IMPROVEMENT
OF ROOT AND TUBER CROPS
BY INDUCED MUTATIONS

CONCLUSIONS AND RECOMMENDATIONS
OF THE FIRST AND SECOND RESEARCH CO-ORDINATION MEETINGS
OF THE FAO/IAEA CO-ORDINATED RESEARCH PROGRAMME
ON IMPROVEMENT OF ROOT AND TUBER CROPS
AND SIMILAR VEGETATIVELY PROPAGATED CROP PLANTS
IN TROPICAL COUNTRIES BY INDUCED MUTATIONS
HELD IN PATTAYA, THAILAND, 17–21 DECEMBER 1984
AND IN VIENNA, 2–6 JUNE 1986

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REPORT OF THE
SECOND RESEARCH CO-ORDINATION MEETING

(VIENNA, 2–6 JUNE 1986)

CONCLUSIONS AND RECOMMENDATIONS
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FOREWORD

The development of improved cultivars of vegetatively propagated crop plants is handicapped by the difficulty (or even impossibility) to perform cross breeding and in this way obtain genetic variants for selection. In some cases cross pollination and seed propagation would be easy but not acceptable, for it would break down the rare genetic structure of a highly appreciated clonal cultivar. Spontaneous somatic mutations (so-called sports) on the other hand are a much too rare event for the determinate plant breeder. Consequently, the application of ionizing radiation and other mutagenic agents seems to be the method of choice for achieving both - production of genetic variants for selection and maintenance of the appreciated clonal characteristics. However, as simple as this sounds, there are a number of problems to overcome e.g. chimeric structure from mutagen treatment of buds; multiple mutations which cannot be separated by segregation; long cycle and large space required for certain plants; difficulties in selecting the rare desired mutant from large populations.

Therefore, a co-ordinated research programme was established by which interested plant breeders were given the opportunity to co-operate in developing the required technology and to gain mutually from their experiences.
EDITORIAL NOTE

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### CROPS

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REPORT OF THE
FIRST RESEARCH CO-ORDINATION MEETING
(PATTAYA, THAILAND, 17-21 DECEMBER 1984)
CONCLUSIONS AND RECOMMENDATIONS
1. INTRODUCTION

The Joint FAO/IAEA Division of Isotopes and Radiation Applications of Atomic Energy for Food and Agricultural Development has established a Co-ordinated Research Programme on Improvement of Root and Tuber Crops and Similar Vegetatively Propagated Crop Plants in Tropical Countries by Induced Mutations.

The crops considered – cassava (= yuca) (Manihot esculenta), sweet potato (Ipomea batatas), potato (Solanum tuberosum), yam (Dioscorea spp.), tannia (= new cocoyam) (Xanthosoma spp.), taro (Colocasia esculenta) and sugar cane (Saccharum officinarum) – are the major sugar and starch producing crops which provide staple food for about 500 million people in developing countries of Africa, the Caribbean, South America, and South East Asia (see Table 1). In Africa, 35-40% of the daily calorie intake in the human diet is provided by roots and tubers.

Table 1: Area and production of the main root and tuber crops and sugar cane in 1983
(Source: FAO Production Yearbook, Vol. 37, 1984)

<table>
<thead>
<tr>
<th></th>
<th>Area, 1000 ha.</th>
<th>Production, 1000 MT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Developed</td>
</tr>
<tr>
<td>Roots &amp; tubers, total</td>
<td>47,523</td>
<td>13,214</td>
</tr>
<tr>
<td>Cassava</td>
<td>14,879</td>
<td>--</td>
</tr>
<tr>
<td>Yam</td>
<td>2,471</td>
<td>8</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>7,914</td>
<td>133</td>
</tr>
<tr>
<td>Taro</td>
<td>1,133</td>
<td>30</td>
</tr>
<tr>
<td>Potato</td>
<td>20,167</td>
<td>13,034</td>
</tr>
<tr>
<td>Sugar cane</td>
<td>15,394</td>
<td>916</td>
</tr>
</tbody>
</table>
The tropical root and tuber crops are important in direct human consumption either as fresh food or as a variety of flours, in their utilization as animal feed and in providing raw materials to industries producing ethanol, textile and paper, adhesive, glucose, dextrine and other foodstuffs, e.g. bread preparation, biscuits and confectionery.

International organizations as well as national governments realize the economic importance of these sugar and starch producing crops and are concerned with the improvement of their performance. Still it appears that to a varying extent, depending on the crop, they have been neglected or received less attention from plant breeders.

Recently, excellent progress in developing improved varieties has been made at international agricultural research centres such as IITA in Nigeria, CIAT in Colombia and CIP in Peru and also in some national programmes. However, due to phytosanitary regulations the exchange and movement of these materials has been very restricted at times. Therefore, the results obtained have benefited the various national programmes to a very limited extent.

The ultimate aim of the FAO/IAEA Co-ordinated Research Programme is to breed cultivars which are more productive, more resistant to diseases and pests, and which have better quality, higher tolerance to various stresses e.g. drought, heat and salinity and improved roots' and tubers' shelf life.

The first Research Co-ordination Meeting was held by the Joint FAO/IAEA Division in Pattaya, Thailand from 17-21 December 1984. The meeting was attended by scientists from Nigeria, Ghana, USA, UK, Australia, Thailand, Pakistan and Japan, holding a research contract or agreement.

After reviewing the individual IAEA sponsored research projects and following discussions on suitable methodology for achieving the breeding objectives, the participants arrived at the conclusions and recommendations summarized below.
2. **CROPS UNDER CONSIDERATION**

The sugar and starch producing crops are usually propagated by tubers (potato), stem cuttings (sweet potato, cassava, sugar cane) or bulbils (yam, taro, tannia) rather than seeds. Normally they are highly heterozygous and polyploid, which makes the breeding of new varieties rather difficult. Additional problems are due to sterility, self-incompatibility, dioecism and a low number of flowers.

The participants felt that in addition to the extensively cultivated potato and cassava crops, there are many other root and tuber plants which have the potential to be cultivated and should be studied more intensively, e.g. *Colocasia esculenta* (taro), *Xanthosoma* spp. (tannia, new cocoyam), *Dioscorea alata* (greater yam), *D. rotundata* (white yam), *Amorphophallus* spp. (elephant yam), *Arracacia xanthorrhiza* (arracacha), *Oxalis tuberosa* (oca), *Ullucus tuberosus* (ulluco), *Helianthus tuberosus* (jerusalem artichoke), *Sphenostylis stenocarpa* (african yam bean), *Canna edulis* (queensland arrowroot), *Maranta arundinacea* (arrowroot), *Tacca leontopetaloides* (east indian arrowroot).

