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Cassava Breeding and Biotechnology Research at the Department of Agriculture, Thailand: *The Present Status and Future Needs*

Peaingpen Sarawat[1], Suchirat Sakuanrungsirikul[1] , Atchara Limsila[2] and Watana Watananonta[3]
[1]Khon Kaen Field Crops Research Center 180 Mitraparp rd. Tumbol Sila Amphoe Muang, Khon Kaen 40000 Thailand. Peaingpen@yahoo.co.uk, suchirat1@yahoo.com
[2] Rayong Field Crops Research Center. Amphoe Muang, Rayong 21150. Thailand
[3] Department of Agriculture, Chatuchak, Bangkok, 10900 Thailand

Introduction

Cassava, *Manihot esculenta* Crantz is an important economic crop of Thailand. In 2004 its planting area covers 6.76 million rais (1600 m^2), producing 21.44 million tons of fresh root (Field Crop Research Institute, 2005). Seventy percent of the production is export mainly to Asian countries (50% and EU (20%). The rest 30 % is domestic uses (Chutharatkul, 2005). Most of cassava production is used as source of energy in the feed industry and as source of starch. The national breeding program has been carried on by the Department of Agriculture (DOA) since 1957 for enhancing the productivity and for expanding the industrial uses. The series of varieties were recommended to farmers including Rayong 7 and Rayong 9, the two varieties released this year. In prospect, cassava genetic improvement will be more progress because of the available of diverse genetic resources and the use of new tools to assist clones selection and evaluation. New approaches i.e. cassava inbreeding and MAS techniques have been introduced into DOA breeding program to improve its efficiency.

Present status of cassava breeding

Breeding objectives

Cassava breeding objectives have been set after the end-use of cassava production. One is to improve cassava for high starch yield. The aim of this objective is to increase productivity for starch industry as well as for ethanol production. Two is to improve cassava for leaf production. The purpose is to diversify the use of cassava as local feed. Dry young shoot cassava is potentially used as a protein source for animal dietary. It has been accepted in dairy farms because the supplementation of protein from cassava hay could reduce milk-production cost.

Genetic resource and germplasm enhancement

Creation of broad genetic variability in cassava population through collection and introduction is essential for successful recombination of certain desirable traits. Cassava germplasm in Thailand was first introduced from Indonesia, Virgin Island, and CIAT. Together with landrace and promising clones from routine cassava breeding, they were conserved totally 266 accessions. In 2004, these clones were reevaluated emphasis on root yield and starch quality. Their root properties are concluded in table 1 (Field Crop Research Institute, 2005)

Table 1 Root properties of 266 cassava accessions evaluated in 2003-2004 at Rayong Field Crop Research Center harvested at 12 month-old.

Root Properties	Range
Fresh Root Yield (t/rai)	1.98-14.85
Starch Content (%) estimated from fresh root using Reiman's Scale	10-32
Biomass (t/ha) fresh weight	5.21-24.92
HCN (ppm)	5.95-177.9
Protein in Leave (%)	19-29
Protein in Root (%)	0-3
Starch Content (%) estimated from hand extraction	6.3-28.9

Recently, CIAT collaborated with DOA to send a duplicate of CIAT cassava core collection to be preserved in Thailand. Four hundred accessions were obtained in the form of *in-vitro* plantlets and conserved at Rayong Field Crop Research Center. Plantlets have been transplanting and evaluating for yield and root properties. The latter germplasm shows higher variation in morphological traits than that of the former. Their root characteristics are expected to be more variation as well. Clones with desirable traits of fulfilling breeding objective will be used to form new segregating populations.

The first set of germplasm has been conserved in fields in RYFCRC. Some accessions were duplicated and grown in field at Khon Kaen and Nakorn Rachsima Field Crop Research Center (KKFCRC and NMFCRC). The second set has been preserved as *in-vitro* plantlets in laboratory and as living plant grown in field at RYFCRC.

Inbreeding cassava

In 2004, CIAT and DOA established a collaborative project of 'Inbreeding Cassava'. The project's aim is to facilitate the production of partially inbred germplasm and to evaluate their performance in search of useful recessive traits. Inbreeding cassava will offer several advantages in cassava improvement. Inbred lines are better progenitors because by definition they carry lower levels of genetic load. Heterotic effects would be effectively exploited from inbred lines. The available homozygous lines would be greatly useful in genetic study and molecular marker analysis (Ceballos *et al.*, 2004).

Elite germplasm has been self-pollinated since 2003 at KKFCRC and RYFCRC. A number of partially inbred seeds have been generated (Table 2). These inbred are continued on self pollination. The S2 and S2 plants will be evaluated and used in the formation of new hybrids.

Table 2 Number of self-pollinated seed produced from elite germplasm at KKFCRC and RYFCRC in 2003-2004.

Parental clones (number)	Number of partially Inbreds	
	S1	S2
5	2267	230
40	2336	0
Total = 40	4,603	230

Breeding method

Cassava is an open pollinated crop with allotetraploids (2n=4x =36). It is generally uniformly grown from stems although some varieties produce a lot of seeds. Most of cassava cultivars are heterozygous in common. A new variety usually developed by crossing two clones then selected for the best and disseminated to farmers.

