceae
Carex gracilis Curtis.	Cyperaceae
Carex lacustris Willd.	Cyperaceae
Coix lacryma-jobi L.	Poaceae
Colocasia esculenta (L.) Schott	Araceae
Cyperus esculentus L.	Cyperaceae
Cyperus alterniflorus Willd. ex Kunth	Cyperaceae
Cyperus articulatus L.	Cyperaceae
Cyperus dubius Rottb.	Cyperaceae
Cyperus flabelliformis L.	Cyperaceae
Cyperus grandis C. B. Clarke	Cyperaceae
Cyperus immensus C. Presl	Cyperaceae
Cyperus involucratus Rottb.	Cyperaceae
Cyperus isocladus L.	Cyperaceae
Cyperus malaccensis Lam.	Cyperaceae
Cyperus esculentus L.	Cyperaceae
Echinochloa polystachya (Kunth) A. S. Hitchc.	Poaceae
Eichhornia crassipes (Mart.) Solms-Laub.	Pontederiaceae
Eleocharis sphacelata R. Br.	Cyperaceae

Species	Family
Epilobium hirsutum L.	Onagraceae
Festuca arundinacea Schreb.	Poaceae
Filipendula ulmaria (L.) Maxim.	Rosaceae
Glyceria maxima (Hartm.) Holmb.	Poaceae
Gynerium sagittatum (Aubl.) P. Beauv	Poaceae
Heliconia psittacorum L. f.	Heliconiaceae
Heliconia rostrata Ruiz and Pav.	Heliconiaceae
Hemerocallis fulva L.	Xanthorrhoeaceae
Hibiscus moscheutos L.	Malvaceae
Hymenocallis littoralis (Jacq.) Salisbury	Amaryllidaceae
Iris pseudacorus L.	Iridaceae
Iris tectorum Maxim.	Iridaceae
Iris versicolor L.	Iridaceae
Juncus effusus L.	Juncaceae
Juncus inflexus L.	Juncaceae
Juncus subsecundus N. A. Wakef.	Juncaceae
Kyllinga erecta Schumach.	Cyperaceae
Lepironia articulata (Retz.) Domin	Cyperaceae
Liatris pycnostachya Michx.	Asteraceae
Lobelia cardinalis L.	Campanulaceae
Lythrum salicaria L.	Lythraceae
Mentha spicata L.	Lamiaceae
Monochoria vaginalis (Burm. f.) C. Presl ex Kunth	Pontederiaceae
Panicum maximum Jacq.	Poaceae
Panicum repens L.	Poaceae
Paspalum distichum L.	Poaceae
Pennisetum purpureum Schumach.	Poaceae
Phalaris arundinacea L.	Poaceae
Phragmites australis (Cav.) Trin. ex Steud.	Poaceae
Phragmites karka (Retz.) Trin. ex Steud.	Poaceae
Phragmites mauritianus Kunth	Poaceae
Phylidrum lanuginosum Banks and Sol. ex Gaertn.	Philydraceae
Pontederia cordata L.	Pontederiaceae
Rudbeckia hirta L.	Asteraceae

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Species	Family
Sagittaria latifolia Willd.	Alismataceae
Scirpus acutus (Muhl. ex J. M. Bigelow) Á. Löve and D. Löve	Cyperaceae
Scirpus americanus Pers.	Cyperaceae
Scirpus californicus (C. A. Mey.) Steud.	Cyperaceae
Scirpus cyperinus (L.) Kunth	Cyperaceae
Scirpus fluviatilis (Torr.) A. Gray	Cyperaceae
Scirpus grossus L.	Cyperaceae
Scirpus lacustris (L.) Palla	Cyperaceae
Scirpus maritimus L.	Cyperaceae
Scirpus pungens Vahl	Cyperaceae
Scirpus sylvaticus L.	Cyperaceae
Scirpus tabernaemontani (C. C. Gmel.) Missbach and E. H. L. Krause	Cyperaceae
Scirpus validus Vahl	Cyperaceae
Silphium perfoliatum L.	Asteraceae
Sorghum halepense (L.) Pers.	Poaceae
Spartina alterniflora Loisel.	Poaceae
Spartina argentinensis Pers.	Poaceae
Spartina densiflora Brongn.	Poaceae
Spartina maritima (Curtis) Fernald	Poaceae
Spartina pectinata Bosc ex Link	Poaceae
Stenotaphrum secundatum (Walt.) Kuntze	Poaceae
Thalia geniculata L.	Marantaceae
Thrinax radiata Lodd. ex Schult. and Schult.	Arecaceae
Thysanolaena maxima Kuntze	Poaceae
Triglochin procerum L.	Juncaginaceae
Typha angustifolia L.	Typhaceae
Typha capensis Rohrb.	Typhaceae
Typha domingensis Pers.	Typhaceae
Typha latifolia L.	Typhaceae
Typha orientalis C. Presl	Typhaceae
Zizania caduciflora (Turcz. ex Trin.) Hand.-Mazz.	Poaceae
Zizaniopsis bonariensis (Balansa and Poitr.) Speg.	Poaceae

Table 1. Synopsis of vascular plants used for constructed greed wetlands with horizontal surface for wastewater treatment.

2.1. Phragmites

It is one of the most used genera. *Phragmites australis* (*Phragmites communis* Trin.) has been tested in Europe, Canada, Australia, many countries of Asia (except India and Nepal, where they use *Phragmites karka*) and many of Africa (except Central Africa, where it is used *Phragmites mauritianus*). In the USA and New Zealand, it has given good experimental results, but actually the utilisation of common reed has been limited because it is considered an invasive plant species [1, 14].