3. **BREEDING SYSTEMS**

In some species, e.g. cassava, potato, sweet potato and sugar cane large germplasm collections which proved valuable for breeding are available. However, in the other species, the available germplasm resources are rather limited and further collection of germplasm should be encouraged.

Genetic improvement through conventional breeding techniques is possible where sexual reproduction is available and a number of cultivars have been obtained through these approaches. Sexual reproduction can be useful in broadening the genetic variability. Pollen and seed irradiation could also be utilized to increase genetic variability.

Clonal selection of spontaneous mutations has been practiced for a long time and produced many of the varieties which are cultivated at present, but it has not been adequate to meet the needs.
Mutation induction can contribute to the improvement of a cultivar without altering most of its desirable characteristics and yield a new cultivar in a shorter period. Usually, bud treatment is practiced; suitable treatment doses are already known for some of the crops considered. For others they can be worked out.

Broadening the genetic variability through "somaclonal variation" following in-vitro cultures of protoplasts, single cells, callus and somatic embryogenesis should also be explored. So far, plants have been regenerated by in-vitro culture in several varieties of sweet potato, potato and sugarcane, but not in the other species. The main difficulties may be encountered in the evaluation and selection of the genetic variability generated. Therefore, development of methods for large scale selection at early stages will be very useful and should be given high priority.

Mutation breeding by using in-vitro technology can be expected to make a significant contribution to root and tuber crops improvement once the methodology is well developed. Initially, suitable treatment doses of chemical and physical mutagens should be determined for different in-vitro systems, in the various crops.

4. GENERAL BREEDING OBJECTIVES

The objectives vary with the crops considered, their utilization and the specific conditions of their area of cultivation. Higher yields, stability in production and disease resistance are the main goals for these crops, as for others.

Diseases and pests are the main yield constraints. In cassava the major diseases are: mosaic virus, bacterial wilt (Xanthomonas manihotis) and anthracnose (Colletotrichum spp.) and pests like mealy bug (Phenacoccus manihoti) and green spider mite (Mononychellus tanajoa) which severely affect the yield in Africa. In sugar cane, mosaic virus, smut (Ustilago scitaminea) and downy mildew (Sclerospora sacchari) are wide-spread in all the cultivated areas.
Other characters amenable to improvement by mutation breeding appear to be:

a) Plant architecture for a better canopy structure, e.g. lodging resistance in sugar cane: bushy type in yam; shorter stems in sweet potato.

b) Product quality, e.g. reduced content of toxic compounds such as alkaloids and glucosides, increased starch content, higher protein content with better amino-acid composition.

c) Improved storability of the roots or tubers (e.g. cassava, yam).

5. CROP SPECIFIC OBJECTIVES AND TECHNIQUES

5.1. Cassava

Cassava is used in the human diet and as animal feed in several countries of Africa (about half of the world’s production), South America and the Caribbean region and also in countries like Thailand, India, Indonesia and Asia.

Cassava improvement through conventional breeding techniques is possible; so far most of it was achieved by these methods. These techniques entail the production of large numbers of seeds and growing large segregating populations. This becomes a constraint in several national programmes because of the limited facilities and funds available.

The major production constraints are:

a) Diseases (e.g. bacterial wilt, cassava mosaic virus) and pests (e.g. mealy bug and green spider mite).

b) Difficulties in harvesting and processing due to variation in the size and shape of the tuberous roots.

c) Low value of "composite flour" for bread baking.

d) Long period from planting to harvest.

e) High perishability of the roots after harvest.

f) Low dry matter content.

g) Plant architecture unsuitable for intercropping systems in some areas.

Some progress in overcoming these constraints has been accomplished. Cultivars with good levels of resistance to bacterial wilt and cassava mosaic diseases are available; low levels of resistance to mealy bug and
green spider mite have also been identified, e.g. varieties like TMS 50395, 30572, 4(2)1425, 91934 and 30001 developed at IITA.

A fairly large collection of cassava germplasm has been evaluated and preserved both at IITA and CIAT; local cultivars have also been collected to a limited extent in certain national programmes such as India, Ghana, Nigeria, Brazil, Thailand. These germplasm collections, although available, have not been exchanged extensively due to quarantine restrictions. With the availability of in-vitro facilities and sterile cultures this difficulty should be solved in the near future.

Mutation breeding techniques could be utilized to modify several of the characteristics which limit cassava production. This approach, as an additional and complementary breeding tool could be used to enhance the limited genetic variability available and to induce desired traits in fairly well adapted heterozygous genotypes (which are normally maintained vegetatively) without losing them through selfing or crossing.

Mutation breeding may be used specifically to improve the following characteristics (priorities will vary with location):

a) Reduce level of cyanogenic glucoside (HCN).
b) Insect resistance, e.g. mealy bugs and mites.
c) Flour quality for baking.
d) Size and shape of roots to facilitate harvesting and processing.
e) Storability.

Experience with mutation breeding of cassava and other vegetatively propagated plants led to the following recommendations:

a) Identify one or a few agronomically superior varieties which are well adapted and require improvement in one or a few characters.
b) Irradiate stem cuttings and/or microcuttings and/or in-vitro shoot tips and plantlets with 2-3 krads of gamma radiation; it should be remembered though that varieties differ in their response to irradiation.
c) A minimum of 500 $M_1 V_1^*$ stem cuttings are needed for treatment to obtain about 8000 $M_1 V_2^*$ and 32000 $M_1 V_3^*$ shoots from which selection can be made.

$M_1 V_1$, $M_1 V_2$ and $M_1 V_3 =$ First, second and third vegetative "generations" (through removal of the respective apical buds and regrowth) after mutagen treatment of the original material.
d) Isolation and selection of desirable mutants should be done in M_{1} V_{2} and M_{1} V_{3}.

Screening and selection could be practiced as follows:

a) Screening for resistance to insects can be accomplished by growing the M_{1} V_{2} and M_{1} V_{3} populations in the areas where their natural infestation levels are high. Scoring is normally done on a scale of 0-5. The techniques of artificial infestation are not available yet; some research should be done in this area, including mass rearing of insects.

b) A rapid and reliable analytical technique to screen for HCN content has been developed at IITA (Ibadan); 300 samples can be analysed per day.

c) Screening for root size and shape is done visually after the harvest.

d) The desired chemical composition of cassava roots which determines their suitability for mixing with wheat flour for bread baking is known. A technique for rapid analysis to handle large numbers of samples should however be developed.

e) More research is required to understand the chemical changes and deterioration which occur in cassava roots during storage. Rapid screening methods for storability should be developed.

