Recombination between recently occurring A1 and A2 isolates of *Phytophthora infestans* in Ireland

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Background

Recent reports have shown a resurgence of the A2 mating type and the occurrence of new genotypes of *P. infestans* in Ireland and the UK with novel SSR (simple sequence repeat) loci. These include aggressive isolates of 13_A2 ('Blue 13'), which exhibits phenylamide resistance and 6_A1 ('Pink 6'). The emergence of these strains has increased the risk of sexual recombination and the subsequent generation of complex genotypes. As the implications for blight control strategies are as yet unknown, it is important to determine whether novel recombinant progeny exhibit fungicide resistance and whether they have an ability to overcome established host resistance.

Objective

(i) Investigate the potential for genetic recombination between traditional (8_A1) and novel (13_A2 and 6_A1) isolates of *Phytophthora infestans* via two crosses: 13_A2 x 6_A1 and 13_A2 x 8_A1

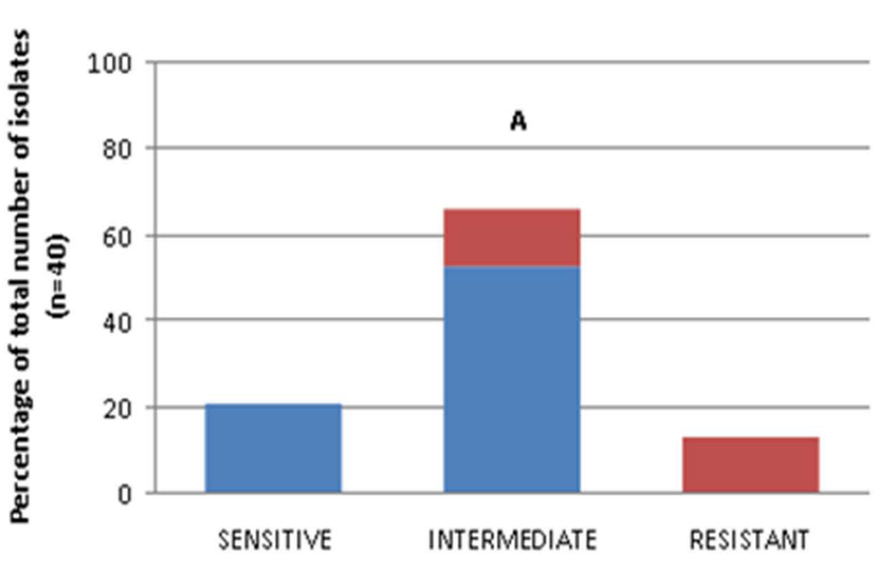
(ii) Characterise the F1 populations for mating type and metalaxyl sensitivity

Methodology

Oospores were harvested from 13_A2 x 6_A1 and 13_A2 x 8_A1 crosses on paired culture plates. A 1:0.5 (v/v) suspension of 2000 oospores/ml and cellulase from *Trichoderma reesei* 1,-4-(1,3:1,4)-β-glucan-4 glucanohydrolase, respectively was incubated overnight to digest residual mycelia. Oospore germination was completed on 0.5% water agar overlaid with rye agar. Pure cultures were raised from single colonies after 10 days (Fig. 1). The 13_A2 parental isolate was metalaxyl resistant in contrast to 6_A1 and 8_A1 which were sensitive. Mating type was tested on F1 progeny by pairing with tester isolates on carrot agar and metalaxyl sensitivity assessed using the floating leaf disc technique at concentrations of 0, 5 and 100 mg/L metalaxyl. Results were scored as resistant when progeny sporulated on leaf discs floated on 0, 5 and 100 mg/L, sensitive when sporulation occurred only on 0 mg/L and intermediate with sporulation on discs floated on 0 mg/L and 5 mg/L metalaxyl only. DNA was extracted from mycelia of each culture (Raeder & Broda 1985) and used for microsatellite genotyping using 10 markers (Lees *et al*., 2006). Genetic similarities between F1 progenies were assessed using UPGMA and Jaccard Co-efficients in Free Tree with 1000 replications (Fig. 3).

