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INVITED REVIEW

Trevor Alleyne Thorpe: His academic life and scientific legacy

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Abstract
This special review is written to commemorate the life and contributions of Dr. Trevor Alleyne Thorpe, who passed away on May 18, 2020, at the age of 83 yr (https://www.sivb.org/InVitroReport/issue-54-2-april-june-2020/in-memoriam-4/). As part of his academic legacy, Trevor had trained an impressive number of laboratory members—15 PhD students, 14 Master's students, 25 postdoctoral fellows, and many visiting scientists—who collectively identify themselves as proud members of "Team Thorpe." This executive summary version of his major scientific contributions was prepared by the team.

Keywords Trevor Thorpe · Scientific contributions · Plant tissue culture · Conifer biotechnology · Developmental plant physiology · Micropropagation · Salinity tolerance

His Academic Life

After completing his early education in Barbados, Trevor started his undergraduate studies in 1956 with a full scholarship at the Allahabad Agricultural Institute, Allahabad, India (presently known as Sam Higginbottom University of Agriculture, Technology, and Sciences). Trevor was proud of his training as an "Aggie" from this institute, which was established in 1910 as the first agricultural institute of India, and Southeast Asia. After graduation, he returned to Barbados and married his wife Yvonne in 1963. Subsequently, he received a Fulbright Scholarship to attend the University of California at Riverside, where he received his Master's and Doctorate degrees. His PhD thesis advisor was Professor Toshio Murashige, well known for the Murashige and Skoog medium (Fig. 1). Professor Murashige was a student of Professor Folke Karl Skoog, famous for his discovery of cytokinins. Trevor carried out postdoctoral research work at the US Department of Agriculture research facility in Pasadena, CA. With such a stellar academic lineage, Trevor built a research career that was focused on physiological aspects of plant morphogenesis using explants cultured *in vitro*. His work contributed to our fundamental understanding of organized plant development *in vivo* and *in vitro*.

Trevor began his independent research after accepting an assistant professorship in 1969, at the Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada. He remained at the university in a teaching capacity until 2000. During that time, he established a renowned lab that attracted graduate students from all over the globe. In his 30 years of service at the University of Calgary, he taught and mentored hundreds of students. Members of "Team Thorpe" remember Trevor as a genuine father figure. The happy memories made during each Team member's stay in Calgary are forever fresh (Fig. 2). One of the numerous positive and happy aspects that helped to build the tremendous team spirit included the twice a year happy gatherings at his home in October and at Christmas. Another important contributor to the evocation of such positive memories is the nature of freedom he gave to his lab members. Trevor embodied one of the hallmarks of good leadership. He was such an open and engaging person, who created an atmosphere in

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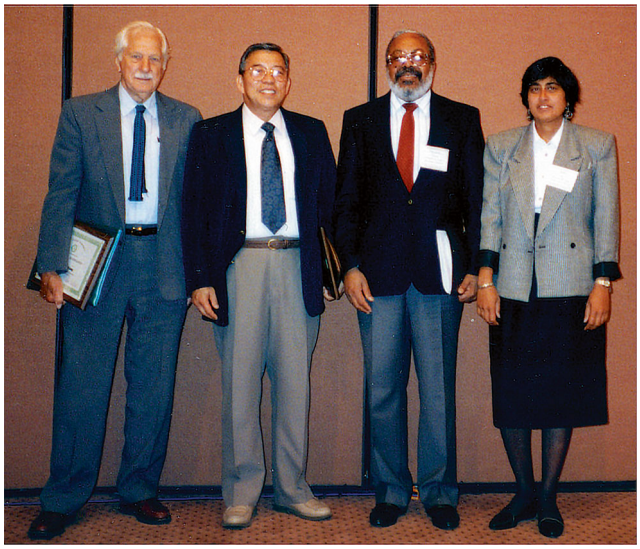

Figure 1. Four Generations of plant biotechnologists. Photographed in 1991 when Toshio Murashige received his Lifetime Achievement Award from SIVB; from the left, Folke Karl Skoog, Toshio Murashige, Trevor Thorpe, and Indra Harry.

which everyone worked well together under an informal and fun environment, but where all members were always aware that they had to live up to his high expectations.

We, the members of Team Thorpe, will never forget that our future careers were made possible because of our work and study in Trevor's lab. He loved to travel for sabbaticals, conferences, delivering lectures, and conducting workshops. Wherever he went, he made friends easily with his hearty laughter, his love for music, dancing, photography, and baseball. Today, practically in every corner of the world, there is someone who fondly remembers Trevor as a pioneering scientist in the field of plant tissue culture and biotechnology—and members of Team Thorpe continue to benefit immensely from this professional network.

