

Phosphoenolpyruvate Carboxylase (PEPc) Presents New Opportunities for Food Security and Space Exploration

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Abstract

The health crisis with the resulting food shortage and economic emergency in the U. S and around the world caused by the Covid-19 Pandemic is enormous, and CNN and MSNBC are other news agencies emphasize the urgency to remedy resultant food insecurity. The national and global 'stay at home' directive makes access to food from shopping centers, grocery stores, farmers' markets, and farms and ranches difficult. Moreover, prices for food and other goods have risen enormously, and prices are too high for most people. The effect is widespread and national, and the situation must be remedied and transformed. The objectives of this study are (1) to present the full advantage of C4, CAM and other improved plants that can be used to fortify our food supply and (2) to determine how best to utilize plants in the critical supply of food with the involvement of homes, households, and corporative bodies in the production process. Plants are essentially the autotrophic organisms that make organic compounds, beginning with fructose, glucose and sucrose, from the inorganic gas carbon dioxide and water. Initially, as much activity will be done away from strangers, but using electronic and Internet communication to ink with groups in association to promote food and agriculture. Personal greenhouses, hydroponics, and small plots would address our selection for growth of crops. Results would take full advantage of the rarely sought after second photosynthetic enzyme, phosphoenolpyruvate carboxylase (PEPc) by selecting for production the more productive C4 species and selected improved C3 and CAM species.

Keywords: *Phosphoenolpyruvate Carboxylase (PEPc); Food Security; Space Exploration*

Introduction

Food is the most important ingredient that perpetuates our communities, but the large rise in grocery prices and the long food lines during the period of the Covid-19 Pandemic are testimony of scarcity and the need for policies that promote national food security [1]. This need was once only earthbound; now, however, with the International space station (ISS) and the newly created Space Force that is now established with directives to work in space and investigate and occupy planet, people's food needs on earth and in space are multiplied.

Today the opportunity exists to address this quandary. When Melvin Calvin and coworkers won the Nobel Prize in 1961 for explaining the uptake and synthesis of carbon dioxide into glucose, using radioisotope carbon-14 which was discovered during World War II, the enthusiasm about plant biochemistry grew rapidly. Soon, thereafter, Kortschak from Hawaii was forced to present his discontent when his attempts to replicate the work revealed in the Calvin cycle produced different results, notably the C4 oxaloacetic acid. This C4 compound

was not the C-3 carbon compound, 3-phosphoglyceric acid (3-PGA), reported in the Calvin cycle, contrasting the reports that the C-3 compound, 3-PGA, was the first product of carbon dioxide uptake in the synthesis of glucose. The enzyme that facilitated the uptake of carbon dioxide was Ribulose1,5-biphosphate carboxylase/oxygenase, notably it could react with either carbon dioxide or oxygen. Work by Drs. Hatch and Slack confirmed Korschack's results with sugar cane, and the recognition of alternative pathways to carbon fixation. Now, it is widely known that sugar cane and corn are C4 plants and that C4 species are the most productive and efficient in the world [2].

In addition to C4 plants, we now also recognize Crassulacean Acid Metabolism (CAM) plants. Notably, C4 and CAM plants have a second photosynthetic enzyme, Phosphoenolpyruvate carboxylase (PEPc). Moreover, species that are C4 and CAM plants are more tolerant to high temperatures and have very high water-use-efficiency.

Objective of the Study

Henceforth, we use this opportunity to pursue the following objectives: 1. More globally select and harness species of C4 and CAM species, characterize more details of their metabolism and productivity and recommended them for use in very productive environments, where water is more scarce, on spaceships, including the ISS, and in efforts to inhabit new planets. 2. Characterize the comparative use of C3, C4 and CAM species as food and feedstock for man, fish and seafood and the smaller and larger domesticated livestock animals in efforts to meet demands as well as provide food security for towns, cities, and other communities as well as for space travel.