Seed germination of *P. australis* reaches germinative percentages up to 96–99% under the pretreatment of soaking the seeds with 0.1% KNO_3, rinsing them with distilled water before they are sown on layers of Whatman grade no. 1 filter paper (pH 7) in 90-mm Petri dishes. They must be moisten for up to 10 days and maintained into an incubator, with alternating diurnal regime of 12-h daylight at 25°C and 12 h of darkness at 15°C [15]. Fourteen hours/25°C and 10 h/20°C regime can also be applied [16]. Watering everyday with 10 ml of 9-mM sulphide solution increases significantly the germination speed [16]. Arbuscular mycorrhizal (AM) fungal inocula of *Funneliformis mosseae* accelerate seed germination of the species and, most important, enhance growth and development of its seedlings, performing as an efficient bio-accelerator, bio-fortifier and bio-enhancer [17]. Other proposals suggest using 1% agar as a germination medium and the following light and temperature conditions: 12-h/12-h photoperiod and 33/19°C (86% germination), 12-h/12-h photoperiod and 26/16°C (93% germination) and 8-h/16-h photoperiod and 35/20°C (95% germination [18]. Chemicals have a differential effect on *P. karka* seed germination. In complete darkness, as well as in 12-h light, 12-h dark photoperiod and different temperature regimes (10/20°C, 15/25°C, 20/30°C), seed germination is significantly promoted by thiourea (10 mM), nitrate (20 mM), proline (0.1 mM), betaine (0.1 mM), GA_3 (3 mM), kinetin (0.05 mM) or fusicoccin (5 μM) [19]. 5- and 10-mM ascorbic acid solutions have also given good results [20].

2.2. Typha

Cattails are very productive plants with maximum above-ground biomass values in constructed wetlands. Treating systems with *Typha angustifolia, Typha capensis, Typha domingensis*, *Typha latifolia* and *Typha orientalis* have been reported in the USA, Central and South America, Asia and several European countries [1]. In Spain, floating systems with these plants have been developed as an interesting innovative green technology, QuarQ Enterprise [2] (**Figure 1**).

Typha seeds and seed heads need to be cleaned in a seed cleaner before they are sown [21]. A strong jet of distilled water can be used for this purpose. Afterwards seeds will be settled in deionised water to select the most viable ones, which sink, whereas non-viable ones float [22]. An immersion for 24 h in slightly saline water with a concentration of up to 1% ClNa and pH 6.5–7.5 has also been proposed [23]. Specialised sources of seeds are recommended by the United States Department of Agriculture (USDA) [21], who indicates typha seeds germinate readily when they are planted in clean, moist seed bed and maintained for about 2 weeks in a greenhouse in pots 1 cm under the soil surface. Greenhouse temperature should be 37 ± 3°C.

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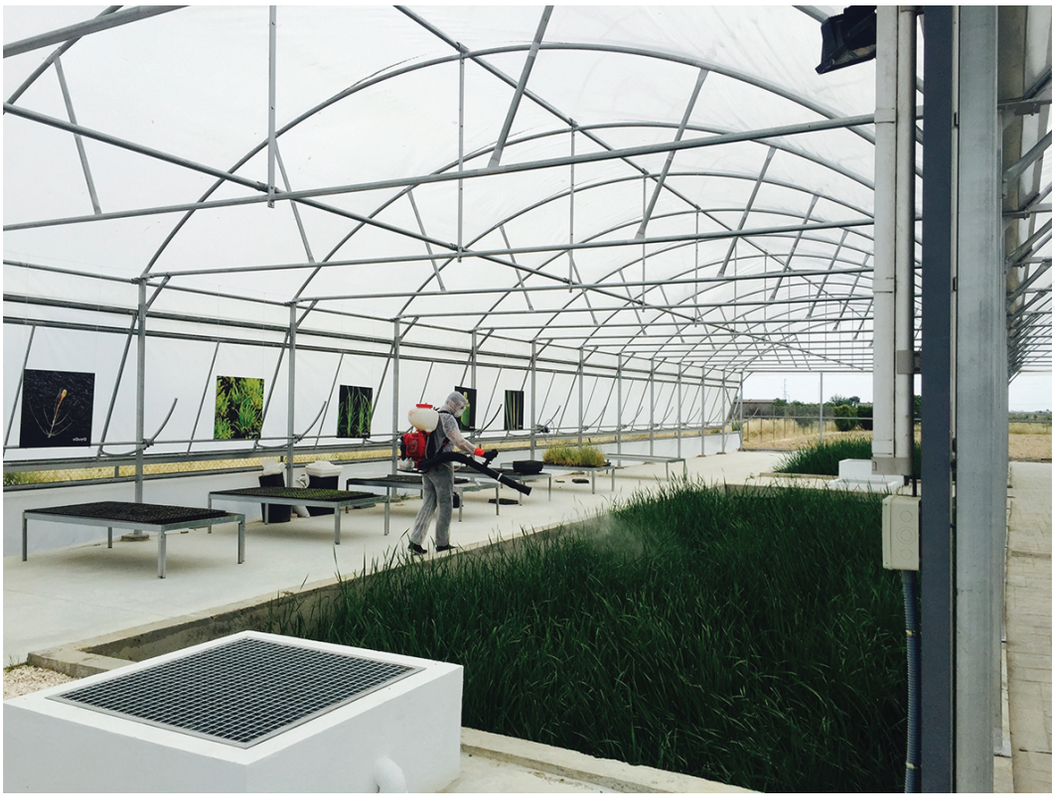

Figure 1. QuarQ Enterprise Water Technologies in Villafranco, Badajoz (Spain).

