

Evolutionary genetic engineering to help the food supply in a warmer and more crowded world

Carretero Vilarroig, María Lidón. Genetics Degree (2010-2014). Faculty of Biosciences. Universitat Autònoma de Barcelona. Barcelona (Spain)

« It is not the strongest of the species that survives, or the most intelligent; it is the most capable of change » Attributed to Charles Darwin

INTRODUCTION

When physiological acclimation and movement to other habitats become exhausted, then there is no way out for species facing global warming other than to adapt evolutionarily. In addition to through the evolution of new genes, it is common in plants to evolve new phenotypes via reorganization of existing gene networks. C4 plants have evolved new anatomical, genetic, metabolic and physiological structures. Whereas C3 photosynthesis occurs completely in the mesophyll, C4 plants have evolved Kranz Anatomy (Fig.2) which allows them to separate carboxylations spatially. The first carboxylation of C4 photosynthesis occurs into mesophyll cells, meanwhile bundle sheet cells develop Calvin's cycle. As a result, C4 plants are more efficient fixing carbon than C3 plants in conditions of lower CO₂ and high temperature.

OBJECTIVES: It is expected that the ongoing global climate warming will foster the expansion of C4 plants. In this new scenario, food supply plants with C3 metabolism, such as rice, will be disfavored. Here we propose a comparative evolutionary analysis of C3 and C4 photosynthesis in order to inform efforts to genetically engineering C4 metabolism in rice.

MATERIALS and METHODS

Searches of the relevant literature, including the *PubMed* database, specialized books, and IRRI's web (International Rice Research Institute). Collection of data from *WorldClimate* database, and prediction of future climate geographical shifts. BLAST searches of C3 and C4 genes in *Oryza sativa* using the NCBI tools.

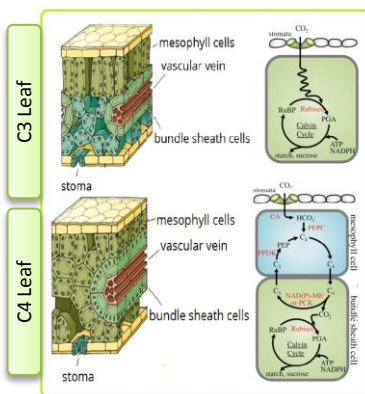


Figure 2 Differences between C3 and C4 anatomy. Ref [2]

ONE OF THE HIGHEST RATES OF CONVERGENT EVOLUTION
C4 evolved from C3 at least 62 independent times
(53% monocotyledon, 47% dicotyledon)

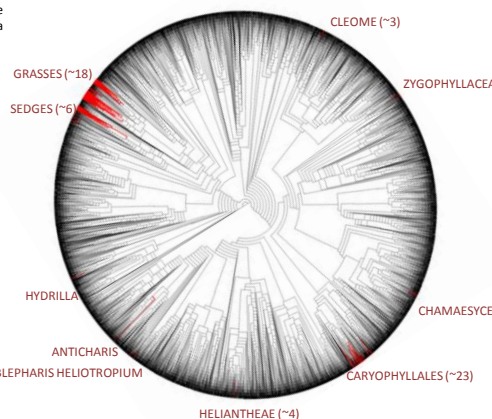


Figure 3. Phylogenetic distribution of C4 lineages is represented by 9412 angiosperm species. Number between names represent the number of different origins. Ref [3]

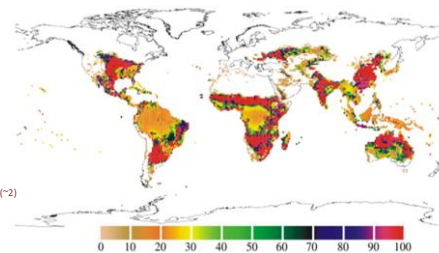


Figure 1. Actual distribution of C4 plants (%) Ref [1]

RESULTS

The evolution of C4 plants involves coordinated gene changes, which affect leaf anatomy, ultrastructure, metabolite transport and enzyme regulation. Despite the complexity of this character, it evolved independently 62 times, constituting one of the most astounding examples of convergent evolution [2] (Fig. 2). C4 plants evolved in 6 geographic regions, which have in common to exhibit arid or semiarid climates. Due to high temperatures and low humidity stomatal conductance decreases, thereby photorespiration becomes more important than photosynthesis. In this conditions, C4 plants evolved a mechanism capable to perform photosynthesis. This evolutionary transition was not discrete, since there are known intermediate forms.

HYPOTHESIS

The Annual Climate Indicators Bulletin indicates that the average temperature of the planet is increasing 0.2C per decade. Currently, the regions where C4 plants live, have an average annual temperature between 25 and 30C. Territories such as Morocco or Turkey exhibit high temperatures, but these are not enough to allow survival of these type of plants. This study demonstrates that, in a period of between 100 and 200 years, such countries will reach temperatures as high as those where there are C4 plants. The increase in temperature is a pre-conditioning for the expansion of C4 plants. This projection, along with information from previous studies, indicates that the current margins of distribution of C4 plants will move towards the poles. However, it must be kept in mind that other factors are also important, so it introduces uncertainty in our prediction.

