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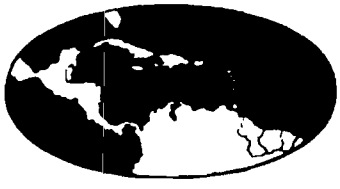

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A NEW APPROACH TO GRAFTAGE

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Grafting is one of the oldest of the Arts of plant craft. Records of mango approach grafting have been found in the old Sandskrit literature showing that the art was practiced since very early times in ancient India. Mention of graftage is made in the writings of Virgil and the elder Pliny describes a cleft-graft in great detail giving numerous precautions many of which are in substantial agreement with modern knowledge.

For several centuries now grafting practices have been widely practiced and studied and great ingenuity has been excercised in devising different methods. The basic fundamentals, however, remain the same and improvements in practice have been very slight indeed. In fact several treatises on grafting which are over 50 years old seem to be just as good as most recent treatments of the subject.

There was, however, a definite break through to a better understanding of the mechanism of graft union in the early 1930's. Previously the old books insisted that the graft union was achieved by new cells origination exclusively from the cambium or according to a later school from the medullary rays. Sass (2) and Sharpless and Gunnery (3) and Mendel (1) showed that all live tissues in the bark and young xylem cells without secondary walls were capable of regeneration and that the grafting union was achieved in 3 basic steps as follows:

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1. A matrix of callus tissue is first layed down by all live tissues from both scion and stock which are exposed by the grafting cuts. This callus fills up the empty gaps between scion and stock elements.

2. Tissue differentiation occurs whereby first the phellogen and then the cambium "bridge in" between scion and stock. This differentiation invariably starts in the cells immediately adjacent to the outer edge of the remaining phellogen or cambium in the scion and stock and extends radially into the callus matrix by a successive sort of "induction" process until the edge originating from the stock meets with and joins the edge originating from the scion thereby bridging the gap in the phellogen and cambium sheats.

3. After the cambium layer "bridges in" it starts to divide tangentially in normal cambium fashion laying down xylem and phloem elements which can thereafter transport water and nutrients between the stock and the scion.

As far as the practical mechanics of grafting go, however, this new, true concept of what takes place hardly makes any difference in established practice. We still have to cut carefully and cleanly and place the scion in careful juxtaposition to enable the cambiums to "bridge in" properly.

It seems almost inconceivable that in this day of tissue cultures, of tremendously advanced knowledge of enzyme chemistry, of so many new ingeneous mechanical devices, of so many advances in human and animal surgery where a host of difficult problems have been solved, we still graft plants the way our grandfathers did.

Part of the difficulty may be in the approach. Experimentation with grafting techniques has mostly consisted of trying some new modification in technique or treatment or selection of material and comparing the results obtained thereby with the results obtained the old way. This criterion is most cumbersome, to say the least. To begin with, the old way of doing things has to be bad enough to allow room for improvement. If you are already getting 90 per cent "takes" you would have to graft a tremendously large number of plants in order to prove statistically that any new modification is advantageous. Conversly also the old way has to be good enough to allow room for harmful practices to manifest their undesirability. An ideal subject for grafting experimentation would, therefore, be one with which you normally attain 50 per cent success. This simple circumstance coupled with a high experimental error has made the use of statistics unpopular in grafting experimentation and this in turn has led to a host of errors and misinterpretations. The graftage literature contains some of the craziest notions in plant science. One old fellow suggested placing the cut shield bud of citrus face down on the tongue when walking to the next rootstock. Another leaves a notch at the top of rose buddings for water to penetrate into the graft. Actually both human saliva and water are slightly toxic and interfere with cell regeneration if allowed to contact the freshly cut surfaces.

