

Willow Breeding Strategies – The Way Forward

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Abstract

The European Willow Breeding Programme was initiated in 1996 to produce new superior willow varieties for biomass energy. To date, the crossing programme has focussed on inter-species combinations between a base population of approximately 100 varieties obtained from the UK National Willows Collection held at IACR-Long Ashton. In total, 750 crosses have been made and over 40,000 unique genotypes have been screened in a seedling nursery. Selected individuals have been evaluated in an extensive array of breeding trials and superior genotypes have been advanced to yield trials. Currently, over 50 genotypes are in advanced observation and yield trials. Of these it is expected that a small proportion will be multiplied for commercial use in the biomass industry from 2004 onwards.

The current methods used to select willows in the field are unsatisfactory and need urgent refinement. Future selection strategies should attempt to combine the breeders' observations with quantitative data and yield estimates. Such information will enable multiple trait selection strategies to be employed. The construction of a genetic map of willows and the identification of markers linked to traits of interest is an extremely important development and is closely tied in with the breeding programme. The use of molecular marker technology will facilitate the rapid selection of desirable genotypes at the seedling stage and accelerate the introgression of valuable traits in backcross procedures.

In future hybridisation schemes, short term breeding initiatives should be coupled with longer-term recurrent selection strategies. Other long-term prospects might include the production of inbred lines by anther culture, polyploid breeding and the application of GM technology.

Key words: *Salix*, willow, Short Rotation Coppice, biomass, breeding, interspecific cross, recurrent selection.

Introduction

The European Willow Breeding Programme (EWBP) was established in 1996 to produce new willow varieties principally for biomass energy production (Lindegaard and Barker, 1997). As with all plant breeding programmes success in willow breeding depends on the creation of variability through hybridisation combined with effective methods of assessment and selection of the progeny in the field. During the first five years of the programme various breeding and selection strategies have been employed. The purpose of this paper is to review the relative success of these strategies and consider where procedures may be improved and whether new approaches are necessary to accelerate the production of varieties yet further.

Crossing Programme and Field Evaluations 1996-2000

Between 1996-2000, over 750 crosses have been made using a base population of 100 individuals belonging to 20 distinct willow species from North and Central Europe, Russia, Asia and North America which are maintained in the UK National Willows Collection held at IACR-Long Ashton. 40,000 unique willow genotypes have been screened in a seedling nursery and selected on the basis of yield estimations, resistance to disease and pests and erect growth habit. Between 5-10% of these selections have been advanced initially to field-based observation trials located at IACR-Long Ashton, from which elite breeding lines have been identified and subsequently included in smaller multi-site observation and yield trials. The breeding scheme being followed is demonstrated in Figure 1 and the progress of the breeding programme to date is illustrated in Figure 2. Already, over 50 genotypes are in advanced observation and yield trials. Of these it is expected that a small proportion will be multiplied for commercial use in the biomass industry from 2004 onwards. It is hoped that these will help increase the average yield to around 15 dry tonnes per hectare per year.

The Way Forward

In only a very short time giant strides have been made in the willow breeding programme. For instance, the success rate of the crosses performed has increased year on year and currently stands at 79.5%, almost four times that achieved in 1996. However, there are a number of shortcomings that have not received sufficient attention. These include field selection strategies and the development of medium- to long-term breeding initiatives. These areas will be considered in turn below along with key research requirements and the potential for novel applications of SRC willows.

Selection Strategies

Until now, field selections from observation trial 1 have been based solely on the value judgements made by the breeder. In future, if we are to be absolutely sure that all the best genotypes are selected for advancement we should not merely rely on “breeders’ eye” alone, but must support this expertise with quantitative data such as measurements of height, stem diameter, number of shoots etc. It is hoped that from 2000 onwards the use of one-hand computer callipers (Savcor Ltd) will provide a rapid non-destructive technique for estimating the yield of genotypes in observation trial 1. The technique will be that performed on the national trial network by Forest Research (see Armstrong, this volume). The information gained from such an exercise will pave the way for more informed selection procedures for SRC willows.