Methodology

Plants are the autotrophic organisms that transition the inorganic carbon in carbon dioxide into organic molecules, and plants are needed, directly or indirectly, to produce all living things, including viruses. As spaceships go farther away from Earth, the requirement for meat, fish, and plants - plants being the most critical - is growing enormously. Research with plants, regarding the two photosynthetic enzymes, and living systems in our changing environment, can be most transformative to the technology and our lives!

Methods of this study rely on our knowledge, published literature, and research we have pursued over the years as well as other research efforts being pursued contemporarily. Work with plants has identified a number of unique metabolic plant groupings, many of which have been similarly researched or confirmed by other studies, hence, lie present a summary; yet, however, we believe that it is important to pursue the study and focus on the process.

Re Literature Review: Corn (*Zea mays*) and sugar cane (*Saccharum officinarum*) are two C4 species of very significant economic value, corn being the largest grain crop produced and being the most productive crop by total volume of edible dry matter produced annually. Sugar cane is the largest producer of sugar, but sugar from corn and sugarcane is frequently processed into ethanol, Brazil producing from sugarcane and the US from corn, with per hectare production from sugarcane being almost two times that from corn.

The cultivation of sugar cane or corn in homers or in spaceships recognizes that plant size is a major disadvantage to both corn and sugar cane for in-home or in space greenhouse cultivation.

The U. S. National Academy of Science surveyed studies from around the world and determined that several significant crop plants were underexploited. One very impressive grain crop is Fonio (*Digitaria exilis*), a C4 species. Fonio is a much smaller plant than corn or sugar cane, and it grows from seed to harvest in an average of 60 days. Corn, in contrast, takes an average of 60 to 120 days from seed planting to harvest, dependent on the type or variety. Consequently, C4 plant types with shorter growth cycles, including Fonio, are a preferable group of plants with which to start. Such smaller species include teff (*Eragrostis tef*) from Ethiopia and Brown Top millet (*Brachiaria ramosa* (L) or *Urochloa ramosa*) that is grown in many states in the US for bird feed. In contrast, however, are some other plants that are not grown for food. They include the pigweed (*Amaranthus retroflexus*) and similar C4 plants that take advantage of their C4 Photosynthetic efficiency to outgrow the competition to dominate the land. Many of the other millets are similarly promising but underutilized [3,4].

Hydroponics and tissue culture

Critical tests with examination of crop species, including their productivity, as well as morphological, anatomical and metabolic properties are continuously being carried out to investigate plants and their cultivation in greenhouses, under hydroponic care, by tissue culture and in laboratories, test plots, and fields, where important discoveries result. During this time of the Covid-19 Pandemic, precaution makes access to many of the facilities untenable, making many analytic techniques unavailable, and results less accessible. However, some openings are available for inquiry into less into less frequently investigated protocols, techniques or subjects.

Confocal microscopy

Microscopy is a magnificent tool for the examination of internal features of plants via microanatomy. Notably, C4 plants absorb carbon dioxide; an uptake that occurs within the spongy mesophyll, facilitated by PEP carboxylase, the enzyme that facilitates the reaction between dissolved CO₂ and phosphoenolpyruvate to synthesize oxaloacetic acid. The oxaloacetate is reduced to malate, a compound that is permeable to the bundle sheath wherein it diffuses. It enters the cells where malate is decarboxylated with the production of pyruvic acid and the release of carbon dioxide within the bundle sheath which is then picked up by the enzyme RuB carboxylase/oxygenase. The picked up CO₂ reacts with ribulose-1,5-biphosphate to form two moles of 3-pgosphoglyceryc acid in the Calvin cycle. The absence of free oxygen in the bundle sheath provides an advantage to the photosynthetic process thereby enabling the Calvin Cycle to go on and produce fructose-6-phosphate more efficiently. Fructose-6-phosphate isomerizes with glucose-6 phosphate and the action of sucrose-6-phosphate synthase catalyzes the reaction of fructose-6-phosphate with UDP glucose to form sucrose-6-phosphate [5]. In plants, the process uses ADP-Glucose as the substrate for sucrose and starch synthesis in the α-1,4 linkage reaction. The two monosaccharide moieties lead to the production of sucrose, the glucose-fructose dimer that moves into the vascular bundle to be transported out of the leaf [5,6].

