Enzyme Engineering
ISSN: 2329-6674

Cañas, Enz Eng 2012, 1:2
DOI: 10.4172/2329-6674.1000e106

Editorial Open Access

New Challenges and Opportunities of Protein Engineering in Plants

Rafael A. Cañas*

Department of Molecular Biology and Biochemistry, Faculty of Science, University of Málaga, 29071 Málaga, Spain

Abstract

The present increase of the human population provokes higher demands for food, combustible and raw materials that may cause short-medium term famine and scarcity. The use of enzyme engineering techniques in plants could contribute to palliate these global problems. Enzyme engineering is changing the chemical and pharmaceutical industries and opens new possibilities, although until now the use of these techniques in plants is very limited. The new techniques employed in Plant Sciences, such as transformation of herbaceous crops and trees, the increasing number of full sequenced plant genomes, and the chemical diversity of plants can support a rapid development of enzyme engineering in plants. In the future, the application of enzyme engineering techniques could increase the crop yield or facilitate the biofuel production.

Enzyme engineering brings our society with an excellent opportunity to advance. The use of these techniques in chemical and pharmaceutical industries has changed our world, and opens new possibilities [1]. Enzyme engineering techniques have been widely used in microorganisms, improving chemical processes due to their diversity in species, lifestyles and metabolic reactions, which make possible the discovery of new enzymes having new interesting characteristics (e.g. an enzyme with a known function more thermostable), or catalyzing new reactions [2]. Moreover, the expression of recombinant proteins in bacteria is routinely done.

But in plants, the use of protein engineering techniques is very limited at this moment. Maybe the main reason of this has been the difficulty to make transgenic crop plants, and the limited number of full sequenced genomes compared to microorganisms until now. Although at present, it is possible to transform almost all crop plants [3], including some trees like poplar [4] and pine [5,6], and the number of full sequenced genomes of plant species is highly increased in the last years; thanks to next generation sequencing techniques [7]. In the present moment, we have enough technology to attend the protein engineering in plants.

The plant domestication constituted the major revolution of all in the human history [8]. Now, the plants are essential for the humanity in different ways. They support the human nutrition, but also are the source of raw materials (wood, pulp, etc), chemicals (biofuels, etc) and pharmaceuticals (acetylsalicylic acid, etc). Nowadays, the human population is increasing, so the demands for food, combustible and raw materials are also increasing, which can cause short-medium term famine and scarcity [9]. The use of enzyme engineering techniques in plants could contribute to palliate these global problems by improving crop yield and qualities.

The Genome Era: Identification of New Genes/Enzymes

The genome era makes possible to identify new genes and protein functions, in a way never thought, until this moment. In plants, it is estimated that the number of protein of unknown function is higher than in the rest of organism, constituting over 50% of the total [10]. A great portion could encode for enzymes and transporters. Many of these proteins of unknown function are part of conserved families shared between bacteria, plants, and other eukaryotes [11]. On this way, the comparative genomics becomes an excellent tool to predict functions and guide experimental validation [10,12].

In plants, this amount of proteins of unknown function can constitute a near unlimited source of new enzymes with new catalytic capacities, which can be used for enzyme and metabolic engineering. It is known that plants have an enormous chemical diversity, which confers a great potential to work with enzymes with new functions [13]. Obviously, the diversity of secondary metabolites in plants must be correlated with an exceptional number of enzymes catalyzing the synthesis and degradation of these metabolites. The modification of these new enzymes involved in the secondary metabolism, and the combination with metabolic engineering techniques could lead to obtaining plants, which can be better exploited by the industry.

Translation from Microorganism Platforms to Plants

Because they are manageable and have a great biomass production capacity (by their capacity to assimilate CO_2), the plants are good candidates to be transformed with modified enzymes from bacteria. So far this possibility has not been much followed, although it is proposed to be used together with metabolic engineering in the production of plants for the use in bioremediation [14]. This methodology has an enormous potential to improve plants, or for the modification and generation of metabolic pathways of interest. These approaches allow the introduction of new routes in plants or the optimization of existing routes, for example, increasing the thermostability or the catalytic capacity of the enzymes. There are some examples of plant transformation with non-modified genes from microorganisms that may confer new or different capabilities. The endogenous tobacco RubisCO in the chloroplasts was replaced by the RubisCO from the α-proteobacterium, *Rhodospirillum rubrum* [15]. The transgenic tobacco plants were fully autotrophic and reproductive, although under CO_2 supplementation. In other case, the *Lactuca sativa* plants have been transformed with gene coding for the asparagine synthetase A (EC 6.3.1.1) from *Escherichia coli* (AS-A) [16]. This enzyme mainly uses ammonium to produce asparagines, a key amino acid in the nitrogen and carbon metabolisms of plants. On the contrary, the

