EXTENDING FRONTIERS OF GENETIC IMPROVEMENT IN CASSAVA

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SUMMARY

Special problems retarding genetic improvement are discussed and methods for overcoming barriers for gene flow are evaluated. The principles discussed have been applied in the cassava breeding programme at Trivandrum and proved valuable. Pachytene analyses for studying the karyotype and chromosomal homology at interspecific level is described and has revealed the very close relationship of cassava and *Manihot glaziovii*. Three clones from variety x variety crosses have been selected after careful testing. These are H-165, H-97 and H-226. They have 2–3 times the yield potential of local 'control' cultivars in regional trials. For future work a return to the diploid level for more intense selection before reforming polyploids from selected diploids is suggested as a promising technique.

RESUME

Les facteurs particuliers liés à l'arriération de l'amélioration génétique ont été abordés et des méthodes pour progresser évaluées. Les principes avancés ont été mis en application par le programme de sélection du manioc à Trivandrum et ils se sont révélés dignes d'intérêt. Des analyses pachytènes pour l'étude de caryotype et d'homologie chromosomique ont révélé la relation très étroite qui existe entre le manioc et *Manihot glaziovii.* Trois clônes H-165, H-97 et H-226 issus de croisements variété x variété ont été sélectionnés après un essai minutieux. Ils ont 2–3 fois le potentiel de rendement des cultivars "temoins" locaux en essais régionaux. L'intensification de la sélection au niveau des diploides avant de réformer les polyploides à partir des diploides sélectionnés a été proposée comme une technique pleine d'espoir dans la poursuite des travaux de recherche.

RESUMEN

Se discuten problemas especiales que retardan el mejoramiento genético y se evaluan métodos para superar barreras al flujo de genes. Los principios discutidos han sido aplicados en el Programa de Hibridación de Yuca en Trivandrum y han probado ser valiosos. El análisis en fase de paquiteno, para el estudio del kariotipo y la homología cromosómica – que se describe- han revelado una relación muh cercana entre yuca y *Manihot glaziovii.* Tres clones de cruzas variedad x variedad se seleccionaron después de cuidadosas pruebas, ellos son: H-165, H-97 y H-226. Estos clones alcanzan 2–3 veces el rendimiento potencial de los cultivares "testigo" locales, en los ensayos regionales.

Se sugiere, como técnica prometedora para futuros trabajos, el regresar al nivel diploíde para hacer una selección más intensa, antes de reformar los poliploídes a partir de los diploídes seleccionados.

INTRODUCTION

Cassava is a multi-purpose crop. Its acknowledged superiority in calorie production and its adaptability to a wide variety of conditions is offset only by its longer crop duration and relatively high labour requirement for cultivation[7,25,26,27]. These facts have ensured its continued cultivation in the face of competition from several other crops (which have been the subjects of comprehensive studies by plant scientists). Much remains to be accomplished in adapting cassava to its various roles and in raising its current status in several parts of the world from a subsistence crop to that of an industrial crop[17]. Understanding the genetic architecture of its important agronomical, biochemical and physiological characters can assist in recombining these characters, employing modern breeding techniques[19]. Recent genetic research opens up new possibilities.

SOME AIMS FOR THE IMPROVEMENT OF CASSAVA

Present day cassava cultivars can be improved upon to meet the growing demands on them, as food, feed and/or industrial crop[5,6,19]. Of primary importance among the needs are:

1. Production of high-yielding cultivars of improved plant types, wider adaptability and responsiveness to modern agronomic practices, particularly the use of fertilizers.

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2. Incorporation of resistance against important diseases e.g. 'cassava mosaic' and pests (*Bemisia* spp., scale insects, red spider mites etc.)
3. Upgrading tuber quality including lowering of cyanide content.
4. Prolonging storage life and enhancing suitability for processing.
5. Reducing maturity.
6. Improving cold and drought tolerance.

SPECIAL FACTORS AFFECTING THE GENETIC IMPROVEMENT OF CASSAVA

A high degree of heterozygosity can be presumed, since monoecy promotes outbreeding and vegetative propagation stabilises this. Non-flowering cultivars (clones) occur frequently, and among those that flower, varying degrees of gametic and zygotic sterility are common[3,14,15]. This imposes a restriction on the exploitation of naturally occurring genetic variability since many clones cannot be used as parents. Many seeds are also inviable.

The possibility of securing a significant level of homozygosity is hampered by severe inbreeding depression[7] which may be explained by a high genetic load of deleterious recessive mutations which are not exposed in heterozygotes.