The opportunity to temporally follow the synthesis and deposit of sucrose and starch, among other molecules of interest, including thioglucosides, glucosinolates, and opiates, for example. With threats from novel viruses and microorganisms, information secured in such studies provide guidelines for moieties and processes for contemporary and future intervention [2].

Confocal microscopy with imaging affords ready examination for evaluation of the photosynthetic process and the effects of growth regulators, light and other abiotic and metabolic factors can be a means for revelations of structures and processes that show functional requisites. There are some magnificent results.

Results

Our results show clear microimages of bundle sheaths around vascular bundles in Fonio and Brown Top millet varieties. Bundle sheaths are clearly evident in the anatomy of these and many other common plant species. Fonio will grow from seed to harvest in 8 weeks, a life cycle that is substantially shorter than that of most varieties of corn. The distribution of stomata on abaxial and adaxial leaf surfaces of folio plants has provided insights regarding some of the adaptations in C4 and CAM species. Such insights have encouraged us to review comparative growth curves of corn, fonio, Brown Top millet, and barley in figure 1, per results reported by McMahan., *et al.* [7], Parker [8], Kering and Broderick [9]. The growth curves provide reference where experiments can be conducted to show which inputs, i.e. boron, manganese, nitrogen, or some other factor would promote even better growth and yield.

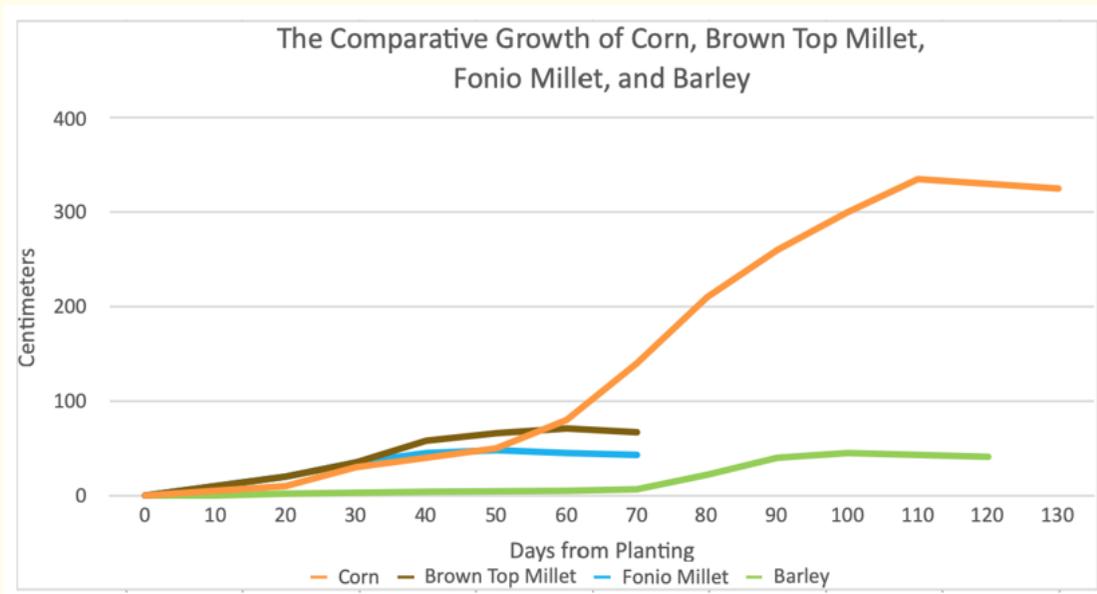


Figure 1: This graph shows the growth curves of barley, corn, folio millet, and Brown Top millet, showing the major differences in the growth curves. Sources: Parker [8], McMahan., *et al.* [7], Kering and Broderick [9].

Crops of corn and various millets abound in fields in diverse environmental conditions. Both types, however are warm season crops. They are temperature sensitive, but they tolerate a higher range of temperatures. Also, despite the high requirements for water, these species show high water-use efficiency. It is notable that while both corn and the millets are C4 plants, they are also monocots. However, there are C4 plants that are also dicots, including species of the *Atriplex* and *Amaranthus* genera [3].

