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Flower induction, microscope-aided cross-pollination, and seed production in the duckweed *Lemna gibba* with discovery of a male-sterile clone

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Duckweed species have a great potential to develop into fast-growing crops for water remediation and bioenergy production. Seed production and utilization of hybrid vigour are essential steps in this process. However, even in the extensively-studied duckweed species, *Lemna gibba*, flower primordia were often aborted prior to maturation. Salicylic acid (SA) and agar solidification of the medium promoted flower maturation and resulted in high flowering rates in *L. gibba* 7741 and 5504. Artificial cross-pollination between individuals of *L. gibba* 7741 yielded seeds at high frequencies unlike that in *L. gibba* 5504. In contrast to clone 7741, the anthers of 5504 did not dehisce upon maturation, its artificially released pollen grains had pineapple-like exine with tilted spines. These pollens were not stained by 2,5-diphenylmonotetrazoliumbromide (MTT) and failed to germinate. Therefore, clone 5504 is male sterile and has potential application with respect to hybrid vigour. Moreover, pollination of flowers of 5504 with 7741 pollen grains resulted in intraspecific hybrid seeds, which was confirmed by inter-simple sequence repeat (ISSR) markers. These hybrid seeds germinated at a high frequency, forming new clones.

Lemnaceae, commonly known as duckweeds, is a family of small aquatic monocotyledonous plants that comprise five genera and 37 species[1,2]. They represent the smallest flowering plants with reduced morphology[3]. Duckweeds can rapidly assimilate nutrients such as nitrogen and phosphate from municipal, industrial, and agricultural wastewaters and have shown great value in wastewater remediation[4–9]. The biomass of duckweeds may contain high starch content and could be used to produce bioethanol and biobutanol[10–14]. Some duckweed clones contain high protein content, and have been used as high quality livestock feed[15–17]. Even the duckweed biomass accumulated on wastewaters may be used for feed production[18–20].

Lemna gibba is one of the duckweed species that has been widely used in research and applications. *L. gibba* is very efficient in removal of organic and inorganic nutrients from domestic and industrial wastewater[21–24], as well as heavy metals such as lead and uranium[25]. *L. gibba* was also found to be efficient in removal of harmful organisms such as *Giardia cysts*, *Cryptosporidium oocysts*, faecal coliforms and coliphage from wastewater[26]. It can also be used in bioassays of toxicity of industrial waste[21].

The potential of duckweeds in commercial applications has brought forward a need for domestication and the breeding of elite varieties serving different purposes. Seed production and application of hybrid vigour have an enormous importance in making duckweed a future crop plant. However, duckweeds reproduce mainly vegetatively. In this process, mother fronds form genetically identical daughter fronds that are therefore called a

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