While fixation of desirable genotypes can be achieved immediately by clonal propagation, quick multiplication of promising clones for practical utilization needs to be worked out.

GENETIC STUDIES

Information on the genetics of the important agronomical, biochemical and physiological aspects is almost totally lacking. In acquiring this information, the possibility of polyploid origin[18] is important because of the gene buffering and interaction to be expected especially in polyploids.

An F_1 population arising from hybridization of heterozygous parents is in the genetical sense more equivalent to an F_2 generation with respect to heterozygous loci common to the parents. Hybridization, therefore, will promote the segregation of parental genes in addition to reshuffling of paternal and maternal chromosomal complements and production of new recombinations arising from crossing over within linkage groups. For maximum efficiency and reliability of hybridization programmes, parents should be genetically diverse and highly fertile to facilitate raising of a sizable F_1 population.

Based on phenotypic diversity and geographical origin, intervarietal crosses were made using a common maternal parent of Madagascar origin and three pollen parents originating from Malaysia, Madagascar and a hybrid between Brazilian and Indian cultivars. The F_1 populations were scored for several contrasting qualitative and quantitative traits and for various biochemical constitutents[23,24]. The F_1 populations of all these three crosses consisted of branched and unbranched plant types even though all the parents exhibited branching. The three populations differed in the frequency of narrow and broad-leaved plants. In crosses involving narrow and broad, the proportion of both the types were nearly equal while narrow x narrow crosses produced narrow leaved plants only. The colour of stipules in F_1 plants of deep purple x deep purple crosses included both green and purple types in some crosses and all purple stipuled seedlings in one cross. In deep purple x green petiole hybrids, almost equal numbers of plants of each petiole colour were produced. All the contrasting parental traits were expressed in the F_1 populations as well as new traits confirming the heterozygosity of the parental genotypes.

The important plant characterisics which affect tuber yield in cassava[1,23] are the number and size of tuber, the size and efficiency of the leaf canopy, the ratio of top to roots and the duration of the period before tuberization. Character association studies for several of these characters in F_1 populations are revealing of their relationships to the yield. Tuber length, tuber circumference and tuber number per plant were all positively correlated with tuber yield, but tuber number per plant was negatively correlated with tuber length and circumference. Hence, simultaneous improvement for all the three yield components is likely to be difficult and a compromise will be essential. The breeder can profitably select for moderate numbers of medium sized tubers of moderate numbers with the expectation of securing a high proportion of marketable tubers and ease of harvesting and handling.

For plant type the breeder was a choice between branched and unbranched types, both of which can be selected from hybrid populations. Unbranched types would probably be preferred when mechanized cultivation is under consideration. Under rainfed conditions it seems that hybrids between cultivars with widely divergent leaf number can be selected that have a high leaf number and associated high yield potential.

SCOPE FOR GENETIC MANIPULATION OF BIOCHEMICAL CONSTITUENTS

Starch, dry matter crude protein and cyanide contents show wider variation in the hybrid populations than exists among the parents[24]. Crude protein values in many hybrids were appreciably higher than found in parents. Character association studies for the above four traits indicated their independent inheritance. The breeder, therefore, can expect to be able to achieve simultaneous improvement for each.

INTENSIFICATION OF CYTOGENETICAL STUDIES

There are other *Manihot* species[29], several of which carry desirable genes for disease resistance (e.g. *M. glaziovii*) and relatively high protein content (e.g. *M. melanobasis, M. saxicola*) which can be crossed readily with cassava[1,7]. There have been cytogenetical studies by the use of pachytene analyses and related cytogenetical studies in both species and species hybrids in order to explore the possibilities for successful interspecific gene transfer.

Pachytene analysis has proved suitable for studies of chromosome homologies in karyotype analysis[4,13,20,21]. In cassava and *M. glaziovii*, all the eighteen pachytene bivalents, which are morphologically distinguishable were identified. In both species several duplicated chromosomal types were found. In the light of the lower chromosome number reported for other Euphorbeacean genera and the behaviour accompanying polyploidization, the presence of three nucleolar-associated bivalent types and duplicated chromosomal types in both cassava and *M. glaziovii* suggested a polyploid origin for the *Manihot* species with $n = 18$[18].

Some chromosomal differentiation between cassava and *M. glaziovii* was apparent from the comparative study of their pachytene karyotypes, and was in agreement with observations on chromosomal homology made at pachytene stage in their interspecific hybrid[20,22]. The major chromosomal differentiation in the two species seemed to involve three chromosomes of the haploid complement.

