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FROM CELL TO SOIL: A BROAD APPROACH TO PLANT LIFE

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I am thankful to Indian Botanical Society for electing me President of the Indian Botanical Society. I wish my father Prof Swami Dayal Tewari could have been alive to witness this as he did his M.Sc. Botany way back in 1944 from Department of Botany, Allahabad University and I am proud to say that his one of the student Prof Umesh Lavania siting in front of me has achieved FNA. Dedication by father and my Ph.D guide Professor H.C. Arya inspired me to remain in the field of Botany instead of joining Indian Police Services when I was finally selected to this post after clearing Indian Administrative Services exam in 1972.

The work on photosynthetic systems of plant tissue culture started under Professor H. C. Arya was presented at 3rd International Plant Tissue culture meeting at Leicester 1974 (Kumar 1974a, 1974b) where I met Professor Dr Karl-Hermann Neumann who invited me to work in his lab with Alexander von Humboldt Fellowship (Germany) to work at the Institut fur Pflanzenernaehrung der Justus Liebig Universitatat Giessen (1977-1979).

I continued work in Photosynthesis in tissue cultures on wheat and maize in India under USDA-ICAR project and worked out on the development of PEPcase and RuBISCO in C3 and C4 plants (Kumar *et al*. 1990, Kumar and Roy 1991). This work was presented at International Photosynthesis Congress, Rhode Island USA. This enabled me to come in contact with Melvin Calvin from USA who advised me to work on biofuels in semi arid and arid conditions akin to Arizona desert of USA .

1. Physiological studies on development of photosynthetic system in plant tissue cultures: Plant tissue culture has several applications in plant studies (Fernandez *et al*. 2010, Kumar and Sopory 2010). The purpose of our investigations was firstly to characterize plastid development in carrot root cells which lacked chloroplasts and contained only chromoplasts at explantation, and secondly to correlate chloroplast developmental stages during culture of carrot root explants with the Hill activity and the activity of the carboxylating system. Electron microscopy, low temperature pigment absorption spectra and fluorescence induction profile recording as well as the determination of the activity of some enzymes (ribulosebisphosphate carboxylase, phosphoenolpyruvate carboxylase) and CO2-fixation experiments were carried out. Based on the results, a sequence of developmental stages of the photosynthetic system was proposed. An ultrastructural analysis of the cells at various stages during 28 days of culture (0, 6, 12, 28 days) indicated that a broad variation in plastidal structures occurred in these cultured tissues. The extent of organization of the internal membrane system was used as an indicator of the developmental stage of the individual plastid and a developmental sequence was arranged from stage 1 to stage 5. It was difficult to trace the origin of stage 1 plastids (typical amyloplasts), from which the following stages conceptually could be

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derived. At about up to 6 days of culture some plastids probably best described by the term 'amylo- chromoplasts' occurred. These 'amylo-chromoplasts' contained starch grains besides traces of carotene crystals. These structures were not observed in cells of the original explants and later developmental stages of the cultures. These 'amylo-chromoplasts' probably represent an intermediate stage between chromoplasts and stage 1 of the chloroplast development. amyloplasts, which could serve as the first stage in the developmental sequences (Kumar *et al*. 1983, 1984, Neumann *et al.* 2009). Another developmental path of plastids could be regarded as an alternative,i.e. from the 6th day on small structures with a double membrane could be observed, which closely resemble proplastids in cells of the shoot apex of other plant systems. Such proplastid-like structures were also found in cells of the shoot apex of young carrot seedlings. Nevertheless, under our standard conditions of culture with continuous illuminations no convincing evidence for a further development of these small structures into chloroplasts could be obtained. In the shoot apex of carrot seedlings grown in the dark such proplastids generally developed into etioplasts. However, when carrot root explants were cultured in the dark for 7-10 days no etioplasts could be detected and the only plastidal structures besides chromoplasts were amyloplasts with heavy starch accumulation as in stage 1. All these observations suggested that these proplastid-like structures hardly contributed to the increase in the plastid population of cultured cells actively dividing. The question of plastid propagation in this carrot root tissue culture system needs further investigation. The occurrence of the various stages of chloroplast development was broadly related to the developmental status of the cultured explants and their con- current nutritional regimes (heterotrophic, mixotrophic and autotrophic) nutrition and with a kinetin supplement (Kumar *et al.* 1999a,1999b).