As with most crops, willow breeding and selection schemes attempt to select for many traits simultaneously. Characters of interest include yield, disease and pest resistance, growth habit and wood quality. Trying to select genotypes that fulfil such diverse criteria from observations alone is extremely demanding and subject to error. Therefore, in future, it would be sensible to consider the utilisation of a multiple trait selection strategy that aims to maximise genetic gain. There are a number of methods that endeavour to deal with this problem, such as index selection, independent culling, tandem selection and principal component analysis (Bisoffi and Gullberg, 1996) as well as cluster analysis (Ares and Gutierrez, 1996). These all require detailed measurements of several traits of interest. Cotterill and Dean (1990) suggest that index selection is the most efficient method for successful tree breeding. However, Bisoffi

Figure 1: The willow breeding scheme followed by the European Willow Breeding Programme (Elaborated from Nilsson (1988) and Larsson (1997)). The cycle from initial cross to registered variety may take as long as 15 years but genotypes showing superior growth can be fast-tracked through the system, reaching the marketplace in 8-10 years.

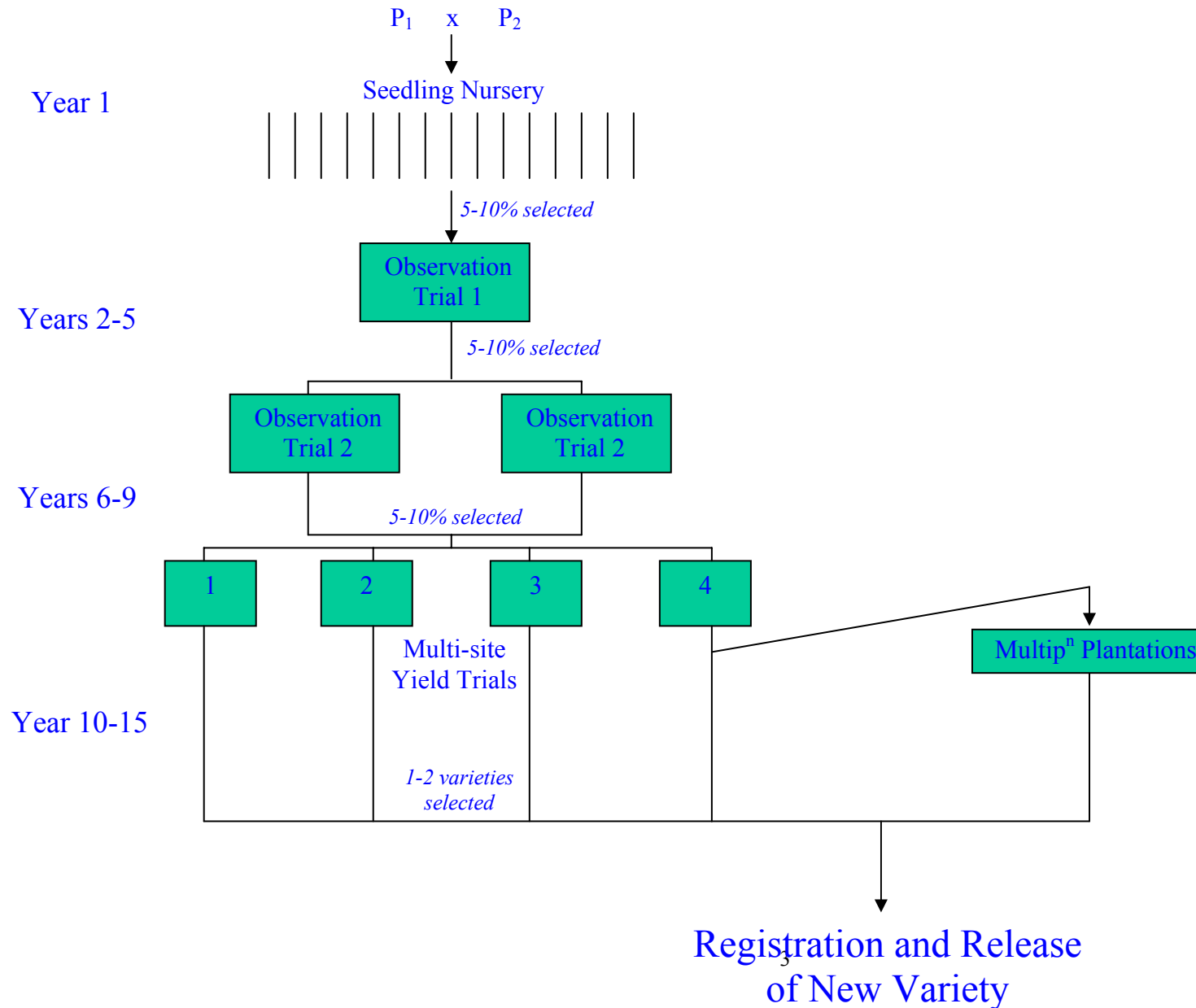
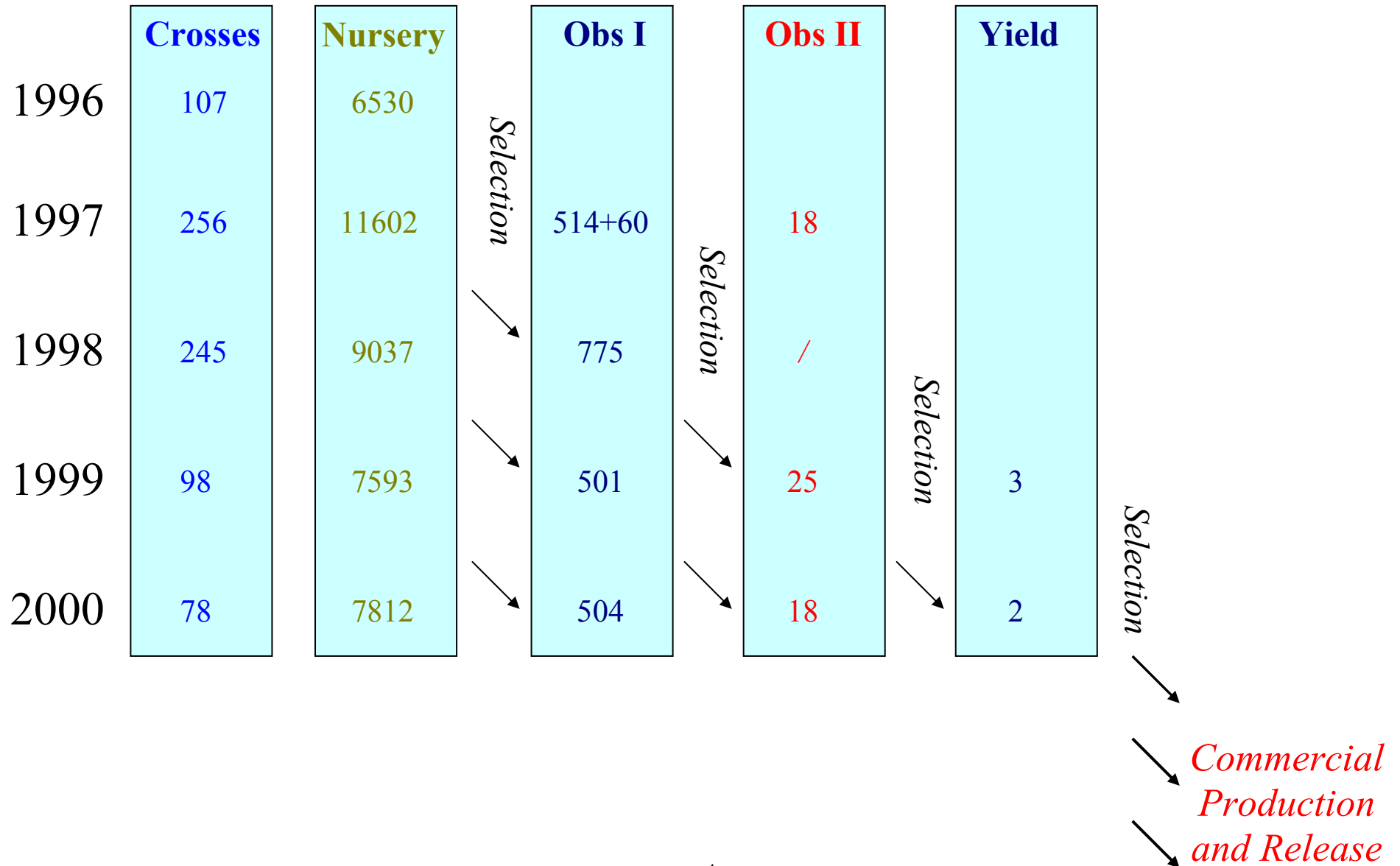


