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AGROFORESTRY BIOTECHNOLOGY FOR SUSTAINABLE AGRICULTURE ON MARGINAL LANDS

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The dramatic increase in the global population combined with rapid industrialization in developing countries has placed great strains on global food and energy supplies. The UN-FAO estimated that the world population will exceed 9.7 billion in 2050. If we use energy and food at the present rate, we will need more than 3.5-times the current energy supply and 1.7-times the current food supply in 2050. To cope with these global crises in food and energy supply as well as environmental problems, the development of new eco-friendly industrial plant varieties for growth on marginal lands is urgently needed to ensure sustainable development. Plant biotechnology can be used as a tool to maximize plant productivity by introducing stress tolerance genes and metabolic genes responsible for increasing yield and improving functions. In the presentation, recent results on transgenic plants (sweetpotato, alfalfa and poplar) with enhanced tolerance to abiotic stresses are introduced. Sweetpotato represents an attractive crop that can be used to help solve the global food and environmental problems as an industrial bioreactor. Alfalfa is one of important legume forage crops on the global marginal lands. Poplar trees provide raw materials, help maintain biodiversity, protect land and water resources, and help mitigate the effect of climate change on global marginal lands.

Keywords: *Sustainable agriculture, agroforestry biotechnology, food security, abiotic stress, sweetpotato, alfalfa, poplar.*

Why biotechnology is essential to cope with three UN environmental conventions?

There are three UN environmental conventions such as Biodiversity, Climate Change and Combating Desertification. For example, the causes of desertification/land degradation derive primarily from human activities such as overgrazing, deforestation and the improper management of soil and water by the local poor people living on marginal land. Thus agroforestry biotechnology will be important to increase local people income and thereby combat desertification/ land degradation in an eco-friendly manner. This concept should be strongly supported as part of the United Nations (UN) "Decade for Deserts and the fight against Desertification" (UNDDD, 2010~2020) and UN Sustainable Development Goals (UN SDGs, 2016~2030).

Our research team is focusing on development of industrial biotech plants (sweetpotato, alfalfa and poplar) for sustainable agriculture on global marginal lands including desertification areas. Industrial biotech plants on marginal lands can produce high value-added biomaterials as well as to cope climate change, biodiversity and combating desertification in a blue ocean manner. Here, our recent results are introduced in terms of possible biotechnological applications for molecular breeding for sustainable development on marginal lands.

Sweetpotato biotechnology

Sweetpotato [*Ipomoea batatas* (L.) Lam] represents an attractive crop that can be used to help solve the world's food and environmental problems in the 21st century; this crop can be used as an industrial bioreactor to produce various high value-added materials, including bio-ethanol, functional feed, and antioxidants, via molecular breeding. Sweetpotato plants have high water use efficiency among starch crops, and help reduce soil erosion. All parts of the sweetpotato plant can be used for human and animal consumption. Due to its rich nutritional content combined with its wide adaptability to marginal lands ranging from tropical to temperate zones, sweetpotato has great potential for preventing malnutrition and increasing food security in developing countries [1].

The nonprofit Center for Science in the Public Interest (CSPI) described sweetpotato as one of ten superfoods for better health, since it contains high levels of low molecular weight antioxidants such as carotenoids and vitamin C, as well as dietary fiber and potassium (2016). Carotenoids benefit human health by acting as dietary antioxidants and alleviating aging-related diseases. Carotenoids also serve as a dietary source of provitamin A, making them essential components of the human diet, since humans are unable to synthesize vitamin A. According to a United States Department of Agriculture (USDA) report, sweetpotato can yield two to three times the level of carbohydrates as field corn, approaching the amount that sugarcane can produce in the US (2008). It would be worthwhile to begin pilot programs to investigate the feasibility of growing sweetpotato for ethanol production on marginal lands. Thus, rational metabolic engineering of low molecular weight antioxidants should contribute to the development of new sweetpotato cultivars with higher levels of nutritional antioxidants and abiotic stress tolerance.

Here, sweetpotato *Orange as* a target gene for regulating carotenoid homeostasis and increasing plant tolerance to environmental stress in marginal lands will be introduced [7]. *Orange* genes, which play a role in carotenoid accumulation, were recently isolated from several plant species, and their functions were intensively investigated. The *Orange* gene (*IbOr*) from sweetpotato helps maintain carotenoid homeostasis to improve plant tolerance to environmental stress. IbOr, a protein with strong holdase chaperone activity, directly interacts with phytoene synthase (IbPSY), a key enzyme involved in carotenoid biosynthesis, in plants under stress conditions, resulting in increased carotenoid accumulation and abiotic stress tolerance (8). In addition, IbOr interacts with the oxygen-evolving enhancer protein 2-1 (PsbP), a member of a protein complex in photosystem II (PSII) that is denatured under heat stress [2]. Transgenic sweetpotato plants overexpressing *IbOr* showed enhanced tolerance to high temperatures (47°C). These findings indicate that IbOr protects plants from environmental stress, not only by controlling carotenoid biosynthesis, but also by directly stabilizing PSII in all plant species [4].