To characterize the photosynthetic activity of the plastids at some stages of cultivation low temperature spectra, fluorescence induction kinetics, activities of some carboxylating enzymes and total CO_2- fixation were determined and compared with data found for leaves.

1.1 Light reaction system: Whereas the low temperature spectrum of freshly cut explants at to showed only absorption in the carotenoid region, after 6 days of culture chloroplasts had developed up to stage 3 and a trace of chlorophyll was found concurrently. This also agrees with the fluorescence induction profiles, which characterizes the extent of electron flow between photosystem II and I. At day 6 some ground but no variable fluorescence occurred, thus indicating the presence of traces of chlorophyll, but no arranged photo-synthetic redox system existed in the chloroplasts of this developmental stage (up to stage 3). The absorption spectra of tissue cultured for 12 (mixotrophic nutrition) and 28 (autorophic nutrition) days were almost identical to that of carrot leaves. Chlorophyll 'a' and 'b' peaks occurred and a shoulder at the chlorophyll 'a' peak around 670nm probably indicated some chlorophyll protein complex typical of 'light harvesting centers'. This suggested the presence of active photosynthetic units. At these developmental stages of the cultured tissue (after 12 and 28 days of culture), variable fluorescence, i.e. photosynthetic electron flow, could be observed and the fluorescence induction profiles of the cultures closely resembled those of mature leaves. This indicated that the chloroplast population, mainly stage 4 chloroplasts, had already a functioning photosynthetic system under mixotrophic conditions which remained essentially unchanged till the end of the experiment at day 28 and which was qualitatively quite comparable to that of leaves.

These data agreed with data on Hill activity as measured earlier by ferricyanide reduction.

1.2 Carboxylation system: An attempt was also made to correlate the development of chloroplasts with their dark reaction system, $14CO_2$-fixation (applied as $NaH^{14}CO_a$) per g fresh weight by leaves exceeded that of tissue cultures severalfold in light and dark, but the ratio of light/dark-fixation was almost the same in both systems (Bender *et al.* 1985). Furthermore, although Ribulose bisphosphate carboxylase (RuBPC*) activity dominated in CO_2-fixation in cultured tissue, at certain stages of the culture Phosphoenolpyruvate carboxylase (PEPC**) also might play an important role and then contribute to the total CO_2- fixation. As also reported by others and in our experiments, no direct correlation of ultrastructural development of the plastids and of the activity of carboxylating enzymes such as RuBPC and PEPC could be found. In *Daucus* cultures apparently RuBPC activity already existed to some extent in the original explants freshly isolated from the carrot root in which no PEPC activity could be detected. Since the cells of the freshly cut explants contained only chromoplasts and no chloroplasts, then RuBPC should exist already in the chromoplasts. From day 21 on, i.e. at the transition from mixotrophy to autotrophy, the activity of RuBPC increased strongly while the activity of PEPC declined during the autotrophic culture. In mature carrot leaves only RuBPC but no PEPC activity could be found (Kumar *et al.* 1983, 1984).

The course of activity of RuBPC as well as that of PEPC after the onset of autotrophy cannot be easily explained. The transformation to autotrophic growth may be initiated by the exhaustion of exogenous sucrose in the nutrient medium. The resulting depletion of the cells of carbohydrates may then lead to an intensification of the calvin cycle, thereby substituting the exogenous sucrose by endogenously synthesized photosynthetates. The reduction of PEPC activity and the increase of the RuBPC activity may be just one indicator of this process.

Our results showed that the light reaction system in carrot tissue cultures was qualitatively quite comparable to that of carrot leaves, whereas some differences existed in the CO_2-fixation mechanism (Kumar *et al.* 1983 see also Neumann, Kumar and Imani 2009).

1.3 Influence of exogenous sucrose and various phytohormones: Many factors have been reported to induce and perpetuate the development of chloroplasts in plants as well as in callus cultures derived thereof. The effects of sucrose concentration on growth during a culture period of 28 days in the presence of several hormone treatments are summarized (Kumar *et al.* 1984). The development of thylakoids and the appearance of the typical absorption bands of chlorophyll 'a' and 'b' at its characteristic wavelength as well as the induction of variable fluorescence (Kautsky effect) upon illumination can only be observed in hormone treated carrot tissue cultures. Therefore, we believe that the hormonal stimulus is essentially required for the development of a functioning photosynthetic system in these cultures. Its quantitative extension, however, is based on excessive chloroplast multiplication as well as maximal thylakoid and chlorophyll formation which requires an ample supply of sucrose besides a supplement of IAA, inositol and kinetin. Besides the hormonal stimulus these processes require therefore a potent source of carbon and energy as is provided by the sucrose supplement. Obviously growth and the developmen of the photosynthetic machinery in freshly isolated carrot root explants are both regulated by co-actions of sucrose supply and hormonal supplements. The regulation of these two processes seems to some extent to be independent of each other (Kumar *et al.* 1984).

carboxylase activity was not required for sugar metabolism. Independent of light intensity, alkalinity and malate concentrations were decreased only in wheat.