Cassava breeding schemes in DOA composes of the series of hybridization, selection, evaluation and recommendation. Hybridization and selection is almost conducted in RYFCRC. Approximately 10,000 seeds is annually produced and grown in fields for selection. First year selection, seeds are germinated and transplanted in field with spacing of 1.50 x 1 meter. The plants have grown for 10-12 months. Superior clones will be selected based on the proportion of root weight and total plant weight. The clones are planted in row with spacing of 1x 1 meter with in 10 plants per row per clone. At that time, recommended varieties are grown as checks in every 10 rows. The out-standing rows are selected based on plant form, row yield and starch content.

The selected rows will be further evaluated for yield potential and yield stability at least 5 years as preliminary trial, standard trial, regional trial, farm trial and field test respectively. Yield evaluation takes long time because of the restricted of planting material. The other result is to ensure that the new recommended varieties give consistent high yield and well adapted to diverse growing environments.

New released varieties

In 2005, two cassava varieties were released by DOA namely Rayong 7 and Rayong 9. Rayong 7 derived from the cross of CMR31-71-25 and OMR29-20-118 which was pollinated in 1992 at RYFCRC. After crossing the progenies were selected and evaluated in 51 plots over cassava growing area in the North and Northeast regions. The average of root yield and starch content was 6.08 t/rai and 27.7 % respectively (Boonseng, 2005). Rayong 7 cuttings are good at germination and well establishment. This variety is high in yield and starch content and well adapted to post rainy planting.

Rayong 9 the newest released is recommended to grow for ethanol production (Field Crop Research Institute, 2005). This variety is derived from the cross of CMR31-19-23 and OMR29-20-118 conducted in 1992 at RYFCRC. The result from 38 trials was concluded that Rayong 9 gave starch and dry root yield of 1.24 and 2.11 t/rai. The ethanol yields were 191, 208 and 194 litr/ton fresh root when harvested at 8, 12 and 20 month-old respectively. Rayong 9 has erect plant and long stem, giving high multiplication rate of 1:8.

Present status of biotechnology research

Molecular marker - assisted breeding for starch content and cyanide

A project to support the DOA's cassava breeding program for selection of high starch content lines was fortified using MAS approach. The study was conducted at Khon Kaen Field Crop Research Center in 2004 where breeding populations and laboratory facilities were available. DNA from bulk samples of high and low starch contents varieties were screened to find out the feasible markers. They were SSR (Mba, 2001), ISSR, RAPD and others. Polymorphic markers in the bulk were then employed to analyze individually of the bulks. Markers that showed consistent polymorphism in the individuals, as well as with bulks, were chosen to analyze the entire family. Preliminary evaluation of putative markers, especially those derived from RAPD markers, encountered the problem of linkage disequilibrium. This may be caused by diverse genetic background of the samples. The evaluation of these selected markers revealed that the bands associated with high starch content in parents, were not appeared the same pattern in their selfing and their crossing populations. Further works are thus required the screening of more markers to eliminate these problems.

Characterization of genetic diversity of local varieties, hybrids and cassava germplasm using DNA markers

Over 600 cassava accessions have been collected and maintained at Rayong Field Crop Research Center. To achieve a better understanding of genetic diversity present in this collection is essential. The assessment of the genetic diversity and genetic differentiation on these resources using DNA marker was initiated in 2004 at Khon Kaen Research Center. The output of this study is expected to provide guidance for conservation and cassava improvement programs. ISSR markers combined with Touchdown PCR were effectively used to quantify the genetic differentiation in *Durio* spp. (Sakuanrungsirikul, 2005). Then this technique has been applied to identify the genetic diversity of cassava germplasm. So far, twenty-five ISSR markers were used to identify the genetic difference of 303 cassava accessions. A high number of alleles (total 340 alleles) and high level of polymorphism (93%) were observed. The polymorphism of 28 accessions was preliminary analyzed for their genetic differences by NTSYS program using Jaccard similarity coefficient. The genetic similarity coefficient among the first sample set was ranged from 0.39 to 0.89. In which the highest similarity pair was Yellow Root and Kaset. Genetic assessment of the remaining accessions is ongoing and will be completed by 2006.

Double haploids

The idea of using double haploids in cassava breeding was raised from CIAT by Dr. Hernan Ceballos. The inbreeding cassava project as mention before involves the development of an anther

culture protocol for the production of double haploids. During CIAT has developed the protocol, a DOA's researcher has preliminary observed on the growth stage of cassava anther. The study on culture media in callus induction are currently undergoing (Field Crop Research Institute, 2005).

Future needs

Cassava breeding is time consuming and laborious while the genetic progress seems slow. We need to make a breeding cycle shorter and genetic progress faster and more consistent. The use of MAS will increase breeding efficiency. Therefore, we need to develop markers related to desirable traits and need improving our technical skills on the use of markers to assist the selection of superior hybrids. More over we also need to be equipped with the knowledge and ability to develop marker sets of our desired traits.

Cassava has been widely utilized only as a source of carbohydrate. In fact, we can expand the industrial uses from cassava by developing new products. Cassava leaf has high protein, beta-carotene and vitamins. The leave is possibly used for producing enzymes in commercial scale (Tham *et al.*, 2005). The deep yellow root flesh contains high beta-carotene (>80 micro gram/g DW) while the pink root flesh contains high lycopene (>90 micro gram/g DW) (Carvanho, 2005). To improve varieties with high value of these traits and with novel starch type we need improving our technical skills on the analysis of leaf and root qualities.

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