Enhancing sugarcane growth quality and productivity through a biotechnology approach

Bambang Sugiharto[1,2*]

[1]Center for Development of Advanced Science and Technology (CDAST)
[2]Department of Biology Faculty of Mathematic and Natural Sciences, Jember University. Jl. Kalimantan No. 37 Kampus Tegalboto, Jember, 68121, Indonesia

Abstract. Sugarcane is a major crop to produce sugar accounting for nearly 80% of sugar production worldwide. Sugarcane is well adapted to warm climates and accumulated high biomass quantities for bioelectricity and second-generation bioethanol. Although Indonesia is one among the cane sugar producers, the produced sugar has been unable to meet the national sugar demand. The study of physiology, molecular biology and genetic is providing a major impetus to develop biotechnological strategies for increasing growth and productivity in sugarcane. Genetic transformation method for sugarcane has been established, including *Agrobacterium*-mediated transformation method. The *Agrobacterium*-mediated transformation has been successfully employed to develop transgenic sugarcane. The overexpression of *SoSPS* gene encoding for sucrose-phosphate synthase (SPS) showed the increases of activity and sucrose content in transgenic sugarcane. Furthermore, field evaluation on growth and productivity of the transgenic sugarcane displayed higher tiller number, plant high, cane yield, percentage of Brix and Pol compared to non-transgenic sugarcane. Furthermore, plants are subjected to a variety of abiotic and biotic stresses, which reduces and limits crop productivity. Plants adapt to water stress with various strategies include change in the gene expression and accumulation of organic compounds called compatible solutes. Genetic transformation of *betA* gene encoding for choline dehydrogenase in bacteria elevated glycine-betaine content as an osmoprotectant and resulted in water stress tolerant of transgenic sugarcane. The drought tolerant of sugarcane was already approved and released by Indonesian Government for commercialization. In addition, mosaic virus is one of the most severe diseases in sugarcane and lead to the constant losses in growth and yield of sugarcane. Pathogen-derived resistance (PDR) and RNA interference (RNAi) technologies have been applied to engineered sugarcane cultivar resistant to mosaic virus, and that the RNAi method produced more resistant against the mosaic virus in sugarcane. Finaly, biotechnology approach of genome editing technology should be exploited to ensure higher sugarcane productivity, and improve the livelihoods of smallholder farmers.

* Corresponding author: sugiharto.fmipa@unej.ac.id

1 Introduction

Sugarcane (*Saccharum spp*) is an industrial crop for sugar production and generates three key by-products, that are bagasse, molasses and filter muds. These products are commonly used for power generation, bioethanol fermentation, papermaking, livestock feed, organic fertilizer, and various cellulose-based industry. Sugarcane production in Indonesia is mainly located on Java Island, Southern Sumatra, and other spotted regions, such as South Sulawesi and West Nusa Tenggara. However, the sugar production has been deteriorating over the years and not met to local consumption in Indonesia. The majority of the problem is associated with the low sugar productivity of sugarcane cultivars.

Sugarcane variety development can be gained by conventional breeding or biotechnological approaches. The complex sugarcane genome, with high polyploidy-aneuploidy chromosomes and poor seed fertility, is the bottlenecks for generating new cultivars using the breeding technique. Introducing desirable traits into sugarcane cultivars by cross-breeding needs laborious work and take time that require a very long breeding duration. However, rapid progress in molecular biology and biotechnology tools have led to the advanced breeding approach. Advanced breeding approaches such as gene overexpression, RNA interference and genome editing have accelerated the breeding program and allowed the development of a new sugarcane cultivars with specific traits. These biotechnological breeding approach have been applied in developing a new sugarcane cultivar.

Sucrose is the end product of photosynthetic carbon assimilation, primary sugar transported in phloem and stored in parenchyma cell of sugarcane stem. Sucrose-phosphate synthase is believed a key enzyme for sucrose biosynthesis during photosynthesis in plant. Some studies demonstrated that overexpression of the gene for SPS enhanced sucrose content and growth rate in transgenic plants. This approach has been applied to enhance sugar content and yield in transgenic sugarcane [1, 2].