***Corresponding author**: Rafael A. Cañas, Department of Molecular Biology and Biochemistry, Faculty of Science, University of Málaga, 29071 Málaga, Spain, E-mail: racanas@uma.es

Received November 15, 2012; **Accepted** November 17, 2012; **Published** November 24, 2012

Citation: Cañas RA (2012) New Challenges and Opportunities of Protein Engineering in Plants. Enz Eng 1:e106. doi:10.4172/2329-6674.1000e106

asparagine synthetase of plants mainly uses glutamine as N donor to form asparagine. The transgenic plants with AS-A had new traits, such as a higher leaf number, wider leaf surface and seed germination, formation and development of leaves, bolting and flowering which occurred earlier than the wild-type [16]. The metabolic profile of the transgenic plants also changed accumulating inulins, a polysaccharide beneficial for human health with industrial and medical uses [17,18].

Only with the use of metabolic engineering, the transformation of plants with non-modified enzymes from microorganisms is enough to obtain interesting results. The use of these techniques, together with the enzyme engineering, could revolutionize the agriculture and the chemical and pharmaceutical industries. An example is the modification of bacterial glyphosate N-acetyltransferases, improving its efficiency four orders and conferring an increased tolerance of *Arabidopsis,* tobacco and maize to the herbicide glyphosate, when the engineered enzymes were expressed in the plants [19].

Crop Yield Increase: Photosynthesis Engineering (RubisCO), NUE

One of the main challenges in biology is to cover the nutritional needs of a human population in constant growth. The enzyme engineering can play an important role as a tool to achieve the crop yield increases needed to meet the created and future demands. In this sense, the modification and improvement of Ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO; EC 4.1.1.39) is a main objective. RubisCO is the key enzyme of the Calvin-Benson-Bassham pathway, the enzyme responsible for the assimilation of atmospheric CO_2 into organic molecules [20]. The carboxylase activity of RubisCO produces two molecules of 3-phosphoglycerate from ribulose 1,5-bisphosphate (RuBP), CO_2 and H_2O. However, the enzyme also catalyzes a competing oxygenase reaction, converting O_2 and RuBP to one molecule of 3-phosphoglycerate and 2-phosphoglycolate. The oxygenase activity decreases the overall performance in CO_2 fixation [21]. Some plants minimize the RubisCO oxygenase activity with systems, to increase the concentration of CO_2 in the chloroplasts of the cells where it acts on RubisCO in C4 plants (e.g. maize and sugarcane) [20]. Although most of the crops are C3 plants with high rates of photorespiration; they expend large resources in photorespiration that slows down their growth and yield. Therefore, the RubisCO engineering, through the increase in its affinity for CO_2, may be crucial for the improvement of the yield of crops with C3 metabolism.

In microorganisms, there have been numerous attempts to improve the RubisCO [21,22]. In plants, the number of works is limited until now. The RubisCO plant transformation is not trivial, since it has two subunits. The large subunit (L) is coded by a gene present in the genome of the chloroplast. The small subunit (S) is coded by several genes present in the nuclear genome [23]. Noteworthy is the work of Whitney et al. [24] who have achieved great results, both in the transformation of plant chloroplasts with genes coding the RubisCO L subunit, and in modifying the properties of RubisCO by directed mutagenesis. They have also linked RubisCO subunits, obtaining an active recombinant RubisCO that could facilitate RubisCO transformation works in plants [23,25]. They also have found that the plastomic replacement of the tobacco RubisCO L subunit gene with one from sunflower produced transgenic tobacco plants with an active hybrid RubisCO, able to support the plant growth [26]. Finally, they have established that the activity of a RubisCO from a very different phylogeny, bacteria, can be integrated into chloroplast photosynthetic metabolism, without prohibitive problems [15].

But, it is not only possible to improve photosynthesis through modification of RubisCO. It is known that plant photosynthesis declines, when the temperature exceeds its optimum range. This reduction in photosynthesis is due to the RubisCO deactivation by the inhibition of RubisCO Activase (RCA), under moderately elevated temperatures. *Arabidopsis* plants transformed with genes coding a modified thermostable RCA had improved photosynthesis and growth rates, under heat stress that could be transferred to crop plant [27].

The improvement of the Nitrogen Use Efficiency (NUE) is also very important for increasing crop yield. The N is a limiting factor for growth and yield, so in the last century, the use of nitrogen fertilizers has been largely increased [28]. As a consequence, a great increase in the production costs and serious environmental problems such as eutrophication have occurred [28]. Therefore, improving NUE has become a fundamental subject of study in Plant Sciences. However, there have been no enzyme engineering approaches for improving NUE, where it could have a very significant role. The change in the characteristics of key enzymes of nitrogen metabolism of plants, such as glutamine or asparagine synthetases, could improve NUE. In this regard, although protein engineering techniques were not carried out, it has been shown that *Lactuca sativa* plants transformed with the gene coding for the asparagine synthetase A from *Escherichia coli* (AS-A), which has different characteristics to the asparagine synthetase of plants, which exhibit an improved NUE [16].

