Strain selection in the edible brown seaweed *Alaria esculenta:* Genetic fingerprinting and hybridization studies under laboratory conditions

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Strain selection in the edible brown seaweed *Alaria esculenta:* Genetic fingerprinting and hybridization studies under laboratory conditions

Contract No. IR.95.MR.011

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TABLE OF CONTENTS

1. INTRODUCTION

The genus *Alaria* presently includes 12 species, 11 of which are located in the cold temperate North Pacific and only one is found in the North Atlantic (Widdowson, 1971). The North Atlantic species *Alaria esculenta* has two northern forms, *A. esculenta* forma *grandifolia* and forma *pylaii* (Lüning, 1990). The study presented here will concentrate on *Alaria esculenta*, the most common North Atlantic species.

1.1 Biogeography

The brown alga *Alaria esculenta*, which literally means 'edible wings', is a large brown seaweed belonging to the family *Alariaceae* of the order Laminariales (Kelp), and can reach a length up to 6 m (Guiry, 1997). Its short stipe gives rise to a long blade with a well-defined rib (Fig. 1). This seaweed forms the main canopy in exposed areas near or below the low-water mark to a depth of 35 m (Lüning, 1990). *Alaria esculenta* is present in the North Pacific and North Atlantic, where it is located North as far as the winter sea ice and as far south as the 16° C summer isotherm, in the European North Atlantic represented by the French coast of Brittany (Lüning, 1990); Fig. 2. Its absence in the southern North Sea and English Channel is due to high summer water temperatures of over 16° C, which it cannot survive (Munda & Lüning, 1977). The species is found all around the Irish coast, where rocky shores as a substratum are available (Sundene, 1962; Widdowson, 1971).

1.2 Utilisation

Alaria esculenta was used in the past in both Scotland and Ireland for human consumption and fodder. It was also gathered and spread on infertile land and used as fertiliser (Newton, 1931; Guiry and Hession, 1996; Guiry, 1997).
It is rich in sugars, proteins, vitamins and other trace metals and contains up to 42% alginic acid (Levring *et al.*, 1969; Indergaard & Minsaas, 1991; Lewallen & Lewallen, 1996). The species can be used for a variety of purposes from human consumption and alginate production to fodder and bodycare products (Guiry & Blunden, 1991; Guiry, 1997). Especially in North America *Alaria esculenta* and *Alaria marginata* are rapidly gaining popularity in the natural foods market (Lewallen & Lewallen, 1996). It also has potential as a foodstuff in aquaculture for herbivorous molluscs, e.g., abalone (Mai *et al.*, 1996).

Young *Alaria esculenta* (the only native species in Ireland) can be used as a substitute for *Undaria pinnatifida* (Wakame), a very popular seaweed in Asian countries with numerous applications and a yield over 300,000 t fresh weight per annum (Nisizawa *et al.* 1987; Indergaard & Minsaas, 1991; Druehl, 1988; Yamanaka & Akiyama, 1993; Lewallen & Lewallen, 1996).

Experiments have shown that within 3 months a harvest of 9 t per hectare is possible (Kain & Dawes, 1987). *Alaria esculenta* is a fast-growing seaweed and can grow at up to 10 cm a day. Commercial potential for *Alaria esculenta* is considerable (Kain & Dawes, 1987; Kain, Holt & Dawes, 1990).

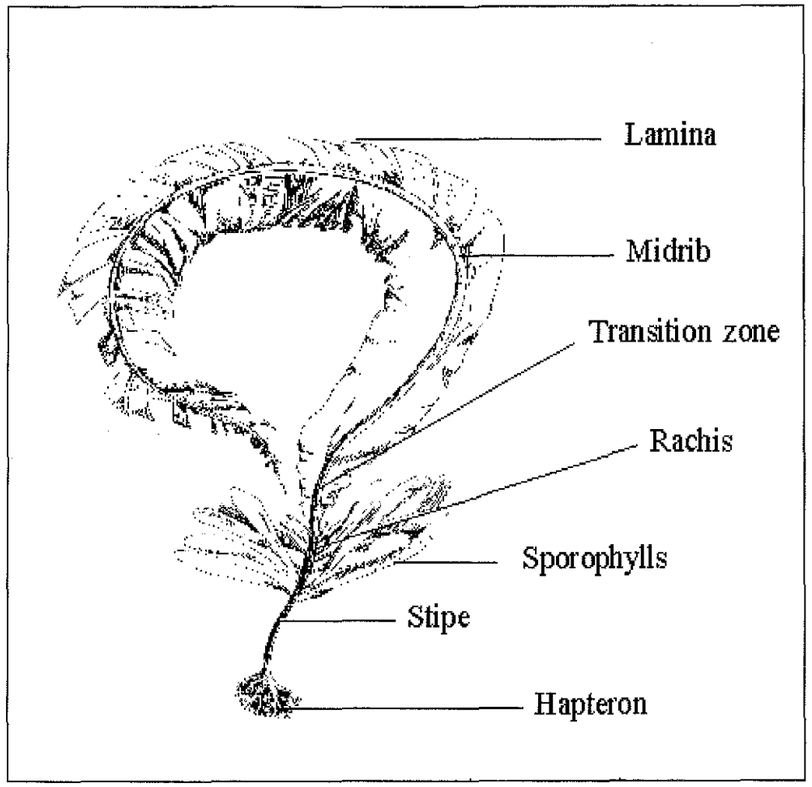

FIG. 1. HABIT OF *ALARIA ESCULENTA* SHOWING SOME MORPHOLOGICAL CHARACTERS.

1.3 Life history

Only during spring and in lesser amount during autumn two rows of ligulate sporophylls form in the upper parts of the stipe, the rachis (Widdowson, 1971). The sporophylls produce haploid spores by meiosis that germinate to form the haploid or gametophytic phase (male and female). Gametophytes produce the gametes (sperm and eggs) which fuse after fertilization to form a zygote. The zygote germinates to form diploid plantlets, the sporophytic phase. Thus the life history is that the large seaweed alternates with a microscopic filamentic phase (van den Hoek, 1996; Fig 3). *Alaria esculenta* is dioecious and has a heteromorphic diplohaplontic life history.

1.4 Hybridization experiments

Members of the Laminariaceae are able to cross inter- and intraspecific (tom Dieck, 1992; Egan *et al.*, 1990), and even intergeneric (Migita, 1984). Bolton *et al.* (1983)

produced hybrids between Atlantic and Pacific *Laminaria* species with different growth rates and morphological characteristics. They concluded that the ability to produce these characters appeared to be genetically determined. Lüning *et al.* (1978) also produced crosses between *Laminaria* species from both sides of the Atlantic. They suggested that blade width in hybrid offspring could be sex linked to the male gametophytes, which is in agreement with the results of a study by Egan *et al.* (1990).

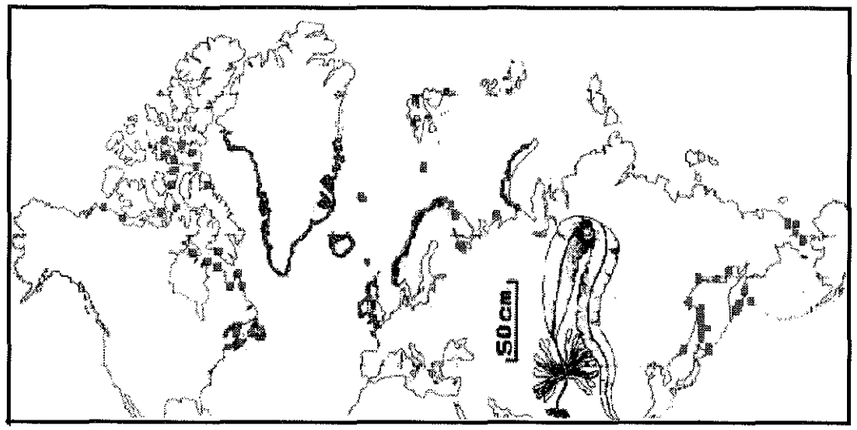

FIG. 2. THE DISTRIBUTION OF *ALARIA ESCULENTA* IN THE NORTH ATLANTIC AND THE NORTH PACIFIC OCEAN (AFTER LÜNING, 1990).

The temperature at which hybridization experiments are carried out plays an important role in the success of the crosses, as temperature will determine the production and release of gametes as well as the healthy growth of the sporophytes (tom Dieck, 1992). It can make a vast difference from which species the female gametophyte is provided as a successful cross may depend on this. Therefore it is important in hybridization studies to perform crosses and reciprocal crosses from every species (Egan *et al.*, 1990).

Lewis *et al.* (1986) produced interspecific hybrids of three *Macrocystis* species. They showed significantly different growth rates in different genetic groups. One of the self-crossed individuals showed the lowest growth rate. They suggested that the observed differences in growth rate between genetic groups was due to genetic adaptation to the conditions of the locality of growth of the parental plants.

Crossing experiments within the Laminariales are plagued with uncertainty because of the ability of female gametophytes to produce parthenogenic sporophytes. Parthenogenesis is a common feature of the members of the Laminariales (Nakahara & Nakamura, 1973; Lüning *et al.*, 1978; tom Dieck, 1992). Although most parthenogeneic sporophytes have an abnormal morphology, become stunted or irregular and do not survive more than a few mm in length (Bolton *et al.*, 1983).

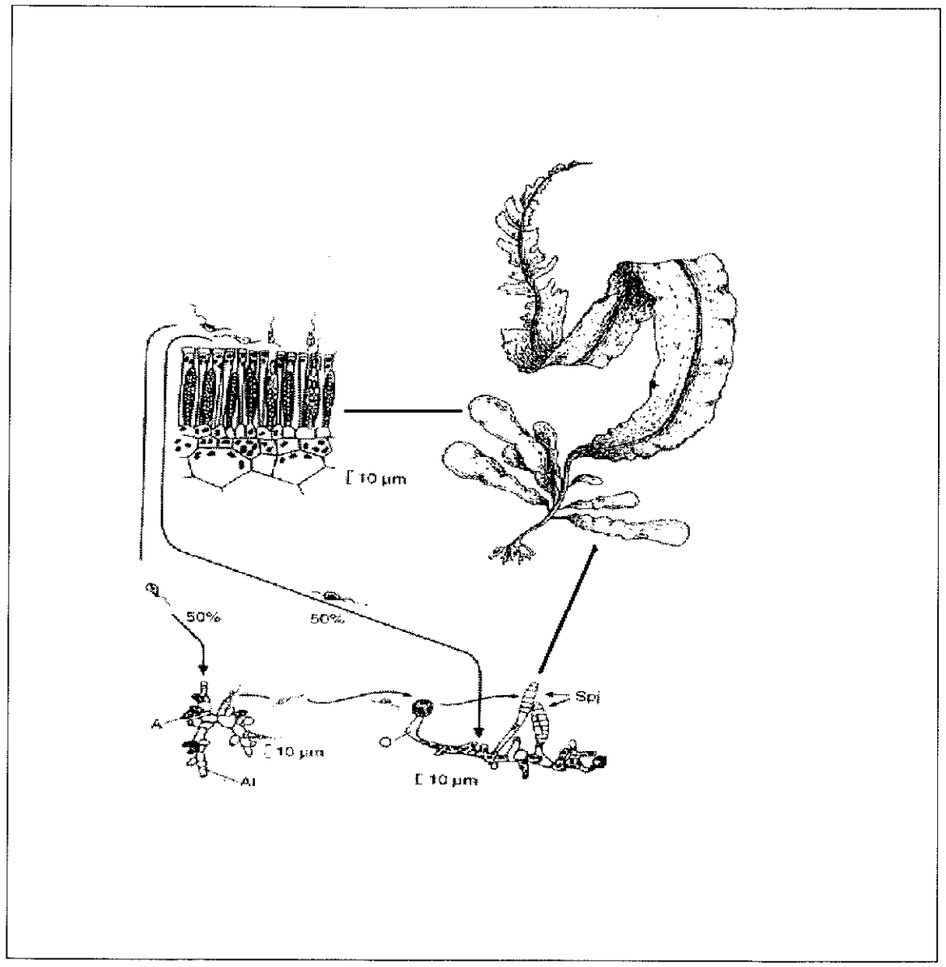

FIG. 3. LIFE CYCLE OF *ALARIA ESCULENTA*. THE SPOROPHYTE (A) PRODUCES SORI ON THE SPOROPHYLLS (B). SPORES (D) ARE PRODUCED IN THE SORI AFTER MEIOSIS IN UNILOCULAR SPORANGIA (C). SPORES (D) CONSISTING OF 50% MALE AND 50% FEMALE SETTLE AND GROW INTO MALE (E) AND FEMALE (F) GAMETOPHYTES RESPECTIVELY. MALE GAMETOPHYTES FORM SPERMS AND FERTILISE THE EGG FORMED ON THE OOGONIUM OF THE FEMALE GAMETOPHYTE. THE FERTILIZED EGG DEVELOPS INTO A ZYGOTE, WHICH DEVELOPS INTO YOUNG SPOROPHYTES (SPJ). AFTER VAN DEN HOEK *ET AL.*, (1996).

Hybridization experiments offer a means of assesing differentiation when correlated to specific environmental differences. However, interpretation of such experiments is not straightforward. Genetic differentiation may evolve without affecting compatibility and intersterility does not neccesarily indicate accumulation of a large number of genetic differences (Innes, 1984).

Increased incompatibility is often associated with greater geographical separation (Rueness, 1973; Guiry and West, 1983) but there are numerous examples where genetic differentiation occurs among populations separated by only short distances (Lewis *et al.*, 1986; Coyer *et al.*, 1997). Hybridization experiments may add valuable information to hypotheses of phylogenetic relationships (Lüning, 1990).

In China, extensive studies on hybridization and inbreeding has led to stable and improved strains of *Laminaria japonica* with higher production and iodine contents. Large-scale production experiments showed that the new varieties yield up to 40% more biomass and iodine contents of 20-58% higher than the control plants. These new varieties are introduced to *Laminaria* farms and grown in large quantities in North China (Chaoyuan & Guangheng, 1987).

1.5 Population genetics

Morphology of lamina and stipe in *Alaria esculenta* can vary widely between specimens mainly, it seems, due to wave exposure, with broader lamina and longer stipes in more sheltered areas (Sundene, 1962; Widdowson, 1971; pers. observ.). Phenotypic plasticity is a common feature of members of the Laminariales (Norton *et al.*, 1982). Chapman (1974) found significant genetic differentiation between populations of *Laminaria* for stipe morphology. By contrast, in a study on the Pacific kelp *Costaria costata* no genetic differences were found between two morphologically distinct populations using restriction fragment length polymormhisms (RFLP) (Bhattacharya & Druehl, 1989). However, it is not known for the kelp *A. esculenta* if there is one variety in the Atlantic ocean (gene flow between populations , no founder effects) or more different varieties (no gene flow between populations). More varieties might indicate varieties with different optimal characteristics suitable for aquaculture.

RFLP is a common applied molecular tool to distinguish morphological similar populations (Hillis *et al.*, 1996). It has been used in numerous algal studies (e.g., Rice & Bird, 1990; Bhattacharya & Druehl, 1989; Bhattacharya *et al.*, 1991; Lehman and Manhart, 1997). To demonstrate possible genetic differences in *Alaria* species with the RFLP technique two non-coding regions in the DNA were amplified. These are the Rubisco spacer which seperates the large and small subunits of the ribulose-1,5-biphosphate carboxylase/oxygenase genes and the nuclear ribosomal DNA (rDNA) internal transcribed spacer regions (ITS1 and ITS2). These non-coding spacers are considered to be more variable than coding genes, because they are under the least degree of functional constraint (Saunders and Druehl, 1993). These spacers have been shown to provide good resolution in examining relationships among and within populations of green algae (Kooistra, 1992; Bakker, 1992) and red algae (van Oppen *et al.*, 1995).