A technique for in-vitro propagation using shoot-tip meristems has been developed. However, more research is necessary in order to make this technique useful in producing the large numbers of plantlets needed in the M_{1} V_{2} and M_{1} V_{3} generations. Early screening of in-vitro plantlets for HCN content should also be explored.

Very little work has been done in cassava on in-vitro plant regeneration from somatic cells, anther culture, protoplast fusion or in-vitro somaclonal variability. It appears that these techniques could also be utilized in generating genetic variability for cassava improvement.

5.2. Yam

The most important cultivated edible species of yam (Dioscorea spp.) grown are white yam (D. rotundata), yellow yam (D. cayenensis), bitter (trifoliate) yam (D. dumetorum) and greater yam (D. alata); all indigenous to West Africa except greater yam which originated in South East Asia. In
Africa, which produces 96% of the world's yam, white yam is the most important species in terms of production and preference, followed by greater yam and yellow yam.

In the past, genetic improvement of yam was limited by lack of knowledge of flowering and seed development and physiology. However, recently, the techniques of hybridization, seed germination and establishment of seedlings have been developed at IITA, Ibadan. Despite these developments, the potential of the conventional breeding approaches in yam is limited because certain varieties do not flower or have only male or female flowers; also, the seed set under both natural and artificial hybridization is very low. Most of the work done so far, therefore, has been through clonal selection in the local varieties.

The major constraints in yam production are:

a) Plant architecture, which at present requires staking.

b) Necrosis and virus diseases.

c) Long life cycle.

d) Poor shelf life of the tubers.

The variability for plant type and disease resistance among the local cultivars is very limited, as shown in IITA and other breeding centres. Only a few studies of the germplasm for length of growing season and storage quality have been conducted. It appears however, that the genetic variability also for these two characters is limited. The flowering and hybridization problems as well as the limited genetic variability available make it desirable to utilize induced mutations in improving yam cultivars.

Mutation breeding has recently been initiated in Ghana and preliminary studies suggest that both tuber pieces and vine cuttings can be treated. However, more studies on the suitable irradiation doses are necessary. Small tuber pieces known as "micro sets" can be used effectively: this gives a better opportunity to recover the induced mutations.

The procedures for irradiation of vine cuttings and of in-vitro plantlets have still to be developed. It is believed that the cutting back system and the sprouting of axillary buds will lead to rapid
multiplication and large populations of $M_1V_2$ plants. In the $M_1V_3$ generation selection of mutants could be performed.

Research is needed in yam to explore the potentials of callus cultures, protoplast fusion, somaclonal variation etc., in generating genetic variability.

5.3. Sweet potato

Sweet potato is an important food and starch producing crop. Also, it has a potential as a source of biomass to produce alcohol. Although sweet potato originated in the Central American-Mexican Centre, the bulk of its production is in Asia.

Conventional cross breeding is possible although there are some cross-incompatibility barriers between varieties. Very recently in-vitro regeneration through somatic embryogenesis from callus has been reported. It may become possible to combine the characteristics of cultivars by protoplast fusion where cross incompatibility exists.

Breeding goals for sweet potato are:

a) High yielding ability.
b) High starch content for starch production (Japan, China).
c) High nutritional value of tops and tuberous roots (carotene, protein) when utilized for animal feeding (China, Oceania, Africa).
d) Improved roots' market qualities such as attractive skin colour and uniform shape and size (China, Japan, US).
e) Low polyphenol content (Japan, where it is used by the food industry to make chips).
f) Shorter stem length (all areas).
g) Early tuberization (all areas).
h) Root-knot nematode and root-lesion nematode resistance (Asia, Africa).
i) Weevil resistance (most of the tropics).
j) Resistance to fungal and viral diseases (all areas).

Germplasm collections are maintained in the following countries: Nigeria (IITA), Taiwan (AVRDC), Japan (Kyushu Agr. Exp. Sta.), China (Dept. of Agric. in each producing province), Papua - New Guinea (Dept. of
Mutation breeding has proved effective in inducing genetic changes in root skin colour and starch content and in stem length.

The mutagens and doses to be applied are:

- Beta rays ($^{32}$P)
  - ca. 30 mCi/root
  - ca. 20 mCi/sprout (20-25 cm long)
- Gamma rays ($^{60}$Co)
  - dormant roots
  - shoot tip sprouts
  - true seeds
- X-rays
- Fast neutrons
- Ethylene imine
- Ethyl methane sulphonate

<table>
<thead>
<tr>
<th>Mutagen</th>
<th>Dose</th>
<th>Method</th>
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<tr>
<td>Beta rays</td>
<td>ca. 30 mCi/root</td>
<td>Soak basal half in 0.3% solution for 3 hours, at 18-25°C, rinse in running tap water overnight (8-12 hr.)</td>
</tr>
<tr>
<td>Gamma rays</td>
<td>6-15 krad</td>
<td>Scarify seeds and presoak for at least 8-12 hrs. at room temperature, till seeds show swelling. Soak seeds in 0.3% solution for 3 hr. at 18-25°C and rinse as above.</td>
</tr>
<tr>
<td>X-rays</td>
<td>10-20 krad</td>
<td>Soak and rinse as for EI, but in 2.5% solution. Presoak, soak and rinse as for EI, but in 2.5% solution</td>
</tr>
<tr>
<td>Fast neutrons</td>
<td>600 rad</td>
<td>Soak and rinse as for EI, but in 2.5% solution. Presoak, soak and rinse as for EI, but in 2.5% solution</td>
</tr>
<tr>
<td>Ethylene imine</td>
<td>Sprouts</td>
<td>Soak and rinse as for EI, but in 2.5% solution. Presoak, soak and rinse as for EI, but in 2.5% solution</td>
</tr>
<tr>
<td>Ethyl methane</td>
<td>Sprouts</td>
<td>Soak and rinse as for EI, but in 2.5% solution. Presoak, soak and rinse as for EI, but in 2.5% solution</td>
</tr>
<tr>
<td>Sulphonate</td>
<td>Seeds</td>
<td>Soak and rinse as for EI, but in 2.5% solution. Presoak, soak and rinse as for EI, but in 2.5% solution</td>
</tr>
</tbody>
</table>

To increase the probability of obtaining the desired mutations, several thousand $M_1V_2$ plants should be analysed. Therefore, much material should be treated and grown in the $M_1V_1$ generation and all the tuberous roots from each $M_1V_1$ plant must be grown in $M_1V_2$.