Team Thorpe was so international that our neighboring lab members used to refer to us as the "mini-United Nations"! Over the years, there were people from Germany, Italy, Spain (+Canary Islands), Sweden, the UK, Russia (USSR), Jordan, Nigeria, Mexico, Costa Rica, Guyana, Trinidad, Brazil, India, Bangladesh, Malaysia, South Korea, Japan, Hong Kong, Taiwan, New Zealand, and of course, a good number of "Canucks" to teach us how to be good Canadians! This diverse and friendly crowd always invited neighboring lab members to come and socialize with us. Of course, it also helped that Trevor's lab had a coffee area that was accessible to all! We did not waste a single excuse to have a birthday cake or wine party—and with so many lab members, there was a birthday to be celebrated practically every month! Younger lab members never missed the opportunity to tease our Major Professor for being so careful as to reduce sugar in his coffee while having a generous slice of cake! Yes, he would enjoy a slice of cake just as much as a single malt whiskey and good dance music!

Exactly how every member of Team Thorpe feels was summed up by one member as his personal reflections of working with Trevor. "The years I spent as part of Team Thorpe were a great experience. I learned a lot from him and met great colleagues – many of whom have now become lifelong friends. With Trevor's support I was able to attend several major conferences and he was also very generous in asking for collaboration on review articles and book chapters – a boon for postdocs! Trevor fostered a relaxed, collegiate atmosphere in which we all worked hard with no need for pressure from the boss. I feel ever-lasting indebtedness and gratitude to him."

Figure 2. Team Thorpe photos. Selected photographs of the team from the late 1970s to 2001.

Trevor was a distinguished and truly international scientist. Besides regular teaching and research, Trevor contributed to the university administration and the scientific community at large. He held academic positions as a department head, assistant, and associate dean at the University of Calgary. Trevor was a pioneering scientist who played leading roles in the International Association for Plant Tissue Culture (IAPTC, now known as the International Association for Plant Biotechnology or IAPB) and the Society for *In Vitro* Biology, USA (SIVB). He was the chair/president of IAPTC from 1974 to 1978 and organized the 1978 IAPTC Calgary conference (https://iapbhome.com/about/). He was the founding Editor-in-Chief of the journal *In Vitro Cellular & Developmental Biology - Plant* and served as a member of the editorial board for several other leading scientific journals, including *Plant Cell Tissue and Organ Culture*, *Tree Physiology*, *Phytomorphology*, and *Physiologia Plantarum*. Also, Trevor edited the Newsletter for IAPTC 1974 to 1978 while serving as a chair of that society. Lastly, but equally importantly, he was an active contributor to various international organizations and trained scientists around the world through workshops he conducted in Mexico, Bangladesh, Malaysia, the Philippines, and Costa Rica.

Despite his phenomenal accomplishments, success never got to his head. Trevor espoused a simple philosophy "Don't forget where you came from"—and he never forgot to follow that! He empowered Team Thorpe to become involved in editorial activities and trained us to be compassionate peer reviewers. He stressed the need to provide constructive feedback, not just criticism by itself, with a view of helping the authors to improve their science and manuscripts!

His Scientific Legacy

This review article was prepared with contributions from his former students, postdoctoral fellows, and collaborators as a tribute to Trevor. It is presented as a "brief biography" of Trevor's research, highlighting just some of his major contributions to *in vitro* morphogenesis, micropropagation and conifer biotechnology, salinity stress, and developmental plant physiology. Trevor published more than 220 scientific works. One of his books, "Plant Tissue Culture: Methods and Applications in Agriculture" (1981; Fig. 3), became an essential *go-to book* for all plant tissue culturists in the pre-internet era. Another remarkable aspect of Trevor is that, over the years, he developed productive research collaborations with numerous colleagues, including some short-term and some long-term collaborations such as those with Professors David Reid and Edward (Ed) Yeung. Obviously, we cannot do justice to all of his scientific contributions or to all his research collaborators in a brief review. We primarily wish to reminisce and highlight some of his many scientific contributions.

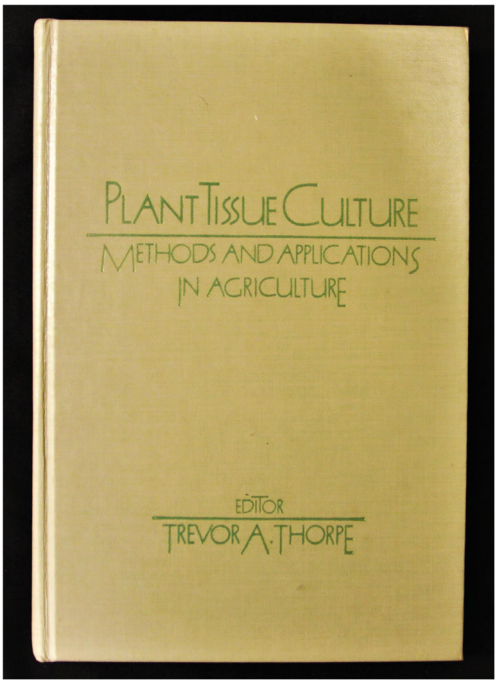

Figure 3. The indispensable reference book for plant tissue culturists during the pre-internet era.

