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AN APPARENT ABSENCE OF HETEROSIS IN HYBRIDS OF GRACILARIA TIKVAHIAE (RHODOPHYCEAE)[1]

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Inbred diploid tetrasporophytes from three different populations of *Gracilaria tikvahiae* were compared with F_1 hybrids in a preliminary attempt to detect heterosis. Growth rates of sporelings and of mature fronds were compared, as were some frond characteristics such as regeneration ability and dry weight. No heterosis was observed for any of the characteristics.

Au cours d'une expérience préliminaire visant à détecter les effets de l'hétérosis, des tétrasporophytes diploides, consanguins, provenant de trois populations différentes de *Gracilaria tikvahiae* furent comparés avec des hybrids F_1. Les taux de croissance des spores germées et des frondes matures ainsi que d'autres caractéristiques des frondes telles la capacité de régénération et le poid sec furent comparées. Les effets de l'hétérosis ne furent détectés pour aucune des caractéristiques étudiées.

Introduction

The term "heterosis" was coined to describe the increased vigor of hybrids over that of their parents (Shull 1911), a phenomenon of great importance in plant and animal improvement. It is, in general terms, the phenotypic expression of gene interactions in hybrids and is always associated with genetic heterozygosity. Thus, it is reduced by inbreeding and restored by hybridization.

In plants, the study of heterosis has been limited, for the most part, to diploid and polyploid angiospermic crop plants. Such research has been rare in lower plant groups, including the algae, some of which are important marine crops. For algal taxa where cultivated fronds are monoploid, such as *Porphyra,* heterosis would have no direct usefulness, although it might have some limited application in improving the growth of the diploid sporophytic phase which is used as spore-stock for generating the harvested haploid phase. Heterosis is potentially a much more important breeding consideration for taxa such as *Laminaria,* which the large diploid sporophytic fronds are cultivated; however, this remains to be demonstrated. Chinese workers have shown that some frond characteristics have strong additive genetic componenets (Fang et al. 1965; Anonymous 1976) but their experiments were not designed to examine heterosis. Chapman and Doyle (1979), on the other hand, found little or no additive genetic component in the transmission of genes for alginate content. Until more data become available, the role of heterosis in all diploid algae remains unknown.

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There is an additional consideration for species such as *Gracilaria tikvahiae* which alternate between isomorphic hapliod and diploid plants growing in the same habitat. For such species, haploid and diploid plants are potentially in direct competition with each other. This situation is very different from that of angiosperms and of algae with heteromorphic life histories where the haploid phase has a different growth strategy and thus does not, or is less likely to, face competition from the diploid phase. It is fair to question whether heterotic gene interactions should be expected in species with isomorphic phases, as heterosis might confer an advantage to diploids that would be to the detriment of the haploid plants and of the smooth functioning of the sexual life cycle.

Recently we decided to determine if any heterosis could be detected in hybrids of *G. tikvahiae* constructed from a limited number of lines that were sufficiently inbred to make the test feasible. The expression of heterosis was sought in the growth rate of sporelings, the growth rate of mature plants, regeneration ability and two other frond characteristics. An absence of any significant heterosis in the hybrids is reported here.

Materials and Methods

Inbred Lines and Hybrids

Partially inbred lines were developed from plants collected from three different local populations of *G. tikvahiae* McLachlan (McLachlan 1979). The collecting sites were: Barrachois Harbour, Colchester Co., N.S.; Pomquet Harbour, Antigonish Co., N.S.; and Malpeque Bay near Lennox Island, P.E.I. Inbred lines were designated as "Barrachois" (B), "Pomquet" (P) and "Lennox Island" (LI). To effect the inbreeding, each generation was initiated from a single diploid tetrasporophyte. For "Barrachois", inbred generation I_5 was attained, for "Pomquet" I_4, and for "Lennox Island" I_6.

The inbred lines above were used to construct age-matched families of inbred and hybrid plants. Families of inbred diploid sporelings were obtained for each of the lines by crossing 3 females with 3 males. More than a single mating pair was used in order to permit a broader sampling of the various genomes. Hybrid families were established at the same time by crossing: 3 Lennox Island females with 3 Barrachois males, 3 Pomquet females with 3 Barrachois males, and 3 Pomquet females with 3 Lennox Island males. The plants used to construct the hybrids were clones taken from the plants used to establish the inbred families. The sporelings obtained from all crosses were maintained under low light for 8 weeks before they were used, at which time they were all approximately 5 mm long.

