Excavating abiotic stress-related gene resources of terrestrial macroscopic cyanobacteria for crop genetic engineering: dawn and challenge

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Genetically engineered (GE) crops with resistance to environmental stresses are one of the most important solutions for future food security. Numerous genes associated to plant stress resistance have been identified and characterized. However, the current reality is that only a few transgenic crops expressing prokaryotic genes are successfully applied in field conditions. These few prokaryotic genes include Agrobacterium strain CP4 EPSPS gene, Bacillus thuringiensis Cry1Ab gene and a bacterial chaperonin gene. Thus, the excavation of potentially critical genes still remains an arduous task for crop engineering. Terrestrial macroscopic cyanobacteria, Nostoc commune and Nostoc flagelliforme, which exhibit extreme resistance to desiccation stress, may serve as new prokaryotic bioresources for excavating critical genes. Recently, their marker gene wspA was heterologously expressed in Arabidopsis plant and the transgenics exhibited more flourishing root systems than wild-type plants under osmotic stress condition. In addition, some new genes associated with drought response and adaptation in N. flagelliforme are being uncovered by our ongoing RNA-seq analysis. Although the relevant work about the terrestrial macroscopic cyanobacteria is still underway, we believe that the prospect of excavating their critical genes for application in GE crops is quite optimistic.

Genetically engineered (GE) crops are crucial for solving future food crisis. Numerous functional genes associated to plant stress resistance have been identified and characterized, in most cases, in the model plant Arabidopsis.¹ However, the current reality is that only a few transgenic crops expressing prokaryotic genes are commercially applied, which is far from safeguarding food supply in the future. One of the points may be that we still lack enough critical genes for application in crop engineering. In this commentary, we summarized several commercial examples of transgenic crops and suggested valuable cyanobacterial bioresources for excavating potentially critical genes for crop engineering.

Food Security and GE Crops

The world population was 7.2 billion in 2012 and is estimated to increase to more than 9 billion in 2050.² To meet the world demands by 2050, it is projected that 70%–100% more food compared with 2007 must be produced from limited cultivation areas.³ Unfortunately, yields of several main crops especially rice are approaching a plateau. Global climate changes, such as increasing temperature and drought, will also exacerbate food security problem. Thus, the sustainability of agricultural production has to be addressed as a high priority in the current world. To overcome this issue, crop varieties are expected to possess resistances to multiple abiotic stresses such as drought, salinity, high and low temperatures.⁴ Genetic engineering presents as an effective technique in creating highly resistant crops, which differs from conventional methods of genetic modification by introducing one or a few well-characterized genes from any species into a plant species.⁵,⁶

Keywords: drought resistance, food security, functional genes, Nostoc flagelliforme, terrestrial cyanobacteria, transgenic crops

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Commercial Cases of GE Crops

Since the first GE-tomato appeared in 1994, crop genetic engineering has attracted increasing attention. According to the ISAAA, GE crops were planted in a record 181.5 million hectares by 18 million farmers in 28 countries in 2014. The principal crops are soybean, maize and cotton, and the principal traits are herbicide tolerance and insect resistance. Recently, the first line of drought-tolerant GE maize has been released by Monsanto Company (http://www.monsanto.com). Most interestingly, functional genes applied in these GE crops all come from prokaryotic organisms like bacteria, which sheds a promising prospect of prokaryotic genes for crop genetic engineering.

The control of weeds is critical for no-till agriculture. The broad-spectrum herbicide glyphosate prevents aromatic amino acid biosynthesis in plant by inhibiting chloroplastic 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). GE soybean, maize, canola and cotton that expressed modified EPSPS gene from Agrobacterium strain CP4 exhibited resistance to glyphosate and achieved growth advantages over weeds. Herbicide-tolerant crops are currently planted in several countries including America, Canada, Argentina and Brazil.

Reducing the use of insecticide is beneficial for protecting the health of workers and preventing environmental contamination. Insect-resistant genes allowed for more precise targeting of insect pests compared to chemical insecticides. Since 1996, GE crops that expressed Bacillus thuringiensis (Bt) Cry1Ab gene have been successfully applied. Bt crops produce Bt toxins that are toxic to Lepidoptera pests, such as Heliothis virescens, Helicoverpa armigera and Pectinophora gossypella. In China, Bt cotton had been planted 3.9 million hectares in 2014. Moreover, insect-resistant maize and canola are also planted over the world.

Because of increasing severity of water scarcity, developing drought-resistant or water-saving GE crops has emerged as a main concern for current agriculture production. The first drought-tolerant crop was released in 2013, in which a bacterial gene encoding chaperonin was introduced into maize inbred lines and conferred drought resistance. The estimated hectares of DroughtGard™ maize planted for the first time in the US in 2013 was 50,000 hectares and increased over 5 fold to 275,000 hectares in 2014 reflecting high acceptance by US farmers. In addition, drought-tolerant sugarcane was developed by Ajinomoto Company by using the beta gene from Rhizobium meliloti. Rice is the principle grain crop in China and the demand for rice is enormous. The development of GE drought-tolerant rice is highly expected in the near future.