Mutations such as skin colour, flesh colour and short stem are identified visually in the field. Natural infections or infestations in the field are used to screen for disease or pest resistant mutants;
individual inoculation of each $M_1V_2$ plant is time consuming. Rapid methods for precise starch content determinations must be devised. Preliminary selection for starch content can be based on dry matter content or specific weight. High carotene content can be recognized easily by flesh colour. Protein content determinations require more laborious analyses.

5.4. Potato

Potatoes are a valuable food and starch crop, providing also high quality protein and vitamins, particularly vitamin C and some of the B vitamins. They also contain important mineral salts, particularly potassium and phosphorus. Most of the world production is in Europe and North America, but recently potato areas have been increasing rapidly in tropical and sub-tropical regions, particularly in China, India and Indonesia.

In contrast with some developed countries, in the developing world potatoes are an expensive crop and are eaten as a vegetable rather than as a staple food. Traditionally the crop has been grown in the mountainous regions of the tropics but this is changing and the trend is towards warmer and drier areas. There has also been an increase in the proportion of the crop grown in warm, humid areas and this has brought with it an increase in disease and pest problems.

Breeding objectives and approaches

The major potato breeding objectives in the tropical regions are:

a) Development of varieties adapted to tropical conditions.

b) Increased yields.

c) Improved resistance to pests, particularly potato cyst-nematode ($Globodera rostochiensis$ and $G. pallida$), potato root-knot nematode ($Meloidogyne incognita$) and potato tuber moth ($Phthorimaea operculella$) and to diseases, particularly bacterial wilt ($Pseudomonas solanacearum$), late blight ($Phytophthora infestans$), potato leaf-roll virus and potato virus Y.

d) Improved storage and germination.

e) Improved market attractiveness.
Potato is an outcrossing plant with a gametophytic self-incompatibility system. Although tetraploid cultivated potatoes are self-compatible, they still have S alleles. The breeding methods used are commonly associated with cross-pollinated crops, in particular the pedigree method. Recurrent selection has proved particularly effective in the development of populations adapted to northern temperate regions from cultivated South American species; it has been used by the International Potato Center (Lima, Peru) to incorporate certain resistance genes in advanced breeding populations.

Breeding at the diploid level has centered largely on the use of dihaploids of common cultivars and the related diploid cultivated species. Dihaploids have the advantage of disomic rather than tetrasomic inheritance. They also provide access to a large number of diploid species, reduce screening efforts for specific traits due to simplified segregations and can give an estimate of parental value since they represent the gametes of the source cultivar. More recently, monoploids have been isolated both by sexual means and by anther culture. They offer the possibility of providing immediate homozygosity.

2n gametes provide another useful alternative to hybridization and gene transfer in potato. They are formed by first or second division restitution (FDR and SDR, respectively), with a preference for the former. Synaptic mutants are now available that result in gametes which can transfer 100% of the diploid genome through the pollen. High levels of 2n egg production by FDR have not been achieved but improvements are being made.

The breeding behaviour and characteristics of this crop offer both advantages and disadvantages. Among the advantages are genetic variability, relative ease of ploidy manipulation and a diverse and rich gene pool. But with these advantages come problems such as male sterility, self-incompatibility, flower and berry drop and various interspecific barriers to hybridization. Much experience in potato breeding, with special attention to tropical areas has been gained by the International Potato Centre (Lima, Peru) and it is suggested that links should be established with it for breeding materials and methodologies.
Induced mutations

Seeds, dormant buds and eyes have been irradiated with gamma rays or treated with chemical mutagens such as ethyl methane sulphonate (EMS) and diethyl sulphate (DES). Such treatments result in chimeras, therefore this technique had limited success and lost its attractiveness. Still, day-neutrality was obtained by induced mutation in India, allowing varieties originally bred for the mountainous north of the country to be grown in warmer, southern regions. Induced nematode resistance has also been reported in India. In Japan, a variety adapted to the north of the country and derived from irradiated Maris Piper is to be released shortly.

High doses of radiation have been applied to pollen grains of a number of crops, to destroy most of the genome and allow only limited gene transfer at pollination. This could facilitate varietal improvement by introducing only a few, hopefully valuable, genes from the pollen parent. This technique is being investigated in potato in Scotland but results have not been published yet.

Tissue culture in potato breeding

Tissue culture has provided some new opportunities for potato breeding. It may facilitate the addition of a single trait to an adapted variety with otherwise good performance, as in classical mutation breeding. A number of tissue culture techniques have been utilized, both for breeding new varieties and for rapid propagation of stocks. Some of these techniques are also applicable in mutation breeding.

Meristem and shoot-tip cultures are now widely used to propagate virus-free and bacteria-free stocks of cultivars. This technique results in insignificant variation in the progeny. However, if the cultivar is chimeric, the chimera may be lost when small meristem cuttings are used. It is somewhat complicated to induce and isolate non-chimeric mutant plants in this propagation system, since a high percentage of chimeras may occur due to mutations within the preformed axillary bud system. Alternative systems are available for mutation breeding, such as adventitious budding from leaf explants, giving rise to a considerable amount of somaclonal variation. Irradiation has been shown to add to this genetic variation.
Protoplast isolation and regeneration does not appear to provide more variation than adventitious budding. However, once appropriate in-vitro screening techniques are developed it can facilitate selection on single-cell basis. Protoplast regeneration though is still problematic, complicated by differences between clones. As far as we are aware, irradiation studies have not been carried out with potato protoplasts; it should be an interesting area for further research.

Anther culture provides a short cut for revealing recessive traits in a given line. It is more valuable for screening dihaploid breeding stocks, leading to a high percentage of homozygous progeny which may be used in crossing programmes. There seems to be no particular reason for applying mutagenic treatments to anther or dihaploid cultures. The need for special pretreatment of anther donor plants and for improved anther culture methodologies might limit its utilization especially in view of the variation between clones.

Protoplast fusion

Protoplast fusion is still another potential means of incorporating new traits into the cultivated potato clones. This technique will facilitate overcoming cross-incompatibility, manipulation of ploidy levels, and the transfer of cytoplasmic traits. It provides an opportunity to form "cybrids", transferring only the cytoplasm from one parent after the nucleus has been inactivated. The value of this technique must still be assessed, although recent fusion products of S. brevidens with diploid and tetraploid cultivated stocks show considerable promise.