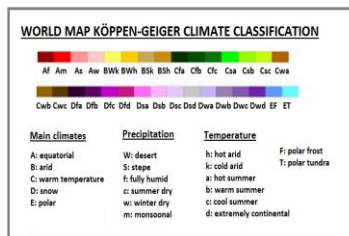
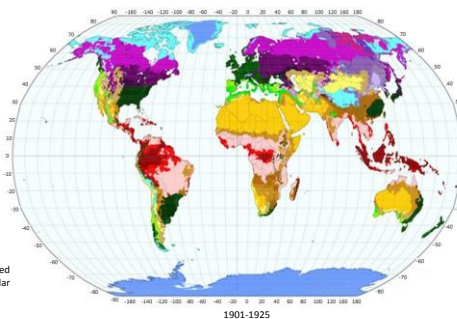


Figure 5. World Map of Köppen-Geiger climate classification calculated from observed temperature and precipitation data for the period 1901-1925 and 2076-2100 on a regular 0.5 degree latitude/longitude grid. Ref [4]



EVOLUTIONARY GENETIC ENGINEERING

The Food and Agriculture Organization of the United Nations predicts that by 2050 we will need 70% more food to feed people, specially Asian people. Using genetic engineering, we can introduce C4 photosynthesis in C3 plants such as *Oryza sativa* and this can help to solve this problem.

How could this be done?

1. Regulation of negatively rice genes by artificial microRNAs (amiRNAs).
2. Introduction of transporters to increase the flow of metabolites.
3. Incorporation of candidate genes drivers of C4 leaf anatomy.
4. Monitoring structure and anatomy to determine any impact on the leaves.
5. Crosses of transgenic plants with significant activity of C4 metabolism.

However, the rice genome already contains genes that are necessary to develop a C4 metabolism (Table 1). In April 2014, Feldman *et al.* proposes to perform directed mutagenesis by point mutations and deletions instead of introducing maize genes with genetic engineering.

Gene	ID	Cell type	Ident	Function in Panicoidae
PEPC	S005789m.g	M	99%	Phosphoenolpyruvate carboxylase activity
NADP-MDH	S013632m.g	M	79%	Malate dehydrogenase (NADP+) activity
PPDK	S021417m.g	M	100%	ATP binding, Pyruvate-phosphate dikinase activity
PPDK-RP	S032116m.g	M	68%	ATP binding, Phosphoryltransfer phosphatase and Protein kinase activity
NADP-ME	S000645m.g	B5	90%	NAD binding, Malate decarboxylating activity
RBCS	S023465m.g	B5	100%	Monooxygenase and Ribulose-bisphosphate carboxylase activity
OMT1	S024403m.g	M	90%	α -ketoglutarate, Malate and Oxaloacetate transmembrane transporter activity
GAPDH	S035707m.g	B5	97%	NAD and NADP binding, Oxidoreductase activity
PGK	S021917m.g	B5	91%	ATP binding, Phosphoglycerate kinase activity

Table 1. Some of the gens that are required for the C4 pathway in mesophyll (M) or bundle sheath (BS) and their identity with *Oryza sativa* (rice) genes. Ref [6]

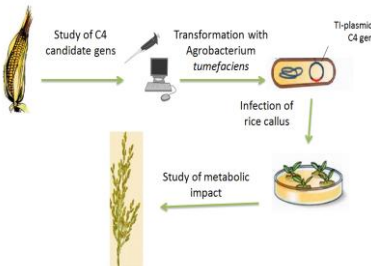


Figure 6. Diagram of how genetic engineering is performed in order to replace a C4 metabolism into *Oryza sativa* (rice). Ref [5]

CONCLUSIONS

- The current climate change is causing a rise in the world temperature, which limits the growth and distribution of plants. Because plants do not have the ability to move searching better weather conditions, plant evolutionary adaptation is expected to be increasingly important to face the progressively stressing conditions.
- The C4 species could have yields up to 50 % higher than C3 plants. We can improve C3-plant performance by engineering C4 metabolism into them. This tool would help us solve demographic problems such as food supply in crowded areas, like Asia. So far there have been different approaches to achieve this goal, but it is still necessary to characterize more genes in order to engineer a C4 metabolism.

REFERENCES

Only relevant references are cited below. Detailed references will be provided upon Committee request.

- [1] Woodward FI, et al. October 2004. Global climate and the distribution of plant biomes. *The Royal Society*. 29:1465-76.
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ACKNOWLEDGMENTS

Thanks to Dr. Francisco José Rodríguez-Trelles Astruga

Contact: lidon.carretero@gmail.com