1.6 Kelp evolution and divergence

The outward morphological diversity of kelps suggests that kelps are an ancient assemblage, however, recent evidence suggest that kelp divergence started in the late

Miocene about (10-15 Ma) or even as late as late Pliocene (3-5 Ma) ago from a common ancestor (Estes & Steinberg, 1988; Saunders & Druehl, 1992). Stam *et al.* (1988) calculated that five *Laminaria* species diverged from a common ancestor 15-19 Ma ago most probably originating in the North Pacific and then invaded the North Atlantic after inundation of the Bering Land Bridge 3.5 Ma ago. This recent divergence event implies that North Atlantic *Laminaria* species probably have close relatives in the Pacific Ocean. Hybridisation experiments have shown this close relationship between Pacific and Atlantic *Laminaria* species (Bolton *et al.* 1983; tom Dieck, 1992) . Stam *et al.* (1988) identified five Pacific *Laminaria* sister species for five Atlantic *Laminaria* species. Relationships between Pacific and Atlantic *Alaria* species are unknown.

OBJECTIVES

There were three main objectives for this study:

1) To isolate, hybridize and grow strains of *Alaria esculenta* and other *Alaria* species from the Atlantic and Pacific to develop varieties with optimal characteristics for aquaculture in Ireland.

2) To identify genetic variation among *A. esculenta* strains from the Atlantic Ocean using the Restriction Fragment Length Polymorphism (RFLP) technique.

3) To obtain phylogenetic information about the relationships between North Atlantic and North Pacific species of the genus *Alaria* by hybridization experiments and fingerprinting

2. MATERIALS AND METHODS

2.1 Cultivation and hybridization techniques

Five individual plants with mature sori at the sporophylls were sampled from the geographical locations given in Table 1. Male and female gametophyte cultures from locations marked with an asterisk were send by the author mentioned.

Zoospores from mature sori were released using techniques described by South (1970) and Nakahara & Nakamura (1973). Seawater with zoospores was divided in sterile replidishes and kept under long-day conditions at 10° C and 20 µmol photons. m^{-2} s^{-1}. After about 10 d zoospores germinated into gametophytes and were visible under a microscope. After 2 - 3 weeks the gametophytes were separated in male and female gametophytes. Within 2 - 3 months full male and female cultures were obtained from the geographical locations listed in Table 1. The gametophytes had been maintained in the vegetative state in red fluorescent light at an irradiance of 10 µmol photons. $m^{-2}.s^{-1}$ in glass dishes containing sterile enriched seawater (Von Stoch 0.25 strength; Guiry & Cunningham, 1984) which was changed every month. Gametophyte cultures were grown

under red light as this prevents the gametophytes from maturing and hence forming eggs and sperm. This was necessary to allow for conducting cross experiments (Bolton *et al.* 1983).
Crosses were made using individual male and female gametophytes previously isolated from spores released by parent sporophytes collected in the sites listed in Table 1. Cross experiments were carried out in two different ways:

- By placing a female gametophyte (about 10 cells long) of a species of *Alaria* with one male gametophyte from another species of *Alaria* and vice-versa in Replidishes.

- By gently grinding the male and female gametophytes of the cross of interest in a mortar to produce a suspension of male and female filaments of 1-10 cells long. The fragments were then poured in Replidishes or small 20 ml Petri-dishes.

The crosses were incubated at 10, 15, 20, and 25 °C, long-day conditions (16:8 h) and 30 µmol photons.$m^{-2}.s^{-1}$ white light to initiate the formation of eggs and sperm (Lüning 1990). As soon as sporophytes appeared they were measured with an binocular. After two months viable healthy sporophytes with a length of 2 cm were transferred to 20 ml universal containers on a shaker. Daylength conditions were changed to longday 13:11 h to mimic spring or autumn conditions and initiate fast growth of the developing sporophytes. The medium was changed weekly or fortnightly. Sporophytes outgrowing the containers were transfered to small tanks with an aeration system under the same conditions.

In addition, self crosses and isolated male and female gametophytes were incubated parallel to each crossing experiment to observe possible parthenogenesis or apogamy (Nakahara & Nakamura, 1973). The plants were examined and measured in length and width weekly in the raply dishes, forthnightly in the sterile containers and once or twice a month in the tank.

2.2 Relative Growth Rates

The relative growth rate of the self crosses and hybrid sporophytes of the different crosses was calculated using the formula for relative growth rate (RGR)

$$RGR = L_n (L_2) - L_n (L_1) / t_2 - t_1$$

Where L2 is the length at time t2 and L1 the length at time t1 in increaments per day. The final length and width of the crosses were determined with image analysing equipment.

2.3 Detection of genetic variation

DNA extraction and purification
Plants from the sites listed in Table 1 were transported to the laboratory in a coolbox wrapped in plastics bags on cooling elements or sent in a similair way by mail. On

arrival, plants were cleaned and directly processed for DNA extraction or quick-frozen in liquid nitrogen and stored at -70° C.

TABLE 1. ORIGIN OF *ALARIA* GAMETOPHYTE CULTURES USED IN THE PRESENT INVESTIGATION.

Species	Code	sex	origin	Collector
Alaria esculenta	IRL	M & F	Ireland (Inis Oírr)	S. Kraan
Alaria esculenta	ACH	M & F	Achill Island Co. Mayo	S. Kraan
Alaria esculenta	CBH	M & F	Corbet Head Co. Down	S. Kraan
Alaria esculenta	SLH	M & F	Slea Head Co. Kerry	S. Kraan
Alaria esculenta	SJP	M & F	John's Point Co. Donegal	S. Kraan
Alaria esculenta	ICL	M & F	Iceland (Tjörnes)	K. Lüning*
Alaria esculenta	NOR	M & F	Norway (Ålesund)	J. Rueness *
Alaria esculenta	HFX	M & F	Canada (Halifax)	C. Bird *
Alaria praelonga	AP	M & F	Japan (Hokkaido)	M. Masuda*
Alaria crassifolia	AC	M & F	Japan (Hokkaido)	M. Masuda*
Alaria tenuifolia	AT	F	Canada (Vancouver)	L. Druehl
Alaria nana	AN	M & F	Canada (Vancouver)	L. Druehl
Alaria marginata	AM	M & F	Canada (Vancouver)	L. Druehl

DNA was extracted from ca. 1 g of blotted wet weight of healthy plant material or ca. 0.5 g of -70°C stored blade material. Extraction as follows: Grind material in liquid nitrogen, add powder to a 2 mL eppendorf tube with 2 µL β-mercapto-ethanol and 900 µL extraction buffer (2% CTAB (v/v), 4M NaCL, 0.5 M EDTA pH 8 and 1M TRIS-HCL pH 8). Incubate for 1 h under slow agitation, add 900 µL CIA (chloroform:isoamylacohol, 24:1 v/v) and mix gently for 3 min. Centrifuge at 14.000 g at 10° C. Transfer upper aqueous phase to sterile new tube, repeat CIA extraction until the interphase has dissappeared. Transfer upper phase to fresh sterile tube, precipitate polysaccharides by adding 0.1 vol 5M K-acetate, mix, 0.25 vol 96% Ethanol, mix, 1 vol CIA, mix, centrifuge as before and collect upper phase in a new tube . Add 1 vol cold (-

20° C) isopropanol, mix gently, and leave to settle for at least 1 h at 4° C. To precipitate the crude DNA ,centrifuge as before for 30 min. Rinse pellet 3 times in 70% Ethanol, air dry and dissolve in 100 µL 0.1 x TE buffer. Add 10 units Dnase-free Rnase (Boehringer Mannheim) and incubate at 37° C for 30 min. Precipitate DNA as described above. Redissolve in 100 µL 0.1 x TE buffer. Average yields were 10-100 µg of high molecular weight DNA.

PCR Amplification

Double stranded amplifications were performed in a Hybaid Omn-E thermal cycler with an initial denaturation step of 95° C for 3 min followed by 35 cycles with the following temperature profile: 1 min 95° C, 2 min 55 °C and 2 min 74° C and ended with one extension step of 72° C for 5 min. The reaction volume was 100 µL comprised of 10-100 ng genomic DNA, 50 µM of each dATP, dNTP, dCTP and dGTP, ca. 0.5 µM of each primer, 10 µL of 10x reaction buffer (Sigma), 6 µL 25 mM $MgCL_2$ (Sigma) and 2.5 units of Taq (Sigma). Primer pair P1 and G4 were used to amplify the region in the ribosomal cistron from position 1542 in the 18S across the ITS1, the 5.8S, ITS2 and to position 42 in the 26S (Saunders and Druehl, 1993). Primer pair s130s and l1105f were used to amplify a small part of the Large subunit across the Rubisco spacer into the small subunit, starting at position 1105 in the Large subunit to position 172 in the small subunit (Valetin and Zetche, 1990).

Amplifications were checked for correct length, purity and yield on 1.5% agarose TAE gels stained with EtBr according the methods of Sambrook et al. (1989). Excess primers, salts and nucleotides were removed from PCR products using the PCR purification kit according the manufacturer's instructions (Boeringer Mannheim). One restriction enzyme reaction could be performed with 15 µL of purified product.

Restriction endonuclease digestion of PCR fragments

Fifteen µL of the PCR amplified Rubisco spacer or rDNA spacers of all isolates listed in table 1 were completely digested with 2-4 units of restriction endonuclease for 4 h as follows: Fil 1.5 ml Eppendorf tube with 15 µL PCR product (cleaned), 3 µL 10x incubation buffer and 2 µL restriction enzyme (Boeringer Mannheim). Mix this 20 µL and spin down for 10 sec. The 8 enzymes used were RSA1, HindIII, CFO1, DRA1, XBA, Dpn1, PST1 and SMA Restriction fragments were run on 3% TAE agarose gels (1% agarose/ 2% MS agarose, Boeringer Mannheim) at 20 V for 20 h and stained afterwards with EtBr. The stained restriction fragments were visualized by UV-fluorescence, captured with a video camera and printed with a thermic printer. Fragment seize was determined according standard methods (Hillis *et al.* 1996) All digestions were performed twice so accurate calculation of the seize of the fragments was possible.

2.4 Data analysis

The data sets of relative growth rates, width, length and biomass showed a departure of a normal distribution and heteroscedasticity was pronounced. Therefore we applied the Kruskal-Wallis non-parametric analysis of variance (with tied ranks and unequal sample

seize) to examine the null hypothesis: similarity between RGR of Atlantic crosses = similarity in RGR between Atlantic with Pacific crosses = similarity between pacific crosses. Multiple comparisons were made as aposteriori testing using Dunn's test if the null hypothesis was rejected (Zar, 1996).

Presence and absence of restriction fragments were scored manually from the thermic prints and a presence/absence matrix was constructed for all individuals. Pairwise similarities were calculated using Dice (Jacquard) coefficient (Sd) = two times the number of bands shared by two individuals, devided by the number of bands displayed by each individual. Sd does not allow for negetive matches which might cause false similarities among those taxa sharing many negetative matches (Coyer *et al.*, 1997). From the bandsharing data a pairwise distance matrix was generated where in the number 1 correspondens to two individuals being indentical and the value 0 corresponds to two individuals that are entirely dissimilar. No further statistical analysis was performed on the restriction fragments because of the low amount of fragments generated.

3. RESULTS

3.1 Hybridization experiments

All successful crosses between Atlantic x Atlantic, Atlantic x Pacific and Pacific x Pacific *Alaria* species are listed in table 2 and 3. Successful crosses were produced at 10° C only. All the (self) crosses and reciprocal crosses between *Alaria esculenta* strains of Atlantic populations proved to be successful except for the Male Iceland x Female Halifax cross. This cross did not produce any viable healthy sporophytes. Although the products of some crosses were small, like the Iceland and Halifax crosses, they looked like perfect healthy sporophytes and were not parthenogenetic. Parthenogenetic sporophytes in the female parallel cultures were easily identified because of their round cell clump-like appearance without haptera. The Irish self cross, Irish x Norway crosses and female Irish x male Halifax cross produced reproductive structures (sporophylls) in culture.

Between crosses of Atlantic *A. esculenta* with other species of the Pacific only the *A. praelonga* x *A. esculenta* and reciprocal cross produced viable healthy sporophytes which formed sporophylls in culture. From *A. crassifolia* only male gametophytes hybridized with *A. esculenta*. The reciprocal cross produced small (± 1 cm long) stunted sporophytes which resembled the pathenogenic sporophytes from the control. From *A. tenuifolia* only female gametophytes hybridized with male *A. esculenta* and produced sporophylls. The reciprocal cross was not tested because no male *A. tenuifolia* gameto-phytes were available. Crosses and reciprocal crosses between *A. nana* and *A. marginata* did not produce any sporophytes.

The control cultures with female or male gametophytes alone produced in some cases parthenogenetically or apogamous derived sporophytes. These sporophytes showed a high mortality rate and never grew >1 cm. It was therefore very easy to recognize healthy

and viable hybrids from parthenogenic or apogamous sporophytes. In some experiments the parthe-nogenic or apogamous sporophytes survived up to a year without growth development.

TABLE 2. HYBRIDIZATION WITHIN 4 NORTH ATLANTIC STRAINS OF *ALARIA ESCULENTA* AND THE DEVELOPMENT OF THE HYBRIDS INDICATED AS AGE, MORPHOLOGY AND MAXIMUM LENGTH THAT WAS ACHIEVED DURING CULTIVATION AT 10° C, 30 µ MOL PHOTONS.$M^{-2}.S^{-1}$ AND LD (13:11). (-) = NO DEVELOPMENT OF SPOROPHYTES. HAPTERA + = WELL DEVELOPED, +/- = HAPTERA OF HALF THE SPOROPHYTES IS WELL DEVELOPED, - = POOR HAPTERA DEVELOPMENT. SP = SPOROPHYLLS FORMED, NT = CROSSING NOT TESTED. FOR ORIGIN CODES SEE TABLE 1

	MALE			
Female	IRL	ICL	NOR	HFX
IRL	Normal, sp 43 cm, Haptera + 7 months	Small/broad 6.5 cm, Haptera - 6 months	Normal, 46 cm, Haptera +/- 6 months	Normal, sp 42 cm, Haptera + 6 months
ICL	Small/broad 8 cm, Haptera +/ - 6 months	Nt	Small, 3 cm, Haptera +/- 16 months	- 12 months
NOR	Long/thin, sp 27 cm, Haptera +/- 6 months	Normal, sp 70 cm, Haptera + 9 months	Nt	Small, 21 cm, Haptera +/- 9 months
HFX	Normal/ Missing midribs, 13 cm, Haptera + 6 months	Small, 18 cm, Haptera + 9 months	Very small, 2.5 cm, Haptera - 9 months	Nt

TABLE 3. HYBRIDIZATION OF NORTH ATLANTIC *ALARIA ESCULENTA* WITH PACIFIC *ALARIA* SPECIES AND WITHIN PACIFIC SPECIES AND THE DEVELOPMENT OF THE HYBRIDS INDICATED AS MORPHOLOGY AND MAXIMUM LENGTH THAT WAS ACHIEVED DURING CULTIVATION AT 10 °C, 30 µ MOL PHOTONS.$M^{-2}.S^{-1}$ AND LD (13:11). (-) = NO DEVELOPMENT OF SPOROPHYTES. HAPTERA + = WELL DEVELOPED, +/- = HAPTERA OF HALF THE SPOROPHYTES IS WELL DEVELOPED, - = POOR HAPTERA DEVELOPMENT. SP = SPOROPHYLLS FORMED, NT = CROSSING NOT TESTED. FOR ORIGIN CODES SEE TABLE 1

	MALE				
Female	IRL	AP	AC	AN	AM
IRL	Normal, sp 43 cm, Haptera + 7 months	Normal, sp 21 cm, Haptera + 6 months	Normal 6 cm, Haptera - 7 months	Few elongated stipes 8 cm Haptera - 6 months	Few elongated stipes 8 cm Haptera - 7 months
AP	Normal, sp 11 cm, Haptera + 6 months	Normal/long, thin, 13 cm Haptera +/- 8 months	Clumps <1cm Haptera - 9 months	Clumps <1cm Haptera - 12 months	Clumps <1cm Haptera - 9 months
AC	Stunted <1cm Bleached Haptera - 7 months	Normal, sp 25 cm Haptera + 9 months	Nt	Stunted 1cm Bleached Haptera - 7 months	Clumps <1cm Haptera - 12 months
AN	Few elongated stipes 6 cm Haptera - 6 months	Normal 5 cm Haptera +/- 6 months	Normal 6 cm Haptera +/- 6 months	Nt	- 20 months
AM	Clumps <1cm Haptera - 14 months	Normal, sp 60 cm, Haptera + 10 months	Clumps <1cm Haptera - 12 months	- 20 months	Long, thin 22 cm Haptera + 8 months
AT	Normal, sp 61 cm, Haptera + 6 months	Normal, 12.5 cm, Haptera + 9 months	Stunted <1cm Haptera - 6 months	Stunted <1cm Haptera - 6 months	Normal 56 cm Haptera + 9 months

Within the Pacific crosses *A. praelonga* male gametophytes hybridized with all other Pacific *Alaria* species. *Alaria tenuifolia* female gametophytes hybridized with *A. praelonga* and *A. marginata* male gametophytes, the latter also producing sporophylls.