T. angustifolia seeds can germinate with maximum success (100% germination) watering with distilled water, maintaining temperatures of 35/20°C and programming an 8-h/16-h photoperiod. Seed scarification does not seem to be worthwhile, because 58% of germination is obtained with this pretreatment and the above-mentioned germination conditions. When using 1% agar medium and germination conditions of 33/19°C, and a 12-h/12-h photoperiod, germination can reach 85% [18]. *T. domingensis* seeds do not germinate without light [24]. Germination up to 100% is obtained [24, 25] using an environmental chamber or an outdoor shade house covered by nylon netting (light: 10,764–32,292 lx). Petri dishes with paper towels or filter paper as substrate [25] and oscillating temperatures of 32/26°C or 29/21°C for 15 days [25]. A constant temperature of 30°C and a 12-h/12-h [23] (light: 5,000 lx) or 14-h/10-h [24] light/dark photoperiod can also be used. In the latter case, 5-mm layer of water-saturated *Sphagnum* peat has been tested with good results (85±13% germinative percentage). pH peat is adjusted to 7.0 by adding calcium carbonate (7.5 g $CaCO_3$ per pet's litre) [24]. To remove possible germination inhibitors and to retard the growth of microorganisms, seeds must be previously washed with running water and sodium hypochlorite solution (10%), commercial bleach [24], and furthermore immersed in a germination activator solution with ammonium and phosphate, pH 6.5–7 [23]. Pretreatments with 0.1% $KMnO_4$ are also recommended [15]. Another medium quite useful to be used is 1% agar. This combined with the 8-h/16-h photoperiod can give very interesting germination results: 90–100% germination maintaining constant temperatures of 20–25°C during the germination period and 80–90% if tempera-

ture is elevated to 30°C [18]. Alternating temperatures of 35/20°C with the same photoperiod and germinative substrate produce 100% germination as well. Changing the photoperiod to a 12-h/12-h rhythm can give good results (86% germination) at a constant temperature of 31°C, and it is excellent (98–100% germination) when a 33/19°C alternating programme is applied [18]. *T. latifolia* seed germination success depends on light, pH and alternating temperatures. It is inhibited by total obscurity and limited at low levels of pH [22] but scarcely affected by anoxic conditions [26]. Germination rates can reach 84% on mesic peat (pH 4.3), 22°C and a 16-h/6-h (day/night) photoperiod [22] and maximum rates when using 20/30°C with 12 h/12 h photo-thermoperiod [27]. Excellent results (97% germination) can be obtained in similar conditions (19/33°C, 12 h/12 h) on 1% agar germination medium [18]. Pre-sowing treatments are also recommended, moist in high humidity over water for 1 day at 20°C, and then removing and chipping the covering structure [18]. After that, seeds can be sown in 1% agar germination medium and 8-h/16-h photoperiod, obtaining 78% germination at a constant temperature of 30°C and 88–84% germination using alternating temperatures of 30/15°C and 30/20°C, respectively [18]. In pre-sowing as above-mentioned and fitting the latter photo-thermoperiod (30/20°C, 8 h/16 h), 76% germination has been reported for a medium containing 1% agar + 101-mg/l potassium nitrate (KNO_3) [18]. *T. orientalis* seeds germinate 100% in 1% agar and 35/20°C, 8-h/16-h photo-thermoperiod [18] (**Figure 2**).

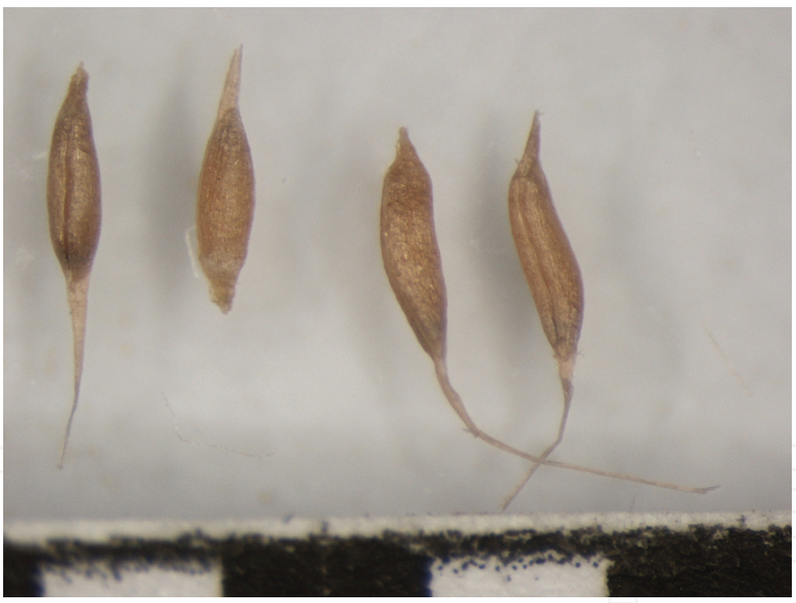

Figure 2. *Typha latifolia* seeds.

2.3. Scirpus

Different species of *Scirpus* (*Schoenoplectus*) have been tested in the USA, China, Australia and New Zealand (*Scirpus acutus, Scirpus americanus, Scirpus californicus, Scirpus cyperinus, Scirpus fluviatilis, Scirpus grossus, Scirpus lacustris, Scirpus maritimus, Scirpus pungens, Scirpus sylvaticus, Scirpus tabernaemontani, Scirpus validus*) [1, 5, 6]. Mexico and France have experimented

with *S. validus* and *S. maritimus*, respectively. Sewages wastewaters have been frequently treated with these plants [1]. *S. grossus* has been tested for heavy metals content in Malaysia [3].

S. americanus seeds need light to naturally break dormancy and germinate [28] and/or to be subjected to cold stratification (3–6°C) for 30–180 days [18]. Germination conditions of 30–32°C, 12 h/12 h or 35/20°C are proposed [18]. When germination processes are taking place at greenhouses, it has been suggested to sow seeds in a cold frame pot standing in three centimetres of water. The seeds germinate quickly. When they are large enough to handle, they must be planted into their permanent positions in early summer [21]. Alternatively, a container (pot or flat) can be used. It should be watered from the bottom as necessary, and it should not be covered after sowing, although a light dusting of soil can be applied. If grown in outdoor beds, seeds are sown on level soil and covered with a single layer of burlap or cotton sheet. Soil dry must be avoided, shading with a window screen set 30 cm [28]. Procedures to maximise seed germination of *Scirpus acutus* have been studied in the laboratory [29]. Pregermination conditions included scarification and stratification at 4±1°C for 84 days [18, 29] while submerged in water [29]. Seeds were placed in night/day temperature regimes of 10/25°C under a 14-hour photoperiod (≈200 μmol m^{-2} s^{-1} photosynthetic photon flux density), and up to 97.5% germination was achieved [29]. At the greenhouse, similar requirements as for *S. americanus* were reported [28]. To germinate seeds of *S. californicus* in greenhouse, they must be introduced in greenhouse in 2.5 × 2.5 × 5 cm pots, 0.5 cm under the soil surface. Soil surface needs to be moisted and maintained at 35–40°C. Seeds begin to germinate after a couple of weeks. Plants are ready in 100–120 days to come out as plugs [21]. *S. cyperinus* seeds should be imbibed on agar 1% for 20 weeks at 5°C as a pre-sowing treatment that has been suggested to have best results [18]. At greenhouses, seeds should be sown in a cold frame as soon as they are ripe in a pot standing in three centimetres of water, and they will germinate easily [21]. A loam, peat and sand wet substrate can also be used [28]. An 8-h/16-h photoperiod is a good option to obtain germinative success; using 1% agar as medium, 100%, 98%, 96% and 90% germination can be reached setting the following alternating temperatures: 35/20°C, 30/15°C or 20/5°C, 25/10°C and 40/15°C [18]. Using 1% agar + 250-mg/l gibberellic acid (GA3), with the same photoperiod, 100% germination is got at alternating temperatures 20/5°C and 93% at 25/10°C [18].