The corn and millet species show stark contrast to C3 species which are increasingly very threatened by the current changing environment [10]. Nevertheless, it is important to take full advantage of all the benefits that can potentially accrue from different types and species of plants.

CAM species, such as the pineapple plant (*Ananas comosus*), although not the focus of this proposal, are also significant. They have no bundle sheaths but they too have this second photosynthetic enzyme, PEPc. These plants typically keep their stomata closed during the day, open them at night when they would not transpire much water but be able to pick up carbon dioxide from the atmosphere. By the reaction of phosphoenolpyruvate with CO₂, phosphoenol pyruvate plus carbon dioxide yields oxaloacetate, which is stored in the vacuole then exported to the chloroplasts where they are used. When oxaloacetate is reduced to malate and malate is broken down to carbon dioxide and pyruvate, the pyruvate exits and the CO₂ is assimilated via the action of RuBPC/o in the Calvin cycle [11]. Two very interesting and useable species of CAM crop plants are the pineapple plant (*Ananas comosus*) and the purslane plant (*Portulaca* spp.) Pineapple is a delicious fruit, and purslane can be used as a vegetable in making tasty stew dishes.

Many C3 species are used to make most of the predominant food crops. No trees have been identified to have C4 or CAM physiology, but they too are important in maintaining atmospheric oxygen levels, producing wood, as well as producing the large diversity of fruits and nuts, among other features. Whereas most vegetable species are C3 plants, including collards, broccoli, radish, other members of the Mustard family (Brassicaceae), and the Capsicum peppers, tomatoes, Irish potatoes, and eggplant of the Solanaceae (Nightshade family), we must recognize the complementary value of these other crops.

Discussion and Conclusion

The indispensable need for plants for food as well as for the many secondary compounds, including medical substances, they produce, is abundant. This fact emphasizes the need these substances they provide for nutritional and health sustenance. This is evident in the food lines that are seen everywhere in the U. S., due to needs caused by the Covid-19 Pandemic. The circumstances are acute for many who are forced to seize all opportunities to contravene the current dilemma of shortages that are due to the emergency need to secure health and have adequate and nutritiously filling food, not ignoring the concurrent enormous economic challenges. Fonio, teff and Brown Top are three C4 grains that can be added to rice, wheat, and corn as major grain crops for the human diet and for use as livestock feed. Additionally, contemporary science and technology must be called upon in addressing current food and feedstock inadequacies. As grains, fonio and teff have low glycemic index, are gluten-free, and rich in methionine-cysteine abundant proteins and fiber [12]. In addition, the recommendation from the U. S. Academy of Sciences is to improve the exploitation of Fonio and some of the other impressive plants. Experiments to discover new uses of plants and compounds or substances they produce as well as with our changing environment, the Covid-19 Pandemic is an appropriate time for innovation and to establish some new and important practices that are productive and which can change and improve our access to food, improve our health, and prosper our Nation.

We present a novel scheme here: Home Farming is the innovation. Every home and every household should continuously grow crops that they can eat and share with neighbors and the needy. They may also sell some, per community demands. Urban and suburban residents can readily adapt the practice. Notably, there is a large variety of fruits and vegetables [7,8] and production techniques have various dimensions, from potted pots of soil through greenhouse culture and hydroponics to biotechnology with *in-vitro* study and production [13,14]. Moreover, short-season tropical plant species have an added value due to their season-long warm temperature requirement [15]. With minimal training, many of these technique and practices can be made amendable and adopted by the general public.

