Molecular Sciences

Editorial

Unifying Approaches in the Plant Sciences: The Importance of Scientific Tolerance

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From its inception, in vitro plant regeneration research has involved intuitive and empirical approaches. Various auxins and various cytokinins, as plant growth regulators or plant hormones, were used in culture (or growth) media to study plant propagation and development in vitro. During its earliest stages, it was determined that high auxin to low cytokinin concentration ratios in growth media can induce callus to form from small pieces of organized plant tissue placed on the growth medium, whereas, conversely, high cytokinin (of any given type) to low auxin (of any given type) concentration ratios in growth media ensued in calli, on such media, producing small plantlets via buds. This is a process referred to as organogenesis or in vitro plant regeneration. It was the concentration used that was the critical factor in promoting in vitro developments, not the particular cytokinin and auxin used, even though some cytokinins were more effective than others in such. As utilized by researchers, such in vitro developments from calli on culture media enabled in vitro plant propagation.

Researchers in the field did not know why the hormone concentration ratio used was the effective parameter. It was effective from the empirical standpoint, and this was all that mattered. There was no unifying theory to explain why or how this happened. This empirical approach was effective with various plants, such as tobacco and carrot, enabling their in vitro propagation. However, with other types of plants, such as conifers, using high cytokinin to low auxin concentration ratios in culture media was not effective with regard to inducing in vitro organogenesis. This type of molecular approach to plant propagation was thus restricted.

In the summer of 1980, I commenced research in a botany laboratory where efforts were being made to induce calli of the pine, *Pinus taeda*, to undego in vitro organogenesis. Using the cytokinins and auxins in various concentration ratios with respect to one another for various culture periods did not ensue in organogenesis in the pine callus culture. The conventional approaches were not effective with the pine in vitro. Through an encompassing theory of natural processes, it had occurred to me, intuitively, that cohesive forces were necessary for the induction of organogenesis in vitro. Such forces, it was conjectured, had to be induced in the callus for organogenesis to ensue, but the currently used growth hormones were not effective in doing such in the pine callus.

Based on the research of Albert Szent-Gyorgyi, who showed that cohesive forces induced in mammalian tumors in vitro could inhibit the growth of such, with their subsequent death, I thought that a variation of his approach could be applied to overcoming recalcitrant plant regeneration in pine callus. In this regard, Szent-Gyorgyi's research demonstrated that a combination of methylglyoxal (MG) with ascorbic acid (AA) in culture media could induce cohesive forces in mammalian tumors in vitro, and consequently, such forces could inhibit their growth, leading to their death, while normal, organized tissue, requiring such cohesive forces, was not affected by the application of MG in low concentration and AA via culture media.

In view of these findings, I thought using MG (in low concentration) and AA in a plant culture medium would ensue in cohesive forces occurring in the pine calli placed on such a medium, inhibiting their growth, while inducing pockets of the organogenic process within any given pine callus. Through this approach, and not using the conventional growth hormones, pine buds did develop from the calli. However, many buds had deteriorated, but some were quite viable. It was thought that too much MG had accumulated in the pine buds, inhibiting their growth, due to the buds extended presence on the MG medium. Later research suggested this possibility.

In subsequent research, with another pine species, *Pinus muricata*, deterioration of developing buds did not occur on the experimental culture media. MG, AA, and a high cytokinin to auxin concentration together in the culture medium ensued in pine calli on

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such a growth medium producing small green buds and a low frequency of small green pinelets, as opposed to controls, where developments did not occur. The period of culturing was three to four weeks under controlled light. Callus growth was greatly inhibited, and the calli were a dark brown in color. Nevertheless, to avoid any future MG accumulation problem, these calli producing the buds and pinelets were sub-cultured onto media without MG, AA, auxin, and cytokinin. Developments were still maintained under these new conditions. This change in protocols avoided any possible, future deterioration problem in the pine cultures and enabled the continued in vitro pinelet developments from the calli of *Pinus muricata*. Another objective of the new protocols was to determine any effect on these developments by those changed culture conditions.

With the green bean, which had a history of recalcitrant development in vitro, bean calli on culture medium containing MG, AA, and a cytokinin plus an auxin underwent a high frequency of somatic embryogenesis, resulting in many, very green bean plantlets. Calli on control media only produced tiny knobs. Deterioration was not a problem with the somatic embryogenesis of bean using this MG medium. After pieces of the bean calli were sub-cultured onto culture medium containing AA and a cytokinin to an auxin at high concentration ratio, the bean calli eventually underwent frequent organogenesis, producing large buds, resulting in many bean plantlets growing from such. Control cultures, on media without AA, produced only exceedingly small buds, which did not develop further.

One of the important conclusions arrived at from this research approach was that chemicals such as MG, AA and the cytokinins can be agencies or avenues of cohesive forces, while other chemicals, such as the auxins, can be agencies or avenues of repulsive or loosening forces. The calli, it was conjectured, were enabled or generated mostly through repulsive forces in plant tissues in vitro due to their exposure to a high concentration of auxin. These chemicals, it was concluded, acting synergistically with one another, could affect, via critical combinations of cohesive and repulsive forces, in vitro plant development. From this perspective, worth further investigation, it is the particular, changing, coordinating, and effectively cohesive, configurations of such forces, mediated through those chemicals, that could enable in vitro plant development, as well as plant development in general.

The success of this approach using MG and AA in plant culture media was based on a unifying theory involving cohesive forces in biological development. And other researchers in India and South Africa have confirmed that MG in low concentration in culture media can induce in vitro organogenesis and somatic embryogenesis in the calli of various plant species, such as sugar cane. Without such a unifying theory, it would not have occurred to researchers in 1980, involved in plant culture research, to have used MG and AA in culture media as agents of the organogenic process, especially its enhancement. At that time, when I advocated for the use of MG and AA as a means to overcome recalcitrant organogenesis in pine callus, my approach was met with skepticism, if not hostility. After all, my colleagues responded, “these are not plant growth hormones,” failing to see the significance of the theory underlying my proposal or hypothesis. In the context of the theory, and empirically, MG and AA became, as agencies of changing cohesive forces, effective plant growth hormones. Had my approach not been undertaken through the years, a deeper, constructive insight in botanical research would have been precluded.

From this, one can see how different, complementary approaches in plant science are invaluable. It is not just a molecular approach verses a holistic approach, involving cohesive, coordinating forces, that is better to use, but it is both approaches used together in a complementary mode that are effective, and thus important in biological and medical research. In general, the continued, constant regeneration of constructive scientific practice requires this perspective. This is a perspective involving the tolerance and encouragement of complementary views, intuitions and unifying theories. The history of research involving in vitro plant regeneration makes this very clear.