Sugarcane growth is significantly affected by various abiotic stresses such as drought stress, temperature elevation, and low soil fertility. Among of them drought stress is the main factor affecting plants growth and productivity. Plants have various strategy to survive under water stress by alteration in gene expression and production of compatible solutes to protect plants from cell damage. The overexpression of transcription factor (TFs) such as DREB gene family and engineering of the level of compatible solutes have been used to produce drought-stress tolerant plants. Furthermore, biotic stress is also another factor that determined plant productivity. Many pathogens have been known cause diseases in sugarcane, including bacteria, fungi, nematodes, and viruses. Mosaic disease is one of the most important sugarcane diseases and the infection leads to reduction of chlorophyll content and photosynthetic activity, subsequent reduction of the productivity. Pathogen-derived resistance (PDR) and RNA interference (RNAi) technologies have been applied to engineered sugarcane cultivar resistant to mosaic virus [3]. This review describes our recent research related to following scopes: (1) *Agrobacterium*-mediated transformation in sugarcane; (2) enhancing sucrose synthesis and cane yield; (3) drought-tolerant; and (4) mosaic virus-resistant. Finally, the future direction of genome editing in sugarcane is discussed.

2 *Agrobacterium*-mediated transformation in sugarcane

Technology for DNA transformation serves as a useful and practical tool to introduce a particular trait or DNA for plant improvement. Several DNA transformation methods have been attempted for introducing DNA into plant cell such as direct and indirect transformation. However, indirect transformation using *Agrobacterium tumefaciens* offer some advantages

and considered more efficient. Although the *Agrobacterium*-mediated method has been applied to monocot plants, the lack of reproducible results has been an obstacle to establish effective transformation protocol in sugarcane.

It was reported that *Agrobacterium*-mediated transformation using explants axillary bud from field growth sugarcane achieved a high transformation efficiency. However, the use of axillary buds is difficult avoid bacterial contamination and need numerous axillary buds. Micropropagation of the apical meristem to generate *in vitro* sugarcane was successfully applied for sugarcane transformation, instead of using the callus explants. Basal segment of the *in vitro* sugarcane shoots was excised, injured, and used for the transformation in the presence of acetosyringone. The basal sugarcane shoots vastly regenerated to new shoots, and produced transgenic sugarcane within 4 months [4]. This method has provided an effective *Agrobacterium*-mediated transformation and used routinely sugarcane transformation.

3 Enhancing sucrose synthesis and yield in sugarcane

Sucrose is the end product of photosynthetic carbon assimilation, and sucrose-phosphate synthase (SPS) is a key enzyme for sucrose synthesis in plants. The important role of SPS has encouraged to clone the gene from various plants, including from sugarcane. Overexpression of sugarcane *SoSPS* gene encoding for sugarcane SPS resulted in a significant increase sucrose content, plant height, and stem number in transgenic sugarcane. However, the increase was followed by increasing activity of soluble acid invertase and the level of glucose and fructose resulted from sucrose degradation [1]. These results support a model in which the sucrose-cleaving enzymes such as invertase play a pivotal role in maintaining the balance between sucrose signalling and metabolism. Sugar-related metabolism is linked to plant development, and the abundance of hexose induces cell division and expansion. In fact, the overexpression of SPS has been reported to increase growth and biomass accumulation in overexpression have also transgenic *Brachypodium distachyon* [5].

The increase sucrose content and growth of transgenic sugarcane overexpressing of *SoSPS* gene encourage to have field experiment in natural environment. The experiment was directed to evaluate the growth and yield, and to assess environmental risks of the transgenic sugarcane under supervision of the Indonesian Biosafety Commission. The transgenic sugarcane lines showed higher tiller number and plant height than the non-transgenic counterpart. The transgenic sugarcane lines have also significantly higher on cane yield, and percentage Brix and Pol. Furthermore, the alteration of sucrose metabolism in transgenic sugarcane did not affect on soil bacterial as well as insect biodiversity [2]. Although the transgenic sugarcane lines have not been approved officially by Indonesian Biosafety Commission, food safety assessments have shown no potential risk of allergenicity and toxicity in this transgenic sugarcane [6], and the nutrition and mineral composition were substantially equivalent to the non-transgenic sugarcane [7].

4 Drought-tolerant sugarcane

Water availability is considered the most critical environment that effects on plant growth and development. Water supply enhances rapid growth and stem elongation, but limited water availability stacks the growth and seriously reduces sugar productivity in sugarcane. Moreover, climate change induces water scarcity, which affects the displacement of sugarcane growing areas into non-irrigated marginal lands. Therefore, most studies on sugarcane have focused on water stress mechanisms and their implications for the development of biotechnological solutions.