DNA manipulation

Vector systems using plasmids from Agrobacterium tumefaciens have been developed for transferring DNA from one genotype to another. Kanamycin resistance was transferred into potato using this technique. Before genetic manipulation can be of real value, however, it is necessary to identify useful genes which can be transferred. In tobacco, portions of a virus genome have been transferred to the plants, and were expressed. It is hoped that this may protect the plants against infection by the same virus. It will probably be some years before DNA manipulation will benefit breeding.
In-vitro screening

In-vitro screening is an attractive possibility since it would facilitate testing much larger populations than those feasible by more conventional means. There are, however, a number of problems to overcome. If screening is carried out at the single cell level, it is necessary to ensure that the desired trait is expressed at this stage. Suitable screening methods must be developed and the results must correlate with those obtained using whole plants in the field. Also regeneration itself may act as a screen but not necessarily a desirable one.

For some characters, in-vitro screening at the level of regenerated plantlets may be a more appropriate method. This may be particularly useful if tissue cultures are used to propagate the breeding material. Screening at this level would probably require less sophisticated techniques or equipment than screening at the single cell level.

True potato seed

The use of true potato seed (TPS) is of considerable interest in developing countries. The TPS are inexpensive, free from most viruses and easy to store, and all the harvested tubers can be consumed. Developing countries will not need sophisticated seed tubers certification schemes, and also will not have to buy expensive seed tubers from abroad. Yields from TPS are currently slightly lower than those from seed tubers, but this may change with the advent of hybrid seed production via 2n gametes or inbreeding. True potato seed may have a significant impact on potato production in the tropics. Research is currently being carried out in a number of countries under the auspices of the International Potato Centre.

5.5. Sugar cane

About 60-65% of the sugar produced in the world comes from sugar cane, making it one of the major agricultural crops in tropical and sub-tropical countries. Also, it is an important export crop earning valuable foreign currency for many developing countries.
Commercial sugar cane clones are complex hybrids of several **Saccharum** species. Their highly polyploid and heterozygous nature impedes sugar cane improvement by sexual means. In addition, in some sugar cane growing countries, it is difficult to utilize conventional breeding approaches because of difficulties in flowering and seed setting. Other limiting factors are the inadequacy of the screening and selection techniques and the large populations which must be analysed.

Large numbers of germplasm collections of **Saccharum** species (officinarum, spontaneum, robustum, barberi, sinensis and hybrid clones) and related genera are available in international germ plasm pools located at the USDA Sugar Cane Field Station, Canal Point, Florida, and at the Sugar Cane Breeding Institute, Coimbatore, India. The germ plasm collections contain genes for resistance to the major diseases and other important agronomic characters.

Induced mutation breeding is an important approach for improving specific characteristics without altering most of the desired agronomic characteristics of the commercial clones. The irradiation of single-eyed pieces of sugar cane "set" is an accepted technique for the induction of mutations. A dose ranging between 2-4 krad of gamma rays from a $^{60}$Co source, depending on the clones should be applied. The methodology for handling the $M_{1}V_{1}$ plants and their further propagation in the $M_{1}V_{2}$ and $M_{1}V_{3}$ generations has been established. With this approach mutant varieties having higher sugar content, better cane yield, resistance to diseases, etc. have already been obtained at Coimbatore, India.

**In-vitro** shoot tip cultures led to a wide genetic variability among the regenerants. Somaclonal variation which arises in cell and callus cultures is expressed in regenerants obtained via somatic embryogenesis. **In-vitro** micro-propagation offers a means for easier recovery of non-chimeric plants.

6. **GENERAL CONCLUSIONS AND RECOMMENDATIONS**

The First Research Co-ordination Meeting convened on "Improvement of root and tuber crops and similar vegetatively propagated crop plants in tropical countries by induced mutations" provided a forum for scientists working on similar crops in different countries, to discuss the problems and develop suitable methodologies to achieve their breeding goals.
The participants presented their research project plans, discussed their implementation and exchanged ideas and information. They made the following general recommendations:

1. There are additional important root and tuber crops which could potentially be cultivated. The collection, exchange and evaluation of improved and unknown germ plasm would be extremely useful and should be strongly encouraged. National and international organizations should support this task.

2. Induced somatic mutations can contribute to the improvement of clonal varieties without altering most of their desired characteristics. To maximize the effectiveness of inducing somatic mutations the following aspects should be studied:
   a) Appropriate techniques and dosages of mutagenic treatments with radiations and/or chemical mutagens, applied to shoots, buds, tubers, roots, cuttings, bulbils, in-vitro explants, single cells and protoplasts of the various plant species concerned.
   b) New evaluation techniques as they become available.
   c) Suitable techniques for identification and isolation of mutated tissues in chimeras. Breeders, pathologists and technologists have to develop methods for the easy and rapid detection of desired mutants.
   d) Selection (at the earliest possible stages), propagation and evaluation of desired mutants.
   e) In-vitro culture applications feasible for mutation induction, mutant selection and propagation. Mutation breeding employing in-vitro technology should make a significant contribution and studies along this line should be encouraged.

3. The Joint FAO/IAEA Division through the Agriculture Laboratory at Seibersdorf, should provide advice to breeders on mutation breeding with in-vitro techniques for the specific crops considered.
APPENDIX

PAPERS ON INDUCED MUTATIONS IN ROOT AND TUBER CROPS AND SUGAR CANE IN SELECTED IAEA PUBLICATIONS


- Heinz, D.J. Sugar cane improvement through induced mutations using vegetative propagules and cell culture techniques. p. 53-59.


- Jagathesan, D. Improvement of sugar cane through induced mutations. p. 139-153.

- Siddiqui, S.H. and Javed, M. Mutation breeding in sugar cane (Saccharum sp. hybrid) by gamma irradiation of cuttings and tissue cultures. p. 155-166.


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1. **INTRODUCTION**

The first Research Co-ordination Meeting in this programme was convened in 1984 in Thailand. It reviewed in detail the status and breeding research needs of the major root and tuber crops and other vegetatively propagated crops, viz. cassava, yam, sweet potato, potato and sugar cane. In the present (second) RCM the participants presented their research achievements and difficulties during the past two years, reviewed their earlier recommendations, exchanged information and ideas, co-ordinated their future research and, based on the experience gained, made more general as well as crop specific recommendations which are presented below. These recommendations should be considered together with those of the first RCM.