Sporeling Growth Comparisons

For the Lennox Island and Barrachois lines, growth rate measurements were made on 30 randomly selected sporelings taken from the inbred sporeling families. For the (LI x B) F_1 hybrid sporelings, these measurements were made on 90 plants. Because the Pomquet line had only attained the I_4 generation, it and its hybrids were examined less critically. For each of: Pomquet inbred, the (P X LI) F_1 hybrid, and the (P X B) F_1 hybrid, growth rate measurements were made on 10 sporelings. Growth comparisons were made over a 4-week interval in a controlled temperature room at 20 C. Illumination was from 40W, cool-white, fluorescent lamps at a quantum irradiance of approximately 35 $\mu E\ m^{-2}s^{-1}$ with a 12 h light period. The growth medium was SWM-3 without soil or liver extract (McLachlan 1973) and it was replaced weekly. The plants were photographed on a photocopy machine at the beginning and end of the growth period. Plants were centered on the machine to avoid distortions that may occur at the edges. Lengths were measured from the

photocopies to obtain specific growth rates as $\ln (L_t/L_o)/t$ where L_o = initial length, L_t = final length and t = the duration of the growth interval in days.

Growth Comparisons for Mature Plants

The growth rate of mature inbred and F_1 hybrid tetrasporophytes was further compared in 10 l tanks of flowing seawater in a greenhouse after allowing at least 2 weeks for the laboratory-grown plants to acclimate to the greenhouse conditions. Due to space limitations it was not possible to grow, as large mature plants, all of the sporelings studied in laboratory culture. Accordingly, the numbers were reduced as follows: Barrachois and Lennox Island inbred lines, 27 plants each, split into three replications of 9 plants; the (LI X B) F_1 hybrid, 36 plants split into 4 replications of 9 plants; Pomquet inbred, the (P X L1) F_1 hybrid, and the (P X B) F_1 hybrid, 10 plants each, as before for sporelings.

The plants were grown for 4 weeks at 20 C under ambient light conditions with nitrogen and phosphate fertilizer added twice weekly so as to reach initial concentrations of 900 μM NO_3– and 90 μM $PO^3{}_4$–. Flushing rate was 5-8 volumes per day. Fragmentation of mature thalli made it impossible to weight individual plants and thus only one growth rate determination could be made for each replicate of 9 plants. In the Pomquet series, where there were unreplicated sets of 10 plants, only a single determination was possible for each set, and one of these, (P X B) F_1 hybrid, was not obtained as the plants became badly fouled by epiphytes. Plants in the Pomquet series were grown in a separate growth trial a few weeks after the others. Results are expressed as specific growth rates based on weight, as $\ln (W_t/W_o)/t$ where W_o = inital weight, W_t = final weight and t = the growth interval in days.

Other Comparisons

Regeneration ability was determined after the sporeling growth measurements, just before the plants were transferred to the greenhouse at the Atlantic Research Laboratory's Seaweed Culture Station. To do this, 5 mm sections of frond were excised from the main axis of each frond 1 cm below the apex. These excised portions were cultured for 4 weeks and the number of regenerated apices was counted, and expressed as the mean number per excised section. After the plants had grown at the Seaweed Culture Station, plant stoutness was measured (= the sum of thickness + width of fronds), and the % dry weight and ash-free dry weight were determined.

Results and Discussion

No evidence for heterosis was obtained from any of the variables measured. Sporeling growth rates for the hybrids did not significantly exceed those for the inbred lines (Table 1). In two comparisons the F_1 hybrids were intermediate to the inbred families and in the third, the very small increase of the (LI X B) hybrid over the best parental line was not significant ($t = 0.075$, $p > 0.9$; Table I). Thus for these combinations, there was no exploitable heterosis in the growth of sporelings. Similarly there was no heterosis in the growth of mature plants. In the two sets for which data were obtained, growth of the F_1 hybrids was intermediate to that of the inbred families (Table I).