3.2 Relative growth rates

All hybrids and selfcrosses showed a similar growth pattern of rapid growth during the first 4 to 5 weeks and a slower growth during the rest of the period. In this report, these growth rates will be called "settlement relative growth rate" (RGRset) and "Relative growth rate" (RGR) respectively. The mean RGRset and RGR for length and width increments per day are presented in figure 4 to 7.

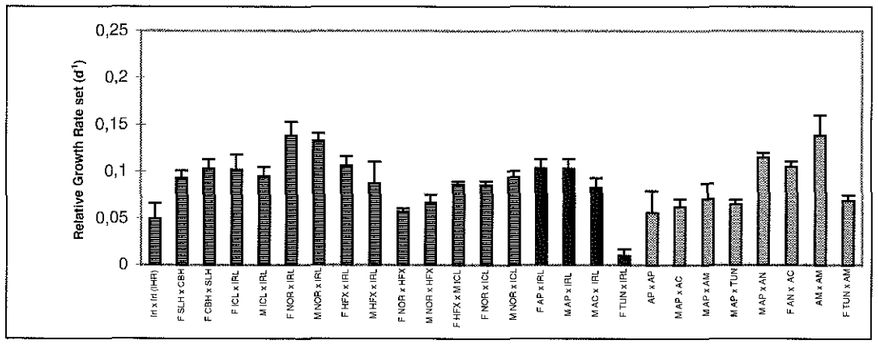

FIG. 4. AVERAGE SETTLEMENT RELATIVE GROWTH RATES FOR PLANT LENGTH (D^{-1} WITH 95% CONFIDENCE LIMITS) WITHIN NORTH ATLANTIC *ALARIA ESCULENTA* CROSSES (STRIPED) WITHIN PACIFIC *ALARIA* CROSSES (GREY) AND BETWEEN PACIFIC AND ATLANTIC *ALARIA* CROSSES (BLACK). FOR ORIGINAL CODES SEE TABLE1.

For Fig. 4 the Kruskal-Wallis test showed significant differences between crosses (H= 38.96535, $X^2$0.05,25=37.652). The null hypothesis was rejected; the settlement relative growth rates for length are different between crosses. Multiple comparisons are given in Appendix I. Significant differences in Appendix I are marked with patterns, vertical striped for Atlantic, black for Atlantic x Pacific and grey for Pacific.

The Irish self crosses and Irish x Norway crosses are in most cases significantly different from other crosses in length elongation.

For Fig. 5 the Kruskal-Wallis test showed significant differences between crosses (H= 82.47678, $X^2$0.05,25=37.652). The null hypothesis was again rejected. The relative growth rates for length are different between crosses. Multiple comparisons are presented in Appendix II. Significant differences in Appendix II are marked with patterns, horizontal striped for Atlantic, black for Atlantic x Pacific and grey for Pacific. The Irish x *tenuifolia*, Irish x Norway and *A. marginata* self cross are in most cases significantly different from the other crosses.

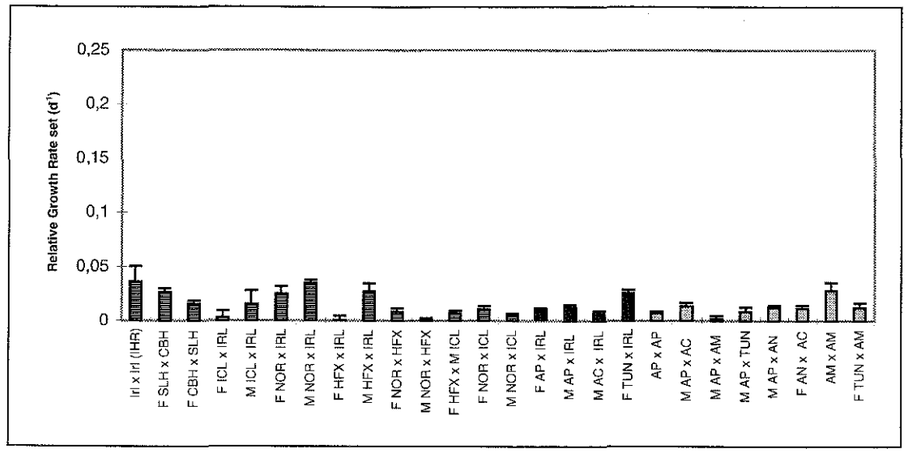

FIG. 5. AVERAGE RELATIVE GROWTH RATES FOR PLANT LENGTH (D^{-1} WITH 95% CONFIDENCE LIMITS) WITHIN NORTH ATLANTIC *ALARIA ESCULENTA* CROSSES (STRIPED) WITHIN PACIFIC *ALARIA* CROSSES (GREY) AND BETWEEN PACIFIC AND ATLANTIC *ALARIA* CROSSES (BLACK). FOR ORIGINAL CODES SEE TABLE 1.

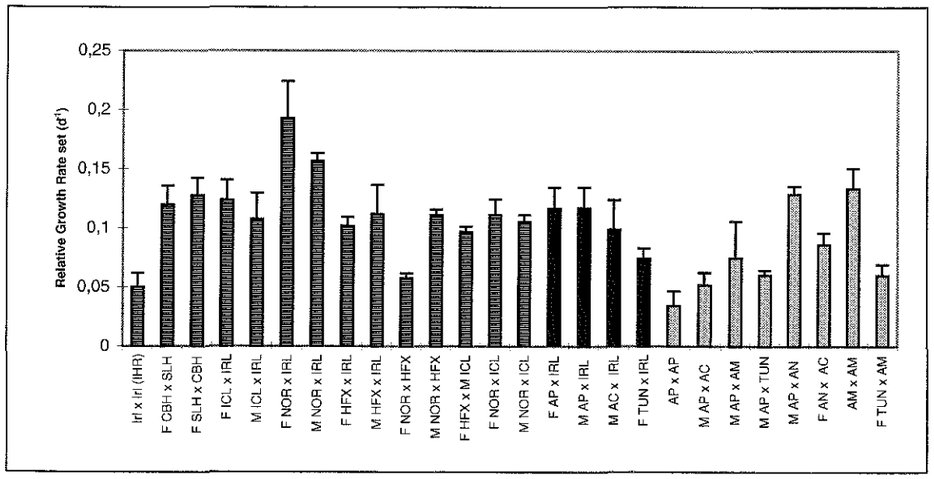

FIG. 6. AVERAGE SETTLEMENT RELATIVE GROWTH RATES FOR PLANT WIDTH (D^{-1} WITH 95% CONFIDENCE LIMITS) WITHIN NORTH ATLANTIC *ALARIA ESCULENTA* CROSSES (STRIPED) WITHIN PACIFIC *ALARIA* CROSSES (GREY) AND BETWEEN PACIFIC AND ATLANTIC *ALARIA* CROSSES (BLACK).FOR ORIGINAL CODES SEE TABLE 1.

For Fig. 6 the Kruskal-Wallis test showed significant differences between crosses (H= 90.85175, $X^2$0.05,25=37.652). The null hypothesis was again rejected. The settlement relative growth rates for width are different between crosses. Multiple comparisons are presented in Appendix III. Significant differences in Appendix III are marked with patterns, striped for Atlantic, black for Atlantic x Pacific and grey for Pacific. Almost all

slow growing Pacific crosses were significantly different from Atlantic crosses.

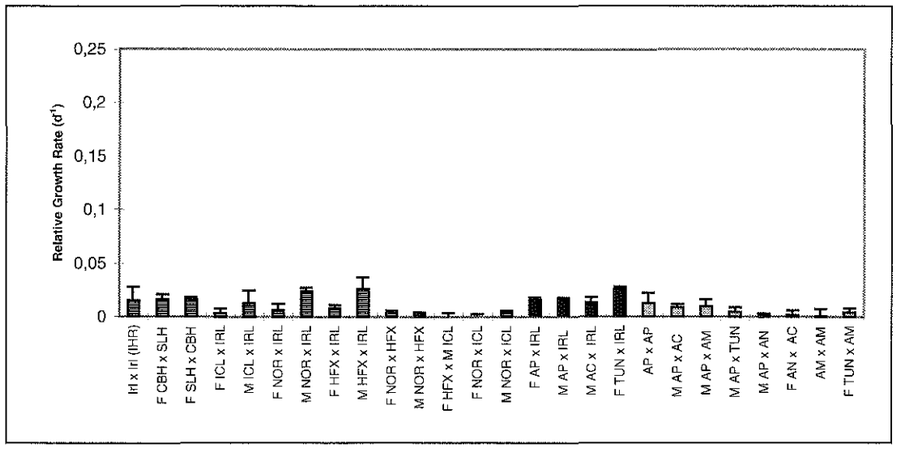

FIG. 7. AVERAGE RELATIVE GROWTH RATES FOR PLANT WIDTH (D^{-1} WITH 95% CONFIDENCE LIMITS) WITHIN NORTH ATLANTIC *ALARIA ESCULENTA* CROSSES (STRIPED) WITHIN PACIFIC *ALARIA* CROSSES (GREY) AND BETWEEN PACIFIC AND ATLANTIC *ALARIA* CROSSES (BLACK). FOR ORIGINAL CODES SEE TABLE 1.

For Fig. 7 the Kruskal-Wallis test showed significant differences between crosses (H= 50.97903, $X^2$0.05,25=37.652). The null hypothesis was again rejected. The relative growth rates for width are significant different between crosses. Multiple comparisons are presented in Appendix IV. Significant differences in Appendix IV are marked with patterns, striped for Atlantic, black for Atlantic x Pacific and grey for Pacific. The female Irish x male Norway, female Irish x male Halifax and female *tenuifolia* x male Irish crosses are in most cases significantly different from all other crosses.

3.3 Length, width and length:width ratio

For reasons of equal comparisons all length and width measurements were taken after 120 (+/- 5) d. The results are presented in Figs. 8 and 9.
The ratio length:width presented in Figure 10 reveals the shape of the sporophytes, where a high number indicates long and thin sporophytes and a small number broad and small sporophytes.

Irish self-crosses from Inis Oírr formed a lot of sporophytes with very long thin elongated stipes which resulted in the large ratio figure. Irish crosses between Slea Head and Corbet Head, Halifax x Ireland, Norway x Ireland, *tunefolia* x Ireland and *A. marginata* self cross produced the longest sporophytes with broad leaves. Most Pacific crosses produced small sporophytes. Crosses of Iceland with other strains did not perform well resulting in small/broad sporophytes.

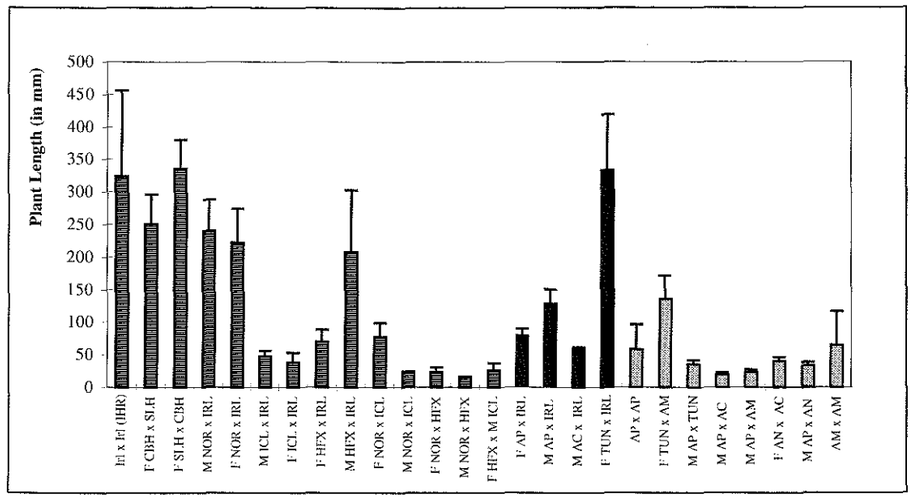

FIG. 8. AVERAGE PLANT LENGTHS (IN MM WITH 95% CONFIDENCE LIMITS) WITHIN NORTH ATLANTIC *ALARIA ESCULENTA* CROSSES (STRIPED) WITHIN PACIFIC *ALARIA* CROSSES (GREY) AND BETWEEN PACIFIC AND ATLANTIC *ALARIA* CROSSES (BLACK). FOR ORIGINAL CODES SEE TABLE 1.

For Fig. 8 the Kruskal-Wallis test showed significant differences between crosses (H= 100.6577, $X^2$0.05,25=37.652). The null hypothesis was rejected. The average plant lengths after 120 d are different between crosses. Multiple comparisons are presented in Appendix V. Significant differences in Appendix V are marked as patterns, striped for

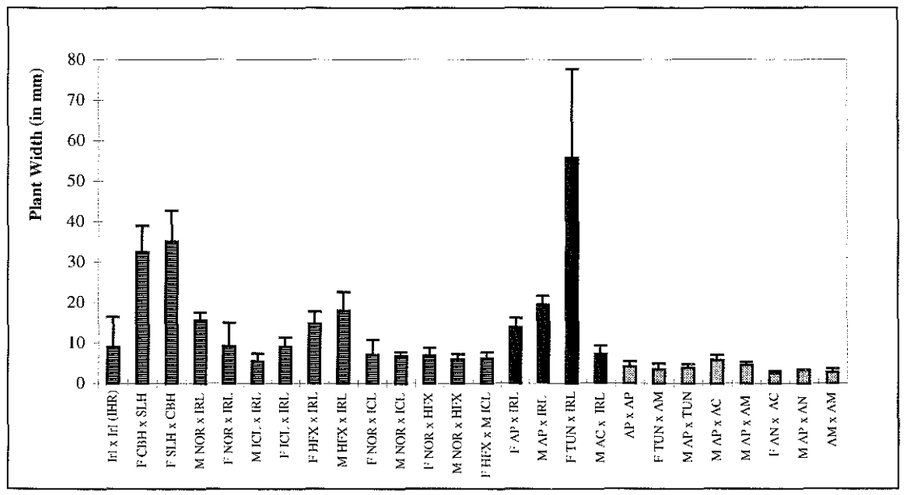

FIG. 9. AVERAGE PLANT WIDTHS (IN MM WITH 95% CONFIDENCE LIMITS) WITHIN NORTH ATLANTIC *ALARIA ESCULENTA* CROSSES (STRIPED) WITHIN PACIFIC *ALARIA* CROSSES (GREY) AND BETWEEN PACIFIC AND ATLANTIC *ALARIA* CROSSES (BLACK). FOR ORIGINAL CODES SEE TABLE 1.

Atlantic, black for Atlantic x Pacific and grey for Pacific. The Irish x *tenuifolia*, *marginata* x *tenuifolia* Irish x Norway and the Irish self crosses are significantly different from the other crosses but do not differ significantly amongst each other.

For Fig. 9 the Kruskal-Wallis test showed significant differences between crosses (H= 90.85175, $X^2$0.05,25=37.652). The null hypothesis was again rejected. The average plant widths after 120 d are different between crosses. Multiple comparisons are presented in Appendix VI. Significant differences in Appendix VI are marked with patterns, striped for Atlantic, black for Atlantic x Pacific and grey for Pacific.
Most Atlantic and Atlantic xPacific crosses differ significantly from Pacific crosses in width. Almost no differences where found between Atlantic and Atlantic x Pacific crosses.