2. **MUTATION INDUCTION PROCEDURES**

2.1 **Objectives**

a) Select objectives by priority and do not set too many objectives in the same programme, since this will require much larger populations. Mutations may be common after treatment, but agronomically useful mutations are rare, say 1 in 1000. Hence it follows that the concurrent selection of two useful mutations will require a population exceeding 1,000,000. Consecutive mutation induction and selection will be much more efficient, requiring \[2 \times (1000^+)\] plants. Such accumulation of mutations has been reported.

b) Mutation breeding can be used to:

- induce mutant alleles not known in the natural germ plasm or in related wild species.
- overcome specific defects or obtain specific traits in well accepted varieties without affecting the rest of the genotype.
- produce new varieties to satisfy evolving agricultural and market needs.

2.2 **Choice of plant material**

a) The material to be treated should be healthy (pathogen-free), typical and not genetically heterogeneous (e.g. do not treat seeds of vegetatively propagated plants). The health of the material under investigation may have an important effect on uniformity within clones. All efforts should be made to produce as
standardized material for selection as possible. Stock plants of
the clones should be maintained in pathogen-free conditions.
b) The material to be treated should be chosen according to the
objectives of the programme.
c) Concentrate on one specific variety and on one specific trait to
be changed at a time.
d) For mutation induction in vivo and in some in vitro cases,
propagation systems which ensure true-to-type multiplication
should be utilized. If these do not lead to the desired results,
systems capable of inducing much wider variability ranges can be
utilized (e.g. through callus phase). However, in the latter it
would be difficult to eliminate the undesirable changes that would
be induced simultaneously.
e) For in vitro shoot tip cultures it is better to treat the material
directly in vitro and not in vivo before it is cultured. For
other in vitro systems, more studies are needed before recommend-
ations can be made.

2.3 Mutagenic agents
   a) Physical mutagens are commonly used because they reach the targets
easily and can be applied precisely.
b) Chemical mutagens can reach the target more easily in vitro than
in vivo. However, applying the desired doses to cell or tissue
cultures is difficult.

2.4 Doses
   a) Preliminary tests are needed to identify for each target and
mutagen the most suitable doses, physiological stage, season and
treatment conditions. In these tests, as many parameters as
possible should be specified in order to improve the precision of
assessment.
b) In mutation breeding programmes, the dose applied should be based
on the preliminary tests, limiting the number of treatments to
very few.
c) The doses should be as low as possible, compatible with efficient
mutation induction, since in vegetatively propagated plants it is
not possible to eliminate undesirable mutations induced
simultaneously with the desired ones.
d) The dose rate recommended is between 1 to 100 Gy per minute.
2.5 **Number of treated buds**

a) Generally, the larger the population the higher the probability of success. The $M_1V_1$ population should be large enough to supply sufficient material after the rejection of grossly abnormal plants, to give enough $M_1V_2$ plants (see below, B IV).

b) The population to be treated should be of reasonable size, e.g. 600 to 1000 $M_1V_1$ buds surviving the treatment.

c) If the character desired can be easily mutated the above population can be smaller.

2.6 **Handling the treated and subsequent materials**

a) Grow $M_1V_1$ (the treated material) under the most favourable conditions possible. The time elapsing between treatment and sprouting must be as short as possible.

b) The $M_1V_2$ propagation material should be chosen according to the species. As the $M_1V_1$ generation of most crop plants will include many mericlinal chimeras, enough propagules must be taken to sample all parts of the stem circumference; this number - up to 5 - can be determined from the phyllotaxy of the plant. In choosing the segment of the treated $M_1V_1$ plant used for propagation to the $M_1V_2$ one should take into consideration that the lowest axillary buds may be chimeras, while the distal parts of the $M_1V_1$ plant will probably only repeat parts sampled in the lower-middle region. The number of cells in the treated buds should be taken into consideration - a small number of cells will give larger mutated sectors.

c) To dissolve chimeras, the $M_1V_2$ generation should be propagated to $M_1V_3$ following the same procedure. Every selected plant should be propagated separately.

d) Plants which underwent the same propagation process as the irradiated material should be used as control.

e) The more the character desired is affected by the environment, the larger the control population needed.
3. SELECTION APPROACHES

3.1 General

All methods of plant breeding include a stage in which genetic variation is produced and a stage of selection within that variation. However, selection following mutation induction differs from that following hybridization in two ways. First, there is often a narrower range of useful variation, with all the potentially useful variants more or less resembling the parent. Secondly, particularly for vegetatively propagated crops, the selected desired clones may be chimeras and thus unstable, and they may also contain undesirable mutations induced simultaneously with the desired ones.

Mutation selection is often within a narrow range of variation, where small differences have to be identified with confidence, so all efforts should be made to produce as standardized material for selection as possible; e.g. cuttings or rootstocks should be graded and uniform, and agronomic conditions should be as good as possible.

3.2 Standards and control

Selection should always be by comparison with both standard and control clones; the standard one being the applicable cultivar (normally the parent) grown by the best agronomic technique, while the control clones are parental plants that have undergone all treatments save the mutagenic one alongside the population to be screened. In this way it should be possible to identify some of the primary effects that are caused by some treatments. If it is possible to multiply clonally the mutated population, it may be worthwhile to sample more than one selection environment. For economic reasons this may have to be delayed till later selection stages.

3.3 Stage

a) The best stage for selection will depend on the crop, trait and propagation technique. If plants can be produced from single cells with little chance for chimera formation, then the M1 V1 stage may be used for selection once the primary effects of the mutagenic
treatment are overcome. In most conditions, however, only gross abnormalities can be rejected at this stage, and even then at some risk of rejecting useful mutations carried as small mericlinal sectors.

b) In the M\textsubscript{1}V\textsubscript{2}, the great majority of the plants will be either homohistont or periclinal chimeras, and hence histologically relatively stable. However, each plant could still be unique so only poor plants should be rejected since one plant is not sufficient to assess most characters, with the exception of those that are clearly expressed, such as resistance to some diseases.

c) The M\textsubscript{1}V\textsubscript{3} generation should be derived from all the M\textsubscript{1}V\textsubscript{2} plants retained, and this should be the main selection stage on a clone basis. The characteristics and stability of the clones selected should still be re-checked during further multiplication. The selection at M\textsubscript{1}V\textsubscript{3} and later stages should be backed by careful measurements and statistical analysis to help distinguish between random and real differences from the control and the standard clone.