I am convinced that much more is to be learned about plant grafting if we split grafting into steps and use some of the knowledge obtained with tissue cultures and in enzyme chemistry. Unfortunately, from our point of view, most workers in the field of plant tissue culture have been primarily interested in nutrition and in Virus diseases. When they achieved success with excised roots or callus tissue they have gone on with those cultures to investigate the needed organic acids, etc. When they achieved success with excised meristems, they have used the technique to obtain virus-free plants and later to propagate orchid plants. No one to my knowledge has cultured differentiated stem tissues in aqueous solutions successfully. Probably the basic reason for this is the fact that differentiated bark tissues of most and possibly all angiosperms contain phenolic compounds and phenolic oxidases. When these tissues are cut and bruised and the surfaces exposed to air, a typical complex browning reaction occurs. The products of these oxidations and polymerizations have tanning properties and inhibit respiratory enzymes. They are therefore toxic and interfere with the regeneration of new cells which normally takes place on the exposed cut surface of live tissue. The enzymes and their phenolic substrates are presumably spatially separated in the intact tissue and a simple cut with a knife and exposure of the surface to air results in limited phenolic oxidation taking place only in the localized areas where some slight exudation and the intimate mingling of these elements occurs.

If the cut surface is wet, however, the phenolic substances, which are soluble in water, are apparently transported over the entire surface, mingle freely with their corresponding enzymes and interfere with regeneration from all tissues which they contact. If the water contains traces of the heavy metals, browning and staining is even further accelerated and increased injury results. This then may explain the non existence, thus far, of cultures of differentiated stem tissues in aqueous solutions. It also accounts for the lethal effect of rain which penetrates recent grafting and for the numerous failures met with when freshly cut scions have been treated with aqueous solutions of different plant metabolites.

Cut stem tissues can, however, be placed in petri dishes or moist growing chambers and regeneration and differentiation may be conveniently observed. In our experiments we, at first, would place bark strips and cut stem pieces with the cut faces upward on 2 per cent agar but later changed to the use of moistened filter paper instead of aggar and found this to be equally as good and much more convenient. Although we are not primarily concerned here with reporting experimental results, we can briefly mention a few of our observations as follows:

1. Working with avocado bark strips we observed the formation of hemispherical thin walled new cells which projected upward from the exposed inner surface of the bark. This was observable three days after the bark strips were removed from the stem and placed in the moist growth chambers. These cells were later observed to continue to grow and divide forming a cushion of callus tissue several cells thick.

2. When juice from macerated avocado bark was placed as individual drops on the exposed inner surface of such bark strips, cells failed to grow in the circular area where the drop was placed and resulted in the formation of a brownish-red, stained, crater-like gap in the cushion of callus tissue. Distilled water and phosphate buffer solution adjusted to pH 6 delayed regeneration but the gap caused thereby would later be filled by lateral expantion and growth of the surrounding regenerating tissue.

3. Over all coverage of the entire exposed inner bark surface with petrolatum did not interfere with the formation of new cells even though such treatment presumably interfered with direct contact with air of the exposed tissue.

4. In agreement with the literature on the subject, the callus tissue appeared to be free of phenolic substances capable of oxidation and polymerization to produce colored products. Bruised callus exposed to air did not undergo browning.

5. Cut young stems of avocado with a young, translucent, live, inner pith regenerated callus tissue from this central cylinder whereas those with white, dead pith did not. The latter consequently delayed a much longer time in developing a callus which never did cover the wood and pith completely.

Our concern with tropical orchard trees and awareness of the difficulty of grafting many of them leads us to attempt the immediate practical application of even these meager finding as well as to anticipate that many more additional useful findings may be achieved with this approach. For example, in top-working rubber trees (Hevea) in Central America years ago we became aware that whenever our work was interrupted by rain the prcentage of "takes" for that day was greatly reduced yet when unwrapping other old buds, after the customary 3 1/2 to 4 weeks, we frequently found that rain water had penetrated many live buddings with no apparent damage. The answer we now realize is that water damage occurs when the fresh cuts are wet; after the first 3 to 5 days the tissues are no longer vulnerable because of the regenerated callus. The obvious practical application is to stress protection from rain during the first week possibly by providing a temporary roof even though the grafts have been waxed. The observed regeneration from the live pith confirms previous grafting experiences with avocado to the effect that young rootstocks and careful selection of scion material with live pith insures excellent success. The confirmation of stain and polyphenol toxicity emphasizes the importance of many existing recommendations regarding grafting technique. Foremost among these is frequent cleaning of the knife particularly when working with succulent material. Ethyl alcohol is an excellent solvent for phenolic compounds and not only cleans the knife but sterilizes as well. Alcohol and paper towelling should invariably be included in the grafting kit. A stainless steel blade of some of the new steels which can hold a good edge will also be most advantageous since it would not accelerate "browning" the way ordinary steel does. Bruising of tissue is to be avoided.

