Seaweed Research at SEAFDEC/AQD

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During the 1987 Aquaculture Development in Southeast Asia (ADSEA) Conference, member countries of the Southeast Asian Fisheries Development Center (SEAFDEC) recommended *Gracilaria* as the number one priority for seaweed research in the following areas: 1) refinement of culture techniques, 2) basic biology, 3) product utilization, and 4) screening and characterization of natural products. However, three years later, *Kappaphycus* was also included as priority research in answer to the needs of the industry.

As an initial action to the above mandate, an inventory of the seaweed resources of Panay was made to determine the distribution and taxonomy. As a result of this activity, a monograph was published by Hurtado-Ponce et al. in 1992. This monograph describes 114 species that include 38 Chlorophyceae, 21 Phaeophyceae and 55 Rhodophyceae. There were 37 species considered as new record for Panay (Luhan et al. 1992). But a revision of this monograph is imperative to determine possible changes in the distribution of the seaweed resources brought by some ecological alterations. Collection of seaweeds around Panay is in progress.

Preliminary screening of the different *Gracilaria* species *(G. arcuata, G. changii, G. coronopifolia, G. eucheumoides, G. firma, G. heteroclada* (= *Gracilariopsis heteroclada), G. manilaensis, G. salicornia,* and *G. tenuistipitata)* in Panay revealed that among the species tested for growth and agar characteristics, *G. heteroclada* showed the best qualities, hence, research and development of this species were further pursued.

There are several accomplishments on seaweed research along these areas:

Production ecology

The reproductive state of *Gracilariopsis heteroclada* is known to be seasonal, the carposporophytes ad tetrasporophytes being high in January (48%) and May (64%), respectively. The results suggest that gathering of natural stock prior to maturity is not advisable.

Likewise, there was seasonality on the year-round biomass of *G. heteroclada* in Jaro, Leganes, Zarraga, Estancia, Iloilo; Ivisan, Capiz and Batan, Aklan (de Castro et al. 1991, Luhan et al. 1992). On the other hand, *G. manilaensis* (April-June) and *G. changii* (January -June) were found to occur only during certain months of the year; that simply means that these seaweeds are dormant during the rest of the year (Pondevida and Hurtado-Ponce 1996). Apparently, salinity is a critical factor for growth and biomass production.

Physiology

Sporelings from fertile cystocarp plants of *G. changii, G. coronopifolia, G. firma* and *Gracilariopsis bailinae* were grown in the laboratory to document their life stages for future molecular studies. Furthermore, viability of sporelings grown under laboratory condition was observed and use for possible outplanting will be tested by MRJ Luhan (pers.com.).

Crop management

The amount of biomass left after the first cropping or gathering of *G. heteroclada* is important in determining the amount of biomass available for the next cropping season. Among the 4 levels of harvest (25, 50, 75 and 100%), 75% provides the appropriate amount of 'seeds' for the next cropping season. The amount of biomass to be harvested during each harvest regime should exceed the amount of biomass available for cropping (Hurtado-Ponce 1993)

Corollary to the above scheme, the use of harvesting tools is also critical in managing the biomass. The use of 'araña' is efficient in areas where there is water during harvesting since it facilitates the dragging of the tool. It leaves a certain amount of biomass (75%) for the next cropping or gathering. However, the use of a rake is very detrimental to the biomass, since it leaves nothing for the next cropping and it takes almost 3 months for the seaweed to regenerate. The use of a pair of scissor and bare hands as tedious and brings a lot of trampling to the substrate, thereby resulting to excessive disturbances and slow recovery of the seaweed.

Farming systems

Tank cultivation. Gracilariopsis heteroclada was studied under tank conditions to determine salinity tolerance, nutrient assimilation (quantity and quality), water exchange and stocking rate. Optimum growth of *G. heteroclada* was obtained at 25 ppt, 500 gm^{-2} stocking rate, water exchange.

Two varieties of *Kappaphycus alvarezii* were studied under tank conditions to determine variations in growth rate, pigments and carrageenan qualities as influenced by different levels of ammonium sulfate as source of nutrient. No significant difference was analyzed between the varieties, but there were significant differences between levels of nutrients and culture period in terms of growth rate, pigments (chl-a, R-PE), carrageenan yield, gel strength, gelling and melting temperature and sulfate content.

Line cultivation. Fixed-off bottom line of cultivating *G. heteroclada* in estuaries showed promising results. Growth rate of 6.7%/day was obtained, suggesting its commercial potential. The constant replenishment of nutrients during spring tide makes the area suitable for culture purposes (Hurtado-Ponce et al. 1997).

The seasonality of two varieties of *K. alvarezii* grown in fixed off-bottom line, hanging long line and a combination of the two methods showed lowest and highest growth rates and yields from July to August and January to February, respectively.

Cage cultivation. The monoculture of *G. heteroclada* using vertical lines at 10 cm interval showed significant growth over those cultivated at 15 and 20 cm interval (Hurtado-Ponce 1990), while the polyculture of the same seaweed with seabass, *Lates calcarifer* proved to be encouraging since high growth rate and production were obtained from the seaweed and seabass, respectively (Hurtado-Ponce 1992).

The broadcast method of culturing *G. heteroclada* in hapa net installed in floating cages showed significant growth and net production during the dry season (Guanzon and de Castro 1992) when grown at different stocking densities.