***In Vitro* Morphogenesis** One of the key aspects of Trevor's research was to use tissue culture as an experimental system to understand morphogenesis. He had spent the summer of 1966 visiting Professor William Jensen's laboratory at Berkeley learning histochemical techniques. Therefore, he understood the importance of plant anatomy as a basic science in plant biology. Accordingly, major efforts were directed at understanding the histological reasons and evidence for how a group of founder cells organized themselves as meristemoids and subsequently went on to develop shoot buds. This was examined with direct bud formation from cultured explants as well as indirectly via a callus intermediate stage. He stressed that there needs to be a direct vascular connection between the shoot and the adventitious roots formed from the bases of *in vitro* shoots. This theme of research can be traced to histochemical observations he made during his PhD thesis research days. Quite a number of the papers on micropropagation from his lab included such histological or histochemical observations (Villalobos *et al.* 1984; Patel *et al.* 1984; Kim *et al.* 1985; Douglas *et al.* 1982; Patel and Thorpe 1984; Rumary *et al.* 1986; Thorpe *et al.* 1991). Excellent histological work was facilitated by Kamlesh Patel, who received earlier training at Yale University in Graeme Berlyn's lab.

For research on anatomy and histology, Trevor had a fruitful and long-term research collaboration with Edward Yeung, who ran an excellent lab next door. Ed's Ph.D. training was under Professor Ian Sussex at Yale University before he joined the University of Calgary in 1978. Ed's recollection

of his interaction with Trevor is as follows: "I met Trevor when I came for an interview at the Department of Biology, The University of Calgary, in the winter of 1978. Trevor picked me up at the airport, after waiting for more than two hours, because my plane's landing gear had gotten stuck and ultimately landed with fire trucks running along-side the plane. Trevor's patience, and my survival, led to a life-long friendship and collaboration." This lengthy research collaboration endured because Trevor and Ed shared a common view, "To successfully solve research problems, one needs to employ an integrated approach of investigating various related parameters. These would include studies on structure, physiology, biochemistry, and cell and molecular biology."

Trevor and Ed collaborated on different projects, but the first project was on radiata pine, *Pinus radiata* (Yeung *et al.* 1981), which built upon work from Trevor's group that optimized a protocol on shoot formation in this species (Aitken *et al.* 1981). In order to understand the very early developmental events associated with the shoot-initiation process, a histological study was performed detailing the events associated with shoot primordium formation. This and several other subsequent studies from Trevor's laboratory demonstrated successes in direct shoot organogenesis from conifer cotyledon explants, leading to future studies on conifer micropropagation and somatic embryogenesis. Figure 4 is a photograph of a radiata pine cotyledon section taken recently from a slide prepared about 40 years ago. The tissue was embedded in glycol methacrylate, a plastic-embedding medium designed for light microscopy studies. As is evident, the utilization of proper techniques can contribute to the quality and successes of such studies. In this example, the use of glycol methacrylate as an embedding medium, in conjunction with improved fixation methods, enabled better preservation of cellular structures (Fig. 4). Thus, better visualization of changes occurring within the cultured explants led to the successful identification of specific cells with morphogenetic potential. Over the years, students from both laboratories benefited by working together and learning from one another using different techniques and approaches.

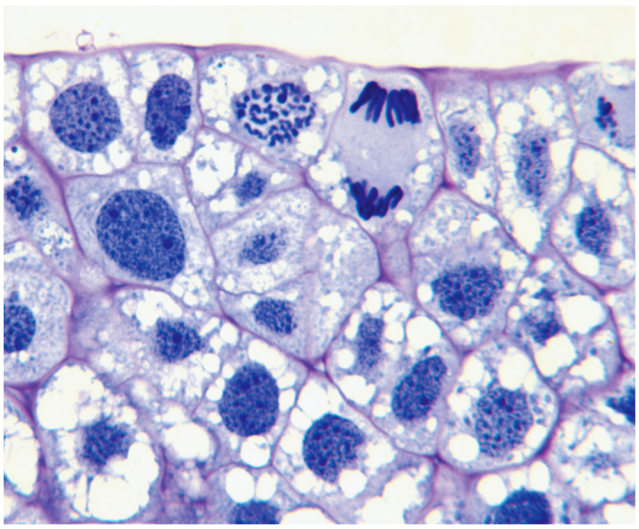

Figure 4. A glycol methacrylate section of radiata pine cotyledon showing positive responses to the benzylaminopurine treatment *in vitro*. Mitotic activities are localized in epidermal and subepidermal cell layers. Photograph taken recently from a slide prepared 40 yr ago.