3.4 Population size

a) The desirable population sizes can be estimated from knowledge of the crop plant concerned and the breeding objectives. The M\textsubscript{1}V\textsubscript{2} generation will probably have the largest number of genotypes. It must contain plants showing as large a proportion as possible of the variation induced in the M\textsubscript{1}V\textsubscript{1}, with little duplication.

b) The size of the M\textsubscript{1}V\textsubscript{3} population will depend on two main considerations. The selection pressure on the M\textsubscript{1}V\textsubscript{2} specifies the number of clones, while the replication of each clone will depend on the uniformity of the M\textsubscript{1}V\textsubscript{3} conditions. The poorer the growing conditions the greater the replication should be, preferably using a statistically designed and blocked layout to reduce environmental effects particularly where subtle differences, such as in yield, are measured.

c) Most of the above considerations apply equally to in vitro and in vivo selection, though the former will often have fewer constraints on population size. However, final selection must take place under the
commercial field conditions of the crop, preferably under a range of applicable environments.

3.5 Early in vitro screening

a) Early screening of plants for their resistance or tolerance to stress or disease agents has been requested by breeders in order to speed up the selection procedure and to allow the handling of a larger number of individual plants within a limited space, by taking full advantage of the possibilities of in vitro cultures. The production of protoclones, calliclones or other somaclones raised the hope to select for given traits in vitro under controlled and disease-free conditions.

b) Simple and reliable methods of in vitro selection of plants have been implemented in selection for tolerance to herbicides, salinity, minerals or toxins. Screening for resistance (or tolerance) to fungi, bacteria, viruses or nematodes is more complex. At first, it was hoped that in vitro screening for tolerance towards toxins produced by disease agents might open the way to the selection of disease resistant material. This technique, however, has not been very successful so far; further work is needed to assess its value with specific pairs of host and pathogen.

c) Selection by in vitro dual culture of host and pathogen, although a more complex proposition, was found meaningful provided adequate experimental conditions are devised. The methodology should be adapted to each cultivar and for any host-parasite combination. The in vitro behaviour of cultivars with known field reaction to various pathotypes should be compared by adjusting culture medium, growth conditions (including light and temperature), and inoculation procedure (inoculum density, application methods, assessment of the results), so as to correlate the in vitro and in vivo reactions. This implies a proper control of all conditions and requires uniform response of the control population.

d) Mother stock plants of each clone should be kept separately from material submitted to the selection procedure. Material selected from in vitro culture should be tested for stability and passed to a standard in vivo selection procedure, taking into account the possible
side-effects of \textit{in vitro} culture. Subsequent selection progress should depend on understanding and overcoming if necessary the physiological changes induced by the previous culture conditions. Comparison with controls and standards will be needed. Changes in maturity, etc., can have effects on disease expression, among other characters, and it is probably best to postpone selection for quantitative characters for one or more \textit{in vivo} generations.

3.6 Selection of plants

a) Selection \textit{in vivo} can generally follow the applicable well-established techniques used following conventional hybridization. Where artificial inoculation techniques can ensure an adequate selection pressure they should be used, particularly at the $M_2$.

b) Where the target is a multicellular structure, chimeras will usually be the result of mutagen treatment. Rarely, homohistont branches or plants may result if considerable cell death occurs in the target meristem. By the $M_2$ or $M_3$ at the latest, all clones should be relatively stable periclinal chimeras. Clones not stable by the $M_3$ are probably best rejected. For many plant species periclinal mutants may have adequate stability. Where this is considered not to be the case selected mutants should be subjected to known chimera breaking techniques such as re-irradiation, and if they are found to be unstable but still valuable, then one of the various techniques to produce homohistont plants should be used, e.g. producing adventitious buds or \textit{in vitro} micro-propagation techniques. However, as such techniques may be mutagenic themselves, or reveal pre-existing mutations, further trials will be needed of the clones produced.

3.7 Genetic studies

a) While the genetic nature of a mutation may be unimportant for selection \textit{per se}, it can have a large bearing on the usefulness and stability of such a change (e.g. disease resistance). Somatic mutations may or may not be of nuclear origin or homohistont, and therefore may behave unpredictably when used as parents. Hence, if this is likely to be important it might be worthwhile to commence genetic studies concurrently with the final steps of selection, e.g. from the $M_3$ stage.
b) The physiological mode of action of mutant alleles can be equally important (e.g. self-fertility or male sterility) and should be examined at a relatively early stage.

4. CROPS

In the first Research Co-ordination Meeting (1984) the participants made detailed assessments of the breeding needs and the potentials of mutation breeding and other approaches for the improvement of cassava, yam, sweet potato, potato and sugar cane. In the present (second) RCM the 1984 crop-specific recommendations were reviewed, and amended where necessary. The additional or modified conclusions and recommendations are given below; they should be considered together with those of 1984.

4.1 Cassava

General

a) The genetic variability in the cultivated cassava is rather limited. Cross-breeding involving wild species brings a lot of undesirable characters and is rather long-term. Mutation breeding techniques could be used to change only certain characters and maintain other desirable traits of adapted, accepted cultivars.

b) Cultivated cassava is quite heterozygous and hence has a good background for mutation induction. In some cassava clones which are obligatory apomicts, induced mutagenesis is the exclusive way to induce genetic variation.

Mutagens, plant material and handling

a) Both physical and chemical mutagens can be used for mutation induction. Doses between 20 and 30 Gy of X-or gamma-irradiation are recommended for treatment both in vitro and in vivo. Experience in chemical mutagenesis is very limited and investigations in this field are needed, both in vivo and in vitro.

b) In each country local, well-adapted and accepted varieties should be the preferred materials for mutation breeding programmes. The materials from International Institutes, e.g. IITA and CIAT could be used, but their suitability to the region must first be established.
c) Common vegetative propagules i.e. stem cuttings, should be used for mutation induction. The matured woody cuttings at a uniform stage of development must be used. The length of the cuttings should be as that used for normal propagation, about 20 cm. The treated cuttings should be sufficiently long to assure good rooting (7-10 buds, depending on variety). The use of very small cuttings must be avoided since plants with morphological irregularities could result from their use.

d) In vitro cultures to obtain healthy virus-free stock should be initiated from apical meristems taken from superior varieties. Single nodal cuttings in cytokinin-rich media should be used for rapid propagation of meristem-derived plantlets. For mutation induction, single axillary buds should be considered as treatment units.

e) To obtain the desired populations, after in vivo treatments, the following procedure is recommended: 500 cuttings should be treated to give 1000 $M_{1V_1}$ shoots. These shoots should be cut back at the end of the growing season, leaving two nodes on the stump of each, thus 2000 $M_{1V_2}$ shoots will be produced. Further cutting back (as above) and regrowth will yield 8000 $M_{1V_3}$ shoots.

f) For in vitro induction the number of treated single bud cuttings should be high, up to 10,000.