Pachytene analyses in back cross plants showed no departure from random assortment of parental chromosomes and confirmed the close relationship between the two species (evident in the intimate synapsis of the parental chromosomes at pachytene stage in the F_1 hybrid) and absence of barriers for gene exchange.[20]

Cytogenetical studies on similar lines might profitably be carried out in other *Manihot* species in order to resolve the impasse in the taxonomic treatment of the genus.

Studies on chromosome morphology in artificially induced triploids ($3n = 54$) and induced tetraploids ($4n = 72$) have thrown light on the nature of chromosome pairing as related to factors affecting fertility and meiotic irregularities[2,10,11]. Efforts are now underway to obtain aneuploids by making triploid-diploid crosses. This should be possible because the triploid produces viable aneuploid gametes that may unite with euploid gametes from the diploid parent.

Comparative developmental studies of several male fertile and male sterile lines of cassava have been conducted[3,14]. Cytological data suggest that meiotic abnormality is not the cause of pollen degeneration in the material studied. In some male sterile lines, degeneration of individual microspores may be due to the failure of the separation of microspores from tetrads, but in other male sterile lines pollen abortion has been attributed to abnormality of the structure and function of the tapetum. Attempts to study the genetics and breeding behaviour of various male sterile lines are underway.

CHOICE OF BREEDING STRATEGY

The approaches described have been followed and their results are apparent in the success of the cassava breeding programme at Central Tuber Research Institute, Trivandrum. By stringent selection and evaluation procedures in the germplasm collection several superior seedling selections were identified. A large scale hybridization programme among diverse genotypes was followed by rigorous selection in the hybrid populations and led to the recovery of promising F_1 hybrid plants as clones. Among those in the advanced stages of evaluation are selections S-1309, S-1310, S-1315, S-2380, S-2331 and S-2480 and hybirds H-165, H-97, H-226, H-86, H-43, H-2057 and H-57. Several selections have proved outstanding in local and multilocation yield trials conducted over three years, recording two or three fold higher tuber yield (ranging from 23.5 to 87.7 t/ha) than the respective regional or local controls (yield range 10.0 to 31.0 t/ha). Evaluation used a block design with four replications, NPK at the rate of 100 kg/ha in two split doses, and spacing of 90 cm x 90 cm. Three hybrids H-165, H-97 and H-226 have been released, backed by a package of recommended agronomic practices for general and commercial cultivation in the states of Kerala, Tamil Nadu and Mysore. These three hybrids all possess good responses to fertilization, a high degree of field resistance to Cassava Mosaic virus disease, scale insects, red mites and mealy bugs and good tuber qualities, namely, desirable tuber size, high starch and low cyanide contents, good cooking quality etc. They are relatively early maturing and are well adapted to a wide range of agro-climatic conditions[12].

Mutation breeding in cassava improvement might be useful when rectification of specific defects in commercial varieties, such as high toxicity, late maturity or disease susceptibility is required. Already, by using a dose range of 4000 r and 7500 r. a mutant of var 300 has shown an increase in starch content to 31.3% (on fresh weight basis) and decrease in cyanide content to 0.01 percent[31].

In view of the polyploid origin of cassava, notwithstanding the practical difficulties[9,16,30], 'analytical breeding' or a 'genome approach' is being pursued for efficiently tackling the problems of intergenomic and intragenomic repatterning[7]. The main aim of this method is to reverse the evolutionary course and regenerate diploids from polyploids with a view to being able to intensify selection before returning to the polyploid level. This approach may also provide promising materials for basic genetic, cytogenetic and evolutionary studies which would be of great value in further improvement of the crop[28].

REFERENCES

1. Jennings, D.L. (1970) Cassava in Africa. *Field Crop Abstracts.* 23, 271-8.

2. Jos, J.S., Magoon, M.L. and Nair, S.G. (1970) A cytological and morphological analysis of triploid cassava. *Genet. Iber.* 22, 27-39.

3. — — — — —, Sadasivaiah, R.S. and Appan, S.G. (1966) Studies on sterility in cassava I. Mechanism of pollen abortion in some male sterile lines. *Indian Jour. Hort.* 23, 177-84.

4. Krishnan, R., Magoon, M.L. and Vijaya Bai, K. (1970) The pachytene karyology of *Manihot glaziovii. Genet. Iber.* 22, 177-91.

5. Magoon, M.L. (1967) Role of root and tuber crops in human nutrition. Proc. Int. Symp. Sub-trop. and Trop. Horticulture, New Delhi. 1, 46-61.