Trevor and Ed completed their final major collaboration in 2011 as co-editors of a book entitled Plant Embryo Culture, a volume in the Springer Protocols Series (Thorpe and Yeung 2011). This reference book "can be used to help others develop their customized methods for different species and different purposes." As showcased in this book, plant embryo development can greatly benefit from both *in vivo* and *in vitro* studies. The use of *in vitro* culture of zygotic embryos has greatly facilitated the study and understanding of embryo development and has allowed for studies not possibly done *in vivo*.

Micropropagation and Conifer Biotechnology Since Trevor was a pioneer in the field of plant tissue culture, Calgary became a magnet for overseas scientists and researchers wishing to develop protocols for micropropagation of various species. One such researcher, Kee Yoeup Paek, arrived in Calgary with seeds of two vegetables especially important to Korean horticulture. They were the Korean varieties of Chinese cabbage (*Brassica campestris* ssp. *pekinensis* cv. Kimjung) and Japanese radish (*Rhaphanus sativus* var. *longipinnatus* cv. Gungjung). For both varieties, reliable and optimized micropropagation protocols were developed by research conducted by Kee Yoeup along with Steve Chandler. They discovered that in the case of Chinese cabbage, supplementing the medium with adenine sulfate was beneficial for axillary shoot formation. For Japanese radish, more extensive media manipulation was required to establish a suitable nitrate to ammonium ratio that would support optimal shoot growth. From this basal medium, growth was further enhanced through the addition of adenine sulfate or sodium phosphate to the medium (Paek *et al.* 1987). Both species rooted easily in culture and acclimation to soil was readily established.

Trevor's lab contributed significantly to micropropagation of woody species. One of the noteworthy contributions to this field where Trevor made a significant impact is the landmark paper entitled "Plant tissue culture media." This paper is one of the highly cited papers authored jointly by four of the undisputed giants in the field, namely, Gamborg *et al.* (1976). During the 1970s through the late 1990s, Trevor's lab developed *in vitro* plant propagation protocols for several conifers that were of importance to the temperate forestry industry. Although early efforts were focused on Canadian conifer species, the scope broadened as more international students and visiting scientists conducted research in his lab using their

native species. The general focus was on micropropagation, including organogenesis and embryogenesis and complementary studies, all of which contributed to the fundamental understanding of organized development *in vitro*.

Arduous studies were conducted using various salt formulations and growth regulators. Of particular importance were the effects of cytokinins and auxins and their ratios in plant development *in vitro*. Numerous meticulous experiments were conducted over many years to understand these effects using various explants from many conifer species (key personnel who worked on these include Jenny Aitken-Christie, Victor Villalobos, Kamlesh Patel, Eng-Chong Pua, Indra Harry, Michael Thompson, Richard Joy IV, Kiran Sharma, and Chin-yi Lu). Thus, a multi-stage approach to micropropagation morphed into a standard protocol, which included induction, bud development, shoot elongation, rooting, and acclimatization of plantlets. For many species, excised embryos from sterilized seeds were used as explants. Occasionally, explants included cotyledons and other seedling parts, but this was empirically determined. Bud induction was accomplished by adding cytokinins to the nutrient medium, and bud development by the addition of activated charcoal in the absence of any growth regulators. Elongated shoots were rooted in media containing auxins or in soilless commercial mixtures. Proper rooting was and remains a challenge for conifer multiplication, and rigorous experimentation had to be undertaken for each species. Occasionally, efforts were placed on increasing shoot multiplication by inducing axillary shoots from preformed meristems.

Using the organogenic route, a range of genera and species of gymnosperms was cloned. These included *Pinus radiata*, *Thuja occidentalis* (Harry *et al*. 1987), *Larix occidentalis* (Harry *et al*. 1991, Fig. 5*a–d*), *Picea rubens* (Lu *et al*. 1991), *Tsuga heterophylla* (Harry *et al*. 1994), *Pinus canariensis* (Pulido *et al*. 1994), *Pinus roxburghii* (Muriithi *et al*. 1993), *Pinus banksiana* (Harry and Thorpe 1994), *Cupressus sempervirens* (Lambardi *et al.* 1995), *Juniperus cedrus* (Harry *et al*. 1995), and *Abies amabilis* (Kulchetscki *et al*. 1995).

