Cocoa Breeding in the 21st Century

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As we stand at the epoch of the 21st century an expanding array of new techniques are becoming available to the plant breeder. It is envisaged that the benefits of molecular genetics will most rapidly be felt in improving the efficiency of the screening process in cacao. A model system for crop improvement in cacao is proposed which utilizes newly established technologies. Many of these techniques still require refinement for their application in cacao. It is argued that the full benefits of these new methods may be restricted in their impact by our limited understanding of the basic genetic control of economic traits in cacao. Furthermore, the need to establish long term conventional breeding programmes is stressed as a prerequisite before more advanced methods are applied. In short it is suggested that the potential advancements available in the 21st century may be restricted in their impact by our failure to properly utilize the techniques of the 20th century.

Introduction

Crop improvement in *Theobroma cacao* can trace its history to the first half of the present century. These early crop improvement efforts often relied solely upon the selection of local clones rather than breeding *per se*.

Posnette (1951) demonstrated inter population heterosis in cacao. This resulted in the majority of improvement programmes relying upon the exploitation of heterosis rather than long term breeding work. With one or two exceptions, material released to farmers has been derived from single generation crosses between upper Amazon Forastero clones and locally selected material. The segregation that results from crossing two such highly heterozygous parental lines is of course considerable. This segregation is partly responsible for the staggering variability observed in many commercial plantings with respect to yield.

During the latter part of the century there has been a great expansion of molecular genetic techniques available to the plant breeder. However, this is not the place to review such advances. Arguably the greatest impact on crop improvement has been in providing a potentially unlimited supply of genetic markers. These markers have the ability to effectively saturate linkage maps. They can also be of use in estimating heterozygosity and can be used as starting points for the identification and isolation of genes. Although much interest is being shown in using these techniques in cacao, they have as yet had no significant impact on crop improvement in cacao. Furthermore, there is little possibility of applying genetic engineering techniques to cacao while it is not possible to reliably regenerate mature plants from tissue culture.

In this paper I consider the possibilities for exploiting the new technologies alongside conventional breeding programmes in the 21st century. Both the potential and the pitfalls will be considered as I propose a model breeding system for cacao.

Per Chance To Dream.

The most costly and time consuming stage of any breeding programme is the evaluation of phenotypes. This is especially true of tree crops which require both a large land area and a long time period for screening. It is considered (King, 1990) that the benefits of molecular techniques will be most rapidly felt in improving the efficiency of the screening process.

Initially, traits controlled by major genes will be most amenable to molecular analysis. An ideal target gene would be one which confers durable vertical disease resistance. However, no such genes are currently known in cacao. This problem is a major stumbling block for the utilization of molecular techniques in cacao. We are in a position of potentially having an unlimited number of genetic markers; but markers to what? The list of agronomically important genes identified in cacao is very limited and perhaps only include the three incompatibility loci (Cope 1962) and the genes identified recently at Penn. State (Fritz *et al.*, 1992). This compares very unfavourably with other crops (Table 1).

Let us consider, therefore the incompatibility mechanism as a target of our proposed model breeding system. Work at the CRU has demonstrated that in abandoned estates at least, self-compatible trees set significantly more pods than do self-incompatible trees (Warren and Kalai 1992). We will assume therefore, that in addition to other yield and resistance traits, we wish to select for cacao trees which are self compatible. This would currently require propagating all material to be screened to the flowering stage (say three years minimum), then selfing flowers on each individual to determine its incompatibility type. However, this is time consuming and expensive on both land and labour.

In contrast the utilization of a RAPD marker linked to the 'S' allele would allow this screening procedure to be performed within a week. It is a relatively simple procedure to extract

Table 1 Identified Genes of Agronomic Importance.

Brassica oleracea	Genes	*Theobroma cacao*	Genes
Disease Resistance		Disease Resistance	
Clubroot	1		
Alternaria brassicae	1		
Peronospora parasitica	1		
Turnip Mosaic Virus	5		
Cauliflower Mosaic Virus	3		
Pest Resistance		Pest Resistance	
Delia radicum	1	Trypsin inhibitor	1
		Chitinase-A	1
Morphology		Morphology	
Node Number	1	Self-incompatibility	3
Tissue Colour	1	Stearoyl-acyl	1
Curd Colour	3	Storage protein	1
Flower Clusters	1		
Rosetting	1		
Self-incompatibility	1		
Total	20	Total	7

After King (1990).