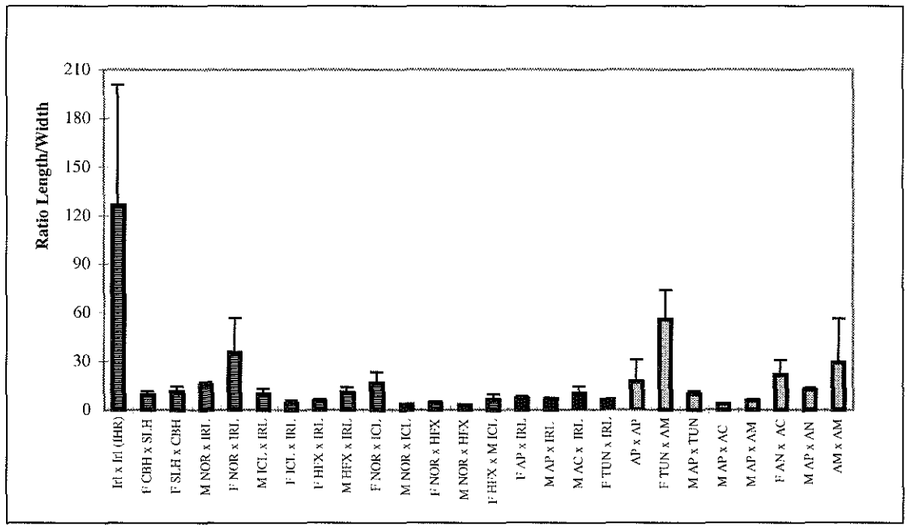

FIG. 10. RATIO LENGTH:WIDTH OF THE EXPERIMENTAL SPOROPHYTES (WITH 95% CONFIDENCE LIMITS) WITHIN NORTH ATLANTIC *ALARIA ESCULENTA* CROSSES (STRIPED) WITHIN PACIFIC *ALARIA* CROSSES (GREY) AND BETWEEN PACIFIC AND ATLANTIC *ALARIA* CROSSES (BLACK). FOR ORIGINAL CODES SEE TABLE 1.

3.4 Genetic variation

Restriction fragment length polymorphisms on the ITS1, 5.8S and ITS2 and the RuBisCo spacer revealed 123 scorable bands of which 23 were variable. The absence and presence of bands are presented in a matrix in Appendix VII. Genetic distances are calculated with the Dice(Jaquard) coefficientindex (Sj) and are presented in Table 4. Fromt Table 4 it is clear that *Alaria esculenta* from Ireland, Scotland, Halifax, France and *A. marginata* from Canada are identical in genetic composition at the spacer level. *Alaria esculenta* from Iceland and Norway are less identical with the above described species because of absence or presence of restriction sites, i.e., the restriction enzyme PST1 recognized a unique restriction site in the ITS spacers of Norway and Iceland not shared with one of

the other species. Enzyme XBA revealed a unique site for *A. crassifolia* and enzym DRA showed one site only present in *A. praelonga*. All *Alaria* species are least related to *Undaria pinnatifida*, a member of the family Alariaceae but not belonging to the genus *Alaria* and chosen as an outgroup here.

TABLE 4. DISTANCE MATRIX FOR PAIRWISE COMPARISONS OF RDNA SPACERS AND RUBISCO SPACER RESTRICTION FRAGMENTS OF *ALARIA* SPECIES BASED ON DIGESTIONS WITH 8 RESTRICTION ENZYMES. PAIR WISE SIMILARITIES WERE CALCULATED ACCORDING THE DICE (JACQUARD) COEFFICIENT. PAIR WISE DISTANCES BETWEEN SPECIES ARE LISTED ABOVE THE DIAGONAL. THE TOTAL NUMBER OF RESTRICTION FRAGMENTS GENERATED IN A SPECIES IS LISTED BETWEEN PARENTHESES ON THE DIAGONAL. THE NUMBER OF RESTRICTION FRAGMENTS SHARED BETWEEN SPECIES IS LISTED BELOW THE DIAGONAL. SCL=SCOTLAND, FRC=FRANCE. FOR OTHER CODES SEE TABLE 1.

	IRL	SCL	NOR	ICL	HFX	FRC	AM	AN	AC	AP	UND
IRL	(11)	1	0.77	0.95	1	1	1	0.82	0.92	0.82	0.45
SCL	10	(11)	0.77	0.95	1	1	1	0.82	0.92	0.82	0.45
NOR	5	5	(8)	0.86	0.77	0.77	0.77	0.67	0.71	0.67	0.29
ICL	10	10	6	(12)	0.95	0.95	0.92	0.82	0.86	0.78	0.43
HFX	10	10	5	10	(11)	1	1	0.82	0.92	0.82	0.45
FRC	10	10	5	10	10	(11)	1	0.82	0.92	0.82	0.45
AM	6	6	5	6	6	6	(7)	0.86	0.92	0.86	0.31
AN	9	9	5	9	9	9	6	(13)	0.8	0.83	0.42
AC	6	6	5	6	6	6	6	6	(8)	0.8	0.29
AP	9	9	5	9	9	9	6	10	6	(13)	0.42
UND	5	5	2	5	5	5	2	5	2	5	(13)

4. DISCUSSION AND CONCLUSIONS

4.1 Atlantic cross experiments

Intraspecific hybridization

The results presented in this study for crosses between *A. esculenta* from different geographical locations in the North Atlantic show that the four strains are interfertile except for the male Halifax x female Iceland cross. Müller (1979) demonstrated in Ectocarpus siliculosus that sterility barriers do occur within species from different geographical locations.

The significant morphological best and fastest growing crosses where produced by the female Iceland x male Norway, female Ireland x male Norway, female Ireland x male Halifax and the Irish selfcross. All except the female Ireland x male Norway cross produced well developed haptera and sporophylls and are therefore most suitable for rope cultivation in Atlantic Irish waters. Slower growing but morphological healthy crosses were produced between female Halifax x male Iceland, female Norway x male Halifax and female Norway x male Ireland. Similar results were found in crosses *of Laminaria digitata* from Halifax and Helgoland (tom Dieck, 1993).

All female Iceland crosses produced poor or no results. Female Halifax crosses produced hybrids that did not develop midribs. The poor hybridization results from Icelandic plants might be caused by ecotypic variation, i.e., different temperature tolerance of the gametophyte and/or sporophyte and temperature responses of growth (Breeman, 1988). However, these results are laboratory results under controlled conditions and do not reflect field conditions.

The winter isotherms are the same for Iceland, Halifax and Norway (0 - 5°C). Only the summer isotherm is different for Iceland (5 - 10°C v.s. 10 -15°C for Ireland, Norway and Halifax (Lüning 1990). Fertilisation, zygote and young sporophyte development in all crosses was induced at 10°C, which is the upper summer isotherm boundary for Iceland. This relatively high temperature for crosses with Iceland gametophytes might prove unfavourable resulting in the observed poor results in Iceland crosses. Tom Dieck & de Oliveira (1992) showed differences in fertility of female gametophytes of five species of *Laminaria* at three different temperatures.

Lüning & Freshwater (1988) showed that little or no variation exists in the upper temperature tolerance of *Chondrus crispus* and *Scytosiphon lomentaria* ranging from Iceland to Spain and of isolates of *Laminaria saccharina* and *Laminaria longicruris* from various locations, although only a few detailed studies are available. There is however a possibility that local and genetically fixed temperature ecotypes have evolved in *Alaria esculenta.*

Genetic analysis

The calculated pairwise distances from the restriction fragment analysis between *Alaria esculenta* isolates from Ireland, Scotland, Halifax and France (Table 4) showed that they were genetically identical. Norway and Iceland showed differences at the DNA level resulting in more or less restriction fragments.

The differences encountered in restriction fragments in respect of the DNA spacers examined suggest that the Icelandic and Norwegian *A. esculenta* populations are more isolated than the other Atlantic *Alaria* species. Probably little or no gene flow occurs between the Icelandic and Norwegian populations and between other populations. The different genotype and therefore genetic make-up of the Icelandic strain might account for the existing fertility barrier and the poor cross results in the Icelandic crosses. However, these genetic results are only preliminary results and serve to illustrate possible existing genetic variation between *A. esculenta* populations rather than to establish conclusive facts regarding the nuclear genome of *A. esculenta.*

Coyer *et al.* (1997) showed distinguishable biogeographic populations along the northeast Pacific coast of the kelp *Postelsia palmaeformis*. They showed decreasing genetic relatedness with increasing distance between populations assessed with M13 Fingerprinting and RAPDs.Bhattacharya et al. (1990) showed with RFLPs 8 distinct populations of the kelp *Costaria costata* over a range of 400 km. Theses populations appeared to be discreet breeding groups.

4.2 Pacific x Atlantic and Pacific Cross experiments

Interspecific hybridization

From the results presented in Table 3 it is clear that *A. esculenta* from the Atlantic does hybridize with other Pacific *Alaria* species, showing a close relationship between *Alaria* species throughout the northern hemisphere. In fact, the best cross produced was that between Atlantic *A. esculenta* and Pacific *A. tenuifolia*, giving rise to the largest and best-developed plants. This cross would be most suitable to be used in closed-tank cultivation.

Crosses between *A. esculenta* and *A. praelonga* produced healthy and well developed sporophytes. Crosses between female *A. esculenta* and male *A. crassifolia* produced slow-growing offspring, whereas the reciprocal cross did not. Crosses and reciprocal crosses of *A. esculenta* with *A. marginata* and *A. nana* where not succesfull, resulting in a few elongated stipes, indicating that various fertility barriers are active. Similar close relationships and fertility barriers have been found in other Atlantic and Pacific kelp species (Bolton *et al.*, 1983; Lewis *et al.*, 1986; tom Dieck & de Oliveira, 1993; tom Dieck, 1993).

Crosses among Pacific species showed that they hybridize in many cases, producing the largest plants in *A. tenuifolia* x *A. marginata* crosses. Only *A. nana* x *A. marginata* crosses did not produce any sporophytes which is in contrast with the observations of Widdowson (1971). He found plants that appear to be hybrids between *A. nana* and *A. marginata* in the wild. Widdowson (1971) considered that *A. marginata* and *A. tenuifolia* intergrade with each other through a series of populations that appear to be ecotypes. The different cross results shown in Table 3 however, support the hypothesis that *A. marginata* and *A.tenuifolia* are two gentically different species.

Fertilisation in *Alaria* species is facilitated by the sex pheromone lamoxirene, which can be found in all species of the Alariaceae, Laminariaceae and Lessionaceae

(Müller *et al.*, 1985), but incompatibility was expressed between several species of the genus *Alaria*. In three crosses (two inter-specific and one intra-specific) no microscopic sporophytes were formed. This in contrast with Laminariales from the North Atlantic and Pacific where microscopic sporophytes were procuced in all crosses but became irregular and stunted during later development (tom Dieck, 1992). Yarish *et al.* (1990) postulated that the sharing of a common sex pheromone is sufficient reason to expect interfertility. This is not true for the genus *Alaria*. Reproductive isolation develops gradually by different processes that might lead to fertility barriers. There are several examples in the Laminariales of gradual reproductive isolation. In several *Undaria* species the succesful formation of hybrids was expressed differently in reciprocal crosses (tom Dieck, 1992). The same results were encountered in the crosses between *A. crassifolia* and *A. esculenta* during the present investigation.

Species belonging to the order Laminariales are able to produce sporophytes from unfertilized eggs (parthenogenic sporophytes), although most parthenogenic sporophytes have an abnormal morphology (Nakahara & Nakamura, 1973; tom Dieck, 1992). In the present study parthenogenic sporophytes did not survive or were clearly recognized as cell clumps without haptera or half-bleached stunted sporophytes up to 1 cm long, resembling the parthenogenic sporophytes in female control cultures.

Genetic analysis

Druehl (1990) found that environmental factors caused distinctieve species morphotypes in *A. nana*, *A. marginata* and *A. tenuifolia*. Using restriction enzyme digestion of the small subunit rDNA and intergeneric spacers he found three restriction map variants indicating distinctive breeding populations of the three species which is in contrast with the findings of Widdowson (1971). Druehl & Saunders (1990) concluded that there is no consistent relationship between morphological and rDNA variation in *Alaria*. After sequencing of the entire 18S ribosomal gene for *A.nana* and *A. marginata* they found a nucleotide divergence of only 0.05%, indicating almost negligible genetic divergence. In this study *A. marginata* and *A. nana* showed different restriction fragment patterns at the intertranscribed spacer level. The differences in results compared with the study of Druehl & Saunders (1990) probably is caused due to the use of the 18S slow-evolving ribosomal gene instead of the fast-evolving spacer regions applied in this study. Cross experiments showed that *A. nana* and *A. marginata* do not hybridise, showing a fertility barrier which is an indication of a large gentic distance between the two species.

In this study *Alaria marginata* is identical to four *A. esculenta* isolates in its restriction patterns. Remarkably *A. marginata* did not hybridize with *A. esculenta* in this study despite of the simalarities in genotype of the ITS 1 and ITS 2. In Table 4, *A. nana* and *A. praelonga* share the same distances, *whereas A. crassifolia* is more closely related to *A. esculenta*. This is also inconsistent with the hybridisation experiments. These results show clearly that eight restriction enzymes, of which five are informative, are not sufficient to solve genetic relationships in the genus *Alaria*. Clearly more enzymes have to be applied to generate a reliable data set as only 2.56% of the entire ITS1 and ITS2 is sampled with the five informative restriction enzymes.

Chromosomes

If we compare chromosome numbers (n) in the genus *Alaria* we can distiguish two groups. One group with n=14 viz., *A. nana*, *A. marginata*, *A. tenuifolia A. fistulosa*, *A.*

taeniata and one group with 22-28 viz., *A. praelonga*, *A. crassifolia*, *A. grandifolia* (subspecies of *A. esculenta* according to Lüning, 1990) and *A. esculenta* (Robinson and Cole, 1971; Lewis, 1996).

Lewis (1996) suggested that the evolution of most taxa of brown algae was accompanied by polyploidy. Nakahara & Nakamura (1973) obtained diploid gametophytes in several members of the Laminariales. When these were crossed with haploid or diploid gametophytes, triploid and tetraploid sporophytes were obtained respectively. They even managed to cross diploid with triploid gametophytes of *A. crassifolia* resulting in pentaploid sporophytes. These laboratory investigations illustrate hypothetical mechanisms by which polyploidy could have arisen in the genus *Alaria*. The existence of polyploids suggests one mechanism by which speciation has probably occured (Lewis, 1996).

In hybridization, two sets of chromosomes from different sources are combined. The succes of hybridization between species by sexual means initially depends on the compatibility of the gametes and then on the compatibility of the genomes. The results presented here (Table 3) show that the *Alaria* species with matching chromosome numbers, i.e., n =22-28, hybridized without difficulty, except for *A. crassifolia* which produced in two different reciprocal crosses stunted and abnormal sporophytes. This might be an indication of gradual reproductive isolation. Species with n=14 produced only two healthy viable crosses with n=22-28 species. Lewis (1996) showed that there are few exceptions in brown algae that it is not necessary for the chromosome number to match to obtain hybrids. Crosses among n=14 species showed even more incompatibility. No viable crosses were produced except for the *A. marginata* x *A. tenuifolia* cross. In n=14 crosses incompatibility is probably caused by incompatibility of the gametes or perhaps by chromosomal mispairing at meiosis. Hybridization results indicate that *A. esculenta*, *A.praelonga* and *A.crassifolia* are most closely related, and to a lesser extent, to *A. tenuifolia and A. marginata* and least related to *A. nana*. These findings are in contrast with the restriction patterns which are probably caused by the few informative restriction enzymes (see discussion on genetic analysis).

