REVIEW OF IN VITRO PROPAGATION AND MAINTENANCE OF SWEET POTATO GERMPLASM

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Propagation

Sweet potato is normally field propagated by the use of stem cuttings, however under in vitro conditions a wide range of methods are available for micropropagation.

Propagation by single node cuttings

Sweet potato germplasm can be introduced into in vitro culture in the form of a nodal cutting. When placed onto an appropriate culture medium the axillary bud is induced to grow and the result in the development of a new in vitro plantlet. It is important to note that this type of propagation involves the growth of an existing morphological structure, the axillary meristem. The hormone/nutrient conditions of the medium simply play a role in breaking the dormancy of the axillary bud and promoting its rapid growth. Several laboratories have developed media for single node propagation of sweet potato (1,2,3). Care must be exercised however that the culture conditions do not allow the formation of callus and subsequent de novo regeneration of plantlets, as will be shown in a later section this has been shown to affect the genetic stability of the genotype (4,5).

Most laboratories grow in vitro plantlets under long day (16 hrs light 3000 lux) conditions at 25-28°C. Under these conditions in vitro propagation rates are rapid and a single node cutting will have grown the full length of the culture tube and be ready for subculture after 6 weeks.

Several laboratories have shown that in vitro plantlets of sweet potato produced from single node cuttings can easily be transferred to non sterile conditions, for example transplant to jiffy pots, and in some cases plantlets have been transplanted directly to the field.

Propagation in liquid culture (stem segments)

From experience gained with other crops, especially potato, it is known that under certain conditions the speed and labour requirements for propagation by single node cuttings limit the potential production. As in potato, it

is possible to micropropagate sweet potato in flasks (250 cm^3) containing liquid culture medium. Whole stem segments 5 to 8 nodes long are prepared by removing the apical tip and roots. This stem segment is then inoculated into the flask so as to be bathed by the culture medium. The liquid medium contains gibberellic acid to break the dormancy of all the axillary buds along the length of the stem segment. Shoots develop from this and in 3-4 weeks the flask is full of vigorously growing sweet potato shoots, (6). The flasks containing shoots can be a source of plant material either for preparation of single node cuttings of stem segments depending on the needs or the program.

Propagation by plantlet regeneration

The sweet potato is developmentally a highly "plastic" plant. It has been possible to regenerate de novo in vitro plantlets from almost all plant parts when placed into culture. Several scientists have been able to successfully regenerate plantlets of sweet potato from cultured stems, petioles, roots and leaf discs, (7). In all cases the first step is the formation of callus at the cut surface, the size and type of callus varies between treatments and genotypes. When given an appropriate hormonal stimulus it is possible to induce the de novo formation of meristems within these calli which then eventually form a regenerating plantlet.

A number of disadvantages exist to using this method as a standard propagation method. Firstly the labour involved in dissecting off these individual plantlets would make the method cost ineffective within a production program. Secondly, callus derived plantlets are likely to have undergone minor or major genetic abberations during the callus stage, (8) thus, the regenerated plantlets would not be genetically the same as the original genotype. In a clonally propagated crop these types of genetic changes would probably be unacceptable to the producer or program.

Somatic embryogenesis

Plantlet regeneration de novo can take place through two possible routes, organogenesis, that is direct organ formation as described in the preceding section or by embryogenesis, the direct formation of embryos from somatic cells. The induction of somatic embryos has been reported in many plants (9). In a few cases somatic embryos are being encapsulated to form 'synthetic' or 'clonal' seeds.

Somatic embryogenesis has been reported in one genotype of sweet potato (10). However, to my knowledge no analysis has been made as tc the genetic stability of these embryos. Since they are also derived from a callus/cell suspension

there is a high probability that the plantlets are not all genetically identical.

The importance of analysis of genetic stability in in vitro propagation (and conservation) programs

This section of this review is equally important both to propagation and conservation. In a clonally propagated crop it is important to know that each propagule is 'true to type', even small modifications could accumulate in the crop from one generation to the next and may affect uniformity and yield. In the case of conservation of clonal germplasm a detailed analysis of genetic stability in culture is vital. Clonal germplasm storage involves the maintenance of specific gene combinations (genotypes). If a plantlet should come out of storage with a different gene combination to that which it entered with, then the validity of the storage method must be questioned.

Our ability to detect genetic changes during propagation and storage are however only as good as the detection methods available.

Many germplasm collections evaluate the stored genotypes routinely on the basis of morphological characters of the plantlets when grown under controlled conditions. If the plants show different morphological characters, ie. leaf form, tuber or storage root colour change, then we know some genetic change has probably taken place. However, if the plants appear the same this does not mean that no change has occurred, it means we cannot detect it. For example a change in a virus resistance gene could not be detected on the basis of morphology.