Results on the mono-and polyculture of *Kappaphycus alvarezii* were encouraging. Among the 3 morphotypes (brown, green and red) of *K. alvarezii,* brown and green gave better growth rates results.

K. alvarezii, when cultured using the horizontal line with grouper, *Epinephelus* sp. showed better growth rate (5.3% day^{-1}) than when cultured using the vertical and cluster technique. After 120 days of culture, 68% of the grouper survived and reached mean weights of 297 g.

The use of growth hormone (IAA) alone in culturing *K. alvarezii* in tanks showed comparable growth with those grown with ammonium phosphate. When the same plants were outplanted, growth of *K. alvarezii* was slightly better (7.1-7.5%) when grown in higher combination levels of ammonium phosphate/IAA (10/1 and 10/5 mg L^{-1}) than in lower combination levels (5/1 and 5/5 mg L^{-1}; 6.8-6.5%). The addition of ammonium phosphate appears more practical than the addition of IAA, a plant growth regulator, because the latter is expensive (Fermin and Hurtado 2001).

Pond cultivation. Polyculture of *Penaeus monodon* and *G. heteroclada* showed good growth at a stocking 5,000 ha^{-1} and 2,500 kg ha^{-1}, respectively when the water temperature was 29.5° C, 24 ppt, transparency of 74% and water depth 83 cm.

Colloid characterization

Several studies were made on the characterization of agar from different species of wild *Gracilaria* in Panay. Results show that each species produce quality agar as influenced by concentration and time of NaOH treatment (de Castro 1993, Luhan 1992). *Gracilariopsis heteroclada,* both cultured in tanks and brackishwater ponds demonstrated superior qualities compared to the species collected from natural population (Hurtado-Ponce 1994, de Castro 1996, Pondevida and Hurtado-Ponce 1996b, Hurtado-Ponce and Pondevida 1997)

The quality of agarose extracted from wild populations of *G. bailinae* showed significant seasonality in yield and gel strength, however, gel strength was inversely proportional to the agar yield. Water temperature, turbidity and pH exhibited no significant correlation with gel properties, however, a slightly positive correlation existed between agarose yield and salinity.

Gel strength of brown and green strains of *K. alvarezii* varied seasonally when grown by fixed off-bottom long line, hanging long line and a combination of the two cultivation methods, however, a higher gel strength was recorded when *K. alvarezii* was grown by a HL-FB method compared with those grown by the two other methods.

The carrageenan properties and pigment levels of two varieties of *K. alvarezii* grown in tanks at different levels of ammonium sulfate as nutrients were studied. Significant differences in growth rate, R-PE, and gel strength were determined between the two varieties as influenced by the levels of ammonium sulfate. Significant differences were detected between the two varieties in terms of carrageenan yield, gel strength, gelling and melting temperatures and sulfate content (Gonzales 1996).

Economics

The economics of cultivating *Gracilaria* and *Kappaphycus* both experimental and actual inputs were analyzed using economic indicators like return on investment (ROI) and payback period. Results showed that farming these seaweeds using the line method, fixed off-bottom, raft (single or multiple) and hanging long line are all profitable, however, production is affected by environmental conditions (typhoon, salinity-temperature) and fluctuating farmgate price (Hurtado-Ponce et al. 1992, 1996 & 1997, Hurtado and Agbayani 2000, Hurtado et al. 2001).

Biotechnology

Young plantlets of *Eucheuma denticulatum* that regenerated from tissue culture after 6 months of laboratory culture resembled wild plants. After 3 months of field growth, the plants were potential sources of 'seedlings' for further planting purposes.

Analysis of molecular variance (AMOVA) suggested that there were significant genetic differences ($P<0.001$) among *E. denticulatum* plants derived from tissue culture, wild *E. denticulatum,* and cultured *K. striatum.* The same analysis also indicated that *E. denticulatum* plants regenerated from calli were genetically distinct ($P<0.001$) from those derived from calli, in which random variation was induced by acute exposure to sublethal amounts of UV radiation.

Techniques on mass production of sporelings from carpospores were established under laboratory conditions in some *Gracilaria* species *(G. firma, G. coronopifolia* and *G. heteroclada).* Protoplast production of the same species is presently pursued.

Polymorphic DNAs are high resolution genetic markers, which can be used to characterize or differentiate various species, strains or populations of seaweed. In the laboratory, polymorphic DNAs, such as RFLPs and RAPDs are being generated to characterize strains of wild and cultured populations. A series of 40 arbitrary primers were screened for ability to generate distinct and highly polymorphic fragments from *G. coronopifolia.* Primers A1, B1Q, B12 and B14 were selected to generate RAPDs for various seaweed species. Discrimination between species was possible with primers B10, B12 and B14. *G. coronopifolia* and *G. firma* share similar Al-primed RAPDs.

Sequestration of heavy metals (Cd, Cu, Pb, Zn) by *G. heteroclada* is directly proportional to the level of concentration and time of exposure. It was shown that heavy metals were retained in the tissues and in the extracted colloid in this order (Cu > Zn > Cd > Pb).

Gracilariopsis bailinae is a good biofilter in an integrated culture system of finfish broodstock. Ammonia concentration in tanks with seaweed was lower than in tanks without seaweed. Nitrogen in the tissue of *G. bailinae* became saturated after five days of culture, suggesting that growth was initiated upon saturation of nitrogen pools in the plant tissue (Luhan, unpublished).

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