• It can be concluded that an enhancement of PEP-carboxylase activity in young shoots of maize supports organic acid metabolism under salt stress.
• Reduced sink activity in growing shoot tissues of maize in the first phase of salt stress may be compensated by increased PEPcase activity (Hütsch *et al.* 2017).

2.1 Role of PEP carboxylase under saline conditions: In growing tissues of young maize shoots, salt stress caused a significant increase of PEP-carboxylase activity by 25-36 % . This is in agreement with measurements on young shoots of different maize genotypes (Hatzig *et al.* 2010, A. Kumar unpublished data). Hatzig *et al.* (2010) concluded that an increase in PEP-carboxylase activity was required as support of organic acid metabolism but not for sugar metabolism as sugar concentrations increased in the saline treatment. This is in agreement with Hu *et al.* (2000), who found a significant increase of sucrose concentration in the elongation zone of wheat leaves under saline conditions. Also in our study, no source limitation was observed under salt stress and one might speculate about the compartmentation of sucrose and the hexoses within the symplast and in the apoplast. For synthesis of cell wall material and other metabolites as well as for the supply of metabolic energy, sucrose has to be cleaved and the hexoses are needed in the cytosol for the different bio-chemical purposes. One possibility is that sucrose and/or hexoses are located in the apoplast, caused either by inhibited acid invertase activity or restricted hexose/H+- cotransport into the sink cell. The inhibited plasmalemma H+-ATPase activity in young maize leaves under salt stress (Zoeorb *et al.* 2005, Pitann *et al.* 2009) could play a key role for both processes. On one hand, its inhibition causes a pH increase in the apoplast with presumably less favourable conditions for acid invertase, and on the other hand, a weaker electrochemical H+-gradient between apoplast and cytosol restricts the H+-cotransport of hexoses into the cytosol. To our knowledge, so far no sucrose transporter has been described which could shift the intact sucrose molecule from the apoplast into the sink cell. Concerning the symplastic pathway of phloem unloading, the plasmodesmata can be considered as a 'bottleneck', restricting the transport into the sink cell (Baluska *et al.* 2001). The regulation of the pore size of these gateways requires cell wall extensibility, which is reduced under salt stress and could thus cause a sucrose accumulation outside the sink cell and make it unavailable for biochemical purposes. Taking the possibilities of sugar compartmentation into account combined with the high metabolic activity of meristematic and expanding cells in the growing tissues investigated, an anaplerotic function of PEP carboxylase supporting the tricarboxylic acid cycle could be beneficial under saline conditions. In maize root tips, it was shown that 33 % of PEP enters the TCA cycle as oxaloacetate pro- duced by PEP-carboxylase action (Dieuaide-Noubhani *et al.* 1995). In our study, no increase in PEP-carboxylase activity was observed in the root tips under salt stress. However, even under saline conditions, the sucrolytic enzyme activities were twice as high as in the growing shoot parts of the control plants (Fig. 3a-c), presumably suffi- ient to meet the demand.

The simultaneous determination of sucrose-cleaving enzyme activities in different sink tissues of young maize plants gives information about the various levels of sink strength and whether the phloem-unloading pathway can be considered as mainly symplastic or apoplastic.

Such autotrophic cultures have been raised successfully in fermenter cultures (Bender *et al*. 2001a, 2001b).