Cyanobacterial Gene Resources for GE Crops

Terrestrial macroscopic cyanobacteria

Cyanobacteria are a group of oxygenic phototrophic prokaryotes that occur in a great diversity of extreme habitats, such as hot springs, deserts and polar regions. The cyanobacterial genus Nostoc includes some species producing dense extracellular polysaccharide (EPS) sheath, presenting as colony forms, such as Nostoc flagelliforme, Nostoc commune, Nostoc spharoides, Nostoc venuccosum, Nostoc zettedtii and Nostoc pratiforme. The former 2 are terrestrial species grown on soil surface, exhibiting extreme resistance to various environmental stresses. N. commune is a cosmopolitan species, while N. flagelliforme is only distributed in arid and semi-arid regions. The habitats of N. flagelliforme are characterized by low rainfall and high evaporation rates, substantial annual and diurnal temperature changes, and intense solar radiation. Among those sequenced cyanobacterial species, N. flagelliforme is most relative to Nostoc punctiforme PCC 73102 and next to Nostoc sp. PCC 7120. Thus, terrestrial macroscopic species might have evolved from microscopic species grown in temperate or aquatic environments. Since cyanobacteria can perform oxygenic photosynthesis, we also infer that their functional proteins might have better cellular compatibility in transgenic plants than those from non-photosynthetic microorganisms. The genomes of these macroscopic cyanobacteria are currently not available. Therefore, the excavation of potentially valuable genes from terrestrial macroscopic cyanobacteria still remains a challenging task.

Abiotic stress-resistant genes and present situation

The dense elastic EPS sheath as well as the fillers in it such as superoxide dismutase, acidic water stress protein (WSPA), mycosporine-like amino acids (MAAs) and syctonemis is critical for terrestrial macroscopic cyanobacteria to resist abiotic stresses in arid environments. Various abiotic stresses also lead to the thickening of EPS sheath. MAAs and syctonemis are ultraviolet (UV)-absorbing pigments. WSPA was induced upon desiccation or UV radiation and secreted into EPS sheath upon rehydration. This protein was proposed to be unique in macroscopic Nostoc species and transgenic Arabidopsis plants that expressed wspA gene exhibited more flourishing root systems than wild-type plants under osmotic stress condition. This transgenic work may represent the first transgenic case in plant with the gene from terrestrial macroscopic cyanobacteria. Because of the extracellular localization property of WSPA, we further propose that stronger stress resistance may be obtained if this protein is guided to incorporate into cell wall of transgenic plants. Plant cell wall is a protective barrier and cell wall architecture is quite important in resistance to abiotic stress. The EPS sheath of macroscopic cyanobacteria is largely similar to plant cell wall. The elaborate regulation of their rigidity and elasticity should be crucially important for the balance of cell growth and stress resistance. A number of functional proteins in plant cell wall were found, including enzymes involved in cell wall formation and reorganization, carbohydrate metabolism and cell wall loosening. However, the types of proteins are relatively less in the EPS sheath of terrestrial macroscopic cyanobacteria. In a SDS-PAGE analysis of extracellular water-soluble proteins of N. flagelliforme, only several (around 5) protein bands were visualized, with WSPA being the most abundant (unpublished). We infer that these few proteins may undertake crucial roles in regulating 3-D architecture of the EPS sheath and thus facilitate coping with repeated desiccation-rewetting processes.
in arid habitats. A deep understanding of their regulative mechanisms in the EPS sheath should provide valuable reference for crop engineering by elaborately modifying cell wall architecture.

In addition, terrestrial macroscopic cyanobacteria should have been endowed with strong genes or new mechanisms involving stress resistance. Thus far, a few reports have provided limited gene or protein information about them. In our ongoing RNA-seq analysis about *N. flagelliforme*, we have found that some genes were expressed in high levels during the whole desiccation process, including the genes encoding alcohol dehydrogenase, cupin, manganese-containing catalse, oxidoreductase domain-containing protein, fatty acid desaturase, CsbD family protein, short-chain dehydrogenase and glycosyl transferase. Their precise functions or potential mechanisms involving drought response and resistance await further investigation. In general, progress achieved but much work remains to be done for excavating critical genes from terrestrial macroscopic cyanobacteria.

Transgenic strategies for GE crops

The critical gene is undoubtedly the most important aspect for the development of GE crops. Nevertheless, transgenic strategies are equally important for enabling critical genes to exert maximum functional effects. Researchers have suggested the crucial importance of spatio-temporal expression of functional genes. Spatio-temporal gene expression is an energy-saving means as compared to constitutive gene expression. The spatio-temporal expression of antioxidant genes is particularly important for the elaborative control of redox homeostasis, in which inapproprate expression may disturb reactive oxygen species signaling and lead to unexpected growth impairments. In addition, cyanobacterial extracellular proteins are suggested to be guided into cell wall to function in transgenic crops as mentioned above. Finally, co-expression of multiple relevant genes in crops such as prokaryotic operons may introduce new metabolic processes and thus contribute to greater resistance to abiotic stresses. In general, the excavation of potentially critical genes from terrestrial macroscopic cyanobacteria and appropriate application of them in GE crops are highly expected in future work.

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No potential conflicts of interest were disclosed.

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