Secondary Metabolism: Lignin

Due to the current energy and global environmental crisis, the discovery of new renewable energy sources and raw materials are essential for today's society. In this context, the production of plant biomass for obtaining biofuels, biopolymers and chemicals is an alternative, which is extensively studied in recent years [9]. The modification in the activity or functions of the enzymes involved in the lignin synthesis could be of great importance to develop the biofuel industry. One of the problems to the efficient use of cellulosic fibers in agricultural and industrial applications is the lignin, particularly in the conversion of cellulosic biomass to liquid biofuels [29]. The use of protein engineering to reduce or modify the content, and/or quality of lignin in the crops used in the biofuel industry, is a major challenge today. At this moment, there is no much work about it except for the article by Zhang et al. [30]. They described catalytic improvement of an artificial monolignol 4-O-methyltransferase, created by iterative saturation mutagenesis. This new enzyme is expressed into *Arabidopsis,* reducing the lignin levels of transgenic plants, which are able of producing higher saccharification yields [30]. This is very interesting because it uses enzyme engineering techniques over the plant, not in a chemical process, which is very promising for the industry.

In this line, the modification of plant secondary metabolic enzymes is not too much explored, although there is enormous chemical diversity of plants. The most successful example is the engineering of the sesquiterpene synthases. In a first work, the plasticity residues were probed in the active site of a promiscuous sesquiterpene synthase, and finally seven specific and active synthases were obtained, producing specific and very different products [31]. In a later work, all the possible substitution combinations of nine amino acid residues in the active site of a sesquiterpene synthase were explored, resulting in the correlation between the substitutions and the sesquiterpene product spectra [32]. Unfortunately, these results were not applied over plants for diversifying plant metabolism.

Conclusion

The enzyme engineering opens new possibilities to solve classic and present problems in Plant Sciences. The new techniques to generate transgenic plants in herbaceous crops and even in trees, the increasing number of full sequenced plant genomes, and the chemical diversity of plants constitute a great source for enzyme engineering works. In the future, the application of enzyme engineering techniques could increase yield of crops (RubisCO activity or NUE increases), or facilitate biofuel production (lignin amount modifications).