2. Role of enzymes in salinity resistance C_3 and C_4 plants: Soil salinization mainly affects less-developed countries in arid climatic zones. Due negative evapotranspiration and precipitation rate in such climatic zone salinization is an irreversible occurrence and development of salt-resistant plant species is imperative. Phosphoenol pyruvate carboxylase (PEPC) has an important role in biosynthesis of OAA and malate. Working with Professor Dr Sven Schubert in Germany (Schubert 2011) who developed salt resistance through normal breeding processes over the years capable of enhanced ATPase activity under salinity stress in hydroponics during initial dosages of 100 mM NaCl studied role of enzymes in salt resistance and the data was presented at Hannover conference(Kumar and Schubert and published lateron. An increased activity of PEPcase in SR03 during first phase of salinity resistance might provide a supplemental way for excess sugar being synthesized due to its activity. In addition to acidification of symplast the PEPcase might have a role in sucrose biosynthesis during generative phase in Maize also. There being reports of PEPcase being active in undifferentiated tissue of Maize and higher amounts of PEPcase being recorded in heterotrophic or mixotrophic youngest developing leaves of Maize in our investigations might suggest different isoforms being transcribed during different phases of growth. This necessitated a closer examination of role of PEPcase in providing resistance to salinity due to contribution of carbon fixation and pH regulation both or either of them. The results were presented at Plant Molecular Biology meeting at Amsterdam 23-25th September (Kumar and Schubert 2019 in press).

Studies with newly developed resistant maize genotypes (Schubert and Zörb 2005, Schubert *et al.* 2009, Hartig *et al*. 2010 and Huetsch *et al.* 2015) documented a better plant growth due to maintenance of cell-wall acidification and thus cell-extension growth. Wheat (*Triticum aestivum* L. cv. Thasos) and maize SR 03 hybrid (*Zea mays*, Schubert and Zörb 2005) were grown in two climate chambers at 26°C for 16 h and 18°C for 8 h.

The effects of salt stress (100 mM NaCl for 6 d) on growing tissues (shoot apex, growing leaf segments, root tips) of young maize plants (*Zea mays* L. cv. Pioneer 3906) were investigated in comparison to an unsalinized control, focusing on assimilate supply from source leaves and the activity of sucrolytic enzymes in the sink tissues.

In these salt-resistant maize genotypes we found that in the young shoots PEPcarboxylase (EC 4.1.1.31) activity was increased during the first phase of salt stress. PEP carboxylase, which catalyzes the irreversible carboxylation of phosphoenolpyruvate in mesophyll cells, plays an essential role in C_4 carbon assimilation. The increase in PEPcase activity depended on hybrid genotypes. The genotypes, which were more resistant, showed greater increase in PEPcase activity.

While glucose and fructose concentrations did not exceed 0.3 mg g^{-1} fresh weight, sucrose concentrations showed ten-fold higher values up to 3.0 mg g^{-1}. In the young shoot, both plant species showed a significant increase in sucrose concentration under saline conditions.

• Analyses of shoot fresh weight revealed a comparable reduction for wheat and maize and suggested salt resistance at a similar level.
• Analyses of sugar concentration showed an increase in the saline treatment for both genotypes independent of the light intensity.
• Results of sucrose concentrations led to the conclusion that an increase in PEP-

One possibility is that sucrose and/or hexoses are located in the apoplast, caused either by inhibited acid invertase activity or restricted hexose/H+-cotransport into the sink cell. The inhibited plasmalemma H+-ATPase activity in young maize leaves under salt stress (Zoerb *et al.* 2005, Pitann *et al.* 2009) could play a key role for both processes. On one hand, its inhibition causes a pH increase in the apoplast with presumably less favourable conditions for acid invertase, and on the other hand, a weaker electrochemical H+-gradient between apoplast and cytosol restricts the H+-cotransport of hexoses into the cytosol.

Regarding the possible compartmentation of sugars in a plant cell and in the apoplast, PEP-carboxylase can have an anaplerotic function supporting the demand for energy and metabolites in sink tissues of young maize plants under salt stress. In root tips, an additional supply of organic acids to the tricarboxylic acid cycle is not needed because of high activity of PEP carboxylase, which can meet the demand of the sink cells.

3. Plant pathological studies: I also remember at this moment Dr G.S. Nathawat who took us to his village Pachkodia to show us the chepa disease of ergot in rainy season which lead to start my research on the subject with a CSIR project on ergot disease of bajra caused by *Claviceps fusiformis.* Significant finding were on radio tracer studies on honey formation and etiiology of ergot of Bajra (Roy and Kumar 1985) Subsequently several of my Ph.D scholars worked on plant pathological problems of leaf blight of wheat *Alternaria triticina* Prasada and Prabhu, *Pyricularia* leaf spot of bajra *Pennisetum typhoides* (Stapf. & Hubb.), Blast disease of rice, mushroom production *Agaricus bisporus* (Log.) Sing**.** cultivation of oyster mushroom *Pleurotus citrionopileatus* Singer. blight of clusterbean *Cyamopsis tetragonoloba* (Linn.) Taub . Studies on dye degradation potential of native micro flora of Sanganer.