Alfalfa biotechnology

Alfalfa (*Medicago sativa* L.), one of the most important legume forage crops, is widely cultivated throughout the world due to its significant economic value and excellent agricultural traits. As one of the highest-yielding forage crops, alfalfa has outstanding nutritional quality, with high levels of protein, minerals, and vitamins as well as well-balanced amino acids. Moreover, the nitrogen-fixing ability of alfalfa reduces the amount of energy required for its production and improves soil structure. In addition, alfalfa has deeper roots than most crops. This deep, vigorous root system allows alfalfa to adapt to various environmental conditions and increases its capacity to prevent soil erosion. Thus, alfalfa is considered to be a potential forage crop for use in areas subjected to environmental stress such as drought and high salinity. However, the pernicious effects of abiotic stress (such as salt and drought stress) still represent major limits to alfalfa production. To improve the adaptability of alfalfa to these environmental stresses, many studies focused on modifying various aspects of alfalfa plants, such as the activation of cascades of molecular networks involved in stress responses.

In an effort to improve the nutritional quality and environmental stress tolerance of alfalfa, we transferred the *IbOr* gene into alfalfa (cv. Xinjiang Daye) under the control of an oxidative stress-inducible peroxidase (*SWPA2*) promoter through *Agrobacterium tumefaciens*-mediated transformation [5]. Among the 11 transgenic alfalfa lines (referred to as SOR plants), three lines (SOR2, SOR3, and SOR8) selected based on their *IbOr* transcript levels were examined for their tolerance to methyl viologen (MV)-induced oxidative stress in a leaf disc assay. The SOR plants exhibited less damage in response to MV-mediated oxidative stress and salt stress than non-transgenic plants. The SOR plants also exhibited enhanced tolerance to drought stress, along with higher total carotenoid levels. The results suggest that SOR alfalfa plants would be useful as forage crops with improved nutritional value and increased tolerance to multiple abiotic stresses, which would enhance the development of sustainable agriculture on marginal lands [9].

Poplar biotechnology

Trees have great values as a source of essential elements for human living. They have unique characteristics, such as perennial growth, developmental phase changes, secondary growth and metabolism, and trees also exhibit resistance systems to extreme environmental conditions. Among the various tree crops, poplar occupies a prominent place as a model system for functional genomics studies. Poplar has many features that make the species a suitable model for forest biotechnology, such as fast growth, a relatively small genome, ease of vegetative propagation, facile transgenesis and tight coupling between physiological traits and biomass productivity. Of particular importance and convenience is the highly efficient genetic transformation system coupled with efficient regeneration of poplar that is unsurpassed by other tree crops.

Nucleoside diphosphate kinase 2 (NDPK2) is known to regulate the expression of antioxidant genes in plants. Previously, we reported that overexpression of Arabidopsis NDPK2 (AtNDPK2) under the control of SWPA2 promoter in transgenic potato and sweetpotato plants enhanced tolerance to various abiotic stresses [6]. Transgenic poplar (*Populus alba* × *P. glandulosa*) expressing the AtNDPK2 gene under the control of a SWPA2 promoter (referred to as SN) was generated to develop plants with enhanced tolerance to oxidative stress. The level of AtNDPK2 expression and NDPK activity in SN plants following MV treatment was positively correlated with the plant's tolerance to MV-mediated oxidative stress. We also observed that antioxidant enzyme activities such as ascorbate peroxidase, catalase and peroxidase were increased in MV-treated leaf discs of SN plants. The growth of SN plants was substantially increased under field conditions including increased branch number and stem diameter. SN plants exhibited higher transcript levels of the auxin-response genes IAA2 and IAA5. These results suggest that enhanced AtNDPK2 expression affects oxidative stress tolerance leading to improved plant growth in transgenic poplar.

The flowering time regulator GIGANTEA (GI) connects networks involved in developmental stage transitions and environmental stress responses in *Arabidopsis*. However, little is known about the role of GI in growth, development, and responses to environmental challenges in the perennial plant poplar. Here, we identified and functionally characterized three *GI-like* genes (*PagGIa*, *PagGIb*, and *PagGIc)* from poplar [3] *PagGIs* are predominantly nuclear localized and their transcripts are rhythmically expressed, with a peak around zeitgeber time 12 under long-day conditions. Down-regulation of *PagGIs* by RNA interference led to vigorous growth, higher biomass, and enhanced salt stress tolerance in transgenic poplar plants. Taken together, these results indicate that several functions of *Arabidopsis GI* are conserved in its poplar orthologs, and they lay the foundation for developing new approaches to producing salt-tolerant trees for sustainable development on marginal lands worldwide.

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