In addition, exhaustive efforts were placed on the use of somatic embryogenesis for rapid multiplication of selected genotypes. Successful protocols circumvented the difficult procedures encountered with the rooting of adventitious shoots. When Chin-yi Lu came to the lab in 1985 from IK Vasil's lab, she brought the expertise of somatic embryogenesis in cereals and grasses. She was able to induce somatic embryos from white spruce (*Picea glauca*) using immature zygotic embryos (Fig. 5*e–j*). Using this system, large numbers of plants were produced. Also, this *in vitro* regeneration system could be used for future genetic manipulation experiments (Lu and Thorpe 1987). Since somatic embryogenesis has several advantages over adventitious shoot formation, this technology has been developed in several conifer species. Additional species propagated via this route in Trevor's lab were *Picea rubens* (Harry and Thorpe 1991), and *Pinus sylvestris* (unpublished, Fig. 5*k–n*).

Overall, Trevor's lab contributed the most exhaustive studies on all aspects of conifer tissue culture. Besides the micropropagation protocols, research from his lab contributed significantly to a clearer understanding of the nature of regeneration and the histological details of the origin of shoot buds or somatic embryos from various explants. Using his approaches, Trevor was able to answer developmental, physiological, and histological questions about *in vitro* morphogenesis, especially somatic embryogenesis. Furthermore, from a practical viewpoint, some of these protocols have been applied directly to commercial production systems.

Salinity Stress One of Trevor's research programs from 1983 to 1988, initiated with Eng-Chong Pua, was aimed at studying the effects of salinity stress on plant cell culture. Most of this work was carried out using tissue cultures of sugar beet (*Beta vulgaris*), *Brassica* sp., and tobacco (*Nicotiana tabacum*) to understand both the effect of excess salinity on metabolism and physiology at the cellular level, and what effects salinity could have on plant regeneration as well as other developmental processes *in vitro*. However, a second objective with a more commercial focus was to try and select for salt tolerance *in vitro*, with a view to regenerating salt tolerant plants (Chandler and Thorpe 1986). A key focus of the group was resistance to sodium sulfate (Na_2SO_4) in *Brassica* species, because dryland salinity in canola (*Brassica napus*) caused by sulfate accumulation at the soil surface was a significant agricultural problem in Alberta and neighboring Canadian provinces. Na_2SO_4 tolerant cultures of canola were selected, and the response of these cultures to osmotic and salt stress was studied (Chandler and Thorpe 1987). Evidence of physiological adaptation, a much higher and stable baseline proline content in salinity tolerant lines than in the unselected control, was established.

Sodium sulfate was found to have an initial inhibitory effect on the growth of tobacco callus reducing both growth (concomitant with an increase in dry weight, water potential, proline concentration, and osmotic potential) and morphogenetic capacity. However, after repeated subculture on medium containing the salt, root and shoot formation was restored. After 3 yr, shoot formation capacity, with or without salt stress, was only retained in cultures that had been grown on Na_2SO_4 during maintenance. The response of these long-term cultures was speculated to be the result of changes in endogenous hormone level and/or osmotic adjustment. Growth and rooting of tobacco shoot cultures was also inhibited by Na_2SO_4 (Chandler *et al.* 1988), and exposure was accompanied by accumulation of proline and a decrease in reducing sugars' concentration. It was not possible to obtain Na_2SO_4-tolerant plants in tobacco even after long-term