Figure 2: Progress of the European Willow Breeding Programme from 1996-2000. Already over 50 varieties are in advanced observation and yield trials.



and Gullberg (1996) report no examples of index selection being used in poplar breeding. Instead the use of principal component analysis (Bisoffi, 1990) and cluster analysis (Ares and Gutierrez, 1996) have shown promise and should thus be considered most relevant to willow breeding.

A distinct, albeit related concept involves the development of a tree ideotype (Dickmann, 1985). Dickmann and Keathley (1996) propose an ideotype selection programme in poplar. In this example, the breeder selects a subset of genetically correlated characters and these are combined as a single quantitative trait. This working ideotype is then treated as one of two to three variables in an independent culling process, in which clones that do not achieve a certain predetermined standard are eliminated. Although there are only a few examples of ideotype breeding in practice, Koski and Dickmann (1992) suggest encouraging results from the use of a modelling approach with SRC willows and poplars.

In the medium term, the construction of a genetic map for willow and the identification of markers linked to traits of interest will facilitate the rapid selection of desirable genotypes at the seedling stage and accelerate the introgression of valuable traits in backcross procedures. This technique, termed marker-assisted selection, will improve the efficiency of the initial stages of the willow breeding process (see Hanley *et al*, this volume).

Medium-Long Term Breeding Initiatives

Figure 3 illustrates the various hybridisation pathways so far utilised in the willow breeding programme. The vast majority of the crosses fall into only three categories: interspecific crosses between two species (type b), three-way species crosses (type f) and four-way species crosses, also known as double crosses (type g). Together these comprise over 90% of the crosses attempted. These types of crosses are beneficial because they maximise heterozygosity and the breeder may select specific gene combinations demonstrating heterosis. Hybrid vigour in such crosses is due to the trapping of dominant and epistatic gene action without eliminating or masking favourable recessive genes (Stettler, Zsuffa and Wu, 1996). However, in many cases this approach will result in barriers to continuing gains from genetic improvement because of differences in ploidy levels and other causes of genetic disharmony. As a result of this, continuing solely with this approach would necessitate retracing successful crosses with progressively poorer parents due to the exhaustion of the germplasm available.

Hence, it is imperative that we back up such productive short-term approaches with longer-term strategies based on population improvement. Long-term breeding strategies aim to increase genetic diversity and create new crossing opportunities for the future (Kang, Lascoux and Gullberg, 1996). This can be effectively achieved using recurrent selection schemes which involve the concentration of desirable alleles during repeated cycles of hybridisation and selection (Jenkins, 1940; Hull, 1945). Several forms of recurrent selection exist but the application best suited for tree crops is termed reciprocal recurrent selection. Baudouin *et al* (1997) reviewed the breeding strategies of six tropical tree crops (cacao, coffee, rubber tree, oil palm, coconut and eucalyptus) and showed that all but rubber tree improvement were based on this method. Also this is the preferred strategy for long term poplar breeding (Stettler, Zsuffa and Wu, 1996; Bisoffi and Gullberg, 1996).