The inbred lines differed in their regeneration ability with Lennox Island plants forming about 4 times as many new apices as Barrachois plants. Again the F_1 hybrid was intermediate (Table II). For this character, Pomquet and Lennox Island lines were similar with the F_1 hybrid not significantly better than the best parental line ($t = 0.175$, $p > 0.8$). Likewise, no heterosis was observed for the other three

Table I Growth rates of inbred and hybrid plants

Genotype	Sporeling Growth Rate $\ln (L_t/L_o)/t$ $n \geq 10$	Mature Plant Growth Rate $\ln (W_t/W_t$ $N = 3$
LI X LI	0.118 ± .003	0.066 ± .009
LI X B	0.120 ± .001	0.063 ± .005
B X B	0.117 ± .002	0.059 ± .002
LI X LI	(0.118 ± .003)[1]	0.046[2]
LI X P	0.116 ± .002	0.051[2]
P X P	0.105 ± .002	0.057[2]
B X B	(0.117 ± .002)	———
B X P	0.112 ± .002	———
P X P	(0.105 ± .002)	———

All values are $\bar{x}$ ±S.D.
[1]values in parentheses are repeated for ease of comparison.
[2]These plants were grown unreplicated in a separate experiment.

Table II Characterization of inbred and hybrid plants*

Genotype	Stoutness of the frond (mm) n 10	Regeneration section^{-1} $n \geq 30$	% dry wt n = 3	% ash free dry wt n = 3
LI X LI(I_7)	2.84± 0.01	3.38± 0.21	9.45	48.8
LB X B (F_1)	2.97± 0.04	1.41± 0.13	9.07	46.8
B X B(I_6)	3.09± 0.07	0.86± 0.20	8.93	46.0
LI X LI(I_7)	3.93± 0.04[3]	(3.38± 0.21)	10.40[2]	53.5[2]
LI X P(F_1)	4.26± 0.04[3]	3.50± 0.43[1]	9.60[2]	48.9[2]
P X P(I_5)	5.02± 0.03[3]	2.80± 0.20[1]	9.69[2]	48.4[2]

Values are reported as $\bar{x}$±S.E. for first two columns. Values in the last two columns are single values or the average of 3 determinations.

*Results are comparable within each subset, but not between subsets, as the plants were grown in two different trials.

[1]n = 10
[2]n = 1
[3]Although only 10 plants were present originally, more thalli were measured at the end of the growth period due to fragmentation.

frond characteristics measured, the hybrids being intermediate in all cases (Table II).

The results of this preliminary study suggest that heterosis is not an important genetic component for the growth of tetrasporophytes of *G. tikvahiae*. The comparisons made for Barrachois, Lennox Island and their F_1 hybrid are reasonably complete and yield no evidence for hybrid vigor. It remains possible that by chance, this is a non-heterotic combination in a system where heterosis plays an important role; however, the similarly negative results obtained from the other two combinations suggests that an absence of heterosis may be the general situation.

The results perhaps complement observations that there does not appear to be any growth superiority of diploid tetrasporophytes over haploid gametophytes

(Edelstein 1977). With the bold assumptions that the same genes control growth of haploids and diploids and that growth reductions caused by reproductive effort are approximately equal for both phases, then the absence of a strong diploid superiority can also be taken to indicate that heterosis is not a large factor in the growth of *Gracilaria tikvahiae*. It appears from data available thus far that breeding approaches based on maximizing hybrid vigor will have no application in this alga.

Failure to demonstrate heterosis in these tests did not result from an absence of heterozygosity in the hybrids. The inbred lines differed from each other in morphological characteristics and were derived from different populations. It is possible that the "inbred" lines retained enough heterozygosity to express a high degree of hybrid vigor and that failure to see heterosis was due to insufficient inbreeding of the control lines; however, this seems unlikely as five or six generations is a significant amount of inbreeding.

It is likely that the traits measured here are determined by additive factors, but this remains to be proven in properly designed quantitative genetic experiments. Some single gene differences can have large effects on plant morphology and growth rate (Patwary & van der Meer 1982), and polymorphism for such genes may also contribute to the total variability observed.

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