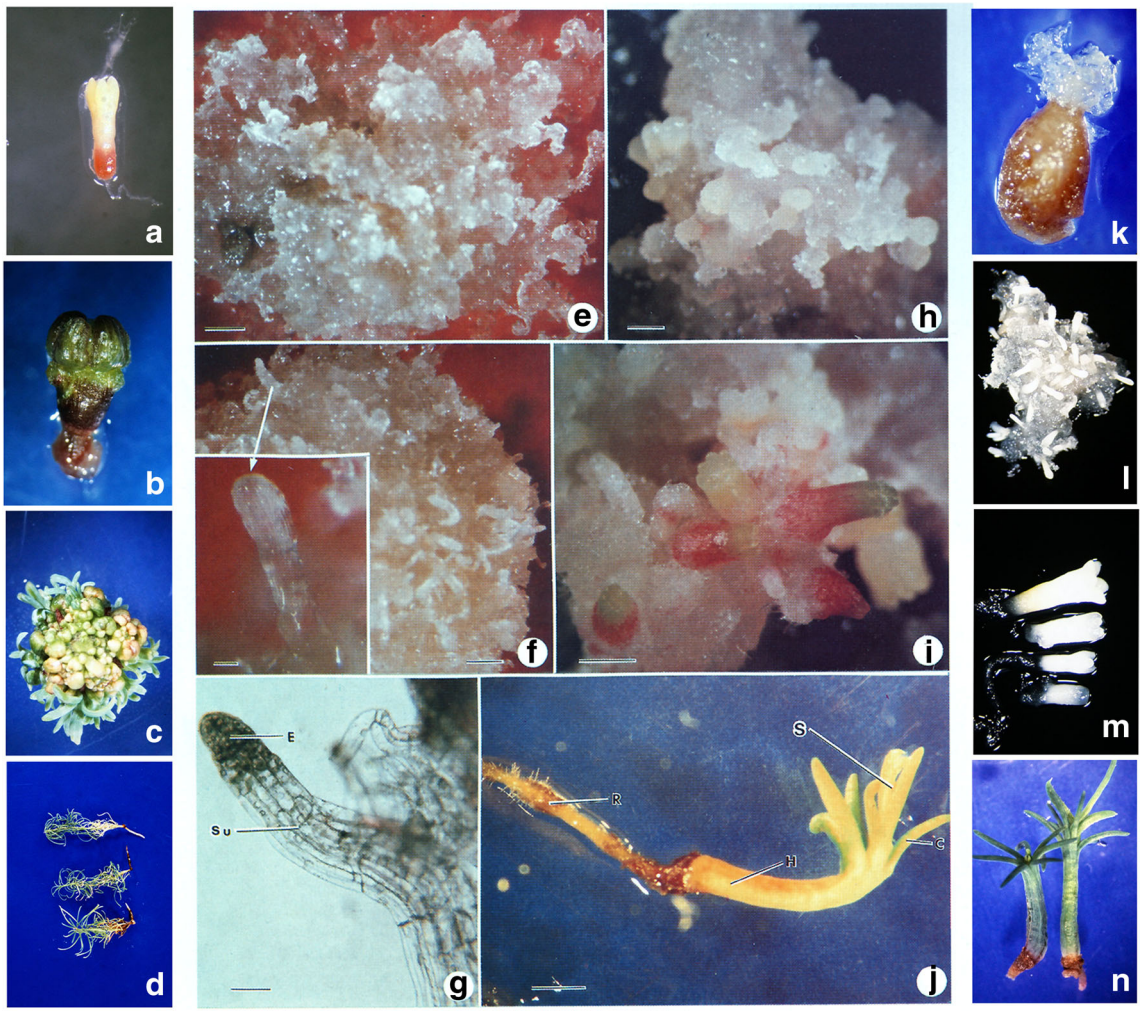

Figure 5. Plant regeneration via adventitious shoot buds and somatic embryogenesis in different conifers. (*a–d*) Adventitious shoot bud regeneration from zygotic embryo explants of larch. (*a*) Explant, (*b*) early stage of adventitious shoot buds, (*c*) well-formed shoots, and (*d*) rooted shoots. (*e–j*) Somatic embryogenesis and plantlet regeneration from cultured immature embryos of white spruce. (*e*) Translucent embryogenic callus. (*f*) Somatic embryos can be observed protruding from the embryogenic callus. An enlarged somatic embryo is shown in the *inset*. (*g*) An embryonal head consisting of small, densely cytoplasmic cells and a suspensor with elongated and vacuolated cells (*E*, embryonal head; *Su*, suspensor). (*h*) Further development of the somatic embryos. The embryonal heads are dense and white. (*i*) Somatic embryos at different stages of maturation. (*j*) Plantlet regenerated from somatic embryo (*C*, cotyledon; *H*, hypocotyl; *R*, root; *S*, shoot) (Figures *e–j* were published previously by Lu and Thorpe 1987. J Plant Physiol. 128:297–302. Copyright Elsevier, 1987). (*k–n*) Somatic embryogenesis in Scots pine (previously unpublished). (*k*) Embryogenic callus initiated from the zygotic embryo explant, (*l*)well-formed somatic embryos from callus, (*m*) isolated somatic embryos at different stages of maturity, and (*n*) plantlets after germination.

subculture with selection pressure. In fact, it was found that shoot cultures that were more sensitive to the salt gave rise to plants whose seeds were more sensitive to K_2SO_4, NaCl, and KCl than seeds from control, non-sensitive shoot cultures. Shoots derived from salt-sensitive seed were also more sensitive to salt during rooting, suggesting that stable selection for salt sensitivity had occurred. Subsequently, callus cultures of Chinese cabbage proved to be more sensitive to salinity than callus cultures of canola, and it was not possible to select tolerant cell lines. Na_2SO_4 was more inhibitory to growth than NaCl, but for both salts, there was a significant accumulation of proline in response to salt exposure (Paek *et al*. 1988). Subsequently, microspore-derived embryos of *Brassica napus* were also used for such salt stress studies (Rahman *et al*. 1995).

