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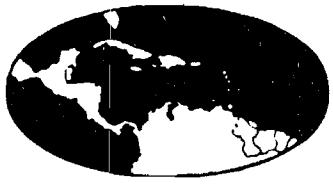

FOURTH ANNUAL MEETING

KINGSTON , JAMAICA

JULY 25 -- AUGUST, 1 1966

VOLUME IV

EMBRYO CULTURE: A VITAL NEW TOOL IN BANANA BREEDING

D. S. Lacy

ABSTRACT

Embryo culture methods are now being used routinely to overcome serious difficulties encountered with the germination of banana seeds under greenhouse conditions. The methods used and the results obtained are discussed.

INTRODUCTION

Despite several significant advances in seed germination technique since the inception of large scale banana breeding in Trinidad and Jamaica in the early nineteen twenties, the general level of performance has remained unsatisfactory. Fortunately, all banana seeds are not equally intractable in this respect. Today, the germination of wild type and semi-wild type material usually presents no difficulty provided a few basic rules are followed in respect of the harvesting and storage of seeds, sowing procedure and greenhouse care of seed flats. It is with seed from the more complex crosses that difficulties are still experienced. When this happens in the diploid breeding programme, it is usually more of an inconvenience than a serious limiting factor: the majority of diploids are reasonably fertile and any germination problems that arise can be surmounted by sheer weight of seed numbers. It is when the germination problem is complicated by poor seed set, such as in the tetraploid and triploid programmes from which plants of commercial potential are expected, that the matter becomes extremely serious. Such plants are very expensive to produce. In fact, N. W. Simmonds has estimated that they are probably the most costly seedlings ever produced on a large scale.

Two years ago embryo culture was finally resorted to in an attempt to provide a solution. From the first, the method proved outstandingly successful and was quickly adopted as routine practice for all the costly seed material.

PROCEDURE AND RESULTS

Prior to embryo extraction seeds are water-soaked for 5 to 10 days and then surfact sterilized in a 1% silver nitrate solution. Both pre-extraction treatments are absolutely essential if the maximum possible yield of seedlings is to be realized.

In a series of trials with a batch of M. balbisiana seed the performance of embryos from non-soaked seeds were compared with embryos from seeds soaked for periods ranging from 1 to 15 days. The 5 to 10 day range appeared the most favourable, although the only really dramatic difference was between no soaking (0 - 10% germination) and 1 days soaking (50 - 70% germination).

Surface sterilization is called for as experience has shown banana seeds to be seriously contaminated superficially with bacteria and fungi. It is possible to obtain plants from embryos removed from non-surface sterilized seeds but losses due to embryo contamination, account for 80% or more unless extreme care is taken during the extraction process. Silver nitrate has been used continually over the last 18 months as a surface sterilant with admirable success: the only possible drawback is its relative expense. Calcium hypochlorite and mercuric chloride have also been tried but appeared to be less effective under the prevailing conditions. It should be emphasized that no attempt is made to obtain completely aseptic seeds: in practice seeds are soaked in the sterilant for about 45 minutes, which period has been found to be sufficient to enable removal of embryos with only rare losses from contamination.

Seeds are carefully rinsed and dried under aseptic conditions before being split with a stout razor blade to facilitate embryo removal. The embryo is a mushroom shaped structure and is easily located as it sits immediately below the micropylar plug. They are of somewhat variable size, M. balbisiana embryos being significantly larger than those of M. acuminata. Tetraploid embryos are also larger than diploid units. Although even the largest embryo is quite a small structure - about 1 mm in diameter at the "cap" - an optical aid is never required for extraction, which is accomplished with a special hook tipped needle.

For routine work culturing is usually done in 6 x 1" test tubes on semi-solid slants of modified Knudsons medium, each tube containing about 20 ml of the material. Smaller tubes, 6 x 1/2" and 4 x 1", containing proportionally less medium are used from time to time through necessity, but the plantlets obtained are frequently of an inferior quality.