Kelp evolution and divergence

The ability of the Atlantic *A. esculenta* to hybridise with several Pacific *Alaria* species indicates a close relationship between the species in the two oceans and is further support of the original hypothesis of Stam *et al.*, (1988) that species of laminariales diverged from a common ancestor 15-19 Ma ago, most probably in the north Pacific, and then invaded the North Atlantic after inundation of the Bering Land Bridge 3.5 Ma ago. It is possible that *Alaria* species with n=14 chromosomes diverged first from a common ancestor of the genus *Alaria*, representing the oldest assemblage of *Alaria* species at the beginning of the miocene 27 Ma ago. Probably during the mid-Miocene steepening of the temperature gradient between high and low latitudes extensive radiation in North Pacific Laminariales took place (Stam *et al*,. 1988). This major cooling step in the Tertiary leading to glaciation of the higher latitudes was possibly the driving force of speciation in Laminariales (Lüning, 1990). The climatic disturbance coupled with the continously changing configuration of the archipelagos in the North Pacific due to plate tectonics and

sea level fluctuation could provide the ecological opportunity in which polyploids (amphiploids) can exploit their inherent advantage (Grant, 1981). Climatic changes in general bring about changes in the distribution of the species, altering genetic isolation. New contacts are made, natural hybridization occurs, and hybrid polyploids are formed (Grant, 1981). It therefore is possible that n=22-28 *Alaria* species are polyploid *Alaria* species and diverged from hybrids of n=14 species and radiated in several new species during the mid-Miocene cooling period. Possible doners for the polyploid hybrids might be *A. tenuifolia* and *A. marginata* because of the ability of these doners to hybridize with the postulated polyploid *Alaria* species (see Table 3). Not untill the opening of the Bering Land Bridge were *Alaria* species and Laminariales in general able to invade the Atlantic Ocean. Because polyploids often exhibit superior vigor, homeostatic buffering and environmental adaptability as compared with their diploid partners they are likely to be the most suitable candidates to invade the Atlantic and adapt to their new environment (Grant, 1981). With our limited knowledge of chromosome numbers in the genus *Alaria*, *A. praelonga*, *A. crassifolia* and *A. esculenta* are the most likely candidates from which only *A. esculenta* has really invaded the Atlantic. There is a possibility that other species might have invaded the Atlantic but did not survive the severe Pleistocene glaciation conditions.

Remarkably, nowadays the species with n=22-28 all occur at the North West Pacific coast/polar circle area or in the case of *A. esculenta* in the Atlantic Ocean as well. The n=14 species are mainly found at the north eastern Pacific coast (Widdowson, 1971). In the geological past, different types of temperature stress have existed on both sites of the Pacific, with more extreme temperature ranges on the West Pacific coasts and more colder gradual ranges on the East Pacific coasts. Nowadays the Asiatic side of the Pacific has serverely compressed isotherms and a wide annual span of seawater tempera-tures (Lüning, 1990). This supports the idea that the hypothised polyploid species are found in the more extreme temperature ranges of the West Pacific coast/polar circle area (polar light regime) due to their possible superior adaptability.

4.3 Summary

Intraspecific crosses of the genus *Alaria esculenta*

The significant morphological best and fastest growing crosses were produced by the female Iceland x male Norway, female Ireland x male Norway, female Ireland x male Halifax and the Irish self cross. All except the female Ireland x male Norway cross produced well developed haptera and sporophylls and are therefore most suitable for rope cultivation in Atlantic Irish waters.

The restriction fragment length polymorphism technique distinguishes genetic different populations of the genus *A. esculenta* in the North Atlantic Ocean.

Interspecific crosses of Alria species

The significant morphological and fastest growing cross was produced between Atlantic *A. esculenta* and Pacific *A. tenuifolia*, producing the largest and best developed plants. This cross would be most suitable to be used in closed tank cultivation.

There is a close realtionship between Atlantic and Pacific *Alaria* species indicating a recent species radiation in the genus *Alaria*.

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Appendices

I Statistical results of the multiple comparisons using Dunn's test for relative growth rates settlement length.

II Statistical results of the multiple comparisons using Dunn's test for relative growth rates length.

III Statistical results of the multiple comparisons using Dunn's test for relative growth rates settlement width.

IV Statistical results of the multiple comparisons using Dunn's test for relative growth rates width.

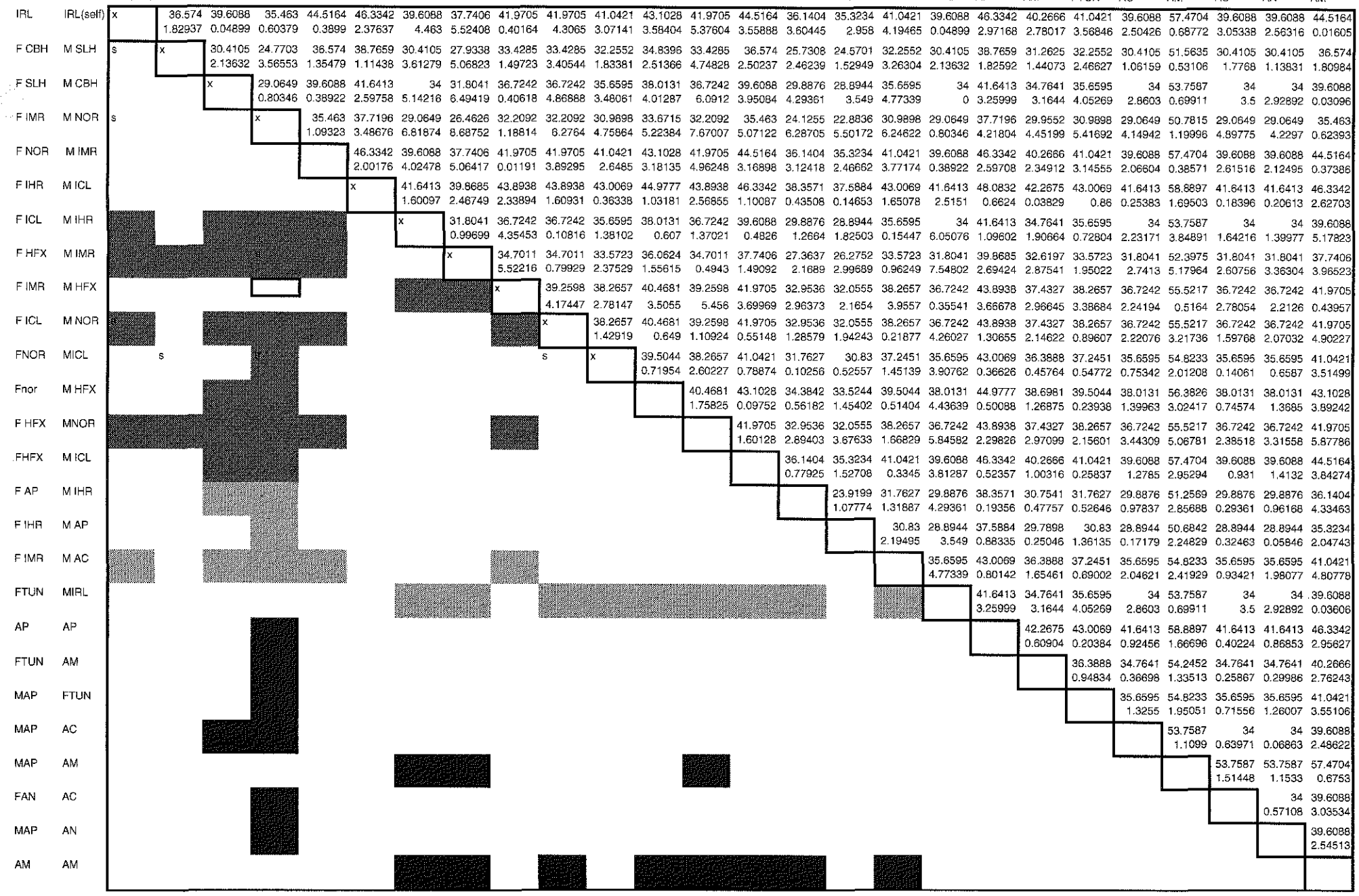
V Statistical results of the multiple comparisons using Dunn's test for plant lengths.

VI Statistical results of the multiple comparisons using Dunn's test for plant widths.

VII Restriction fragment data matrix for the ITS and RuBisCo spacer of *Alaria* species.

	IRL	F CBH	F SLH	F IMR	F NOR	F IHR	F ICL	F HFX	F IMR	F ICL	FNOR	FNOR	F HFX	FHFX	F AP	F IHR	F IMR	FTUN	AP	FTUN	MAP	MAP	MAP	FAN	MAP	AM
	IRL(self)	M SLH	M CBH	M NOR	M IMR	M ICL	M IHR	M IMR	M HFX	M NOR	MICL	M HFX	MNOR	M ICL	M IHR	M AP	M AC	MIRL	AP	AM	FTUN	AC	AM	AC	AN	AM
IRL	IRL(self)		36.39086	39.88327	35.70877	44.82493	46.65527	39.88327	38.00218	42.26135	42.26135	41.32654	43.40155	42.26135	44.82493	36.39086	35.56823	41.32654	39.88327	46.65527	40.54568	41.32654	39.88327	57.86874	39.88327	39.88327	44.82493
F CBH	M SLH	4.32123		3.004303	6.620176	5.478456	2.768278	3.631132	4.254102	1.906504	3.336757	2.106913	0.041555	0.494654	2.453992	4.416783	4.299956	1.981086	3.865148	0.515942	0.803327	0.47116	0.413409	0.637732	4.406311	5.051945	5.172504
F SLH	M CBH		30.09478		24.29273	36.39086	38.62297	30.09478	27.55333	33.18195	33.18195	31.98286	34.62246	33.18195	36.39086	25.28467	24.08566	31.98286	30.09478	38.62297	30.96727	31.98286	30.09478	51.61212	30.09478	30.09478	36.39086
F IMR	M NOR		1.243798	3.257975		2.426933	0.727507	0.413089	0.160123	2.31095	0.489344	2.194357	4.594036	4.109116	1.298492	0.137525	0.179005	2.356944	0.102958	3.448254	4.026245	4.307989	4.677395	2.331787	0.614221	1.469851	2.050073
F IHR	M ICL		29.26632	39.88327	41.92988		34.23561	32.02448	36.97868	36.97868	35.90661	38.27657	36.97868	39.88327	30.09478	29.09467	35.90661	34.23561	41.92988	35.00504	35.90661	34.23561	54.13125	34.23561	34.23561	39.88327
F ICL	M IHR		3.983313	3.152951	0.222594	0.730234		1.306618	1.061422	0.573153	0.912088	3.177531	2.674965	0.246254	1.359342	1.138366	1.056909	1.002855	2.283574	2.492498	2.79475	3.0183	1.531771	1.63329	2.385431	2.80909
F HFX	M IMR			35.70877	37.98099	29.26632	26.64598		32.43245	32.43245	31.20457	33.90482	32.43245	35.70877	24.29273	23.04215	31.20457	29.26632	37.98099	30.16279	31.20457	29.26632	51.13348	29.26632	29.26632	35.70877
F IMR	M HFX			0.256886	2.823612	3.129089	2.80467	4.804661		2.940958	4.785418	7.025607	6.644382	3.5397	3.114835	3.621908	4.95206	2.810179	5.590347	6.757562	6.951767	7.514106	3.901428	2.072698	1.192847	0.127173
F ICL	M NOR				46.65527	39.88327	38.00218	42.26135	42.26135		41.32654	43.40155	42.26135	44.82493	36.39086	35.56823	41.32654	39.88327	46.65527	40.54568	41.32654	39.88327	57.86874	39.88327	39.88327	44.82493
FNOR	MICL				2.495252	2.526122	2.207932	3.904277	2.474023	3.835307		5.699681	5.316126	3.024465	2.33138	2.60428	3.961135	2.292105	4.747588	5.253335	5.47106	5.743845	3.605862	1.750943	1.105309	0.305952
Fnor	M HFX					41.92988	40.14481	44.19798	44.19798	43.305	45.28945		44.19798	46.65527	38.62297	37.84889	43.305	41.92988	48.41646	42.56044	43.305	41.92988	59.29781	41.92988	41.92988	46.65527
F HFX	MNOR					0.37364	0.809829	1.210202	0.268363	0.952155	3.024093	2.390181		0.433385	0.676789	0.615878	1.249266	0.577301	2.506168	1.994845	2.577119	2.601701	2.200102	0.785583	1.725102	2.449384
FHFX	M ICL						32.02448	36.97868	36.97868	35.90661	38.27657	36.97868	39.88327	30.09478	29.09467	35.90661	34.23561	41.92988	35.00504	35.90661	34.23561	54.13125	34.23561	34.23561	39.88327
F AP	M IHR						0.525965	1.737488	0.102912	1.561711	3.965123	3.451083	0.909732	0.430223	0.203603	2.091725	0.320792	3.362891	3.27875	2.989515	3.666139	3.005482	0.903056	1.046838	2.542257
F IHR	M AP							34.94157	34.94157	33.80495	36.31234	34.94157	38.00218	27.55333	26.45733	33.80495	32.02448	40.14481	32.84575	33.80495	32.02448	52.76061	32.02448	32.02448	38.00218
F IMR	M AC							2.320839	0.590967	2.134814	4.678346	4.028452	1.528332	0.025739	0.249656	2.099715	0.272577	5.200591	3.818782	4.440158	3.616335	3.798373	0.416297	1.243515	1.330386
FTUN	MIRL								39.53187	38.5309	40.74852	39.53187	42.26135	33.18195	32.27764	38.5309	36.97868	44.19798	37.69216	38.5309	36.97868	55.90651	36.97868	36.97868	42.26135
AP	AP								1.529005	0.164424	2.083762	1.548541	0.763765	2.080384	1.776024	0.032885	1.74115	1.702733	1.487098	1.58574	1.732981	0.987979	2.52484	3.138174	4.09116
FTUN	AM									38.5309	40.74852	39.53187	42.26135	33.18195	32.27764	38.5309	36.97868	44.19798	37.69216	38.5309	36.97868	55.90651	36.97868	36.97868	42.26135
MAP	FTUN									1.400031	3.504899	2.947619	0.761153	0.498703	0.301679	1.49612	0.310896	3.524339	3.359739	3.154467	3.367556	2.355563	0.921205	1.569447	2.456585
MAP	AC										39.77818	38.5309	41.32654	31.98286	31.04364	37.50323	35.90661	43.305	36.64097	37.50323	35.90661	55.20326	35.90661	35.90661	41.32654
MAP	AM										2.234265	1.717236	0.554815	1.782368	1.5939	0.162587	2.097478	1.969805	1.755593	1.802512	1.965748	1.15845	2.419878	3.050849	4.032286
FAN	AC											40.74852	43.40155	34.62246	33.75676	39.77818	38.27657	45.28945	38.96629	39.77818	38.27657	56.77333	38.27657	38.27657	43.40155
MAP	AN											0.55728	2.743745	3.744891	4.469508	2.416784	4.504542	0.766513	1.018314	0.630244	0.459842	1.01128	3.920155	5.217116	5.874093
AM	AM												42.26135	33.18195	32.27764	38.5309	36.97868	44.19798	37.69216	38.5309	36.97868	55.90651	36.97868	36.97868	42.26135
													2.108197	4.21391	3.979183	1.888821	3.458263	0.082185	0.302787	0.038761	0.119438	0.432682	3.503177	4.791005	5.474888
														36.39086	35.56823	41.32654	39.88327	46.65527	40.54568	41.32654	39.88327	57.86874	39.88327	39.88327	44.82493
														1.394046	1.207308	0.680642	1.068436	2.154501	1.659589	1.940372	2.004316	1.802788	1.621334	2.256421	2.948641
															24.08566	31.98286	30.09478	38.62297	30.96727	31.98286	30.09478	51.61212	30.09478	30.09478	36.39086
															0.323376	2.465667	0.218502	3.538285	3.318209	4.561561	4.509991	3.871629	0.469238	1.354307	2.363421
																31.04364	29.09467	37.84889	29.99627	31.04364	29.09467	51.03543	29.09467	29.09467	35.56823
																2.28937	0.04169	3.404866	4.012845	4.299443	4.395545	3.988257	0.446676	0.951227	1.546286
																	35.90661	43.305	36.64097	37.50323	35.90661	55.20326	35.90661	35.90661	41.32654
																	2.013093	1.334719	1.345488	1.663857	1.820928	0.814565	1.700383	3.331328	4.177106
																		41.92988	35.00504	35.90661	34.23561	54.13125	34.23561	34.23561	39.88327
																		3.102402	3.473309	3.750935	4.021155	2.166032	0.630435	1.382576	2.269636
																			42.56044	43.305	41.92988	59.29781	41.92988	41.92988	46.65527
																			0.199716	0.106223	0.180857	0.216422	3.61715	4.23127	4.453639
																				36.64097	35.00504	54.62113	35.00504	35.00504	40.54568
																				0.357523	0.459458	0.079334	4.089887	4.825495	4.915092
																					35.90661	55.20326	35.90661	35.90661	41.32654
																					0.083086	0.315803	4.352031	5.069169	5.139208
																						54.13125	34.23561	34.23561	39.88327
																						0.37717	4.65159	5.403731	5.399984
																							54.13125	54.13125	57.86874
																							2.564754	3.04045	3.368872
																								34.23561	39.88327
																								0.752141	1.407082
																									39.88327
																									0.761448

