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COMMENTARY

MicroRNA, a new target for engineering new crop cultivars

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ABSTRACT

Global sustainable development depends on, at least partially, the sustainable development of crop reproduction that provides food, cloth and bioenergy as well certain drugs. During thousands of years of human history, many crops have been domesticated for feeding the world. Perfectly, in the past 2 decades, scientists have innovated biotechnological tools for improving crop yield and quality. For sustainable development, more targets and tools are needed to develop. Among these, microRNA (miRNA) is becoming an emerging target for engineering new crop cultivars with high yield and quality as well tolerance to environmental abiotic and biotic stresses. miRNAs are an extensive class of small regulatory RNAs, which play essential roles in all plant biological and metabolic processes, not only in plant development and growth but also in compound biosynthesis and response to various environmental stress. miRNA-based biotechnology is becoming a new strategy for crop improvement, which will play important role in future agricultural sustainable development.

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Global human population is continuously increasing, which requires more food, clothes and energy. To face this challenge, sustainable agricultural development is required. Among all strategies of sustainable agricultural development, sustainable development on crop reproduction is critical and all others are based on it because food and clothes is the first need for all human beings as well as wild life. During long human history, scientists have been making huge efforts on domesticating plant species and modifying these crops for agricultural purpose. The first and second green revolution is great evidence on improving crops, both contribute to high yield. However, as the limited germplasm and climate change, new strategies are needed to develop for facing the increasing need of the global population. One of them is engineering technology that provides significant contribution to crop yield and quality as well as tolerance to environmental stresses, including insects and diseases also climate change-caused drought, salinity and temperature change by targeting an individual gene cross different species. Transgenic crops have been widely cultivating around the world and generating huge benefits for our human and the environment.¹

Identifying and cloning interested genes are always the first step for engineering crops. Thus, scientists in both academics and industries are exploring new genes from different resources. Recently, microRNAs (miRNAs) are becoming a novel target for engineering new crop cultivars.² miRNAs are an extensive class of small regulatory RNA molecules with about 20–24 nt in length, which are newly discovered in almost all eukaryotes, including both plants and animals.³ miRNAs is an extensive class of gene regulators, which negatively regulate gene expression at the post-transcriptional levels and many miRNAs are evolutionarily conserved from species to species.³ This suggests that miRNAs do not directly regulate plant growth and development but indirectly control plant development by involving in a miRNA-target gene network. Evaluating evidence shows that miRNAs play an essential role in almost all biological and metabolic processes in plants that provide a new target for crop improvement through plant genetic engineering.² In the past couple of years, several studies demonstrated that manipulating a single miRNA gene can significantly increase crop yield and biomass as well improving crop tolerance to environmental stresses.²

Manipulating miRNAs for increasing crop yield and biomass

For many crops, yield is the final goal to harvest a plant. Because yield is normally controlled by multiple genes and is involved in complicated gene network, currently there are not too many reports on directly increasing crop yield by genetic engineering although many transgenic plants can indirectly increase their yield by gaining back the yield lost caused by insect, disease and abiotic stress. The traditional engineering targets on protein-coding gene, which only can target limited gene number. However, for miRNAs, it will be another case. miRNAs are negative gene regulators, which may target multiple genes at a same time and get involved in a complicated gene network. Thus, miRNAs may become a novel target for directly increasing crop yield. Actually, several recent studies show that manipulating a single miRNA gene can significantly increase crop yield and biomass.

Zhang and colleagues (2013) found that overexpressing miR397 increased the overall grain yield of up to 25% in rice; this is the first study for directly improving crop yield using miRNA-based biotechnology. This increase may due to that miR397 enlarged grain size and promoted panicle branching.⁴ Transgenic rice with overexpressing miR397 shows a substantial increase in grain length and width by more than 10% as well thickness which lead to increase the weight of 1000-grains by about 10%.⁴ Additionally, transgenic rice with overexpressing miR397 increased the number of primary and secondary panicle branches and effective grains per main panicle.⁴ miR397 controlled grain yield majorly due to that miR397 downregulated its targets, LAC. LAC is a lacase-like protein that is involved in the sensitivity of plants to plant hormone brassinosteroids.⁴ miR397 is an evolutionarily conserved miRNA, which widely expressed in both dicot and monocot plant species; the results of Zhang and colleagues suggest that miR397 may be a novel target for improving crop grain yield at least for monocot plant species, such as switchgrass and sorghum.

Several studies also show that a single miRNA may control plant branch and further increase plant biomass, including yield. An early study using model plant species *Arabidopsis* shows that overexpressing miR156 with 35S promoter significantly increased the number of plant branches and further increase plant

biomass by more than 300%.⁵ Additional studies show that overexpressing miR156 also significantly increased plant biomass in switchgrass, the transgenic plants produced 100% more biomass yield than control.⁶ The major reason for increasing plant biomass by manipulating miR156 expression is that miR156 controls apical dormancy and promote plant branching that may further increase grain number and grain yield. However, suitable agricultural practices are needed to perform on cultivating these transgenic plants. The major reason is that more nutrients may need to feed more branches of these transgenic plants.