6. — — — — — (1967) Recent trends in cassava breeding in India. Proc. Int. Symp. Trop. Root and Tuber Crops (Trinidad). 1, 100-116.

7. — — — — — (1968) Some immediate problems, possibilities and experimental approaches in relation to genetic improvement of cassava. *Indian Jour. Genet.* 28A, 109-26.

8. — — — — — (1970) Problems and prospects in the genetic improvement of cassava in India. Proc. 2nd Int. Symp. Trop. Root and Tuber Crops, Honolulu, Hawaii, U.S.A. 1, 58-61.

9. — — — — —, Cooper, D.C. and Ramanujam, S. (1962) Cytogenetical studies in relation to the origin and differentiation of species in the genus *Solanum. Caryologia* 15, 151-2.

10. — — — — — *et al.* (1969) Cytomorphological studies in induced polyploids of cassava. *Genet. Iber.* 21, 27-48.

11. — — — — — (1970) Cytogenetics of induced polyploids of cassava. Tropical Root and Tuber Crops Newsletter 3, 18-20.

12. Magoon, M.L., Appan, S.G., Krishnan, R. and Mandal, R.C. (1970) Some promising high yielding hybrids and selections of cassava. S.A.B.R.A.O. Newsletter (Japan) 2, 19-26.

13. — — — — —, Jos, J.S. and Appan, S.G. (1966) Cytomorphology of the interspecific hybrids between cassava and ceara rubber. *Chromosome Information Service (Japan).* 7, 7-10.

14. — — — — —, Jos, J.S. and Nair, S.G. (1969) A morphological, embryological and cytological study of male sterility in *Manihot esculenta.* Tropical Root and Tuber Crops Newsletter, 2, 10-11.

15. — — — — — and Vasudevan, K.N. (1968) Male sterile cassava. *Nucleus.* 11, 1-6

16. — — — — —, and Khenna, K.R. (1963) Haploids. *Caryologia,* 16, 191-235.

17. — — — — — and Krishnan (1971) Genetic improvement of tuber crops (other than potatoes) in India. Proc. 2nd Int. Symp. Sub-trop. and Trop. Horticulture. 2, 1-20.

18. — — — — — (1971) Problems on the elucidation of genomic evolution in some important tropical tuber crops. Proc. Symp. Genome Concept in Eukaryota, Indian Sci. Congr. Calcutta.

19. — — — — — (1972) Plan and prospects for the improvement of tuber crops (other than potatoes) Proc. All India Symp. Adv. in Cytogenetics. 1, 1-19.

20. — — — — — and Vijaya Bai, K. (1969) Cytogenetics of F_1 hybrids between cassava and ceara rubber and its back cross. *Genetica,* 41, 425-36.

21. — — — — — (1970) Morphology of the pachytene chromosomes and meiosis in *Manihot esculenta* Crantz. *Cytologia.* 34, 612-26.

22. — — — — — (1971) Chromosomal differentiation between cassava and ceara rubber. Tropical Root and Tuber Crops Newsletter. 4, 23-5.

23. Magoon, M.L., Krishnan, R. and Lakshmi, K. (1972) Association of plant tuber characters with yield in cassava. Tropical Root and Tuber Crops Newsletter. 5.

24. Magoon, M.L., Maini, S.B. and Krishnan, R. (1972) Breeding for tuber quality in cassava. Tropical Root and Tuber Crops Newsletter. 5.

25. — · — — – and Appan, S.G. (1966) Cassava — a food for the millions. *Indian Farming* 16, 12-13.

26. — – - — — (1966) Industrial utility of cassava. *Industrial and Labour Review* 4, 1-8.

27. Magoon, M.L. and Sinha, S.K. (1967) Non-conventional foods — importance of subsidiary root and tuber crops in India. Proc. Joint Symp. Science and India's Food Problem Natl. Inst. Sci. and I.C.A.R., New Delhi. 1, 318-31.

28. Magoon, M.L. and Ramanujam. S. (1960) Nature of chromosome pairing in the genus *Solanum* section *Tuberarium. Indian J. Genet.* 20, 22-41.

29. Rogers, D.J. (1965) Some botanical and ethnological considerations of *Manihot esculenta. Econ. Bot.* 19, 369-77.

30. Swaminathan, M.S. and Magoon, M.L. (1961) The origin and cytogenetics of commercial potato. *Advances in Genetics (U.S.A.)* 10, 217-56.

31. Vasudevan, K.N., Jos, J.S., Magoon, M.L. and Nair, S.S. (1967) Radiation-induced mutations in cassava. *Ind. Jour. Hort.* 24, 95-8.