Results

Twenty-four and 40 F1 progeny were isolated from the 13_A2 x 6_A1 and 13_A2 x 8_A1 crosses, respectively. Corresponding segregation of mating type (A1:A2) was 13:11 and 24:16 from 13_A2 x 6_A1 and 13_A2 x 8_A1, respectively. The metalaxyl phenotypes (Fig. 2) of the F1 population segregated into 3:16:3 (sensitive:intermediate:resistant) and 6:25:5 for 13_A2 x 6_A1 and 13_A2 x 8_A1 pairings. There was segregation in 7 of the 10 SSR loci in F1 progenies with the occurrence of new alleles at Loci D13 and G11.



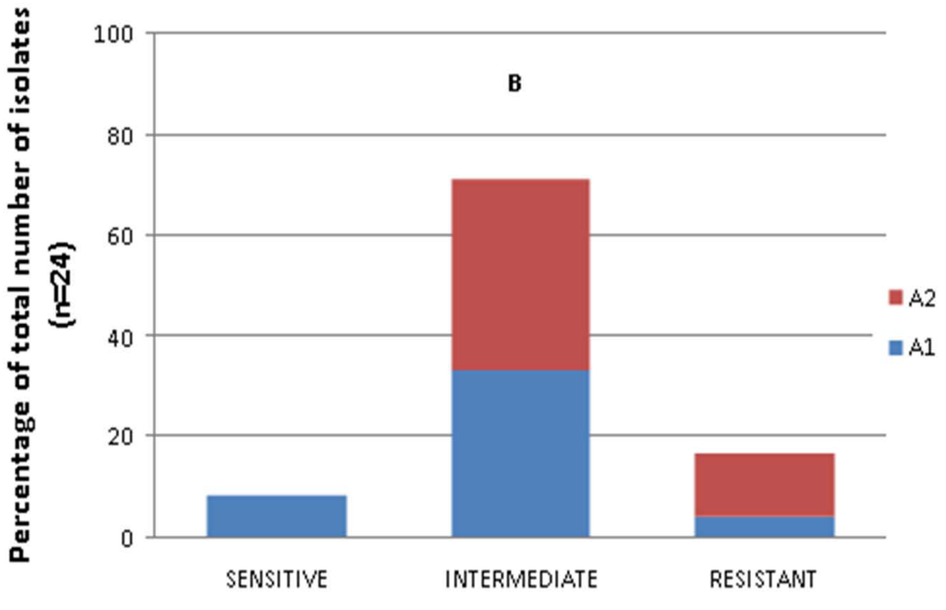


Fig. 2: Metalaxyl sensitivity and mating type profiles of recombinant progeny derived from 13_A2 x 8_A1 (A) and 13_A2 x 6_A1 (B) crossings. Data presented as % of total no. of isolates collected per cross.

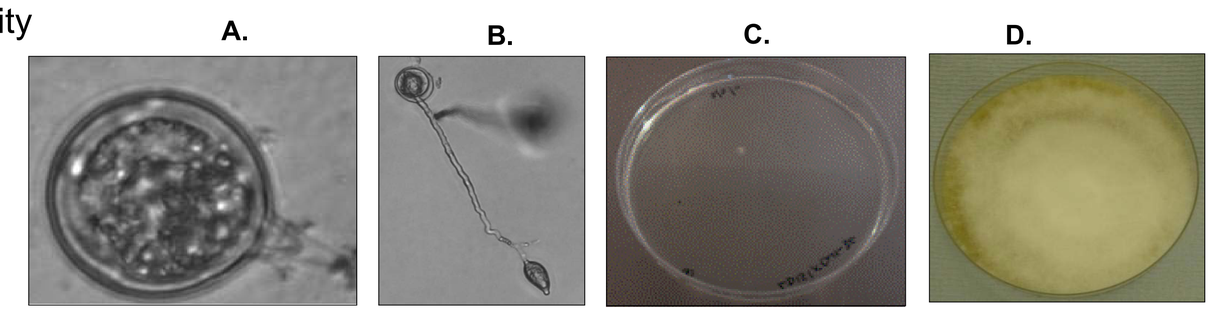


Fig 1 : Images of digested oospore wall (A), germinating oospore post-cellulase treatment (B) single spore colony on 0.5% water agar with rye agar overlay (C) and pure culture from single colony (D).