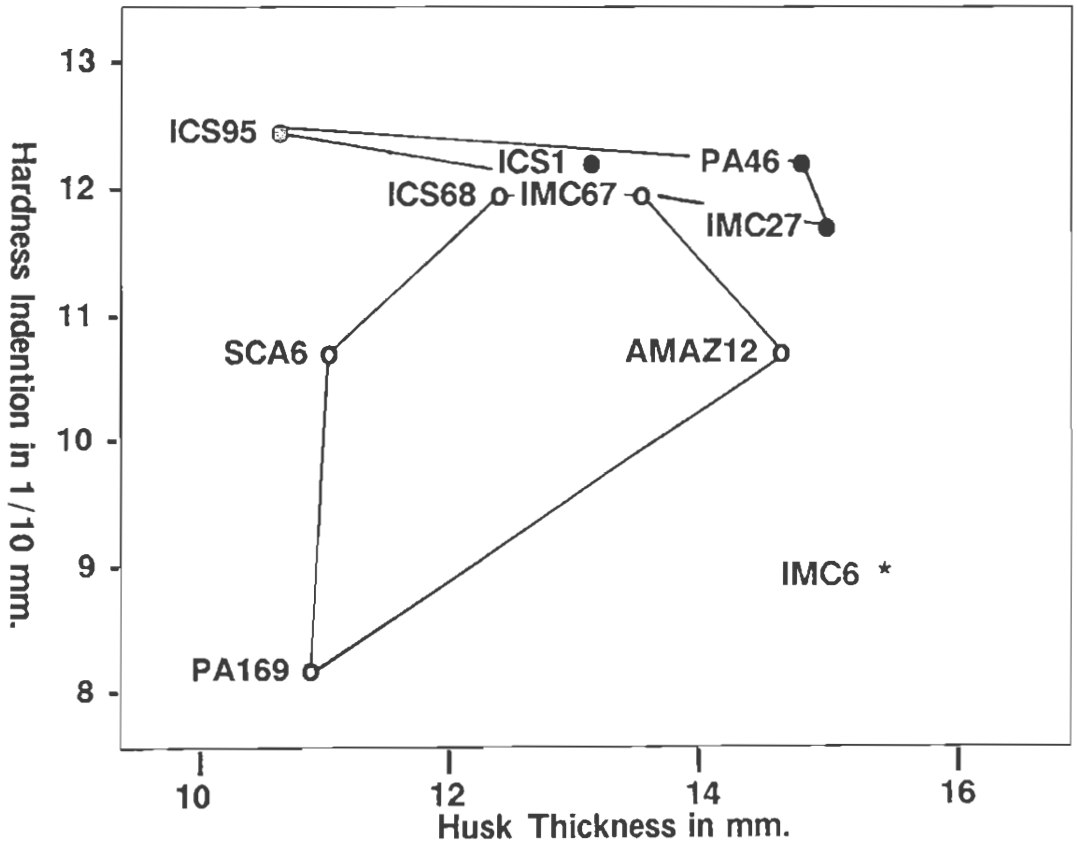
DNA from part of a cotyledon. Thus progeny segregating for incompatibility type could be screened even before the beans themselves germinate. The extracted DNA sample would undergo PCR and the fragments would be separated on a gel using electrophoresis. Progeny containing the RAPD band linked to self-compatibility would be retained and germinated to pass into a conventional screening programme for yield etc. Progeny containing RAPD phenotypes consistent with incompatibility would be discarded. This technique could considerably reduce the number of plants (and hence cost) that require conventional long term screening.

It is possible to extend our model system further to include additional qualitative traits. Let us assume we have identified a single major gene responsible for making pods unattractive to rodents (Warren and Emamdie 1992). Conventional screening for pod traits is even more long term than the selection of incompatibility type, as the trees must be maintained to fruit bearing age. However, as with the 'S' allele controlling incompatibility we should be able to identify RAPD marker associated with the rodent resistant phenotype. The screening procedure would then be identical to the one outlined above. Material would be selected before germination and only selected stock would be retained to enter long term screening trials. Planting material now entering the long term trials could be preselected as being self-compatible and rodent resistant. However if the markers for self-compatibility and rodent resistance occurred only in separate individuals, we would require a further generation to combine them. Here again new technologies may be able to help. In many tree species the time to flowering has been dramatically reduced by the use of plant growth regulatory substances. For example, to aid breeding work for commercial conifers, time to first flowering has been reduced from 20 to 2 years by the application of GAA and NAA (Tompsell, 1978). This area remains underexploited in cacao. The key to flowering appears to be related to jorquetting, but little else is known.

Inducing early flowering in a seedling of a year or so, may only partly solve our problem of reducing generation time. Although such precocious flowering plants may act as male parents they are unlikely to be able to support their pods through to maturity. Physiological constraints are likely to result in pod abortion via cherelle wilt. However, if the tree can be encouraged to support its pods to this early stage, then the developing embryos can be excised from the cherelle and cultured to produce mature plants using embryo rescue techniques. Such tissue culture techniques are already used to propagate material from pods around the cherelle wilting stage. Workers in this area see no reason why the lower age limit could not be reduced even further. So far this goal has not been a target of their efforts. If these two technologies could be refined and modified for cacao then it may become possible to reduce generation time in breeding programmes to around a year. This would allow us to combine markers such as those for self-compatibility and rodent resistance in a minimal time period. This methodology will be limited in its application to the combining of major gene traits. However, the rate limiting step in cacao improvement is likely to be the screening of polygenically controlled yield phenotypes, which must be recorded from mature trees.