Another salinity tolerance study revealed that sugar beet callus selected previously by culturing on medium supplemented with 210 mM Na_2SO_4 could tolerate up to 560 mM NaCl, but the non-selected callus could not survive such high levels of salt in the medium (Pua and Thorpe 1986). Also, tobacco callus grown in the light on shoot-forming medium in the continuous presence of Na_2SO_4 for over a year became acclimated to the high salt medium and retained shoot-forming ability even after 18 mo of culture (Pua *et al*. 1985). However, shoot regeneration from callus grown in the absence of Na_2SO_4 began to decline after 14 mo in culture, and shoot

regeneration capacity was completely lost after 18 mo. Because selection for Na_2SO_4 tolerance using *in vitro* shoot cultures had proved to be ineffective in sugar beet, callus cultures were used for physiological characterization of *in vitro* salt tolerance (Chandler *et al*. 1989). It was found that the response of the tolerant cultures to Na_2SO_4, as measured by ions, proline, and accumulation of quaternary ammonium compounds such as glycinebetaine, was the same as that of unselected control cultures. As tolerance (measured by growth) to Na_2SO_4 was lost after removal of selection, it was concluded that the development of tolerance in cultured cells under selection was due to physiological adaptation. Glucose metabolism of salt-tolerant and salt-susceptible varieties of wheat was also studied (Krishnaraj and Thorpe 1996).

From these research efforts, important insights on salinity tolerance were obtained. In essence, the physiological response to salinity in cell and shoot culture was comparable with that found at the whole plant level and that callus cultures physiologically adapt to salinity. The latter phenomenon negated the possibility of selecting genetically tolerant cell lines *in vitro*.

Developmental Plant Physiology Trevor identified "Developmental Plant Physiology" as one of his key research areas. During his doctoral thesis research under the supervision of the illustrious Professor Toshio Murashige, Trevor performed comparative histochemical studies of changes in endogenous starch content within tobacco callus grown under different conditions. Using a histochemical approach, he showed that starch accumulation preceded in the cells of the callus that will eventually give rise to adventitious shoot buds; more importantly, treatment of the callus with gibberellin prevented starch accumulation in the founder cells, and such callus failed to regenerate shoot buds (Thorpe and Murashige 1968). Subsequently, he continued with this theme of research on tobacco callus system under the shoot-forming and non-organ-forming conditions, but expanded it to include other key metabolites such as nucleic acids, proteins, and various carbohydrates (Thorpe and Murashige 1970; Beaudoin-Eagan and Thorpe 1985). Additionally, his research group examined the roles of various hormones such as gibberellic acid, abscisic acid, auxin, and cytokinins as well as starch on *in vitro* cultures. The idea was to better understand how both intrinsic and extrinsic factors exert their influence on shoot bud regeneration.

During the early years at the University of Calgary, Trevor's group continued to use tobacco callus and radiata pine cotyledon cultures for developmental physiology and tissue culture studies. Various physiological parameters associated with *de novo* shoot bud regeneration were characterized using tobacco callus. These included metabolism of carbohydrates, nucleotides, phenylalanine, tyrosine, and the osmoticum of the culture medium. Among his contributions are the highly cited observations on membrane permeability (rate of ^{86}Rb leakage from the preloaded leaf discs), lipid peroxidation, and activities of superoxide dismutase (SOD) and catalase during *in situ* senescence of leaves (Dhindsa *et al*. 1981). Insights were obtained on how compounds such as adenosine phosphate, NADH, and shikimate pathway intermediates influence tobacco callus proliferation. Trevor's group was one of the first to apply state-of-the-art technology of $^{14}N/^{15}N$ NMR spectroscopy to study how embryogenic white spruce cultures metabolize inorganic nitrogen from the medium into various organic metabolites (Joy IV *et al*. 1997). Another contribution from Trevor's group was the observation that ethylene and carbon dioxide in the headspace gas of culture vessels influence shoot bud regeneration in several species of plants (Huxter *et al*. 1981; Kumar *et al*. 1987, 1998, Nour and Thorpe 1994). Efforts of Ludwig Bender, who came from Germany to complete his Habilitation Thesis, also helped to decipher how sugar metabolism (Bender *et al*. 1987) and carbon dioxide assimilation via phosphoenolpyruvate carboxylase become important for adventitious shoot regeneration in radiata pine cotyledon cultures (Kumar *et al*. 1988). Clearly, Trevor's research contributed towards a better understanding of the physiological processes underlying *de novo* shoot bud regeneration in tissue cultures. Changes at the protein level were also investigated in the radiata pine culture system, and it was identified that almost 50% of the nearly 60 polypeptides that were differentially expressed in response to cytokinin treatment appeared to be those related to maturation (Thompson and Thorpe 1997). Thus, one of the molecular level actions of cytokinin in promoting adventitious shoot bud induction seems to be preventing maturation-related changes in the shoot-forming cultures.