S. fluviatilis seeds germinate after a period of moist, cold stratification. They need to be mixed with equal amounts or more of damp sand, vermiculite or other sterile media and introduced in a plastic bag and maintained at 0–3°C for 3 months. Some seeds may sprout in the storage bag if moist stratified too long. If sprouting occurs, seeds must be immediately planted. Another method of breaking dormancy for this species at temperate climates or latitudes is to sow seeds outdoors in the fall so they may overwinter [28]. *S. lacustris* germination is reported to occur when using a pre-sowing treatment of cold stratification for 80 days and a later germination phase with alternating temperatures of 30/5°C [18]. Presoaking seeds in sodium hypochlorite and performing cold stratification under light conditions are presented for consideration as a good tool to improve germination results with this species [30]. *S. maritimus* cold stratification for 80 days is profitable before planting seeds to germinate under 30/5°C [18]. Stratification period can be reduced to 28 days if seeds are presoaked in sodium

hypochlorite and kept under natural light conditions [30]. Mechanical pretreatment of the seeds to evade physical dormancy has also been stated [31]. *S. pungens*, opposite to the above-mentioned Scirpus species, does not seem to respond so positively to the mechanical scarification (by squeezing with tweezers) or the stratification in cold water, although further studies on this taxon must be performed [32]. *S. sylvaticus* germination works very well (80% germination), imbibing for 56 days the seeds on 1% agar at 6°C and maintaining them afterwards on a 1% agar medium and a thermo-photoperiod of 33/19°C, 12 h/12 h [18]. Even better results (89% germination) can be obtained replacing the pretreatment by adding to the 1% agar, gibberellic acid (GA3) 250 mg/l [18]. *S. tabernaemontani* pre-sowing treatments have been proposed: (a) cold stratification for 80 days [18], (b) imbibing the seeds for 56 days in 1% agar at 5°C [18] and (c) introducing them for 42 days into a sodium hypochlorite solution under low temperatures and natural light conditions [30]. During the germination period, fluctuating temperatures are recommendable [30], 30/5°C [17] and 35/20°C [30]. In the latter conditions, the use of 1% agar as medium and a photoperiod of 8 h/16 h assert germinative percentages up to 84% germination [18]. *S. validus* seeds germinate after a period of [28] moist cold stratification of 180 days [18]. They need light to naturally break dormancy [27] and germination thermic conditions of 30–32°C [18]. They can easily be sown in flat which will be watered from the bottom as necessary. A light dusting of soil can be applied slightly, covering the seeds. If they are grown in outdoor beds, they can be sown on level soil, covering it with a single layer of burlap or cotton sheet, shading it with a window screen (set 30 cm) [28].

2.4. Cyperus

Several species from *Cyperus* genus (*Cyperus alterniflorus, Cyperus articulatus, Cyperus dubius, Cyperus esculentus, Cyperus flabelliformis, Cyperus grandis, Cyperus immensus, Cyperus involucratus, Cyperus isocladus, Cyperus malaccensis*) have been tested in Asia (China, Thailand), America (Nicaragua, Brazil), Africa (Kenya) and New Zealand among other countries [1]. Some species, such as *C. alterniflorus*, have recently been used in a pilot scale in Italy [7]. Most of them are not sufficiently studied in terms of the seed technology.

C. alterniflorus germinates 100% utilising 1% agar medium and alternating temperatures of 25/10°C or 35/20°C and a photoperiod of 8 h/16 h [18]. *C. articulatus* seed germination is easy going if a photoperiod of 8 h/16 h is fixed and a basic 1% agar medium is used for the process. No pretreatments are required. Maximum results are obtained at different alternating temperatures: 100% at 40/25°C, 98–100% at 35/20°C, 96% at 30/15°C and 88% at 25/10°C. By adding gibberellic acid (GA3) 250 mg/l to the agar medium, we can obtain 100% germination at 30/15°C, 98% at 40/25°C, 96% at 25/10°C and 92% at 35/20°C [18]. *C. dubius* can be successfully germinated (76%–85%) sowing the seeds in 1% agar medium, an 8-h/16-h photoperiod and alternating temperatures of 35/20°C and 30/15°C, respectively [18]. *C. esculentus* germination of the seeds is significantly influenced by both light and temperature. It is highest at 35°C, and poor germination was observed at other temperatures (27 and 45°C). The plant growth regulators enhance the seed germination and radical length to a different degree [33]. *C. flabelliformis* germination percentage can reach 100% when using 1% agar as medium and temperature and light conditions of 35/20°C, 8 h/16 h. Cold-wet stratifications as

pretreatments should not be planned because they reduce germination to 80% [18]. *C. involucratus* can be propagated by seeds in temperate latitudes at 18 to 21°C in spring in constantly moist seed compost [34]. *C. malaccensis* germinative process is mediated by arbuscular mycorrhizal colonisation, which is influenced at the same time by pH and moisture [35].