The model cacao breeding system outlined above requires the effort of 4 groups of cacao scientists for its development. Firstly, geneticists are required to identify major gene effects of economic importance. As pointed out, we are currently in the embarrassing position of having an unlimited supply of markers but we have nothing to mark. We could `easily' map the cacao genome but the map would be an empty grid with nothing of importance on it. Possible target traits include butterfat hardness and quantity, certain aspects of taste and perhaps disease resistance. This area of investigation is best performed at the major germplasm collection centers where large numbers of individuals can be screened in the search for such desirable genes, which may be rare in natural populations.

Figure 1 Scatter Plot of pod thickness and hardness



Once major genes of importance have been identified, the next stage is for the molecular geneticists. It will be their responsibility to identify markers so that individuals can be screened for the trait of interest without the requirement of long term trials. This work is likely to be performed at several research centers around the globe. Collaboration in this area is essential. The alignment of separate linkage maps will require the sharing of markers, primers and probes. Access to common reference genotypes is also important. Here again is a role for the germplasm collection centers. Currently isozymes are often used as sign post markers in the aligning of separate linkage maps. This may remain true until the stability of RAPD banding patterns between labs and thermocyclers is better established.

If generation time is to be reduced to facilitate the more rapid combining of genes, then more physiological research into the stimuli to flowering is required. This may include the investigation of plant growth regulatory substances along with grafting techniques. Finally, embryo rescue techniques may need to be developed to salvage very young embryos if the necessity arises.

By concentrating upon the development and utilization of new technologies, I have not stressed enough, the need to develop stable, long term conventional breeding work, based on established sound scientific procedures. It is imperative that conventional long term breeding programmes be maintained and indeed expanded for quantitative traits such as yield and horizontal disease resistance. The reasons for this are three-fold

(1) all the desirable genes in a polygenic system cannot be assembled in a single individual in a single generation

(2) It is impractical to screen using gene markers when many genes producing small effects upon the trait are involved

(3) Quantitative traits tend to be greatly influenced by genotype/environment interactions, thus screening for such traits has to be done locally.

It is envisaged that the utilization of the International Cocoa Germplasm Database (I.C.G.D.) will make an important impact on conventional breeding during the 21st century. Again let me illustrate with a possible example. Figure 1, Pod wall thickness data extracted from the I.C.G.D is correlated to observed rodent susceptibility (Emamdie and Warren, 1992). Thus, if selecting for polygenic horizontal resistance to rodents, the breeder could utilize the database records in selecting parents.

A possible major stumbling block lies in the path of the conventional cacao breeder; that of flavour. Crop improvement in many other species has been criticized for neglecting flavour. Strong selection for traits such as yield and disease resistance has allowed unselected characters such as taste to be greatly altered by random processes. This oversight, if it occurred in a crop such as cacao where the end product is processed could be far more important. It is possible to seduce the consumer into buying a large attractive banana or apple etc., only later to find it has no taste. However, this does not hold true for chocolate since it is taste which sells one brand of chocolate over another. Furthermore, the problem is confounded by the many environmental factors involved. It is a relatively simple procedure to evaluate the flavour of a fruit crop. However, much more effort is required in sampling the taste of a new cacao variety. I am not proposing that flavour 'improvement' should be a part of breeding programmes, only that it should not be forgotten and allowed to change through random genetic drift.

The Take Home Message

As we stand in the doorway of the 21st century the majority of the world's cacao production is still produced by material not significantly different from its wild progenitor. The recent advancements in molecular genetics and the many more which are to come, seem likely to be restricted in their impact on crop improvement in cacao by our limited knowledge of the basic genetics of the crop. We are as yet still unable to apply the techniques of genetic engineering to cacao. However, there seems little point at the moment in concentrating on this new science, which alters only small fragments of the genome, while neglecting conventional selection methods. The cacao crop of today should be considered as being as far removed from the cacao crop of the future as a crab apple is from a modern commercial apple variety. No one would consider improving the apple crop by inserting genes into crab apples, so why then should we consider 'fine tuning' the cacao genome, until we have improved the entire genome by conventional selection? Indeed, some would argue that in the immediate future the application of molecular biology may in fact hinder advancement in breeding by taking resources from it.

The bottom line for cacao improvement in the 21st century must surely be that, to gain the maximum benefits from the available new technologies we must first effectively apply the established techniques of the 20th century in producing improved varieties and in understanding the genetic control of economically important traits.

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