References

1. Bornscheuer UT, Huisman GW, Kazlauskas RJ, Lutz S, Moore JC, et al. (2012) Engineering the third wave of biocatalysis. Nature 485: 185-194.
2. Molina N, van Nimwegen E (2009) Scaling laws in functional genome content across prokaryotic clades and lifestyles. Trends Genet 25: 243-247.
3. Meyers B, Zaltsman A, Lacroix B, Kozlovsky SV, Krichevsky A (2010) Nuclear and plastid genetic engineering of plants: comparison of opportunities and challenges. Biotechnol Adv 28: 747-756.
4. Gallardo F, Fu J, Canton FR, Garcia-Gutierrez A, Canovas FM, et al. (1999) Expression of a conifer glutamine synthetase gene in transgenic poplar. Planta 210: 19-26.
5. Trontin JF, Harvengt L, Garin E, Lopez-Vernaza M, Arancio L, et al. (2002) Towards genetic engineering of maritime pine (*Pinus pinaster* Ait.). Ann For Sci 59: 687-697.
6. Breton D, Harvengt L, Trontin JF, Bouvet A, Favre JM (2006) Long-term subculture randomly affects morphology and subsequent maturation of early somatic embryos in maritime pine. Plant Cell Tiss Organ Cult 87: 95-108.
7. Thudi M, Li Y, Jackson SA, May GD, Varshney RK (2012) Current state-of-art of sequencing technologies for plant genomics research. Brief Funct Genomics 11: 3-11.
8. Vigouroux Y, Barnaud A, Scarcelli N, Thuillet AC (2011) Biodiversity, evolution and adaptation of cultivated crops. C R Biol 334: 450-457.
9. Ragauskas AJ, Williams CK, Davison BH, Britovsek G, Cairney J, et al. (2006) The path forward for biofuels and biomaterials. Science 311: 484-489.
10. Hanson AD, Pribat A, Waller JC, de Crécy-Lagard V (2009) 'Unknown' proteins and 'orphan' enzymes: the missing half of the engineering parts list--and how to find it. Biochem J 425: 1-11.
11. Galperin MY, Koonin EV (2010) From complete genome sequence to 'complete' understanding? Trends Biotechnol 28: 398-406.
12. Bradbury LM, Niehaus TD, Hanson AD (2012) Comparative genomics approaches to understanding and manipulating plant metabolism. Curr Opin Biotechnol.
13. De Luca V, Salim V, Atsumi SM, Yu F (2012) Mining the biodiversity of plants: a revolution in the making. Science 336: 1658-1661.
14. Jez JM (2011) Toward protein engineering for phytoremediation: possibilities and challenges. Int J Phytoremediation 13: 77-89.
15. Whitney SM, Andrews TJ (2001) Plastome-encoded bacterial ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) supports photosynthesis and growth in tobacco. Proc Natl Acad Sci U S A 98: 14738-14743.
16. Giannino D, Nicolodi C, Testone G, Frugis G, Pace E, et al. (2007) The overexpression of *asparagine synthetase* A from *E. coli* affects the nitrogen status in leaves of lettuce (*Lactuca sativa* L.) and enhances vegetative growth. International Journal of Plant Breeding, Springer.
17. Sobolev AP, Segre AL, Giannino D, Mariotti D, Nicolodi C, et al. (2007) Strong increase of foliar inulin occurs in transgenic lettuce plants (*Lactuca sativa* L.) overexpressing the Asparagine Synthetase A gene from *Escherichia coli*. J Agric Food Chem 55: 10827-10831.
18. Sobolev AP, Testone G, Santoro F, Nicolodi C, Iannelli MA, et al. (2010) Quality traits of conventional and transgenic lettuce (*Lactuca sativa* L.) at harvesting by NMR metabolic profiling. J Agric Food Chem 58: 6928-6936.
19. Castle LA, Siehl DL, Gorton R, Patten PA, Chen YH, et al. (2004) Discovery and directed evolution of a glyphosate tolerance gene. Science 304: 1151-1154.
20. Zabaleta E, Martin MV, Braun HP (2012) A basal carbon concentrating mechanism in plants? Plant Sci 187: 97-104.
21. Yoshida S, Atomi H, Imanaka T (2007) Engineering of a type III rubisco from a hyperthermophilic archaeon in order to enhance catalytic performance in mesophilic host cells. Appl Environ Microbiol 73: 6254-6261.
22. Parry MA, Andralojc PJ, Mitchell RA, Madgwick PJ, Keys AJ (2003) Manipulation of Rubisco: the amount, activity, function and regulation. J Exp Bot 54: 1321-1333.
23. Whitney SM, Sharwood RE (2007) Linked Rubisco subunits can assemble into functional oligomers without impeding catalytic performance. J Biol Chem 282: 3809-3818.
24. Whitney SM, Sharwood RE, Orr D, White SJ, Alonso H, et al. (2011) Isoleucine 309 acts as a C4 catalytic switch that increases ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) carboxylation rate in Flaveria. Proc Natl Acad Sci U S A 108: 14688-14693.
25. Whitney SM, Kane HJ, Houtz RL, Sharwood RE (2009) Rubisco oligomers composed of linked small and large subunits assemble in tobacco plastids and have higher affinities for CO_2 and O_2. Plant Physiol 149: 1887-1895.
26. Sharwood RE, von Caemmerer S, Maliga P, Whitney SM (2008) The catalytic properties of hybrid Rubisco comprising tobacco small and sunflower large subunits mirror the kinetically equivalent source Rubiscos and can support tobacco growth. Plant Physiol 146: 83-96.
27. Kurek I, Chang TK, Bertain SM, Madrigal A, Liu L, et al. (2007) Enhanced thermostability of *Arabidopsis* Rubisco activase improves photosynthesis and growth rates under moderate heat stress. Plant Cell 19: 3230-3241.
28. Hirel B, Le Gouis J, Ney B, Gallais A (2007) The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. J Exp Bot 58: 2369-2387.
29. Weng JK, Li X, Bonawitz ND, Chapple C (2008) Emerging strategies of lignin engineering and degradation for cellulosic biofuel production. Curr Opin Biotechnol 19: 166-172.
30. Zhang K, Bhuiya MW, Pazo JR, Miao Y, Kim H, et al. (2012) An engineered monolignol 4-o-methyltransferase depresses lignin biosynthesis and confers novel metabolic capability in *Arabidopsis*. Plant Cell 24: 3135-3152.
31. Yoshikuni Y, Ferrin TE, Keasling JD (2006) Designed divergent evolution of enzyme function. Nature 440: 1078-1082.
32. O'Maille PE, Malone A, Dellas N, Andes Hess B Jr, Smentek L, et al. (2008) Quantitative exploration of the catalytic landscape separating divergent plant sesquiterpene synthases. Nat Chem Biol 4: 617-623.