Figure 4: A generalised model for reciprocal recurrent selection for general hybridising ability (RRS-GHA) as used in the Italian poplar breeding programme (Bisoffi, Figure 3: Pathways used in the crossing programme 1996-2000: intraspecific crosses (a); interspecific crosses producing F₁ hybrids (b); full-sib crosses producing F₂ hybrids (c); first generation backcross (d); second generation backcross (e); three-way species cross (f); four-way species cross or double cross (g). (Based on Figure 2, page 100, from Stettler, Zsuffa and Wu,1996).

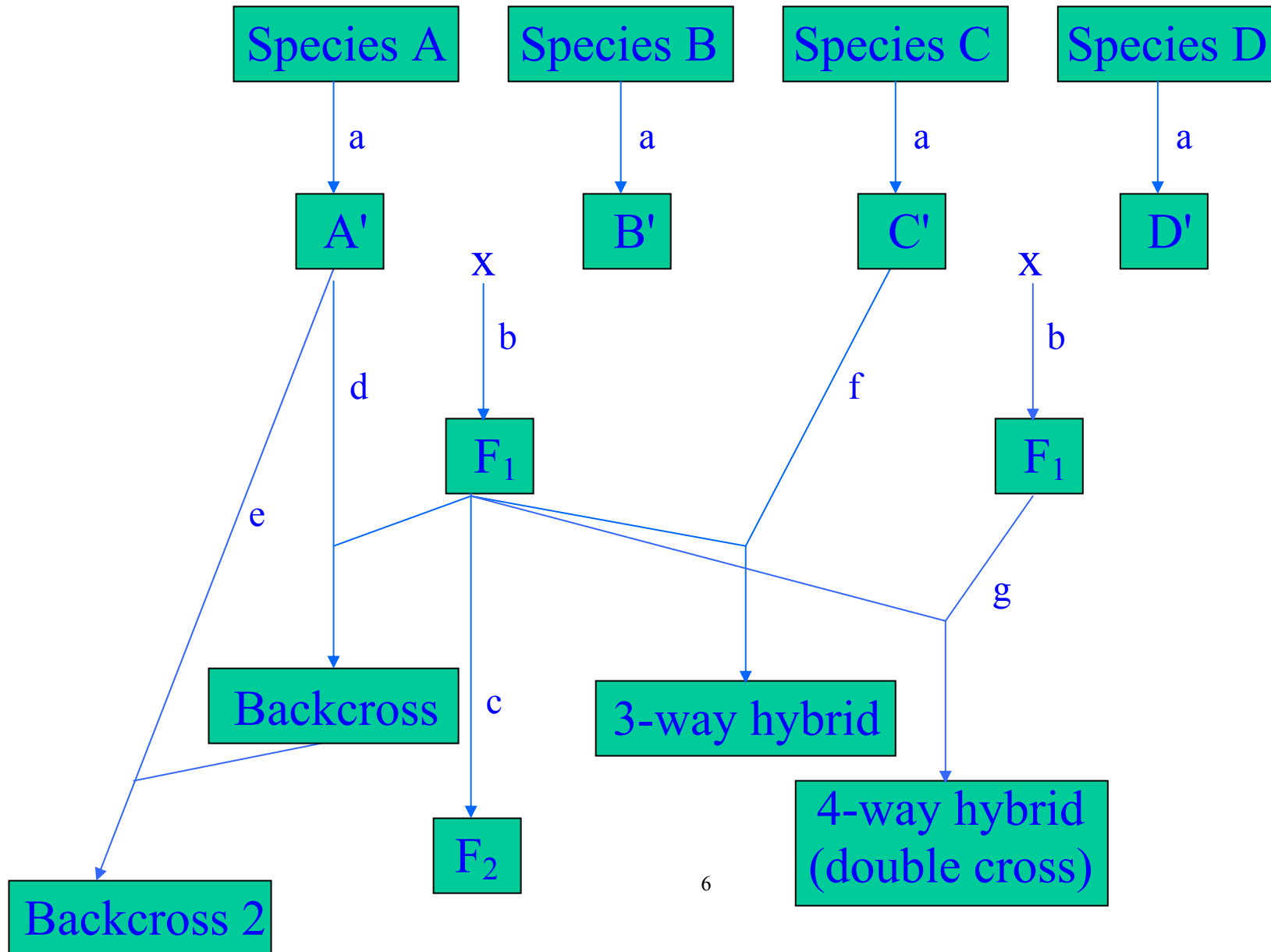
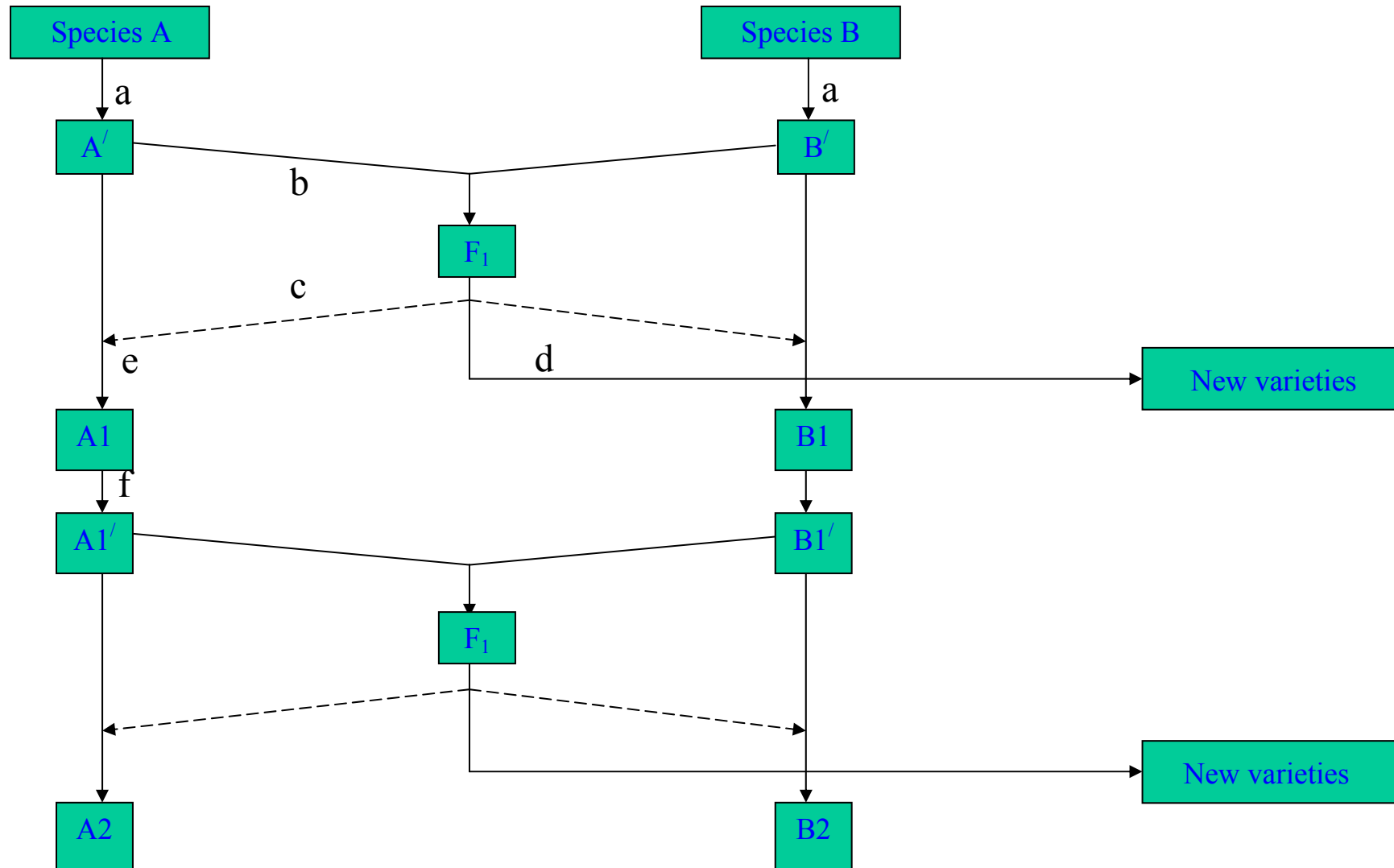