A number of biochemical methods are currently used in both potato and sweet potato to study genetic stability, these being soluble protein patterns and isoenzyme analysis. Although these are highly effective methods for looking for variation in gene products they do not look for changes in the genes themselves. Novel methods such as restriction length polomorphism analysis is now being investigated as a more sensitive way of looking for genetic changes. It is important that major germplasm repositories and seed programs use the most sensitive methods available to determine the genetic fidelity of their storage and propagation systems. In the case of CIP, morphological, soluble protein and enzyme analysis is routinely performed on both potato and sweet potato collections. If and when a more sensitive restriction enzyme method for gene analysis becomes available its inclusion in CIP's routine methods should be considered.

In vitro conservation of sweet potato germplasm

A number of clonal in vitro sweet potato collections exist in many national programs and international organizations (11,12). There are many advantages to placing vegetatively propagated germplasm in an in vitro rather than field maintained form and these have been described previously (13).

A number of techniques exist for in vitro conservation, each with certain advantages or drawbacks.

Limiting growth media

Many years of research has gone into the development of propagation media for sweet potato where the objective has been to optimize rapid in vitro growth. In the case of conservation the objective is to limit growth to a minimum while maintaining viability of the cultures. By this means it is possible to maximize the time between transfers (sub cultures) of the in vitro plantlets. At CIP, for example, in the case of potato in LTS (long term in vitro storage) transfers are needed only once per year by most clones and in some cases only once every three years.

Experiments to limit sweet potato growth in vitro, in our own and in other laboratories have depended on the use of hormonal growth retardants, ie. ABA (Abscisic acid), growth inhibitors ie. B995, CCC (chloro choline chloride), or osmotic regulators such as high sucrose concentrations or the addition of osmotic sugars such as mannitol or sorbitol (14). The difficulty with these types of studies is that different genotypes will react differently under these conditions. When a large germplasm collection has to be maintained in vitro the objective of the studies must be the development of a conservation medium that is widely applicable to a broad range of genotypes. Several storage media have been reported for sweet potato, however again it should be emphazised that storage media should not allow induction of callus that may lead to genetic abberations.

Reduced temperature storage

The growth rate of in vitro plantlets can obviously be restricted by reducing the incubation temperature. The optimal growth temperature for sweet potato in vitro appears to be between 28-30°C. If the cultures are moved to a temperature of 8°C we have found that survival time is less than 1 month. The optimal reduced temperature for genotypes studied to date would appear to be 15°C, but this needs further confirmation. As in the case of other crops

maintained in vitro, ie. cassava, potato, yams, etc., it is possible to apply both reduced temperatures and osmotic/hormonal growth retardents at the same time. At the present time I believe the use of osmotic stress and reduced temperature (5°C) to be the most realistic and cost effective way to maintain a large sweet potato germplasm collection.

Cryopreservation

In the last decade there has been much interest in the use of cryopreserving (-196°C) plant materials in liquid nitrogen as a way of conserving germplasm (15,16).

This type of cryo-conservation is used routinely for storing animal cells and bacteria. The situation with plants is however more complex. It has been possible to freeze and thaw in viable condition plant cells from many plants, however these single cells then pass through a callus to regenerate whole plants causing genetic abberations. If intact plant structures such as meristems or embryos are frozen, their size (multicellular) leads to a problem of ice crystal formation within the tissue. Survival rates of frozen multicellular structures are low and little or no study has been made on the stability of the regenerated plant (with the exception of cassava). The concept of cryopreservation is one that would revolutionarize plant germplasm storage and as such deserves more investigation. However, in the short to medium term I do not see this as a viable option to sweet potato clonal in vitro repositories.

Phytosanitary status of sweet potato germplasm collections

The successful introduction of a sweet potato plant to in vitro culture would imply that the plantlet is probably free from bacterial, fungal and mycoplasma infections. The plantlets may still however be infected with viruses or viroid. The maintenance of the plantlets in vitro (rather than in the field) does, however, limit further viral degeneration of the collection that could result from cross contamination. It is therefore possible to enhance the phytosanitary status of a collection by in vitro introduction. It is also beneficial to virus/viroid eradication programs to already have the germplasm in in vitro. When germplasm is to undergo evaluation is in obviously optimal phytosanitary status is required.

Management of sweet potato in vitro germplasm collections

The size of any given germplasm collection will vary and the problem of managing a collection of several thousand

accessions are distinct from those with fifty accessions. However, with any in vitro germplasm colleciton care and consideration must be given to the following points: 1) How many replicates should be kept of each accession. 2) Should duplicate accessions be held by another institution. 3) What safeguards can be made against errors in data maintenance. 4) How often should the collection be checked. 5) Which accessions are the most valuable in case of emergency. In the case of CIP a computer data base is being established on the sweet potato collection. This should allow us (using the potato collection as a model) to maintain continual records of the in vitro collection and make the maximum amount of information and material available to developing country national programs. It should also facilitate inter-centre information flow on the status of any or all in vitro accessions.

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