APPENDIX I (RGR-set-length)



		IRL	F CBH	F SLH	F IMR	F NOR	F IHR	F ICL	F HFX	F IMR	F ICL	FNOR	Fnor	F HFX	FHFX	F AP	F IHR	F IMR	FTUN	AP	FTUN	MAP	MAP	MAP	FAN	MAP	AM
		IRL(self)	M SLH	M CBH	M NOR	M IMR	M ICL	M IHR	M IMR	M HFX	M NOR	MICL	M HFX	MNOR	M ICL	M IHR	M AP	M AC	MIRL	AP	AM	FTUN	AC	AM	AC	AN	AM
IRL	IRL(self)	x	36.574	39.6088	35.463	44.5164	46.3342	39.6088	37.7406	41.9705	41.9705	41.0421	43.1028	41.9705	44.5164	36.1404	35.3234	41.0421	39.6088	46.3342	40.2666	41.0421	39.6088	57.4704	39.6088	39.6088	44.5164
			1.82937	0.04899	0.60379	0.3899	2.37637	4.463	5.52408	0.40164	4.3065	3.07141	3.58404	5.37604	3.55888	3.60445	2.958	4.19465	0.04899	2.97168	2.78017	3.56846	2.50426	0.68772	3.05338	2.56316	0.01605
F CBH	M SLH	s	x	30.4105	24.7703	36.574	38.7659	30.4105	27.9338	33.4285	33.4285	32.2552	34.8396	33.4285	36.574	25.7308	24.5701	32.2552	30.4105	38.7659	31.2625	32.2552	30.4105	51.5635	30.4105	30.4105	36.574
				2.13632	3.56553	1.35479	1.11438	3.61279	5.06823	1.49723	3.40544	1.83381	2.51366	4.74828	2.50237	2.46239	1.52949	3.26304	2.13632	1.82592	1.44073	2.46627	1.06159	0.53106	1.7768	1.13831	1.80984
F SLH	M CBH			x	29.0649	39.6088	41.6413	34	31.8041	36.7242	36.7242	35.6595	38.0131	36.7242	39.6088	29.8876	28.8944	35.6595	34	41.6413	34.7641	35.6595	34	53.7587	34	34	39.6088
					0.80346	0.38922	2.59758	5.14216	6.49419	0.40618	4.86888	3.48061	4.01287	6.0912	3.95084	4.29361	3.549	4.77339	0	3.25999	3.1644	4.05269	2.8603	0.69911	3.5	2.92892	0.03096
F IMR	M NOR	s			x	35.463	37.7196	29.0649	26.4626	32.2092	32.2092	30.9898	33.6715	32.2092	35.463	24.1255	22.8836	30.9898	29.0649	37.7196	29.9552	30.9898	29.0649	50.7815	29.0649	29.0649	35.463
						1.09323	3.48676	6.81874	8.68752	1.18814	6.2764	4.75864	5.22384	7.67007	5.07122	6.28705	5.50172	6.24622	0.80346	4.21804	4.45199	5.41692	4.14942	1.19996	4.89775	4.2297	0.62393
F NOR	M IMR					x	46.3342	39.6088	37.7406	41.9705	41.9705	41.0421	43.1028	41.9705	44.5164	36.1404	35.3234	41.0421	39.6088	46.3342	40.2666	41.0421	39.6088	57.4704	39.6088	39.6088	44.5164
							2.00176	4.02478	5.06417	0.01191	3.89295	2.6485	3.18135	4.96248	3.16898	3.12418	2.46662	3.77174	0.38922	2.59708	2.34912	3.14555	2.06604	0.38571	2.61516	2.12495	0.37386
F IHR	M ICL						x	41.6413	39.6685	43.8938	43.8938	43.0069	44.9777	43.8938	46.3342	38.3571	37.5884	43.0069	41.6413	48.0832	42.2675	43.0069	41.6413	58.8897	41.6413	41.6413	46.3342
								1.60097	2.46749	2.33894	1.60931	0.36338	1.03181	2.56855	1.10087	0.43508	0.14653	1.65078	2.5151	0.6624	0.03829	0.86	0.25383	1.69503	0.18396	0.20613	2.62703
F ICL	M IHR							x	31.8041	36.7242	36.7242	35.6595	38.0131	36.7242	39.6088	29.8876	28.8944	35.6595	34	41.6413	34.7641	35.6595	34	53.7587	34	34	39.6088
									0.99699	4.35453	0.10816	1.38102	0.607	1.37021	0.4826	1.2664	1.82503	0.15447	6.05076	1.09602	1.90664	0.72804	2.23171	3.84891	1.64216	1.39977	5.17823
F HFX	M IMR								x	34.7011	34.7011	33.5723	36.0624	34.7011	37.7406	27.3637	26.2752	33.5723	31.8041	39.8685	32.6197	33.5723	31.8041	52.3975	31.8041	31.8041	37.7406
										5.52216	0.79929	2.37529	1.55615	0.4943	1.49092	2.1689	2.99689	0.96249	7.54802	2.69424	2.87541	1.95022	2.7413	5.17964	2.60756	3.36304	3.96523
F IMR	M HFX									x	39.2598	38.2657	40.4681	39.2598	41.9705	32.9536	32.0555	38.2657	36.7242	43.8938	37.4327	38.2657	36.7242	55.5217	36.7242	36.7242	41.9705
											4.17447	2.78147	3.5055	5.456	3.69969	2.96373	2.1654	3.9557	0.35541	3.66678	2.96645	3.38684	2.24194	0.5164	2.78054	2.2126	0.43957
F ICL	M NOR	s									x	38.2657	40.4681	39.2598	41.9705	32.9536	32.0555	38.2657	36.7242	43.8938	37.4327	38.2657	36.7242	55.5217	36.7242	36.7242	41.9705
												1.42919	0.649	1.10924	0.55148	1.28579	1.94243	0.21877	4.26027	1.30655	2.14622	0.89607	2.22076	3.21736	1.59768	2.07032	4.90227
FNOR	MICL		s								s	x	39.5044	38.2657	41.0421	31.7627	30.83	37.2451	35.6595	43.0069	36.3888	37.2451	35.6595	54.8233	35.6595	35.6595	41.0421
													0.71954	2.60227	0.78874	0.10256	0.52557	1.45139	3.90762	0.36626	0.45764	0.54772	0.75342	2.01208	0.14061	0.6587	3.51499
Fnor	M HFX													40.4681	43.1028	34.3842	33.5244	39.5044	38.0131	44.9777	38.6981	39.5044	38.0131	56.3826	38.0131	38.0131	43.1028
														1.75825	0.09752	0.56182	1.45402	0.51404	4.43639	0.50088	1.26875	0.23938	1.39963	3.02417	0.74574	1.3685	3.89242
F HFX	MNOR														41.9705	32.9536	32.0555	38.2657	36.7242	43.8938	37.4327	38.2657	36.7242	55.5217	36.7242	36.7242	41.9705
															1.60128	2.89403	3.67633	1.66829	5.84582	2.29826	2.97099	2.15601	3.44309	5.06781	2.38518	3.31558	5.87786
FHFX	M ICL															36.1404	35.3234	41.0421	39.6088	46.3342	40.2666	41.0421	39.6088	57.4704	39.6088	39.6088	44.5164
																0.77925	1.52708	0.3345	3.81287	0.52357	1.00316	0.25837	1.2785	2.95294	0.931	1.4132	3.84274
F AP	M IHR																23.9199	31.7627	29.8876	38.3571	30.7541	31.7627	29.8876	51.2569	29.8876	29.8876	36.1404
																	1.07774	1.31887	4.29361	0.19356	0.47757	0.52646	0.97837	2.85688	0.29361	0.96168	4.33463
F IHR	M AP																	30.83	28.8944	37.5884	29.7898	30.83	28.8944	50.6842	28.8944	28.8944	35.3234
																		2.19495	3.549	0.88335	0.25046	1.36135	0.17179	2.24829	0.32463	0.05846	2.04743
F IMR	M AC																		35.6595	43.0069	36.3888	37.2451	35.6595	54.8233	35.6595	35.6595	41.0421
																			4.77339	0.80142	1.65461	0.69002	2.04621	2.41929	0.93421	1.98077	4.80778
FTUN	MIRL																			41.6413	34.7641	35.6595	34	53.7587	34	34	39.6088
																				3.25999	3.1644	4.05269	2.8603	0.69911	3.5	2.92892	0.03606
AP	AP																				42.2675	43.0069	41.6413	58.8897	41.6413	41.6413	46.3342
																					0.60904	0.20384	0.92456	1.66696	0.40224	0.86853	2.95627
FTUN	AM																					36.3888	34.7641	54.2452	34.7641	34.7641	40.2666
																						0.94834	0.36698	1.33513	0.25867	0.29986	2.76243
MAP	FTUN																						35.6595	54.8233	35.6595	35.6595	41.0421
																							1.3255	1.95051	0.71556	1.26007	3.55106
MAP	AC																							53.7587	34	34	39.6088
																								1.1099	0.63971	0.06863	2.48622
MAP	AM																								53.7587	53.7587	57.4704
																									1.51448	1.1533	0.6753
FAN	AC																									34	39.6088
																										0.57108	3.03534
MAP	AN																										39.6088
																											2.54513
AM	AM																										

APPENDIX II (RGR-length)

		IRL	F CBH	F SLH	F IMR	F NOR	F IHR	F ICL	F HFX	F IMR	F ICL	FNOR	Fnor	F HFX	FHFX	F AP	F IHR	F IMR	FTUN	AP	FTUN	MAP	MAP	MAP	FAN	MAP	AM
		IRL(self)	M SLH	M CBH	M NOR	M IMR	M ICL	M IHR	M IMR	M HFX	M NOR	MICL	M HFX	MNOR	M ICL	M IHR	M AP	M AC	MIRL	AP	AM	FTUN	AC	AM	AC	AN	AM
IRL	IRL(self)	x	34.96161	38.09853	34.11084	42.81906	44.56749	38.09853	36.30162	40.37019	40.37019	39.47722	41.45937	40.37019	42.81906	35.68255	35.17944	40.37019	38.09853	44.56749	38.73129	39.47722	38.09853	55.27916	38.09853	38.09853	42.81906
			5.097427	4.159015	6.895427	5.670039	2.900898	3.036925	3.299735	1.75892	3.801131	3.62198	0.181761	3.688286	3.001	4.25923	4.369333	2.881861	1.247705	0.442347	0.529623	0.354273	0.162798	0.985044	2.264804	4.942072	0
F CBH	M SLH		x	28.98864	23.50302	34.96161	37.08239	28.98864	26.5829	31.91544	31.91544	30.77814	33.28246	31.91544	34.96161	25.73105	25.02871	31.91544	28.98864	37.08239	29.81536	30.77814	28.98864	49.44319	28.98864	28.98864	34.96161
				0.681712	2.424986	1.846924	1.319456	2.156428	2.197976	3.359075	0.77586	1.144597	5.128183	0.918598	1.421968	1.019551	0.979019	1.938655	4.507924	5.337535	5.289263	5.335884	5.93377	2.503113	3.171193	0.3474275	5.097427
F SLH	M CBH			x	27.95668	38.09853	40.05356	32.70359	30.59141	35.32392	35.32392	34.29982	36.56373	35.32392	38.09853	29.85416	29.25098	35.32392	32.70359	40.05356	33.4386	34.29982	32.70359	51.70892	32.70359	32.70359	38.09853
					2.745548	2.213558	0.728192	1.307196	1.263971	2.475502	0.141547	0.450926	4.127496	0.270512	0.786182	0.216795	0.162103	1.192142	3.391574	4.448211	4.125155	4.211878	4.655452	2.011258	2.206689	0.912234	4.159015
F IMR	M NOR				x	34.11084	36.28138	27.95668	25.45359	30.98113	30.98113	29.8082	32.38761	30.98113	34.11084	24.56261	23.82585	30.98113	27.95668	36.28138	28.81303	29.8082	27.95668	48.8453	27.95668	27.95668	34.11084
						0.222127	2.919489	4.274699	4.534648	5.300029	2.63891	3.093883	7.029635	2.785953	3.128296	3.388428	3.420574	3.836772	6.712996	7.026278	7.451344	7.421552	8.191473	3.700589	5.326923	1.678421	6.895427
F NOR	M IMR						44.56749	38.09853	36.30162	40.37019	40.37019	39.47722	41.45937	40.37019	42.81906	35.68255	35.17944	40.37019	38.09853	44.56749	38.73129	39.47722	38.09853	55.27916	38.09853	38.09853	42.81906
							2.5467	3.335649	3.388278	4.255065	2.212854	2.52804	5.67423	2.325698	2.669039	2.544817	2.532018	3.132124	5.124869	5.889944	5.738841	5.795748	6.209775	3.40695	4.10777	1.4305015	5.670039
F IHR	M ICL						x	40.05356	38.34836	42.22016	42.22016	41.36714	43.26279	42.22016	44.56749	37.76286	37.28783	42.22016	40.05356	46.24987	40.6559	41.36714	40.05356	56.64429	40.05356	40.05356	44.56749
								0.339129	0.247729	1.519694	0.572396	0.32449	2.943157	0.453302	0.01861	0.509215	0.6468	0.347149	1.936279	3.720019	2.35185	2.835997	2.975389	1.868332	0.759123	1.4730283	3.227821
F ICL	M IHR							x	30.59141	35.32392	35.32392	34.29982	36.56373	35.32392	38.09853	29.85416	29.25098	35.32392	32.70359	40.05356	33.4386	34.29982	32.70359	51.70892	32.70359	32.70359	38.09853
									0.13348	1.265274	1.068681	0.772376	3.062137	0.967773	0.350009	1.027003	0.997632	0.0214	2.330406	3.833569	2.910671	2.539516	3.274659	1.785724	0.899493	1.403691	3.53791
F HFX	M IMR								x	33.37797	33.37797	32.29221	34.6874	33.37797	36.30162	27.52418	26.86874	33.37797	30.59141	38.34836	31.37593	32.29221	30.59141	50.39962	30.59141	30.59141	36.30162
										1.461377	1.008649	0.695069	3.362997	0.872166	0.269857	0.928131	1.016389	0.094884	2.624965	5.191906	2.974199	3.458487	2.961882	2.082275	1.037402	2.2391912	2.376718
F IMR	M HFX									x	37.76286	36.80668	38.92506	37.76286	40.37019	32.70359	32.15391	37.76286	35.32392	42.22016	36.00547	36.80668	35.32392	53.40475	35.32392	35.32392	40.37019
											2.183215	1.906047	1.680811	2.116162	1.562001	2.199933	2.124667	1.200474	0.581425	2.774075	1.570414	1.510008	1.834608	0.392124	0.424318	3.1863182	2.010194
F ICL	M NOR										x	36.80668	38.92506	37.76286	40.37019	32.70359	32.15391	37.76286	35.32392	42.22016	36.00547	36.80668	35.32392	53.40475	35.32392	35.32392	40.37019
												0.284369	3.748656	0.117034	0.641036	0.038986	0.006841	0.982741	2.623635	5.295035	4.13447	3.693223	4.168564	2.344851	1.865457	0.946386	4.344149
FNOR	MICL											x	37.99814	36.80668	39.47722	31.59467	31.02535	36.80668	34.29982	41.36714	35.00132	35.82499	34.29982	52.73296	34.29982	34.2998	39.47722
													3.564648	0.160599	0.366939	0.227839	0.271676	0.843321	3.021079	5.149602	3.947505	3.504799	3.987873	2.140185	1.619939	1.264484	4.168702
Fnor	M HFX													38.92506	41.45937	34.03898	33.51121	38.92506	36.56373	43.26279	37.22259	37.99814	36.56373	54.23278	36.56373	36.56373	41.45937
														3.631622	3.10762	3.484	4.294341	3.196499	1.175123	0.813161	0.387252	0.192473	0.034254	1.283148	1.820271	4.8559220	0.198318
F HFX	MNOR														40.37019	32.70359	32.15391	37.76286	35.32392	42.22016	36.00547	36.80668	35.32392	53.40475	35.32392	35.32392	40.37019
															0.505245	0.094281	0.147198	1.012491	2.684148	4.464999	3.399738	3.819257	4.039598	2.673668	1.482967	1.093974	4.045375
FHFX	M ICL															35.68255	35.17944	40.37019	38.09853	44.56749	38.73129	39.47722	38.09853	55.27916	38.09853	38.09853	42.81906
																0.658029	0.716632	0.301181	2.005546	3.890288	2.423	2.569458	2.7441	1.911829	1.089927	1.543602	3.255042
F AP	M IHR																26.02625	32.70359	29.85416	37.76286	30.65755	31.59467	29.85416	49.95557	29.85416	29.8541	35.68255
																	0.066493	1.089755	3.498489	4.546649	3.481388	4.501156	4.613999	3.086842	2.079289	1.2160975	5.090753
F IHR	M AP																	32.15391	29.25098	37.28783	30.0705	31.02535	29.25098	49.59746	29.25098	29.2509	35.17944
																		1.162205	3.629792	4.650981	4.429515	4.503575	4.754445	3.393333	1.359445	0.6971123	3.099165
F IMR	M AC																		35.32392	42.22016	36.00547	36.80668	35.32392	53.40475	35.32392	35.32392	40.37019
																			1.947846	3.222526	2.661492	2.780895	3.117969	1.158865	0.562788	2.0367063	3.293555
FTUN	MIRL																			40.05356	33.4386	34.29982	32.70359	51.70892	32.70359	32.70359	38.09853
																				1.679002	0.80813	0.978139	1.263877	0.133762	1.184885	4.303808	1.453532
AP	AP																				40.6559	41.36714	40.05356	56.64429	40.05356	40.05356	44.56749
																					0.989457	0.814656	0.64705	1.309341	2.646456	5.193047	0.442347
FTUN	AM																					35.00132	33.4386	52.17689	33.4386	33.4386	38.73129
																						0.186486	0.427967	0.650468	1.96697	5.0173370	0.529623
MAP	FTUN																						34.29982	52.73296	34.29982	34.2998	39.47722
																							0.226921	0.767388	2.107883	5.0816590	0.354273
MAP	AC																							51.70892	32.70359	32.70359	38.09853
																								0.933108	2.448762	5.5676860	0.162798
MAP	AM																								51.70892	51.70892	55.27916
																									0.615626	2.5882060	0.985044
FAN	AC																									32.70359	38.09853
																										3.1189232	2.264804
MAP	AN																										38.09853
																											4.942072
AM	AM																										