Figure 4: Reciprocal Recurrent Selection for General Hybridising Ability (RRS-GHA). This breeding strategy involves the phenotypic selection of suitable parent plants from two populations' (a) followed by interspecific hybridisation (b). The F₁ families are tested to identify progeny with high GHA (c) and elite lines are selected and advanced through the breeding scheme possibly leading to new varieties (d). F₁ hybrids with high GHA undergo full sib mating (e) and the progeny are selected on the basis of phenotype (f). The process is then repeated several times until there is no further genetic gain. (Based on Figure 1, page 99, from Stettler, Zsuffa and Wu,1996).



1990). This breeding system has the advantage of capturing both additive and non-additive gene effects together with the simultaneous improvement of two base populations and their hybrid offspring. The downside of this strategy is that it is labour intensive and demanding of field space.

Other long-term initiatives are also required for the continued success of the breeding programme. These include developing protocols for the efficient chromosome doubling of triploid hybrids, improving the crossability of recalcitrant cross combinations using embryo rescue techniques, the development of inbred lines using anther culture and the possible application of GM technology.

My own unpublished results suggest that the triploid hybrid variety *S. x stottii* 'Ashton Stott' could be doubled with colchicine using a root immersion technique based on that performed by Morgan (1975). This and other methods, such as that carried out by Zhang *et al* (1997) should be tested more fully.

Research Requirements

Now for the first time the opportunity exists for researchers to test the near-market varieties produced by the EWBP. Such collaboration will provide detailed information on elite varieties prior to their commercial release. This is far more sensible than carrying out research on established varieties or older non-registered clones. The result of the latter is that by the time the experimental data is acquired the varieties are liable to be outclassed or obsolete and therefore unlikely to be planted by biomass growers.

The superior selections created by the breeding programme should form the bedrock of future disease and pest research such as the work on mixed clonal plantations. A major flaw exists in our current breeding strategy; the breeding process is aimed at identifying superior genotypes on the basis of their performance in pure stands. Once superior individuals are identified we advise that they should be planted in mixtures. A high yielding clone may be completely unsuitable for a mixture because it may be too competitive or, if it is slow to establish, not competitive enough. Research is needed to identify genotypes that are high yielding but also possess characters conducive to synergism.

Other crucial questions remain unanswered. For instance we have sparse knowledge on the physiological basis of heterosis. Fundamental research is required into the physiological composition of the highest yielding varieties, such as their water use efficiencies, leaf area indices, respiration rates and concentrations of plant growth regulators.

Novel Industrial Uses

Willow varieties that are unsuitable for use as a biomass crop may have attributes that can be exploited in other novel industrial uses that include:

- ◆ raw materials for the forest products industry (Deka, Wong and Roy, 1992;1994),
- ◆ natural dyes (Stott, Bridle and Timberlake, 1971)
- ◆ pharmaceuticals and other biologically active compounds (Gebhardt, 1992; Meier, 1992; Julkunen-Tiitto and Meier, 1992),
- ◆ livestock feeding (McCabe and Barry, 1988), and
- ◆ pollution control (Riddell-Black, Pulford and Stewart, 1997; Edwards, 2000).

The anticipated benefits from such novel applications are numerous and include the potential to provide the willow grower with value-added products, the provision of sustainable sources of natural products, the diversification of UK arable farming, rural employment opportunities and numerous environmental benefits.

Conclusion

The European Willow Breeding Programme has achieved a great deal in its first five years. By the end of the next five years the first products of this initiative will be in the marketplace. Nevertheless, many important strategies and research initiatives need to be implemented now to ensure the long-term success of the breeding programme and the biomass industry as a whole.

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