Future possibilities to improve grafting through this approach appear to have a tremendous potential. It is doubtless quite probable that some suitable formulation may yet be devised to enable us to wet and therefore treat freshly cut stem tissue with promising metabolites such as Kinetin, sugar, and Sebacic Acid. In the meantime, however, we can supply such metabolites by means of bottle grafts or by pre-treatment of budwood, allowing the stems to take up solutions through their basal ends after the fashion of cut flowers. A third, highly promising possibility is to allow the cut scions to regenerate some callus in a moist growing chamber and then immerse them in aqueous solutions for appropriate intervals before final grafting.

Apart from such treatments, this two step method of grafting in itself offers some good possibilities. We can cut a large number of scions in advance, store them in growing chambers and later graft them to the stocks which can also be precallussed if this proves to be desirable. Such grafting would capitalize on the absence of phenolic substances in the callus tissue which can therefore be cut, or crushed, or wet, presumably without deleterious consequences.

I have started some work along these lines but primarily with the intent of doing grafting in a sort of mass-production assembly line fashion. It is also an attempt to get improved results with some subjects with which I have had difficulty. I refer specifically to macadamia, rambuttan, and quenepa all of which have very hard wood and also sapodilla which not only has hard wood but also exudes a latex which contains phenolic substances and is very troublesome. I have with me a special carpenter's plane. I have always insisted that grafting hard wooded species is basically carpentry work and we should use the best available tools. In this special plane number 60 1/2 made by Stanley, the cutting blade is placed at very acute angle compared to other carpenter's planes and its cutting action approaches that of a sliding microtome. When I first used it as it came from the factory, its base became badly stained very quickly. I have since had it nickel, plated and now only the steel blade becomes stained and this can be cleaned periodically with a rag wet in ethyl alcohol. The technique consists of shaping several hundred scions at a time and placing them in moist growing chambers. I am using discarded plastic petri dishes which are normally thrown away. The scion cutting can be done while sitting down confortably at a desk or work-bench. The final grafting can be done that same day or several days later and one can pick and choose the scion to obtain the best fit for the individual stock. The impressive feature of scions cut in this way is the remarkable flatness and evenness of the cut and this can be done very easily even with such hard woods as macadamia and sapodilla. With sapodilla it has the added advantage of quickly draining off the latex by many successive cuts which keep the latex flowing so that when the final cut is made the laticifer system is well drained and little if any latex exudes from the final cut. This work is still in the early stages and I am not ready as yet to report on the results. I can assure you, however, that it works and I have high hopes for it.

SUMMARY

Despite the fact that graftage is a very ancient plant craft, little progress has been made in plant grafting techniques since early times and remarkably little has been accomplished in the past century. The craft needs a new approach.

An approach to grafting consisting of the use of moist growing chambers is suggested. This constitutes not only a good research tool by which the effect of scion treatments may be observed quickly and conveniently but can also be put to good advantage in routine grafting by a two step method.

This two step method of grafting is described. It consists first, of placing cut and shaped scions in moist growing chambers and secondly, inserting the scions in the rootstocks. The period of time for keeping the scions in moist chambers may be varied from a few hours to a few weeks. The short period simply serves to keep the scions in good condition while many scions are shaped in assembly-line fashion. The longer periods allow the scions to become pre-callused thereby obtaining several possible advantages based on the fact that callus tissue is free of polyphenolic products with "browning reaction" capacity.

Research observations made with moist growing chambers are mentioned and the use of a modified carpenter's plane for grafting hard wooded species is recommended.

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