Life cycle-based selection of elite germplasm in industrially important red alga *Gracilaria dura*: implications for commercial farming

Santlal Jaiswar and Vaibhav A. Mantri

Agar-yielding seaweed commonly known as agarophytes are sourced for commercial exploitation from countries of the Middle East, Japan and to a certain extent from the Republic of Korea[1]. This industrially lucrative gel commands a high prize (US$ 18 kg^{-1}) globally due to its natural high gelling strength and low gelling temperature compared to other seaweed hydrocolloids such as alginates (US$ 12 kg^{-1}) and carrageenans (US$ 10.4 kg^{-1})[2]. The unique phycocolloid is being extracted from the cell walls of red seaweed species mostly from *Gelidium*, *Gelidiella* and *Gracilaria*. The agar trade annually requires about 125,200 dry tonne of biomass for the production of 14,500 t agar worth US$ 246 million[3]. The Moroccan government has now put restrictions on legal annual harvest of *Gelidium* to negate the decline of wild populations and thereby enforcing trade limits on export. This development has triggered agar scarcity in the global market. A recent trend indicates a shift, wherein *Gracilaria* is preferred as the raw material source consisting of over 91% of material supply chain (i.e. 114,100 dry t) with only about 9% (i.e. 11,100 dry t) represented by *Gelidium*[3]. Nevertheless, unlike other phycocolloids, where only a couple of species dominated the trade, several regional species are important in agar business, contributing immensely to the local economy[4].

There are around 32 species of *Gracilaria* reported from the Indian waters[5], of which only *Gracilaria edulis* has been industrially exploited for agar of food grade, while *Gelidiella acerosa* for bacteriological-grade agar[6]. *Gracilaria dura* has been reported to directly yield agarose. The studies reported 20–25% agarose yield on dry weight basis with a gel strength of >1900 g cm^{-2} (1% gel) and gelling temperature of 35°C (ref. 7). The patented green and energy-efficient process was further developed by CSIR-Central Salt and Marine Chemicals Research Institute (CSIR-CSMCRI), Bhavnagar, Gujarat, using surfactants for precipitating out the agarose[8]. The Council of Scientific and Industrial Research (CSIR), New Delhi has acquired 'Trade mark – Sagarose' number 2123313; dt. 30 March 2011 for this product from the Office of the Registrar of Trade Marks, Government of India. The niche applications make agarose the preferred choice in biotechnology, molecular biology, medical and forensic science research[7]. However, scanty biomass availability and limited distribution only to the northern western coast of India have prevented its industrial utilization. The efforts towards experimental farming of *G. dura* through carpospores[9] and via vegetative fragments[10] reported encouraging trend to undertake farming at conducive locations in India. The National Fisheries Development Board, Hyderabad has granted a project to CSIR-CSMCRI to initiate large-scale farming of this species along with capacity building of fishermen in Gujarat.

G. dura is a red alga belonging to the family Gracilariaceae (Rhodophyta). The plants are 10–18 cm tall, 1 mm in breadth, greenish-brown to reddish-brown in colour, irregularly branched, cartilaginous with discoid holdfast. Like other species of the family Garcilariace, it has typical *Polysiphonia*-type three-stage isomorphic sexual life cycle[11]. The knowledge of life cycle is critical for the development of commercial farming. This was well evident by the discovery of 'conchocelis phase' helping in establishing successful farming of *Porphyra umbilicalis*[12]. Further, confirmation of presence of the minute female gametophyte in commercially important *Palmaria palmata* aided its cultivation[13]. Three life-cycle phases in *Gracilaria* include male gametophyte, female gametophyte and tetrasporophyte. The research confirmed that there exists ecological differences between haploid gametophyte and diploid tetrasporophyte in several species of this genus[14–16]. The differences ranged from survival, growth, yield and gel strength of agar. The gel strength was found to be variable among the life-cycle stages of *Gracilaria*[14]. The higher yield and superior agarose characteristics from tetrasporophyte over the male and female gametophyte were reported in *G. cylindrica*[15]. However, in *G. bursapastoris* the vegetative plants showed higher gel strength than carposporic and tetrasporic plants, with no significant differences in yield and intrinsic viscosities[16]. Nevertheless, no variation in agar yield and gel strength was observed between gametophyte and tetrasporophyte of *G. bursa-pastoris* and *G. cornopifolia*[17]. Similarly, higher growth rate (21.1% day^{-1}) was recorded for gametophytes of *G. tenuistipitata* than tetrasporophyte (24.01% day^{-1})[18]. The higher growth rate in females could be attributed to the fact that selective propagation inhibits their reproductive maturity; as a result the growth is vegetative only. It was also observed that female gametophyte in *Gracilariopsis lemaneiformis* (as *Gracilaria sjoestedtii*) reported highest growth rate[19]. Further our preliminary experiments confirmed that tetrasporophyte of *G. dura* reported highest yield of 19% ± 0.24% with gel strength 1800 ± 8.76 g/cm^{-2}, whereas male and female gametophyte showed 12.5% ± 0.80% and 18.5% ± 0.52% of yield and gel strength of 1200 ± 10.5 and 1500 ± 7.87 g/cm^{-2} respectively[20].