When Trevor hosted Professor Hiroshi Ashihara, an expert in the area of nucleotide metabolism in plants, Claudio Stasolla and Natalia Loukanina (from Ed and Trevor's labs, respectively) teamed up with him and carried out a series of studies dealing with nucleotide metabolism. They examined the role of purine and pyrimidine nucleotide metabolism during maturation and germination of somatic embryos and shoot organogenesis in a variety of species, including spruce, pine, carrot, and tobacco. This resulted in over 15 publications (*e.g.*, Ashihara *et al*. 2001, 2005; Stasolla *et al*. 2001a, b). The key findings include the observation that although both purine salvage and degradation pathways are operative during all stages of the maturation process, the salvage activity was identified as a metabolic switch delineating the arrest of cell proliferation of the embryogenic tissue and the initiation of embryo development. The onset of embryo maturation was characterized by rapid utilization of both adenine and adenosine for nucleotide and nucleic acid synthesis, and high activities of the salvage enzymes adenine phosphoribosyltransferase (APRT) and adenosine kinase (AK). Alterations in adenosine metabolism were also critical during the imposition of embryo

desiccation, an obligatory step terminating the maturation process and initiating germination. Embryo desiccation was accompanied by a decline in adenine and adenosine incorporation and by a rapid induction in the activity of APRT. Similarly, the *de novo* pyrimidine synthesis pathway was active throughout the somatic embryo maturation process. During this period, uridine was found to be a better precursor for nucleotide and nucleic acid synthesis than uracil. The extensive catabolism of uracil was attributed to the low activity of uracil phosphoribosyltransferase (UPRT) relative to that of uridine kinase (UK). Nevertheless, the utilization of both uracil and uridine did not correlate to the changes in the activities of these two enzymes, thus suggesting the presence of other mechanisms controlling pyrimidine metabolism during embryo maturation. The late phases of embryo maturation and desiccation were delineated by a rapid rise in the UK activity.

These studies were also important in demonstrating the presence of the *de novo* and salvage pathways of deoxyribonucleotides during embryogenesis. Specifically, during carrot somatic embryogenesis, radio-labeled cytidine was recovered in the DNA fraction. Tracer experiments conducted with thymidine and deoxy-cytidine also revealed that the salvage synthesis of deoxyribonucleotide is operative during embryogenesis. Both precursors were rapidly incorporated and metabolized.

The onset of embryo germination is characterized by an "un-metabolized phase," possibly ascribed to low water content, followed by a rapid salvage phase. Production of purine nucleotides via the salvage pathway was demonstrated by the rapid salvage of adenine by APRT, which was predominant over the alternative route of adenine salvage catalyzed by nucleoside phosphorylase and AK. Adenosine salvage via AK was also induced at the onset of germination, while the other route of adenosine salvage through adenosine nucleosidase (not measurable in fully dried embryos) becomes functional later on, during germination. Collectively, these studies demonstrate that the salvage pathway is the main contributor to the generation of nucleotides before the reactivation of fully functional *de novo* biosynthetic pathways.

There was no previous information related to the metabolism of purine and pyrimidine nucleotides during organogenesis until Trevor's group conducted studies using shoot bud formation from epicotyls of white spruce and cotyledons of radiata pine. Shoot formation is characterized by elevated activity of the salvage pathway with adenine and adenosine being salvaged at higher rates under shoot-forming conditions

Figure 6. Memorable photographs of Trevor from various conferences. (*a*) With Atsushi Komamine and Prakash Kumar (PK), (*b*) with Ed Yeung, (*c*) with PK, Indra Harry and Claudio Stasolla (left to right), (*d*) with PK and Yvonne Thorpe (Trevor's wife), and (*e*) with PK, Oluf Gamborg, Greg Phillips, and David Altman (*left* to *right*).

relative to non-shoot-forming conditions. Elevated salvage synthesis, which for purines was ascribed to the high activities of APRT and AK after only a few days in culture, was also observed for uracil and uridine. From all these studies, it became clear that changes in the metabolism of purines and pyrimidines delineate important morphogenic events during *in vitro* development, and that perhaps manipulations of metabolism can redirect growth and development.

Summary

Trevor Alleyne Thorpe led a tremendously productive, fulfilling, and active life (Fig. 6). He was a very successful teacher and scientist, an effective administrator, editor, and outstanding mentor for numerous people from around the world. Despite his noteworthy accomplishments, he remained a humble, warm, and caring person throughout. Without exception, all his former associates cherish the tremendous opportunity to conduct research in his lab and fondly recall the time spent in Trevor's lab. For most of his students, the time spent with him was one of the best periods of their careers. Members of Team Thorpe feel immensely privileged to have been associated with Trevor. The values he instilled in us are a legacy that will live on. He will not be forgotten.

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