4. Ethnobotanical studies: Award of JSPS visiting Professorhip to Japan (1999-2000) and work with Professor Watanabe and Professor Komatsu at the Natural Products Laboratory Toyama Medical and Pharmaceutical University Toyama enabled me to know the value of traditional knowledge in correlation with modern science and several of my students worked on this aspects. Ethnobotanical studies on plants of semi-arid and arid regions including families: Fabaceae, Euphorbiaceae, *Bacopa moninieri* (L.) Pennell (Brahmi), *Acacia* spp and *Prosopis* spp. *Citrullus* spp etc.

5. Environmental studies on pollution, green house gas mitigation and climate change: Biodegradation of xenobiotics by soil microorganisms in Sanganer area was studied (Nitika and Nidhi).

5.1 Biofuels: Greenhouse Gas Mitigation and Global Warming: Increasing level of population growth, industrialization and prosperity is leading to extensive use of energy. In addition to natural climatic variability, the anthropogenic climate change is taking place due to emissions of greenhouse gases which cause environmental damage to any given area (IEA 2007; IPCC 2007; Stocker *et al.* 2013; IPCC 2014**;** Kumar 2018;Kumar *et al.* 2019). The use of fossil fuels produces major share of green house gases (GHG). (IPCC 2014).Almost 88 percent of this energy comes from burning of fossil fuels.This is contributing to the increase in CO_2 levels. The CO_2 level in 2012 was about 40% higher than it was in the nineteenth century. CO_2 is major contributor of green house gases. Besides this three major non-CO_2 group of gases– CH_4, N_2O and fluorinated gases (F-gases), including CF4, HFCs and SF6 also contribute to the GHG emissions. The increased levels of green house gas emissions are leading to climate change and its adverse effects are reported to cause floods, droughts, forest fires, melting of

glaciers at a faster rate besdies other natural calamities. During Conference of the Parties (COP21), at Paris Climate Conference, (2015) a legally binding and universal agreement on climate change was achieved, with the aim of keeping global warming below 2 °C. Achieving this goal will require drastic emission reductions to stabilize GHG concentration in the atmosphere. Replacement of fossil oil with biofuel derived from plant biomass has the potential to greatly reduce greenhouse gas emissions (see Kumar *et al*. 2018 https://doi.org/10.1007/978-81-322-3763-1).

Bioenergy is renewable energy from biological sources. Biofuels are fuels which can be produced from biomass are renewable as compared to fossil fuels (Kumar 2008). Biomass can be defined as the collection of all organic matter composing biological organisms, but the main components utilized for biofuel production are sugars (starch, simple sugars, and lignocelluloses) and lipids (Kumar 2001, Kumar 2013, Kumar *et al.* 2018a,2018b, 2019). Kumar and Roy (2018) described in details effects of different factors affecting yield of *Jatropha curcas* which could be increased by experimental manipulations and deposited high yielding accessions at NBPGR, New Delhi in a Department of Biotechnology, Govt of India funded project. Lactiferous plants growing in arid and semi-arid region are rich in triterpenoids which can be converted in to biofuel (Kumar 2018). Terpenoids comprise the largest family of natural products which have widespread applications. Extensive studies have been carried out on laticiferouss plants growing in arid and semi-arid regions: *Euphorbia antisyphilitica, Euphorbia lathyris, Euphorbia tirucalli, Calotropis procera* (Kumar 2018b), *Pedilenthus tithymaloides* (Kumar 2013, Kumar *et al*. 2018a,b).

Recent findings that plant metabolic pathways can be reconstituted in heterologous hosts and metabolism in crop plants can be engineered to improve the production of biofuels, has given a new hope for molecular biological approaches in improving food and biofuel production. The de novo engineering of genetic circuits, biological modules and synthetic pathways is beginning to address these crucial problems and is being used in related practical applications.

There is growing appreciation for the need for solidarity in the protection of human rights to overcome the narrow pursuit of economic nationalism. However the risks to human rights of climate inaction and of climate impacts far outweigh the risks to human rights posed by climate action consistent with meeting the 1.5 °C goal set in the Paris Agreement (see Kumar *et al*. 2018 https://doi.org/10.1007/978-81-322-3763-1).

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