Despite the fact that specific life-history phases could be selected as elite gemplasm for commercial farming, little attention has been paid to research in this direction. It has been shown that diploid individuals dominated farmed populations of *G. chilensis* from Chile. Further, it was evident that involuntary selection operated during domestication reduced genetic diversity[21]. This finding paves way to conscious efforts of life history-based selection of elite clone for farming. CSIR-CSMCRI has undertaken a DST sponsored project to ascertain the extent of variation within populations of life-cycle stages pertaining to growth and agarose yield, out-planting of promising strains, ascertaining their performance in open sea and characterization of agarose (Figure 1). Further trait-specific molecular markers will be developed to select best performing strains. The clonal propagation protocol has already been developed for this alga; therefore selected elite germplasm will be quickly multiplied to distribute this elite gemplasm to

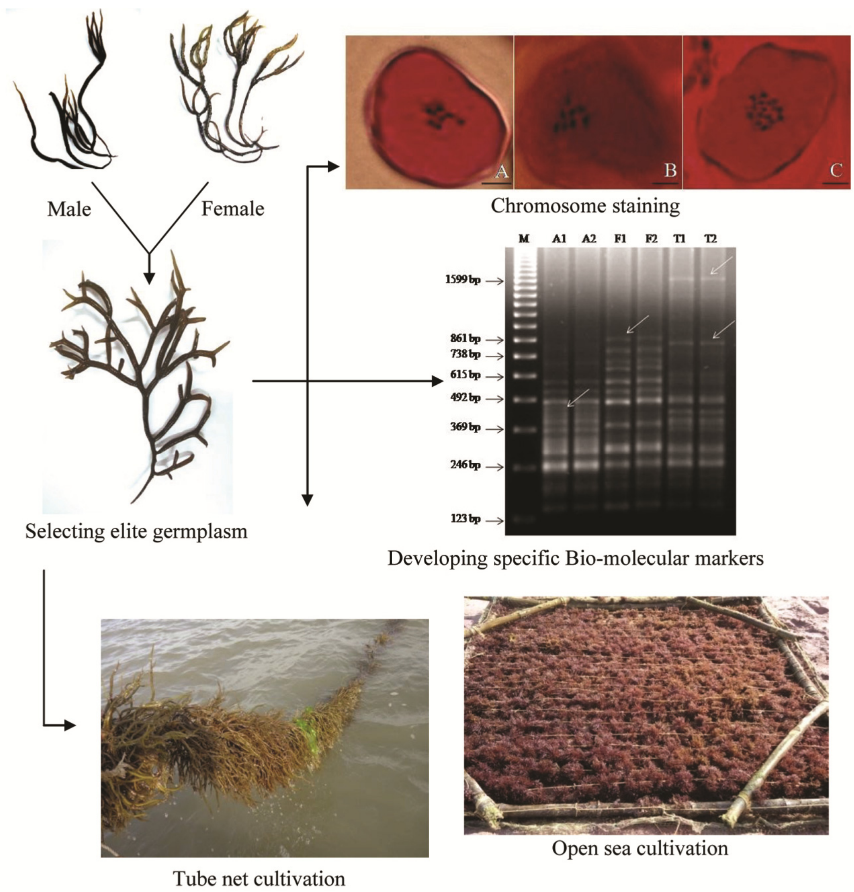

Figure 1. Selection of elite germplasm based on life-cycle phases for commercial farming (adapted from Gupta *et al.*[21]).

the prospective farmers under the CSIR outreach programme. Since there is no production of agarose in the country and the entire domestic requirement is met from imports, there is immense potential for manufacturing agarose indigenously to save foreign exchange. The industries are willing to undertake the production, if sustainable biomass supply is assured. Further, these efforts will successfully provide diversification of livelihood for coastal fishermen who are battling with dwindling fisheries resource. The present study also assumes significant importance considering the species facing long-term extensive exploitation through domestication and farming.

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Santlal Jaiswar and Vaibhav A. Mantri are in the Applied Phycology and Biotechnology Division, CSIR-Central Salt and Marine Chemicals Research Institute (CSIR-CSMCRI), Bhavnagar 364 002, India; Vaibhav A. Mantri is also in the Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201 002, India.*
**e-mail: santlal@csmcri.res.in*