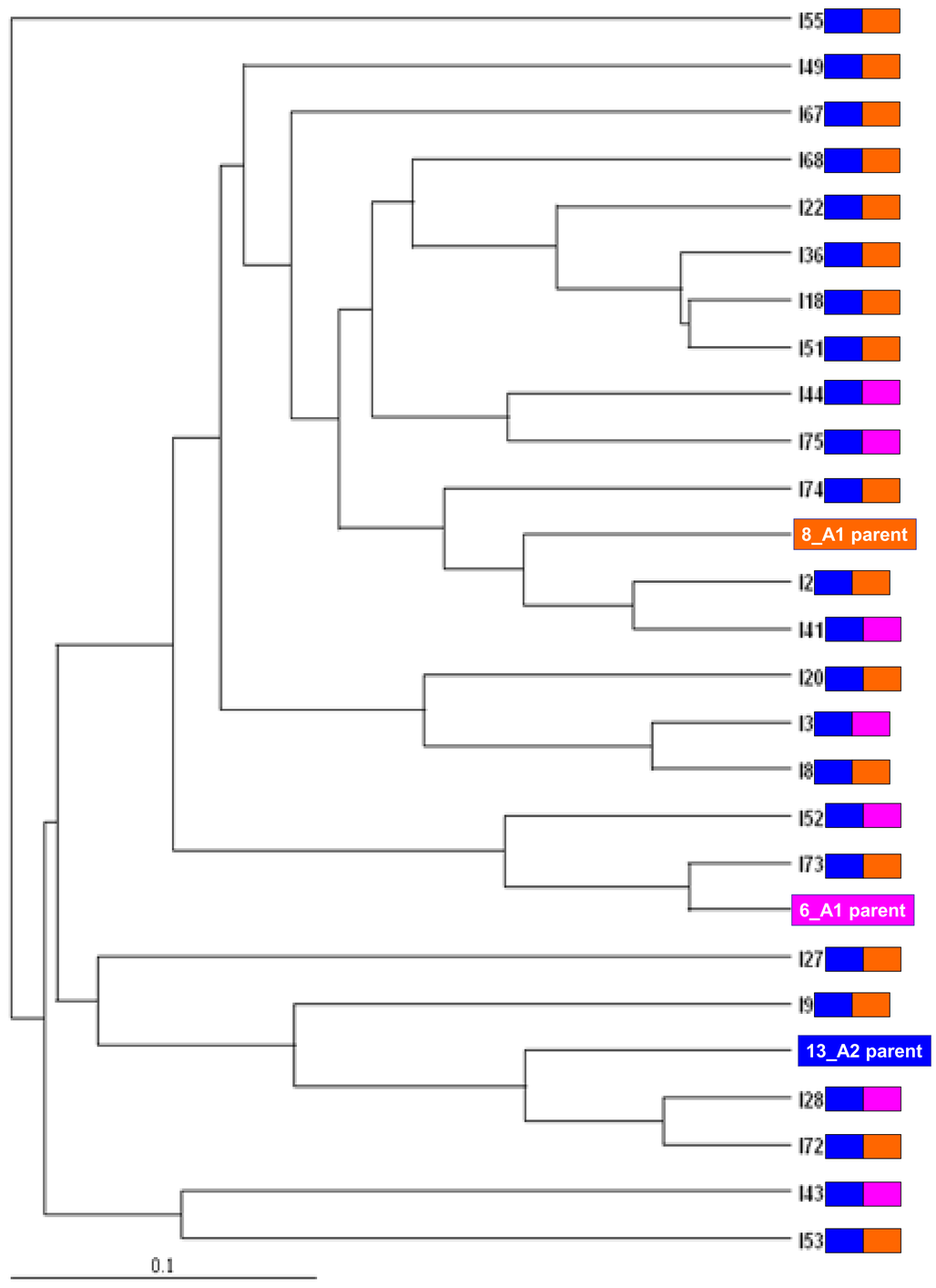


Fig. 3: Dendrogram illustrating genetic diversity within F1 populations from 13_A2 x 6_A1 and 13_A2 x 8_A1 crosses. Bar illustrates genetic distance. Colour bars show genetic contribution to progeny from each respective parent (13_A2 = blue; 6_A1 = pink; 8_A1 = orange).

Conclusion

Isolates of the 13_A2 genotype will mate with isolates of the opposite mating types of 6_A1 and 8_A1 genotype, producing a segregating F1 population. The A1:A2 ratio from 13_A2 x 6_A1 and 13_A2 x 8_A1 crosses did not differ significantly from a 1:1 ratio. In both crosses, the segregation in metalaxyl response produced a low frequency of F1 progeny with sensitivity and resistance, and a high frequency of those with an intermediate phenotype. The F1 populations are currently being tested for haplotype and aggressiveness.

References

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Acknowledgements

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