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Modifying Metabolism for an Improved NUE

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Editorial

Nitrogen (N) is a very important limiting factor for plant growth and development. At agricultural level its fact is translated into the nitrogen fertilization dependence of yield. Since the middle age the farmers have employed culture techniques to increase the useful nitrogen in the soil as the crop rotation with legumes. However until the XIX century the inorganic nitrogen fertilization was not employed and until the beginning of the XX century the industrial production of nitrogen fertilizers was not carried out. The intensive use of industrial fertilizers together with other practices has changed the agriculture promoting the Green Revolution (GR). In the past 50 years this set of agricultural practices has led to a huge increase of crop yield to feed expanding populations, the production of cereal crops tripled during this period, with only a 30% increase in land area cultivated [1]. However the intensive agriculture promoted by GR has led to a number of environmental problems with high impact in the water quality, biodiversity and climate change [2]. One of the most important contaminant factors is the nitrogen fertilization that provokes water eutrophication and greenhouse gasses emissions [3]. At this point the 16 improvement of the nitrogen use efficiency (NUE), in other words the yield per supplied nitrogen amount, becomes in a crucial research aim.

Nowadays, with the current biotechnology skills, a great number of efforts are being developed to increase crop NUE. They are mainly based on the metabolic engineering through the obtaining of new transgenic lines with increased expression of genes coding for the key enzymes in the plant nitrogen metabolism [4]. Nevertheless a variety of divergent results have been obtained since negative effects over the grain yield or biomass accumulation to modifications of only one component of the NUE as nitrogen uptake or transport [4,5]. At this moment the best results has been achieved with transgenic lines overexpressing genes coding for the glutamine synthetase (GS, EC 6.3.1.2) [4,6,7]. This enzyme is the final responsible of the incorporation of the inorganic 28 nitrogen into the organic molecules (NH_4^+ + glutamate + ATP ⇒ glutamine + 29 ADP + P) and is involved in the nitrogen primary assimilation but also in the ammonium recycling during photorespiration or lignification [8]. These transgenic lines usually have higher grain and/or biomass yields than Wt plants at the same nitrogen nutrition level that is the principal objective to reach for the NUE improvement [4]. An alternative approach involves the use of genes coding for enzymes from other species, such as bacteria, which have different capacities than the plant endogenous gene. It is the case of asparagine synthetase (AS, EC 6.3.5.4). There are several works producing transgenic lines in plants through the introduction of NH_4^+ dependent asparagine synthetase (AsnA) from Escherichia coli [9,10,11]. In plants the AS uses preferentially glutamine instead of ammonium which can be used with lower affinity. This type of approximation is supported by the idea that plants transformed with the E. coli AsnA could have an improved ammonium detoxification capacity helping to the GS. In line with this assumption these transgenic lines usually have increased nitrogen status.

Alternatively, the engineering of different metabolic pathways involved in nitrogen metabolism could lead to improve the NUE in crop plants as described with carbon assimilation metabolism. In this regard the relationships between carbon and nitrogen metabolisms are very strong. In C3 plants the photorespiratory flux of ammonium can be 10 times higher compared with that originating from the nitrate reduction [12]. We can assume that improving the RUBISCO (EC 4.1.1.39) amount, or better its affinity for CO_2, could reduce the photorespiration and therefore the nitrogen needs for this pathway [5].

The idea of manipulating the potential of some key enzymes of the nitrogen metabolism in order to obtain crops with an improved NUE results very attractive. However, the genetic network involved in the use of nitrogen is more complex that we thought and even it seems that the levels of nitrogen assimilation enzymes do not limit primary nitrogen assimilation and hence yield [5,13]. In this context, alternative approaches could be needed. One of the answers could be the engineering of metabolism for generating alternative pathways absent in the crop of interest. For example, works aimed to introduce the atmospheric nitrogen fixation into cereals promoting a type of legume symbiosis or transforming the crop plants with genes coding for the nitrogenase (EC 1.18.6.1) [14,15]. Interestingly the latest biotechnology approaches converge in any way with the old agricultural practices.

In summary, the improvement of the crop NUE persists as a social and research problem, biotechnological definitive solutions has not been reached. In this context the metabolic and enzyme engineering have to pass a significant way to achieve crops that require decreased N fertilizer levels promoting a 'Second Green Revolution'.

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