APPENDIX III (RGRset-Width)

	IRL	F CBH	F SLH	F IMR	F NOR	F IHR	F ICL	F HFX	F IMR	F ICL	FNOR	Fnor	F HFX	FHFX	F AP	F IHR	F IMR	FTUN	AP	FTUN	MAP	MAP	MAP	FAN	MAP	AM
	IRL(self)	M SLH	M CBH	M NOR	M IMR	M ICL	M IHR	M IMR	M HFX	M NOR	MICL	M HFX	MNOR	M ICL	M IHR	M AP	M AC	MIRL	AP	AM	FTUN	AC	AM	AC	AN	AM
IRL	IRL(self)	35.33958	38.51041	34.47961	43.28197	45.0493	38.51041	36.69407	40.80663	40.80663	39.904	41.90759	40.80663	43.28197	35.33958	35.55977	40.80663	38.51041	45.0493	39.15001	39.904	38.51041	55.87678	38.51041	38.51041	43.28197
		0.850254	0.538672	2.448015	1.254234	0.328212	1.727733	0.867939	2.133173	1.441954	2.949221	1.838228	2.48209	2.425953	1.515905	1.260253	0.733619	2.714788	0.013742	1.997312	1.766382	0.639283	0.291458	2.218942	2.461301	0
F CBH	M SLH		29.30203	23.75711	35.33958	37.48328	29.30203	26.87028	32.26047	32.26047	31.11087	33.64228	32.26047	35.33958	24.98886	25.29929	32.26047	29.30203	37.48328	30.13769	31.11087	29.30203	49.97771	29.30203	29.30203	35.33958
			0.076786	2.288114	2.386371	1.196089	3.296131	2.303505	1.766868	2.75535	4.748608	3.182999	4.071029	3.821427	0.941372	0.583679	0.003444	2.542486	0.818142	3.591596	3.231453	1.865627	0.92708	3.941706	4.260228	0.850254
F SLH	M CBH			28.25892	38.51041	40.48657	33.05715	30.92213	35.7058	35.7058	34.67063	36.95902	35.7058	38.51041	29.30203	29.56721	35.7058	33.05715	40.48657	33.8001	34.67063	33.05715	52.26794	33.05715	33.05715	38.51041
				1.843983	2.24831	1.162937	2.989772	2.074431	1.533364	2.552495	4.325948	2.958232	3.741222	3.565208	0.726018	0.423329	0.066127	2.185609	0.813027	3.268997	2.964565	1.721766	0.929505	3.562013	3.844353	0.838672
F IMR	M NOR				34.47961	36.67361	28.25892	25.72877	31.31606	31.31606	30.13045	32.73775	31.31606	34.47961	23.75711	24.08343	31.31606	28.25892	36.67361	29.12453	30.13045	28.25892	49.37336	28.25892	28.25892	34.47961
					4.022445	2.704732	5.341404	4.518475	0.084335	4.574262	6.707245	4.931381	5.929619	5.493293	1.297934	1.643965	1.739366	0.712732	2.318442	5.582972	5.140724	3.858097	2.039405	6.01081	6.341089	2.448015
F NOR	M IMR					45.0493	38.51041	36.69407	40.80663	40.80663	39.904	41.90759	40.80663	43.28197	35.33958	35.55977	40.80663	38.51041	45.0493	39.15001	39.904	38.51041	55.87678	38.51041	38.51041	43.28197
						0.876817	0.318096	0.611475	3.463489	0.111638	1.588813	0.542861	1.151774	1.171719	3.052021	2.786858	2.063935	4.124426	1.191287	0.610705	0.405974	0.770354	0.680068	0.809305	1.051664	1.254234
F IHR	M ICL						40.48657	38.76294	42.6766	42.6766	41.81435	43.7305	42.6766	45.0493	37.48328	37.69095	42.6766	40.48657	46.74987	41.09543	41.81435	40.48657	57.25666	40.48657	40.48657	45.0493
							1.278202	0.440176	2.62708	1.032312	2.411158	1.488723	1.978025	2.113905	1.517385	1.590042	1.186551	2.796224	0.34991	1.356348	1.355382	0.235166	0.037049	1.234209	1.975964	0.3652
F ICL	M IHR							30.92213	35.7058	35.7058	34.67063	36.95902	35.7058	38.51041	29.30203	29.56721	35.7058	33.05715	40.48657	33.8001	34.67063	33.05715	52.26794	33.05715	33.05715	38.51041
								1.121769	4.301355	0.215496	1.43254	0.29407	1.002289	1.040728	3.363799	2.891426	3.292339	5.786252	1.846105	0.352695	0.097563	1.240134	1.449354	0.572241	0.540484	2.012748
F HFX	M IMR								33.73881	33.73881	32.64132	35.0624	33.73881	36.69407	26.87028	27.15922	33.73881	30.92213	38.76294	31.71514	32.64132	30.92213	50.94449	30.92213	30.92213	36.69407
									3.524008	0.800059	2.544177	1.339333	2.05809	2.241079	2.436218	2.272235	1.683779	5.076085	1.149855	1.373688	1.24951	0.186497	0.490696	1.642218	2.035355	0.625155
F IMR	M HFX									38.17111	37.20459	39.34588	38.17111	40.80663	32.26047	32.50153	38.17111	35.7058	42.6766	36.39472	37.20459	35.7058	53.9821	35.7058	35.7058	40.80663
										3.821972	5.363568	4.298626	5.062099	5.161933	0.899786	1.073386	1.496187	0.428852	2.717464	5.084143	4.127031	3.12741	2.421312	4.739698	4.887389	2.437912
F ICL	M NOR										37.20459	39.34588	38.17111	40.80663	32.26047	32.50153	38.17111	35.7058	42.6766	36.39472	37.20459	35.7058	53.9821	35.7058	35.7058	40.80663
											1.581645	0.462423	1.078752	1.173153	2.944968	2.71555	2.325785	4.003979	1.804754	0.595465	0.305059	0.95845	0.997164	0.73118	0.966129	1.647947
FNOR	MICL											38.40894	37.20459	39.904	31.11087	31.36077	37.20459	34.67063	41.81435	35.37971	36.21229	34.67063	53.30305	34.67063	34.67063	39.904
												1.058347	0.440606	0.317906	4.291728	4.072273	4.745036	7.143269	3.762886	1.259246	1.268661	2.684308	2.425005	0.911068	0.632382	3.394392
Fnor	M HFX												39.34588	41.90759	33.64228	33.8735	39.34588	36.95902	43.7305	37.625	38.40894	36.95902	54.81908	36.95902	36.95902	41.90759
													0.616329	0.71073	3.116551	3.621931	3.179696	5.397475	2.255943	0.034218	0.193367	1.332202	1.643713	0.192467	0.471761	2.005672
F HFX	MNOR													40.80663	32.26047	32.50153	38.17111	35.7058	42.6766	36.39472	37.20459	35.7058	53.9821	35.7058	35.7058	40.80663
														0.091022	4.800213	4.528762	4.037417	5.392386	2.637248	0.604932	0.862605	2.147177	2.380566	0.371007	0.178597	2.722398
FHFX	M ICL														35.33958	35.55977	40.80663	38.51041	45.0493	39.15001	39.904	38.51041	55.87678	38.51041	38.51041	43.28197
															4.487077	4.213028	3.30673	5.135137	2.71046	0.595019	0.766145	1.784289	2.266009	0.4993	0.260901	2.631315
F AP	M IHR															25.29929	32.26047	29.30203	37.48328	30.13769	31.11087	29.30203	49.97771	29.30203	29.30203	35.33958
																0.346142	0.732628	1.739681	1.445724	3.515333	4.116345	2.513284	2.245425	4.468656	5.063033	1.82825
F IHR	M AP																32.50153	29.56721	37.69095	30.39559	31.36077	29.56721	50.13365	29.56721	29.56721	35.55977
																	0.457756	2.020256	1.205418	4.046939	3.676568	2.214019	2.066478	2.598388	2.784557	0.893896
F IMR	M AC																	35.7058	42.6766	36.39472	37.20459	35.7058	53.9821	35.7058	35.7058	40.80663
																		2.089607	0.715979	2.971071	2.699189	1.527919	0.856251	2.13754	3.493052	0.838421
FTUN	MIRL																		40.48657	33.8001	34.67063	33.05715	52.26794	33.05715	33.05715	38.51041
																			2.597569	5.406565	5.048461	3.907375	2.311806	5.747622	6.029961	3.162633
AP	AP																			41.09543	41.81435	40.48657	57.25666	40.48657	40.48657	45.0493
																				1.887698	1.670878	0.592789	0.273622	2.095345	2.325874	0.013742
FTUN	AM																				35.37971	33.8001	52.74096	33.8001	33.8001	39.15001
																					0.217896	1.585077	1.173833	0.214721	0.490854	1.997312
MAP	FTUN																				34.67063	53.30305	34.67063	34.67063	39.904	
																					1.322926	1.016827	0.431681	0.700881	1.766382	
MAP	AC																				52.26794	33.05715	33.05715	38.51041		
																					0.159435	1.840247	2.122587	0.639283		
MAP	AM																				52.26794	52.26794	55.87678			
																					1.32331	1.501877	0.291458			
FAN	AC																				33.05715	38.51041				
																					0.282339	2.218942				
MAP	AN																				38.51041					
																					2.461301					
AM	AM																									

APPENDIX IV (RGR-Width)