Embryo orientation on the nutrient substratum is not material to success, however, any submergence is highly detrimental. Presumably embryos require to be well aerated for proper germination to take place.

Culturing is usually done singly but occasionally in pairs. The latter procedure is avoided whenever possible as embryos often germinate at widely differing rates, in which event it becomes necessary to effect separation. Mass culturing of 5 to 10 embryos in large flat-bottomed containers of liquid medium is also highly effective, provided a very shallow depth of medium is used. There is no real advantage to be gained in mass culturing, however. Apart from the embryo growth rate differences already mentioned, there are a number of drawbacks related to working with a liquid medium; furthermore, contamination of a single flask can be disasterous.

The amount of agar incorporated in the culture medium greatly influences results. In a series of tests it was found that germination and root growth increased steadily as the agar content was reduced in steps from 1% to 0.4%. Agar is a complex and largely inert polysaccaride used in microbiological media to achieve solidification: the possibility of it having any direct effect on embryos must be remote. Some physical side effect is probably involved.

Almost all banana embryos show some external polyphenol oxidase activity which results in the accumulation of pigmented materials in the medium surrounding them. These substances are definitely toxic and in extreme cases embryos and even plantlets have to be transferred to fresh medium to avoid severe retardation and possible death from their self fouled activity. The semi-solid state of the culture medium is distinctly advantageous in this connection, as gentle manual aggitation of test tubes results in dispersal of the toxic substances and minimization of their effects.

At best embryo germination occurs in 4 days and test tube plants transferred to greenhouse flats a mere 2 weeks after extraction. This is at least equivalent to and probably faster than traditional greenhouse methods, despite culturing on a simple, artificial nutrient medium.

Of the embryos that fail to germinate, only a small proportion remain completely static and lifeless; the majority produce a considerable amount of callus growth but from neither root nor shoot. These are presumed to be inherently defective.

Test tube grown plants are more delicate than those grown from intact seeds and special care has to be taken after transplanting them into flats to buffer the change from test tube to greenhouse environment. Large humidity chambers are used for

this purpose and plants spend 1 to 2 months in them before being hardened off and potted. When done efficiently, which is usually the case, this phase of the process can be accomplished with only negligible losses.

The success of the programme can rightly be described as spectacular. Highgate seed germinations have gone up from a yearly average of 20-25% to 60-65% and the output of plants of commercial potential at least doubled. Triploid germinations have increased similarly. Furthermore, it has been found possible to obtain plants of diploid crosses from which it was scarcely possible to obtain any progeny previously. For example, Paka x Sikuzani germinations have increased from a greenhouse average of 2% to over 30% by embryo culture.

Progress in the breeding of a new variety of banana to replace the much esteemed Gros Michel has always been severely retarded by three factors:

1. Poor seed set
2. Hopelessly inadequate seed germination
3. Failure to transmit adequate Panama Disease resistance to progeny.

Today, it can rightly be claimed that at least the second of these limiting factors has been eliminated.

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INBRED-HYBRID METHOD OF MAIZE IMPROVEMENT

S. M. Sehgal

ABSTRACT

In spite of its limitations, the inbred-hybrid breeding system is the most extensively and successfully employed method of corn improvement in the United States. The breeding procedure consists of (1) isolation of inbred lines by selfing or inbreeding a few plants in each of the source populations which are open pollinated varieties or races, mass selected populations, synthetics or composites; (2) evaluation of inbred lines in top and single crosses; (3) crossing the chosen inbreds to produce highly vigorous and productive hybrids. Selection for desirable agronomic characteristics is practiced during the inbreeding process.

In our breeding programme in Jamaica, we are using this method to develop hybrid varieties which are adapted to low land tropical conditions. The selection emphasis is on the following traits:

1. High yield
2. Short plant stature with relatively low ear height
3. Ears with tight husks and strong shank
4. Resistance to lodging
5. Resistance to various leaf diseases like rust and blight
6. Resistance to chemical burns by herbicides and insecticides.