2.5. Carex

Some sedges (*Carex acutiformis, Carex gracilis, Carex lacustris*) have also been employed as phytoremediators in temperate regions (East Europe) [1], with recent applications in the east of Europe countries [12]. Very few species have been studied to date, and little is known about the technologies to improve the germination of their seeds.

It is reported that *C. acutiformis* seeds should be undergone to a pre-sowing treatment consisting in maintaining them for 2–6 months at 4°C. After that, they are placed of moist filter paper at germination conditions of 22/10°C, and an up to 65% germination is expected [18]. *C. gracilis* cold-wet stratification is suggested for a successful germination process (more than 80% germination). For that, seeds can be wetted with distilled water and introduced in 100-cc plastic vials, which will be sealed, wrapped in aluminium foil and stored at 4°C in a refrigerator for 6 months. After that, seeds must be placed at an incubator with alternating temperatures of 22/10°C [36]. *C. lacustris* has conditionally dormant seeds which require cold stratification [37]. The species is very sensitive to storage conditions [38]. Germination temperature regimen of 27/15°C ensures (at least 50%) discrete results [37]. Better results can be obtained by using seeds produced earlier in the same growing season. They must be situated into highly humid soils. Suitable soil amendments should be added to adjust the proper organic matter content to levels found in natural sedge meadows [38].

2.6. Other Cyperaceae

Eleocharis sphacelata, Kyllinga erecta and *Lepironia articulata* have been locally used in Australia, Tanzania and China [1]. For *Eleocharis sphacelata*, pretreatment, timing and water depth available for germination play an important part on the sexual multiplication of this plant, but there is still much to know about the specific requirements [39]. *Kyllinga erecta* seeds fail to germinate in darkness [40]. 85% germination has been recorded by the application of a pre-sowing treatment (imbibing on 1% agar for 12 weeks at 5°C), a germination medium of 1% agar and germination conditions of 35/20°C and photoperiod 8 h/16 h [18]. For *Lepironia articulata* a 50% germination was achieved in seeds buried 4 months, exhumed and incubated in anoxic/dark conditions [41].

2.7. Bambusoid-like species

Some bambusoids as *Arundo donax, Brachiaria mutica, Coix lacryma-jobi, Gynerium sagittatum, Pennisetum purpureum, Phylidrum lanuginosum, Thysanolaena maxima, Zizania caduciflora* and *Zizaniopsis bonariensis* have been utilised in Morocco, El Salvador, Costa Rica, Jamaica, Central

America, Australia, Mozambique, China and Brazil [1, 9]. But the multiplication by seeds of most of these species is barely developed.

Arundo donax seed germination is much better known than the rest of the bambusoid-like species. Maximum results (100% germination) are got using 1% agar medium and thermic and light conditions of 35/20°C, 8 h/16 h. Several pre-sowing treatments (cold shock −80°C, smoke and heat shock) have demonstrated not to be recommendable, especially if combined with constant temperature regimes of 20°C [18]. On the other hand, hydro-seeding has emerged as a reliable way to be employed. It consists in mixing selected seeds with colloidal substances in an aqueous solution usually along with mulch of various origins and fertilisers. Commercial names and composition are the following: Provide Verde (complete mixture of soil microorganisms), *Penicillium* sp, algae, polysaccharides, mulch (1 L ha^{-1}), vegetal glue, organic fertiliser (4.4% N, 2.2% P_2O_5, 1.1% K_2O, 2.1% Mg, 2.7% Ca), Envitotal-1 (total nitrogen 1.7% , organic carbon 11%, zeolites 38%, vegetal mulch mixture of cellulose 33% and vegetal glue), Envitotal-2 (total nitrogen 1.7% , organic carbon 11%, zeolites 38%, vegetal mulch mixture of cellulose 33%, soil N-fixing microorganisms 2.6%, vegetal glue), and Cellugrun (cellulose fibre mulch of 80% cellulose content, pH 7.5, bulk density 20–35 g L^{1-}, average fibre length 1400 μm, average fibre thickness 45 μm) and an adhesive hydrocolloid compound as soil control [42]. For an excellent (100%) seed germination result in the case of *Coix lacryma-jobi*, they need to be placed in Petri dishes (11 cm in diameter) containing silica sand and 10-mL distilled water into a climate-controlled incubator at 25°C, for a 10 days period, with a light (10 h) and dark (14 h) cycle [43]. Alternating temperatures 35/20°C are not recommended for this species [18].

Pearl millet (*Pennisetum glaucum*) seeds need to be washed previously with liquid soap solution and bavistin (fungicide) for 3 min and rinsed twice with deionised water [44]. Then they must be placed in Petri dishes (90 mm in diameter), containing one moisted piece of filter paper Whatman no. 1, and after that they have to be covered and maintained on a lab bench at room temperature (30 ± 5°C) [44]. An incubator programme of 30/19°C 16 h/8 h has also been successfully used [45]. Better results (100% germination) can be offered if the germination medium is 1% agar and the conditions are constant temperatures of 21°C or 26°C and photoperiod 12 h/12 h or 20°C and photoperiod 8 h/16 h [18]. With the same medium, 80% germination is obtained fixing 33/19°C, 12 h/12 h, and 75% germination with 25°C, 8 h/16 h [18]. Maximum success (100%) is aimed by removing the seed coat and performing the germinative process at 25°C and an alternating light regime of 8 h/16 h [18]. Finally, in selected lines of this species (`MS 841A', `MS 841B' and `D 23'), some pretreatments have been investigated to ensure at least 75% germination. Thus storage containers (cloth bags, polylined cloth bags), storage environments (ambient, controlled) and seed dressings (carbendazim, captan, thiram, *Trichoderma viride* and *Pseudomonas fluorescens*) were tested. The germination of seeds stored under controlled conditions (82.25%) (temperature 20°C and relative humidity 40%) and in polylined bags (76.62%) was significantly higher than seeds stored under ambient condition (66.43%) and in cloth bags (72.05%). Treatment with bioagent *Trichoderma viride* gave a germination percentage of 77.37%, *Pseudomonas fluorescens* treatment 59.58% and untreated control 74.27%. The incidence of seed mycoflora was 32.39, 28.26 and 29.14% in seeds of 'MS 841A', 'MS 841B' and 'D 23', respectively. Seeds stored under controlled conditions germinat-

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ed 35.31%, under ambient condition (24.55%) and maintained in cloth bags 31.21% and in polylined cloth bags 28.66%. The incidence of contamination with seed mycoflora was 40.24% in the untreated control, 6.90% in thiram treatment, 11.65% in captan treatment, 34.07% in *Trichoderma viride* treatment, 39.46% in carbendazim treatment and 47.28% *Pseudomonas fluorescens* treatment [46].