Animal husbandry is rare nowadays in towns and cities, but new developments could make it necessary to raise chickens (*Gallus gallus domesticus*) for eggs, Guinea pigs (*Cavia porcellus*) and rabbits (several species in the Leporidae family) for meat. Rabbit-meat which is reportedly quite tasty. Guinea pigs supply meat to some 2 billion people per year in Peru. Animal feeding has grown to become a major specialty and Jurgens (2002) presents a comprehensive text that covers the scope of animal nutrition. Other than results from direct nutrition studies, plant chemistry presents lots of compounds in diets that present enormous advantage to various activities and facilities in our lives. Aspirin and morphine are two medicinal compounds, and cotton and rubber find utility in too many ways. Remember, every carbon atom in the leaf, the egg, milk, cheese, or meat comes directly or indirectly from the plant, and the biology and biophysical chemistry of plants hold millions of secrets that need to be revealed. In the study of capsaicin, the active ingredient in hot pepper (*Capsaicin chinense*), Broderick and Cooke [16] using confocal microscopy images, examined the storage of capsaicin in patches on the placenta of plants fruits (See figure 2).

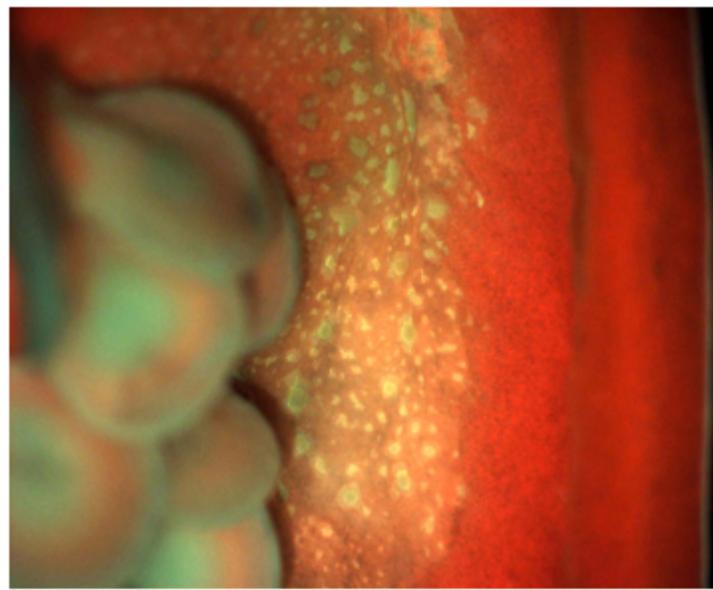


Figure 2: Confocal microimage from the fruit of the hot pepper plant (*Capsicum chinense*) illustrating the accumulation of capsaicin-rich deposits on the placenta of the fruit. Source: Broderick and Cooke [16].

Last, space expeditions are the final frontier. President John F. Kennedy committed the United States to land a man on the moon before the end of the decade of the 1960's. He was successful in accomplishing that mission when Neil Armstrong walked on the moon in 1969. Since - in the years that followed - the U. S. has led several countries in the establishment and operation of the International Space Station (ISS). Despite usually being at loggerheads, the Americans and the Russians have found friendship in space.

The enormous success of investments in space has inspired many, and President Donald Trump on February 19, 2019, launched the Space Force. This initiative for the Space Force is under the auspices of the U. S. Air Force. There are major investments from the private sector in space ventures, and currently, major attention is being paid to space travel and research on the ISS. Also, preparations are underway to travel to inhabit other planets; currently, however, virtually all food consumed on the ISS is supplied from Earth. Experiments on the ISS have made some gain, reportedly producing edible lettuce (*Lactuca* spp.), but that level of production cannot sustain human

livelihood. Moreover, reports of any livestock production in space for the crew and passengers is rare. Selected crops and similar adaptive practices can contribute and make significant breakthroughs.

Based on the strong desire for space expeditions, with the adoption of C4 grain plants such as Folio, Teff, and Brown Top millet, as well as selected C3 and CAM plants, producers will secure a great promise via selection, breeding, and gene manipulation via genetic engineering and biotechnology. Per research results from collaborating scientists producers would secure better yields and profits, and plant scientists would celebrate their successes.

Our desire also is to have more people attend our universities to pursue careers in the plant sciences. Notably, however, the plant sciences have been among a group of disciplines that show major underrepresentation, but the study of plants is indispensable to life. Similarly, as we remember results from late 18th century 11 investigations of plants (around 1775), Antoine Lavoisier and Joseph Priestley discovered oxygen [17-29].

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