Selection and evaluation

a) Screening for foliage and shoot traits in $M_{1V_2}$ and $M_{1V_3}$ can be performed without detaching them from the original rooted $M_{1V_1}$ stock.

b) In order to screen for traits of the tuberous roots, the $M_{1V_2}$ and/or $M_{1V_3}$ shoots must be detached and rooted.

c) Induction of resistance to diseases and pests, e.g. cassava mosaic disease, cassava bacterial blight and green spider mite, would be very important.

d) To screen for cassava mosaic disease, the $M_{1V_{12}}$ and $M_{1V_{13}}$ populations should be planted in heavily infested areas. Scoring
symptom-free plants should also be made in subsequent "generations" to establish the stability of the character.

e) Promising mutant(s) should be further tested in different environments for stability of the new traits. The overall agronomic performance in field trials should be evaluated and promising materials should be identified for rapid propagation. In vitro micro-propagation is recommended at this stage. The promising mutants could be used also in cross-breeding programmes.

4.2 Yam

a) Local yam varieties that are well adapted should be used in mutation breeding programmes.

b) There is limited experience with gamma irradiation and none with chemical mutagens. Studies in this field need to be carried out.

c) The use of small tuber pieces known as "mini-sets" or "micro-sets" are recommended as material to be treated; and about 500 such "mini-sets" could be treated. The \( M_{1V1} \) and \( M_{1V2} \) material must be sliced into "mini-sets" before planting, to give rise to the \( M_{1V2} \) and \( M_{1V3} \) populations, respectively.

d) The procedures for the irradiation of vine cuttings and in vitro plantlets still have to be developed.

e) Exploration of the potentials of callus cultures, protoplast cultures, somaclonal variation etc. in generating genetic variability in edible yams is necessary.

4.3 Sweet Potato

Treatment

a) Stem (shoot) cuttings which are easily rooted and handled thereafter are the preferred material for treatment.
b) Based on available experience, the useful gamma- and x-rays irradiation doses to be applied to the shoot cuttings are in the range of 100–200 Gy. Preliminary studies should be performed to assess the radio-sensitivity of specific genotypes.

Handling

a) In vivo. Usually, treat shoot cuttings which produce $M_1 V_1$ plants and proceed as usual to $M_1 V_2$. In some cases storage roots can be treated; adventitious sprouts arising from them which originate from one or a few cells are $M_1 V_2$.

b) In vitro. A system for micro-propagation which is already available could facilitate faster induction and recovery of somatic mutations. Since some preliminary data on in vitro plant regeneration through callus are available (Taiwan) the use of somaclonal variation should be explored.

Selection

a) Detect variants either in root and/or shoot of the $M_1 V_2$ plants. Selection for clear morpho-physiological characters (e.g. plant type, storage root skin colour, branching type etc.) is relatively easy. Large scale screening for some biochemical characters (e.g. sugar content, dry matter content, carotene content in storage root) is feasible.

b) For selection for disease resistance (e.g. black rot = Ceratocystis fimbriata Ell. & Halst. and scurf = Monilochaetes infuscans (Ell. & Hals.) Harter) artificial and/or natural selection pressures are available.

c) In vitro selection procedures for environmental stresses, disease resistance, etc. should be investigated.
4.4 Potato

General

a) Mutation breeding can be used for the improvement of specific characters and correcting defects in existing successful varieties, and for the production of new varieties to satisfy the needs of an evolving market and new production systems.

b) Specific needs and improvements identified include disease and pest resistance; dormancy; maturity time; tuber quality, size and reducing sugar accumulation; and tolerance of environmental stresses.

c) Mutation breeding, including somaclonal variation, can be expected to meet some of the specific targets defined by growers and the potato processing industries.

d) The possibilities of the emerging techniques of breeding using true potato seed should be explored.

e) It should be stressed that new technologies should complement, not replace, conventional breeding systems. Whereas potato is a very valuable model crop for many new biotechnologies including culture from single cell, somatic fusion or gene transfer through specific vectors such as Agrobacterium tumefaciens, the more conventional methods of obtaining genetic variation should not be neglected. Close co-operation is necessary between laboratories developing new methods, those producing mutants and potato breeders.

Material and treatment

a) Commencing with healthy vegetative material is very important.

b) Possible targets for mutagenic treatments include:
   i) Tubers - these should be avoided if possible due to chimera problems.
   ii) Axillary buds on cuttings (preferrably in vitro). This has proved successful with doses of up to 30 Gy of gamma-rays.
iii) True potato seeds (=TPS) are at present too heterozygous; TPS research may result in more homozygous lines of agronomic interest. Then they could be considered as possible source material for mutagenesis.

c) Somaclonal techniques may be considered mutagenic, but at present their use should be restricted to specialized laboratories.

d) There is little published information on chemical mutagenesis; further work is needed on this as on irradiation techniques.

Selection

a) Screening techniques for selecting potato material obtained from conventional hybridization are well established, the same methods can be applied in mutation breeding programmes. The process may be speeded up by the use of rapid propagation procedures either through in vitro systems or by simple stem cutting techniques where suitable tissue culture facilities are not available.

b) There is a need for further in vitro selection techniques. Some have already given clones with resistance to such characters as resistance to late blight (Phytophthora infestans), common scab (Streptomyces scabies), wart disease (Synchytrium endobioticum), early blight (Alternaria solani) and nematodes. In vitro selection has also been used for the selection of desirable tuber colour and shape and for resistance to toxic substances, including herbicides. Suitable techniques for selection for tolerance to environmental stresses need to be developed.

4.5 Sugar cane

General

a) In sugarcane, recovery of useful genotypes through sexual reproduction is very low due to the high degree of heterozygosity. The chances to recover desirable plants may be increased through induced mutation.

b) Methods to select in $M_{1}V_{2}$ and $M_{1}V_{3}$ for disease resistance, high sugar content etc. are available.
Material and treatment

a) Appropriate doses for treatment of "sets" should be established by preliminary studies. Survival and plant growth can serve as indicators. At least 50 "setts" for each dose are recommended. As established in previous reports, 20-40 Gy of gamma-rays are recommended.

b) The initial \( M_1 \) population for a mutation breeding programme should be at least 1000 shoots.

c) In vitro, shoot micro-propagation is not available in sugarcane. But somaclonal variation is obtained from callus derived from apical dome and unexpanded leaves.

d) Variation present in somatic cells arising through chromosomal mosaicism should be exploited. If possible it should be compared with radiation induced variability.
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