2.8. Non-cane-like Poaceae

In this group we have included species as *Echinochloa polystachya, Festuca arundinacea, Glyceria maxima, Panicum maximum, Panicum repens, Paspalum distichum, Phalaris arundinacea, Sorghum halepense, Spartina alterniflora, Spartina argentinensis, Spartina densiflora, Spartina maritima, Spartina pectinata* and *Stenotaphrum secundatum*, which are reported to be employed in Ecuador, Alabama, Kentucky and Washington (USA), New Zealand, Germany, Czech Republic, Jordan, Italy and Portugal [1]. Although there is some information on the seed technology of these taxa, many of them still require further development of proper methodologies adapted to each species.

Some interesting improvements have been made with *Festuca arundinacea*, for example. To ameliorate seed germination performance of it, hydropriming can be advised. Good results can be obtained by maintaining them in a germinator at 15 to 25°C for a period of 8 h of darkness and 16 h of light with a light intensity of 38 μmol m^{-2} s^{-1} provided by cool-white fluorescent lamps [47]. *Glyceria maxima* seed technology for germination has been more profusely tested. 100% germination has been obtained by sowing in 1% agar medium and maintaining one of the following regimes: 20°C, 8 h/16 h; 15°C, 8 h/16 h; 25/10°C, 8 h/16 h and 23/9°C, 12 h/12 h [18]. Using the same medium 95–96% germination was obtained when employing 21°C, 12 h/12 h, and 25°C, 8 h/16 h, respectively, and 85% germination if the conditions were 21/11°C, 12 h/12 h [18]. The latter result has been improved (94% germination) repeating the conditions but pretreating the seeds by imbibition on agar 1% for 4 weeks at 2°C [18]. Several tests adding 101-mg/l potassium nitrate to the agar medium did not give extremely advisable results, neither imbibing the seeds on agar 1% agar for 8 weeks at 6°C [18]. Opposite to this, an imbibition on 1% agar for 1 day at 20°C and then for 2 days at 4°C (germination conditions of 20/15°C, 12 h/12 h) has been reasonably recommended for it ensures 90% germination [18]. This species can also be germinated on damp filter paper into a Petri dish covered with a colourless plastic to allow light penetration and to prevent rapid moisture loss. They will be introduced in a grow chamber with a temperature range of 21–23°C with a 12-h/12-h (light/dark) cycle. If cold stratification at 4°C for 8 weeks has been implemented, more than 80% germination is reported [48].

For *Panicum maximum* recommended method for best (100%) seed germination is to sow them in 1% agar medium and introduce them in a room chamber at a constant temperature of 21°C and 12 h/12 h or a combined cycle 35/20°C, 8 h/16 h [18]. In the latter case, seeds must previously be immersed in 10% Domestos solution for 5 min [18]. Other scarifying procedures (shallow incisions, mechanical removing) decrease germination in this species [49] from percentages to 92%, 85%, 78%, 72% or even 40% [18]. If the available light cycle is 12 h/12 h, the former conditions should be maintained; increasing temperature to 26°C lightly decreas-

es the percentage of germination to 90%, and changing to alternating 33/19°C reduces it to 75% or 80% (in the particular case that a solution of 101-mg/l potassium nitrate has been added to the agar medium) [18]. *Panicum repens* germinates 80% if seed coats are removed and seeds are germinated on 1% agar medium and photothermic conditions are 35/20°C, 8 h/16 h [18]. Some authors have pointed out [50] that *Phalaris arundinacea* germination does not occur in the dark [50] and that this species is photoperiod insensitive in the range of 12–16 hours. Upon them, the highest germination percentages (up to 80) can be obtained under white light and red light (11.0 μmol s^{-1} m^{-2}) and up to 40 with high red: far-red ratios [50]. On the other hand, 96–98% germination has been reported [18], scarifying the seeds and sowing them in 1% agar at germination conditions of 23/9°C, 12 h/12 h [18], and 90% at an 8-h/16-h photoperiod and 20°C [18]. Without removing the coat, 90% germination can be reached on 1% agar medium, fixing the incubator conditions at 25/10°C, 8 h/16 h [18], and 80% by adding to the agar a solution of 101-mg/l potassium nitrate and choosing the cycle 23/9°C, 12 h/12 h [18].

Sorghum halepense germinates perfectly (100% germination) on 1% agar medium at a constant temperature of 26°C and a day/light rhythm of 12 h/12 h or at an alternating 35/20°C, 8 h/16 h, if seed coat has previously been removed [18]. *S. halepense* has neutral photoblastic seeds and presents mechanical-type dormancy [51]. Different sorts of scarification have been proposed: sandpaper [51], scalpel, pericarp excision from along proximal to distal ridge above embryo and previous imbibition on 1% agar for 18 weeks at 20°C, or at 25/10°C, or for 8 weeks at 6°C [18]. In the 12-h/12-h light regime, alternating temperatures (23/9°C) reduced germination to 80%, so it seems better to maintain it constant at the cited value (26°C) [18]. If an 8-h/16-h photoperiod is of our convenience, we should consider that the thermic alternation of 25/10°C lowers germination to 89%, even for scarified seeds [18]. As well fixing temperatures to 20°C or 30°C brings germination percentages down to 85%, despite the scarifying [18]. Adding 101-mg/l potassium nitrate to the agar medium has not improved the germination results in many tests made in these conditions. It is not a best choice, for it may get down germination even to 78% [18]. *Spartina alterniflora* germination of the seeds is not affected by light or dark, and the optimal temperature 16/26°C (night/day) gives a germination rate >90% [52]. Salinity does not reduce seed germination of this halophilous species [53] when it does not exceed to 450-mM NaCl [54]. Improved smooth cordgrass cultivars of easy germination, 'St. Bernard' (LA12-101) (Reg. No. CV-268, PI 665014), 'Las Palomas' (LA12-102) (Reg. No. CV-269, PI 665015) and 'Lafourche' (LA12-103) (Reg. No. CV-270, PI 665016), are available for implementation [55]. Good results have been obtained in climate chambers at 25°C constant temperature or alternating 20/30°C, much better [56]. In the case of the close taxa *S. pectinata*, 91% germination has been reported scarifying the seed before sowing and doing it on 1% agar at 25/10°C, 8-h/16-h conditions [18].