	IRL	F CBH	F SLH	F IMR	F NOR	F IHR	F ICL	F HFX	F IMR	F ICL	FNOR	Fnor	F HFX	FHFX	F AP	F IHR	F IMR	FTUN	AP	FTUN	MAP	MAP	MAP	FAN	MAP	AM
	IRL(self)	M SLH	M CBH	M NOR	M IMR	M ICL	M IHR	M IMR	M HFX	M NOR	MICL	M HFX	MNOR	M ICL	M IHR	M AP	M AC	MIRL	AP	AM	FTUN	AC	AM	AC	AN	AM
IRL	IRL(self)	37.68443	41.80712	38.44411	49.0232	49.0232	40.47957	39.95724	42.66922	41.80712	41.80712	43.72294	42.66922	41.08832	37.9126	38.44411	49.0232	40.47957	46.74179	41.08832	41.80712	40.47957	41.08832	39.95724	40.47957	45.04151
		1.134863	0.363973	1.231097	1.008026	3.182507	4.803863	3.119521	1.75966	5.426507	3.066862	5.149852	5.98597	4.640963	2.831189	1.986969	2.794935	0.68759	3.536946	1.464878	4.339372	5.821866	5.431942	4.471928	4.73181	3.578974
F CBH	M SLH		31.35534	26.70714	40.47957	40.47957	29.5621	28.84272	32.49591	31.35534	31.35534	33.86764	32.49591	30.39033	25.93621	26.70714	40.47957	29.5621	37.68443	30.39033	31.35534	29.5621	30.39033	28.84272	29.5621	35.55362
			0.878638	0.170807	0.16428	2.797707	5.131288	2.838871	0.994484	5.871408	2.725213	5.385672	6.543901	4.867427	2.489611	1.258862	2.328335	0.505151	3.25466	0.573298	4.421894	6.525247	5.936846	4.712427	5.032626	3.331186
F SLH	M CBH			32.26438	44.34315	44.34315	34.66464	34.05322	37.19816	36.20603	36.20603	38.4023	37.19816	35.3736	31.62919	32.26438	44.34315	34.66464	41.80712	35.3736	36.20603	34.66464	35.3736	34.05322	34.66464	39.8971
				0.99527	0.771258	3.175237	5.170735	3.213522	1.609399	5.845711	3.121027	5.467121	6.457308	4.960556	2.912532	1.895917	2.74676	0.363964	3.592689	1.271364	4.590395	6.359507	5.879321	4.800405	5.086596	3.659055
F IMR	M NOR				41.18773	41.18773	30.52459	29.82843	33.37389	32.26438	32.26438	34.71095	33.37389	31.32739	27.02813	27.76876	41.18773	30.52459	38.44411	31.32739	32.26438	30.52459	31.32739	29.82843	30.52459	36.35784
					0.0507	2.63885	4.820045	2.592124	0.831635	5.564595	2.507044	5.123405	6.23506	4.576219	2.220253	1.046457	2.177548	0.638669	3.071686	0.410534	4.155921	6.170049	5.61365	4.403767	4.724494	3.132033
F NOR	M IMR					51.20306	43.0938	42.60352	45.15686	44.34315	44.34315	46.15382	45.15686	43.66612	40.69207	41.18773	51.20306	43.0938	49.0232	43.66612	44.34315	43.0938	43.66612	42.60352	43.0938	47.40482
						2.081907	3.36572	1.765834	0.568389	4.001745	1.77705	3.807919	4.561876	3.235294	1.423399	0.654821	1.710835	0.500845	2.366227	0.246707	2.976784	4.321968	3.979578	3.034238	3.298038	2.358109
F IHR	M ICL						43.0938	42.60352	45.15686	44.34315	44.34315	46.15382	45.15686	43.66612	40.69207	41.18773	51.20306	43.0938	49.0232	43.66612	44.34315	43.0938	43.66612	42.60352	43.0938	47.40482
							0.892046	0.736306	1.899686	1.568975	0.626929	1.559429	2.153668	0.767829	1.114799	1.956878	0.461302	2.503431	0.218129	1.954733	0.581687	1.796219	1.558756	0.519149	0.833851	0.120336
F ICL	M IHR							32.4096	35.69963	34.66464	34.66464	36.95263	35.69963	33.79426	29.85241	30.52459	43.0938	33.05143	40.47957	33.79426	34.66464	33.05143	33.79426	32.4096	33.05143	38.50376
								2.154019	3.343872	0.907806	1.855528	0.885869	1.758516	0.101994	2.440379	3.493821	1.924189	5.458714	0.673918	4.062424	0.322179	1.219389	0.828629	0.477209	0.086307	1.026114
F HFX	M IMR								35.10624	34.05322	34.05322	36.37968	35.10624	33.1668	29.14019	29.82843	42.60352	32.4096	39.95724	33.1668	34.05322	32.4096	33.1668	31.75479	32.4096	37.95423
									1.411832	2.911711	0.104813	2.951828	3.840143	1.939375	0.475807	1.374689	0.37294	3.322356	1.366791	1.512974	1.751618	2.778451	2.971103	1.586883	2.064025	1.102155
F IMR	M HFX									37.19816	37.19816	39.33908	38.16451	36.38843	32.76023	33.37389	45.15686	35.69963	42.66922	36.38843	37.19816	35.69963	36.38843	35.10624	35.69963	40.79958
										4.080399	1.428386	4.034698	4.84791	3.107843	0.867096	0.033146	1.622799	1.29849	2.757409	0.446275	2.354755	4.498179	3.471028	2.847129	3.130755	2.412323
F ICL	M NOR										36.20603	38.4023	37.19816	35.3736	31.62919	32.26438	44.34315	34.66464	41.80712	35.3736	36.20603	34.66464	35.3736	34.05322	34.66464	39.8971
											2.724684	0.044268	0.743445	0.94206	3.213303	4.045345	2.415442	5.626607	1.942825	5.165984	1.024961	0.253861	0.087958	1.362055	0.975666	1.894273
FNOR	MICL											38.4023	37.19816	35.3736	31.62919	32.26438	44.34315	34.66464	41.80712	35.3736	36.20603	34.66464	35.3736	34.05322	34.66464	39.8971
												2.524589	3.419524	1.766083	0.590241	1.4652	0.278224	3.173756	1.176129	2.108433	1.199734	3.099701	2.271688	1.426749	1.749018	0.951567
Fnor	M HFX												39.33908	37.61849	34.12134	34.71095	46.15382	36.95263	43.72294	37.61849	38.4023	36.95263	37.61849	36.37968	36.95263	41.90034
													0.768956	0.876413	3.132208	4.360304	2.583428	5.783283	1.721359	4.752889	1.260409	0.2275	0.053508	1.063075	0.893842	1.665637
F HFX	MNOR													36.38843	32.76023	33.37389	45.15686	35.69963	42.66922	36.38843	37.19816	35.69963	36.38843	35.10624	35.69963	40.79958
														1.778787	4.520083	5.46484	3.547683	5.03984	1.993053	4.323314	2.072851	0.553227	0.902734	1.79827	1.755366	2.532767
FHFX	M ICL														30.6728	31.32739	43.66612	33.79426	41.08832	34.5211	35.3736	33.79426	34.5211	33.1668	33.79426	39.14324
															2.717446	3.648633	1.229162	3.729575	0.747841	3.176085	0.225678	1.094649	0.941453	0.347715	0.024688	0.833588
F AP	M IHR															27.02813	40.69207	29.85241	37.9126	30.6728	31.62919	29.85241	30.6728	29.14019	29.85241	35.79536
																1.145121	0.729355	2.663249	1.531917	1.243606	2.415135	4.057294	3.662808	2.25577	2.820675	1.804366
F IHR	M AP																41.18773	30.52459	38.44411	31.32739	32.26438	30.52459	31.32739	29.82843	30.52459	36.35784
																	1.472026	1.590649	2.315814	0.517051	3.255274	4.936695	4.809307	3.26547	3.675839	2.70738
F IMR	M AC																	43.0938	49.0232	43.66612	44.34315	43.0938	43.66612	42.60352	43.0938	47.40482
																		2.533621	0.579318	1.759425	1.001282	2.289193	1.973446	0.954269	1.279824	0.561234
FTUN	MIRL																		40.47957	33.79426	34.66464	33.05143	33.79426	32.4096	33.05143	38.50376
																			3.398833	0.957443	4.430548	6.288179	5.780748	4.654565	4.953139	4.035197
AP	AP																			41.08832	41.80712	40.47957	41.08832	39.95724	40.47957	45.04151
																				2.561002	0.38271	1.735443	1.406062	0.332086	0.645387	0.093564
FTUN	AM																				35.3736	33.79426	34.5211	33.1668	33.79426	39.14324
																					3.427055	5.192518	4.72175	3.572745	3.886822	2.580598
MAP	FTUN																				34.66464	35.3736	34.05322	34.66464	39.8971	
																					1.564995	1.180901	0.080191	0.292084	0.50666	
MAP	AC																				33.79426	32.4096	33.05143	38.50376		
																					0.369213	1.758145	1.33504	1.933949		
MAP	AM																				33.1668	33.79426	39.14324			
																					1.341809	0.936482	1.583594			
FAN	AC																				32.4096	37.95423				
																					0.396665	0.460647				
MAP	AN																				38.50376					
																					0.787957					
AM	AM																									

APPENDIX V (Blade Length)

		IRL	F CBH	F SLH	F IMR	F NOR	F IHR	F ICL	F HFX	F IMR	F ICL	FNOR	Fnor	F HFX	FHFX	F AP	F IHR	F IMR	FTUN	AP	FTUN	MAP	MAP	MAP	FAN	MAP	AM
		IRL(self)	M SLH	M CBH	M NOR	M IMR	M ICL	M IHR	M IMR	M HFX	M NOR	MICL	M HFX	MNOR	M ICL	M IHR	M AP	M AC	MIRL	AP	AM	FTUN	AC	AM	AC	AN	AM
IRL	IRL(self)	x	37.61398	41.72897	38.37223	48.93155	48.93155	40.4039	39.88254	42.58945	41.72897	41.72897	43.6412	42.58945	41.01151	37.84172	38.37223	48.93155	40.4039	46.6544	41.01151	41.72897	40.4039	41.01151	39.88254	40.4039	44.95731
			3.289557	3.148492	1.945979	0.251031	0.466298	0.480564	1.958797	1.950796	0.030355	0.466502	0.075426	0.390681	0.256949	1.715015	2.613588	0.102524	3.550606	1.121723	1.180563	1.162662	0.441377	1.117388	2.323972	1.838725	1.958737
F CBH	M SLH		x	31.29672	26.65721	40.4039	40.4039	29.50683	28.7888	32.43516	31.29672	31.29672	33.80433	32.43516	30.33352	25.88772	26.65721	40.4039	29.50683	37.61398	30.33352	31.29672	29.50683	30.33352	28.7888	29.50683	35.48715
				0.244435	1.840469	2.758397	3.627125	3.535339	1.58435	1.25327	3.994028	4.575559	3.757655	4.32778	3.731696	2.272669	0.879466	3.186574	0.668489	4.680884	5.67524	5.503771	4.797758	5.589826	7.51748	6.711157	5.968156
F SLH	M CBH			x	32.20406	44.26025	44.26025	34.59983	33.98956	37.12862	36.13834	36.13834	38.3305	37.12862	35.30747	31.57006	32.20406	44.26025	34.59983	41.72897	35.30747	36.13834	34.59983	35.30747	33.98956	34.59983	39.82252
					1.761013	2.690902	3.483939	3.236047	1.566997	1.300883	3.670616	4.174237	3.51352	3.986742	3.42266	2.105926	0.965534	3.081772	0.34899	4.402617	5.092407	4.978092	4.312642	5.019026	6.592296	5.944393	5.510522
F IMR	M NOR				x	41.11072	41.11072	30.46752	29.77267	33.3115	32.20406	32.20406	34.64606	33.3115	31.26882	26.9776	27.71685	41.11072	30.46752	38.37223	31.26882	32.20406	30.46752	31.26882	29.77267	30.46752	36.28987
						1.517566	2.371358	1.813567	0.115886	0.252518	2.358033	2.923179	2.250277	2.741109	2.051043	0.362243	0.924263	1.938381	2.257708	3.309813	3.936453	3.825239	3.03618	3.853594	5.621178	4.889246	4.484202
F NOR	M IMR					x	51.10734	43.01323	42.52387	45.07243	44.26025	44.26025	46.06753	45.07243	43.58448	40.61599	41.11072	51.10734	43.01323	48.93155	43.58448	44.26025	43.01323	43.58448	42.52387	43.01323	47.3162
							0.68679	0.16584	1.548271	1.570805	0.306144	0.717348	0.338091	0.641683	0.040048	1.295445	2.140704	0.338503	3.049643	1.320552	1.392697	1.373693	0.700172	1.333252	2.468478	2.012753	2.120687
F IHR	M ICL						x	43.01323	42.52387	45.07243	44.26025	44.26025	46.06753	45.07243	43.58448	40.61599	41.11072	51.10734	43.01323	48.93155	43.58448	44.26025	43.01323	43.58448	42.52387	43.01323	47.3162
								0.981868	2.373689	2.490366	0.478119	0.075689	0.441141	0.134103	0.740021	2.012546	3.030971	0.432977	3.253447	0.686223	0.52318	0.589659	0.112592	0.534931	1.603076	1.210496	1.516809
F ICL	M IHR							x	32.34901	35.63289	34.59983	34.59983	36.88354	35.63289	33.73108	29.7966	30.46752	43.01323	32.98964	40.4039	33.73108	34.59983	32.98964	33.73108	32.34901	32.98964	38.43177
									1.814743	1.786739	0.580456	1.09122	0.656313	1.042073	0.240725	1.276418	2.397568	0.820004	4.071275	1.668091	2.056201	1.681356	1.104323	1.885628	3.398114	2.778101	3.322395
F HFX	M IMR								x	35.04061	33.98956	33.98956	36.31167	35.04061	33.10479	29.08572	29.77267	42.52387	32.34901	39.88254	33.10479	33.98956	32.34901	33.10479	31.69542	32.34901	37.88328
										0.141594	2.265613	2.871131	2.395249	2.787935	1.988373	0.364144	0.632621	2.511372	2.246344	4.381708	2.975704	3.914756	2.405944	3.744097	5.025299	4.711534	5.019857
F IMR	M HFX									x	37.12862	37.12862	39.26553	38.09316	36.3204	32.69898	33.3115	45.07243	35.63289	42.58945	36.3204	37.12862	35.63289	36.3204	35.04061	35.63289	40.7233
											2.271832	2.76202	2.326373	2.685859	1.953896	0.489763	0.438193	2.312751	1.662289	4.141312	3.947585	2.919745	2.832121	3.026785	4.839408	4.238644	4.802947
F ICL	M NOR										x	36.13834	38.3305	37.12862	35.30747	31.57006	32.20406	44.26025	34.59983	41.72897	35.30747	36.13834	34.59983	35.30747	33.98956	34.59983	39.82252
												0.50362	0.05283	0.401044	0.307967	1.78207	2.735245	0.101	4.098991	1.617566	1.464101	1.067549	0.478808	1.067822	2.589232	2.020707	2.508476
FNOR	MICL											x	38.3305	37.12862	35.30747	31.57006	32.20406	44.26025	34.59983	41.72897	35.30747	36.13834	34.59983	35.30747	33.98956	34.59983	39.82252
													0.421988	0.076162	0.849807	2.38946	3.3918	0.457712	5.160743	1.041071	0.898955	0.656345	0.047206	0.631674	2.073761	1.517087	1.982462
Fnor	M HFX													39.26553	37.54817	34.05755	34.64606	46.06753	36.88354	43.6412	37.54817	38.3305	36.88354	37.54817	36.31167	36.88354	41.82201
														0.339922	0.352206	1.816088	3.041349	0.05065	4.308884	1.415505	1.302457	1.305343	0.31566	1.1532	2.048391	1.890905	2.211499
F HFX	MNOR														36.3204	32.69898	33.3115	45.07243	35.63289	42.58945	36.3204	37.12862	35.63289	36.3204	35.04061	35.63289	40.7233
															0.748251	2.493595	3.575894	0.348895	3.551999	0.791935	0.705038	0.894617	0.033521	0.819099	1.785583	1.587339	1.923601
FHFX	M ICL															30.61546	31.26882	43.58448	33.73108	41.01151	34.45656	35.30747	33.73108	34.45656	33.10479	33.73108	39.07007
																1.775614	2.870314	0.356883	3.04972	1.863896	1.437512	1.439951	0.691787	1.635788	2.995766	2.461927	2.792537
F AP	M IHR																26.9776	40.61599	29.7966	37.84172	30.61546	31.57006	29.7966	30.61546	29.08572	29.7966	35.72844
																	1.311833	1.721386	2.636516	3.097969	2.994467	3.704527	2.620598	3.507275	4.991597	4.671365	5.133427
F IHR	M AP																	41.11072	30.46752	38.37223	31.26882	32.20406	30.46752	31.26882	29.77267	30.46752	36.28987
																		2.561518	1.41689	3.977421	4.755724	4.620718	3.667939	4.795761	6.171487	5.583226	6.023532
F IMR	M AC																		43.01323	48.93155	43.58448	44.26025	43.01323	43.58448	42.52387	43.01323	47.3162
																			3.451845	0.966997	0.995767	0.982823	0.29797	0.936322	2.011478	1.629085	1.930635
FTUN	MIRL																			40.4039	33.73108	34.59983	32.98964	33.73108	32.34901	32.98964	38.43177
																				4.845861	5.688375	5.548438	4.889161	5.611564	7.299891	6.600556	7.017895
AP	AP																				41.01151	41.72897	40.4039	41.01151	39.88254	40.4039	44.95731
																					0.095502	0.091463	0.853878	0.158677	1.011785	0.543471	0.794669
FTUN	AM																					35.30747	33.73108	34.45656	33.10479	33.73108	39.07007
																						0.002832	0.906681	0.075193	1.337245	0.767097	1.014661
MAP	FTUN																						34.59983	35.30747	33.98956	34.59983	39.82252
																							0.886806	0.076214	1.299494	0.744946	0.992977
MAP	AC																							33.73108	32.34901	32.98964	38.43177
																								0.82987	2.313906	1.711396	1.827295
MAP	AM																								33.10479	33.73108	39.07007
																									1.415509	0.843908	1.080975
FAN	AC																									32.34901	37.88328
																										0.568618	0.122122
MAP	AN																										38.43177
																											0.358242
AM	AM																										

APPENDIX VI (Blade Wdth)

RESTRICTION FRAGMENT DATA MATRIX (PRESENCE=1, ABSENCE=0) FOR THE ITS AND RUBISCO SPACERS OF 6 *ALARIA ESCULENTA* ISOLATES AND 5 OTHER *ALARIA* SPECIES. FOR ABBREVERATIONS SEE TABLE 1.

	rDNA spacers								Rubisco spacer			
	RSAI	CFO1	PST	BGL	SMA	DRA	HIND	XBA	RSA	DRA	HIND	XBA
IRL	0-1-0-0-1-1	1-1-1-1-0-0	0	0	0	0-0	0	0	0-1-0-0-0	1-0-1	0	1
SCL	0-1-0-0-1-1	1-1-1-1-0-0	0	0	0	0-0	0	0	0-1-0-0-0	1-0-1	0	1
NOR	0-1-0-0-1-1	NA	1	0	0	0-0	0	0	1-0-0-0-0	1-0-1	0	1
ICL	0-1-0-0-1-1	1-1-1-1-0-0	1	0	0	0-0	0	0	0-1-0-0-0	1-0-1	0	1
HFX	0-1-0-0-1-1	1-1-1-1-0-0	0	0	0	0-0	0	0	0-1-0-0-0	1-0-1	0	1
FRC	0-1-0-0-1-1	1-1-1-1-0-0	0	0	0	0-0	0	0	0-1-0-0-0	1-0-1	0	1
AM	0-1-0-0-1-1	NA	0	0	0	0-0	0	0	0-1-0-0-0	1-0-1	0	1
AN	0-1-1-1-1-1	1-1-0-1-1-0	0	0	0	0-0	0	0	0-1-0-0-0	1-0-1	0	1
AC	0-1-0-0-1-1	NA	0	0	0	0-0	0	1	0-1-0-0-0	1-0-1	0	1
AP	0-1-0-0-1-1	1-1-0-1-1-0	0	0	0	1-1	0	0	0-1-0-0-0	1-0-1	0	1
UND	1-0-0-0-1-0	0-1-1-1-1-1	0	0	0	0-0	0	0	0-0-1-1-1	0-1-1	0	1

APPENDIX VII