Manipulating miRNAs for improving crop tolerance to abiotic stress

Climate change and global warming as well environmental pollution is a major irreversible problem faced by our human beings, which result in more serious issues, including drought, salinity and pollution stresses in soil as well high and low temperature. These abiotic stresses significantly impact plant growth and development and become a big challenge for sustainable agricultural production. In the past decade, miRNAs became a new important player in plant response to environmental abiotic stress and in improvement of plant tolerance to these stresses.⁷ Increasing evidence show that a various abiotic stresses induced the aberrant expression of many miRNAs in all tested plant species, including the major agricultural and bioenergy crops, such as cotton,⁸ corn,⁹ wheat⁹⁻¹³ and switchgrass.^{14,15} Manipulating a single miRNA significantly altered plant tolerance to abiotic stresses. Transgenic plant overexpressing miR319 significantly enhanced plant tolerance to drought and salinity stress in creeping bentgrass (*Agrostis stolonifera*).¹⁶ Transgenic tomato with overexpressing miR169 significantly enhanced tomato plant tolerance to drought stress.¹⁷ Studies shows that these miRNAs reduced the water loss by inhibiting stomatal open and increased water retention and cell membrane integrity in transgenic plants.^{16,17} Overexpression of miR319 also enhanced plant tolerance to cold stress in transgenic rice evidenced by high survival rate and increasing cold acclimation.¹⁸ Transgenic plant with overexpression of miR408 enhanced plant tolerance to salinity, cold and oxidative stress, but increased sensitivity to drought and osmotic stress in *Arabidopsis*.¹⁹

Manipulating miRNAs for improving crop tolerance to biotic stress

Biotic stresses, including diseases, nematodes and insects, is also a challenge to almost all crops. Up to now, many miRNAs have been identified, which are responsive to disease and insect invasion. For example, Inal and colleagues (2014) employed microarray to identify a number of miRNAs, including miR169, miR869 and miR2592, with highly aberrant expression during fungi (*Fusarium culmorum* and *Bipolaris sorokiniana*) infection.²⁰ In another study, Wu and colleagues (2014) identified at least 4 miRNAs (miR408, miR829, miR845 and miR811) responsive to Northern leaf blight in corn, which is caused by the fungus *Exserohilum turcicum*.²¹ As time going, more and more studies show that disease pathogens cause the aberrant expression of certain miRNAs in different plant species; these disease-responsive miRNAs target disease-related genes, including LRR and peroxidase. These disease-related miRNAs provide new targets for engineering plants for enhancing plant tolerance to fungal and virus infection.

miRNAs also involve in plant and nematode interaction; to test this, Hewezi and college (2008) examined susceptibilities of *Arabidopsis* dicer-like (dcl) mutant and they found that the dcl mutant reduced the expression of miRNAs and caused *Arabidopsis* more sensitive to nematode infection.²² Their further study suggests that nematode infection caused the aberrant expression of miR396 that acts as a developmental regulator in the reprogramming of roots cells during nematode infection.²³ A recent study also demonstrated that plant resistance to root-knot nematode (RKN) is mediated a miR319/TCP4-mediated gene network, which may be also involved in plant hormone regulation.²⁴ All of these evidences strongly support that nematode infection induces the differential expression of specific miRNAs, which in turn regulates gene expression, allowing plants to acquire tolerance to nematodes. This also provide a new strategy for improving crops for managing nematode damage.

Manipulating miRNAs for altering metabolite biosynthesis

Recent studies demonstrate that miRNAs also play an important role in plant metabolite biosynthesis, which provides a new target for engineering new plant natural products, including plant-generated drugs. For

example, Li and colleagues (2015) identified 4 tobacco-specific miRNAs targeting nicotine biosynthesis pathway; regulating these miRNAs affect nicotine contains in tobacco and further affect tobacco quality.²⁵ Using deep sequencing technology, Boke and colleagues (2015) also identified several miRNAs regulating alkaloid biosynthesis.²⁶ Phytosterols are membrane components or precursors for brassinosteroid²⁷ biosynthesis; a recent study show that miR1848 regulates phytosterol biosynthesis by targeting obtusifoliol 14 α -demethylase gene OsCYP51G3 in rice.²⁸

In summary, miRNAs are becoming new targets for engineering new plant species not only for improving crop yield, quality and tolerance to both biotic and abiotic stress as well for improving plant metabolism pathway for producing compounds for agricultural and biomedical purpose.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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