2.9. Juncoid species

We include here plants belonging to Juncaceae (*Juncus effusus*, *Juncus inflexus*, *Juncus subsecundus*), Juncaginaceae (*Triglochin procerum*) and *Hemerocallis fulva* (Xanthorrhoeaceae) that have been utilised for wastewater treatment in Australia, Kentucky (USA), Portugal, Germany, Canada, Slovenia and Spain [1, 10].

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Juncus effusus seeds need light, moisture and heat for germination. To decrease the time the seed takes to sprout, two methods have been proposed: soaking the seeds [21] and imbibing them on 1% agar for 8 weeks at 2°C [18]. To grow seeds in greenhouses, they will be placed on soil surface, pressing lightly to assure good soil contact, not covering them and keeping the soil moist. Greenhouse should be kept hot (32–38°C). Seeds begin to germinate in approximately 1 week. Soil moisture has to be maintained until plants are to be transplanted [21]. In incubators, to ensure 97% germination, the medium suggested is 1% agar + 250-mg/l gibberellic acid (GA3). Germination conditions are alternating temperatures and photoperiod of 23/9°C, 12 h/12 h. If no scarification is made and no GAE is used, germination may be reduced to 91% (35/20°C), 8 h/16 h, or even 88% (30°C, 8/16) [18]. *J. inflexus* seed germination is a challenge, and the best published results about their seed germination reach just 96%. Basic 1% agar has been repeatedly used as germination medium. Germination cycles of 23/9°C, 12 h/12 h, and 35/20°C, 8 h/16 h, let to reach 95%. With the same photoperiod and medium, changes in the thermic programme decrease the germinative success to 88% (25/15°C, 8 h/16 h), 86% (25°C, 8 h/16 h) and 70% (25/15°C, 8 h/16 h), respectively. Moving the light plan to a 12-h/12-h cycle and the temperatures to 23/9°C has reduced the germination to 50% [18]. Pretreatments have also been tested. Chipping with scalpel has given good results (94–96% germination) when combined with adequate photothermic programmes: 26°C, 12 h/12 h. Other proposals have been to water the seeds in high humidity over water for 1 day at 20°C and then imbibing on 1% agar for 8 weeks at 5°C. In this case 85% germination has been got by applying the photothermic routine 20/10°C, 8 h/16 h, and 95% by just changing the photoperiod to 12 h/12 h. It does not seem so recommendable to use another pre-sowing treatments published, vg. 1% on agar imbibition for 8 weeks at 5°C at 20/10°C, 8 h/16 h, or agar imbibition during 6 weeks at 20/10°C, 8 h/16 h, using 25°C, 8 h/16 h, and 25/10°C, 8 h/16 h, Respectively, bacause they let to reach 88% and 75%. On this occasion, even using GA3 gibberellic acid (250 mg/l) as a complement of the agar medium [18]. Finally to germinate *J. subsecundus* seeds, 1% agar medium is suggested, maintaining 20°C, 8 h/16 h, as to reach to 75% germination [18].

2.10. Flowering ornamental monocots

Decorative perennial herbs are easily available and can grow well under local climatic conditions. Among monocotyledons, many species have been used, especially for on-site treatments where aesthetic or look of the place is an important factor. Some of those species are *Acorus calamus, Hymenocallis littoralis, Colocasia esculenta, Canna glauca, Canna indica, Canna x generalis, Heliconia psittacorum, Heliconia rostrata, Iris pseudacorus, Iris tectorum, Iris versicolor* and *Thalia geniculata*. There are reports of their use in Tanzania, Ohio, Kentucky (USA), Mozambique, Brazil, Mexico, Colombia, El Salvador, Czech Republic, Estonia and Portugal [1, 7, 8].

Acorus calamus is an emergent macrophyte with great potential for use in wetland restoration in North America. Seed germination occurs only in full light at 15/25°C or 20/35°C and seeds fully submerged [7]. In temperate climates, they also can be planted in a greenhouse during the fall or winter. A 5-cm deep tray filled with an organic-soil mix can be used. Seeds