Water stress alters physiological and biochemical characters in plants, including accumulation of secondary metabolites called compatible solutes. The metabolites act as osmoprotectant to protect the damage of membrane cell and maintain osmotic potential during drought stress. Glycine betaine (GB) is one of the secondary metabolites that play important role to protect plant cell under drought stress condition. This GB has been engineered in plant to enhance tolerance under salt and drought stress. The GB is synthesis from choline by action of choline dehydrogenase (CDH) or choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH) in bacteria and plant cells. Introduction of bacterial *betA* gene for CDH increased GB level, since the enzyme is capable to convert choline into BG. Overexpression of the *betA* gene encoding for CDH enhanced GB accumulation and resulted in drought and salt of transgenic tobacco and maize. Therefore, the *betA* gene from *Rhizobium meliloti* was constructed into a binary vector under strong promoter CaMV35S (Australian Patent Office, Patent No. 737600 – Inventor(s); Naoki Katsurada, Tsushi Hayakawa, Haruhumi Miwa), and introduced into sugarcane. The *Agrobacterium*-mediated transformation was conducted using explant BL sugarcane cultivars by PT Perkebunan Nusantara XI Indonesia in collaboration with Ajinomoto company and University of Jember. The GB content was sharply increased (180-880 ppm), but almost not detected in non-transgenic counterpart. Morphology observation showed that transgenic sugarcane lines display stay-green and have longer root architecture under drought stress compared to the wildtype [8]. Moreover, the transgenic lines significantly increased cane yields in under drought stress of non-irrigated soils. After completion of Biosafety Assessments and received certification for environment, food, and feed safeties, the transgenic sugarcane was released by Indonesian government, named drought-tolerant sugarcane NXI-4T cultivars.

5 Mosaic virus resistant sugarcane

Mosaic is one of the most important sugarcane diseases caused at least by three viruses, sugarcane mosaic virus (SCMV), sugarcane streak mosaic virus (SCSMV), and sorghum mosaic virus (SrMV). The infection leads to the manifestation of mosaic-like symptoms, which develop as irregular patterns of light and dark green or yellow patches or streaks. Observation of the mosaic virus infection showed that SCMV was one of the most destructive viruses in field sugarcane, although SCSMV was considered more widely spread in the Indonesia sugarcane plantation. Recently, the mixed infection with two or more viruses were found in Indonesia and significantly reduced chlorophyl content and photosynthetic capacity [9].

Genetic engineering is considered as the most effective to illuminate the virus infection compared to the use of pesticides. The gene encoding for coat protein (CP) virus is widely used to induce resistance against virus infection in plants. Expression of *Cp* gene to induce the resistance based on protein, referred as pathogen-derived resistance (PDR) or RNA silencing called RNA interference (RNAi) have been applied in sugarcane. Overexpression of *Cp* gene increased the RNA transcripts and CP protein level in transgenic sugarcane, and that the increases resulted in resistance against SCMV infection. Although, the mechanism by which is not well known, overexpression of full-length *Cp* gene is required for the induction of resistance [10]. In addition, the RNAi is gene regulatory mechanism that limits gene expression by employing a complex of small interfering RNA (siRNA) and a sequence-specific RNA degradation process. The *Cp* gene has been constructed into a vector for silencing of SCMV transcript with sense and antisense orientation, and introduced into sugarcane. The RNAi method is successfully inducing resistance to SCMV infection in transgenic sugarcane [11]. Comparison of these methods revealed that the RNA method effectively produces more resistances against the SCMV infection in sugarcane compared to

the PDR approach [12]. Therefore, we propose the RNAi approach to induce dual resistance against SCMV and SCSMV infection in sugarcane for near future experiment.

6 Future prospects

Biotechnology approach has become an effective method for developing new sugarcane cultivars. The efficient *Agrobacterium*-mediated transformation has been established in sugarcane, and that complete sugarcane genome sequence has been also assembled. Therefore, the rapid progress in breeding approaches such as genome editing be able to accelerate for development of sugarcane cultivars with beneficial traits. The genome editing technology, which enable targeted precise changes to genomes, can improve a wide range of crop plants. The genome editing method has been successfully applied in rice, maize and other crops. However, the ploidy level of the sugarcane genome is an obstacle, and the challenge in sugarcane genome editing should be elaborated. Trial experiment has been conducted in sugarcane to engineered DREB (dehydration responsive element binding) gene encoding for transcription factor protein that activate transcription of genes responsible for drought stress tolerances. The CRISPR/Cas12 guide RNA targeting for deletion and insertion of a region between amino acid residues 136 and 165 have been successfully introduced in the sugarcane genome, but proper mutation was not found in the targeted nucleotide sequences. Reducing of sugarcane production and climate changes adversely impact sugar production and farmer income. The development of biotechnology on genome editing technology should be exploited to ensure higher sugarcane productivity and improve the livelihoods of smallholder farmers.

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