should be scattered sparsely on the surface and pressed firmly into the soil, burying them no further than 0.3-cm deep. Soil needs to be moist to saturation. Seed does not require stratification and germinates in less than 2 weeks. When plants get enough size, they must be transplanted [21]. *Colocasia esculenta* germ plasm can be conserved as seed for at least 2 years at constant 5°C and −20°C when seed moisture content is reduced to 10–12% and at ambiental room temperature (21.5–34.4°C, mean 27.2°C) when seed moisture content is reduced to 7.3% [57]. *Canna indica* is an herbaceous species with ornamental and medicinal value, having seeds with a hard seed coat. Seed germination is good at 10–40°C, being the optimal temperature range between 13.84 and 34.41°C, determined by the enthalpy of activation [58]. To open the imbibition lid, a raised incubation temperature of 50°C during 24 hours in wet surroundings is enough. This hydration induces germination. As a result, the integumentary part of the seed coat softens, making it possible for the germinal root to emerge from the seed. This water-regulating mechanism, combining an impermeable palisade layer and imbibition lid, is a unique feature of the Cannaceae. The seed coat is mainly of chalazal origin, and the main mechanical layer is formed by the exotesta. Other families of the Zingiberales, in contrast, open by an operculum formed by all seed coat layers. Moreover, the seed coat in those families is of integumentary origin, and the main mechanical layer is formed by the exo- and/or endotesta [59]. *Heliconia rostrata* seed germination is well known as difficult and problematic, because the embryo is not yet well differenced when the seed matures, and it has a hard testa which does not allow water to get into it. It can take from 3 months to 3 years [60]. *Iris versicolor* seeds germinate 58% in the greenhouse using cold stratification; this is storing seeds in wet paper towels at 4–5°C for 3–4 weeks [61]. *Iris pseudacorus* germinates 80–86% in an incubator at 12-h/12-h light regime and 30/20°C alternating temperatures or constant temperature of 26°C. In the latter case, seeds must be pretreated by immersion in 10% Domestos solution for 5 min and then imbibed on 1% agar for 8 weeks at 6°C, and then the seed is scarified (covering structure removed and seed coat chipped). After that, a solution of 250-mg/l gibberellic acid (GA3) must be added to the 1% agar medium.

2.11. Megaforbics and other groups

We have included here species as *Asclepias incarnata, Hibiscus moscheutos, Filipendula ulmaria, Liatris pycnostachya, Lobelia cardinalis, Lythrum salicaria, Mentha spicata, Rudbeckia hirta* and *Silphium perfoliatum* [1] that have been tested in Ohio, Kentucky and Minnesota (USA).

To germinate *Asclepias incarnate* seeds, they must be placed into plastic bags filled with moist perlite or vermiculite and stored for 4–12 weeks in a cold (1–3°C) place. Good germination results have been reported without stratification by soaking the seed twice in 87°C for 12 hours. Germination trays can also be used, filling the cells with a commercial seedling mixture or a mix of *Sphagnum* peat moss and vermiculite and moistening them very well. Seeds should be gently pressed into the soil, three seeds per cell, and covered with a very thin layer of soil. It is recommended to keep the soil moist during germination by spraying or misting. Ambient temperatures should remain between 18 and 23°C. This species requires light for germination [21]. *Filipendula ulmaria* is successfully germinated (95%) using 1% agar as germination substrate and incubator conditions of 25/10°C, 8 h/16 h, or 23/9°C, 12 h/12 h

[18]. *Liatris pycnostachya* germinates in 1% agar medium with maximum percentages at a light-alternating cycle of 8 h/16 h, maintaining a constant temperature of 15°C and 93% if temperature is elevated to 20% [18]. Stratification is needed to obtain efficient germination in this genus. Benzyladenine and thiourea are considered good agents for breaking dormancy. Fixing the germination chamber at 20°C and stratification at 4°C for 10 weeks produced 98% germination. Similar good results were obtained with the application of the following pretreatments: aqueous solutions of benzyladenine at 10 or 100 mg/l are applied to blotter paper; dry seeds treated for 3 minutes in benzyladenine at 0 to 1126 mg/l and dissolved in Acetone; a 3-minute acetone permeation of seeds with benzyladenine at 225 or 1127 mg/l; or seeds immersed in thiourea at 0.76 or 7.61 mg/l for 24 hours. Nevertheless gibberellic acid GA3 at 1, 10 or 100 mg/l in H_2O did not show significant efficiency [62]. *Lobelia cardinalis* seeds will germinate without cold stratification, but they need light. For this reason they can be sown in a flat with a damp fine grade peat light mix. If we keep the flats moist and under lights or in a greenhouse, they should green up in a few weeks. Afterwards they can be transplanted in 4–6 weeks into individual pots [21]. At an incubator, 92% germination is reported for the germination medium 1% agar and the germination conditions 15°C, 8 h/16 h [18]. *Lythrum salicaria* pre-sowing treatments have been fully described. Imbibition on 1% agar for 8 weeks at 6°C, 5°C and 2°C and further cultivation on 1% agar medium have given excellent results: 100% germination (21/11°C, 12 h/12 h, or 20/10°C, 8 h/16 h) and 95% germination (21/11°C, 12 h/12 h). Similar results have been reported even without that pre-sowing treatments but fixing the specific photothermic programmes of 35/20°C, 8 h/16 h (100%), or 20°C, 8 h/16 h (95%). Lower levels (84%) are obtained at 25/10°C, 8 h/16 h. The addition of 250-mg/l gibberellic acid (GA3) to the agar germination medium does not seem to have a clearly positive effect, although 92% germination is reached when using 12 h/12 h and 21/11°C and 88% if applying 8 h/16 h and 20/10°C [18]. *Mentha* spp. cultivation has been studied from different perspectives. Recent studies have explored the influence of high-frequency pulsatile electromagnetic fields and ultrasound pulsatile fields to improve mint seed germination. Further studies are needed, but there are promising first results [63]. In *Mentha spicata*, optimal temperature for seed germination is 30°C. It could be affected under different light qualities and full darkness conditions. White light is the most conductive to seed germination, but full darkness is the least conductive. After soaking treatment with GA 3 0.25% KNO_3, seed germination percentage is significantly promoted [64]. *Rudbeckia hirta* can be easily multiplied by seeds cultivating them on 1% agar medium in an incubator at 21°C, 12/12 (100% germination) or at 10°C, 15°C and 25°C and 8-h/16-h light cycle (84%, 89%, 89%, respectively) [18]. Finally *Silphium perfoliatum* seeds germinate 79% in 1% agar, alternating temperatures of 25/10°C and photoperiod 8 h/16 h [18].

3. Final comments

Seed technology for multiplication helophytes needs further experimental work. Significant efforts have been done, but it is a challenge to have a set of available information ready to be transferred to the productive sectors that are interested in this type of plants. Plants used in

constructed wetlands with horizontal subsurface flow have a promising future as sustainable elements of the upcoming sewage equipment. For this reason improvements on the biotechnology of these species are a worthwhile researching line, where seed technology can play an important role.

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