PRE-BREEDING AND GENE DISCOVERY
FOR FOOD AND RENEWABLE ENERGY SECURITY

Plant breeding has been significantly contributed to agricultural productivity through developing new superior crop varieties. The success of plant breeding depend, among others, on the availability of genetic resources, discoveries of genes that control important traits within such genetic resources and pre-breeding. Pre-breeding refers to all activities designed to identify desirable characteristics and/or genes from unadapted materials that cannot be used directly in breeding populations and to transfer these traits to an intermediate set of materials that breeders can use further in producing new varieties for farmers. It is a necessary first step in the use of diversity arising from wild relatives and other unimproved materials.

In additon to crop improvements, elucidating important genes in microbes is the key aspect for further application in industrial agriculture, such as biofuel, bioactive compounds, enzyme production, or as a gene source for biotechnology process such as marker genes or in developing superior plant such as high yield, resistant to pest and diseases, and adapting in marginal climate such as dry, submerged, saline, high temperature etc. Genome sequences of potential microbes using NGS technology reveal potential genes for industrial and plant engineering purposes.

This book contains selected papers on pre-breeding and discoveries in plants and microbes presented at the International Conference on Pre-breeding and Gene Discovery (ICPGD), held in Bogor, Indonesia, August 13–15, 2014. It consists of four chapters providing current status of pre-breeding and gene discovery in plant and microbes in Indonesia and some other countries. Chapter 1 provides a policy and supporting activities to pre-breeding and gene discoveries, which includes genetic resources management, public-private partnership and program on genetic resources utilization, in particular through the application of advance techniques. Chapter 2 consists of six papers related to pre-breeding and gene discoveries in plant. The content of this chapter is primarily on the emerging concept and understanding of advanced technology of pre-breeding and gene discovery in plants, such as the progress of genome sequences and molecular markers development and their application in plant breeding program, and strategies for gene discoveries as important for sustainable agriculture. Chapter 3 consists of six papers related to gene discoveries in microbes, primarily addressed current status of gene discovery in microbes in Indonesia, strategies of gene discovery for renewable energy development and crops productivity, and environmental metagenomic and microbial genomics. Annex contains selected abstract of papers presented at the conference in order to enrich readers' information on the topics.

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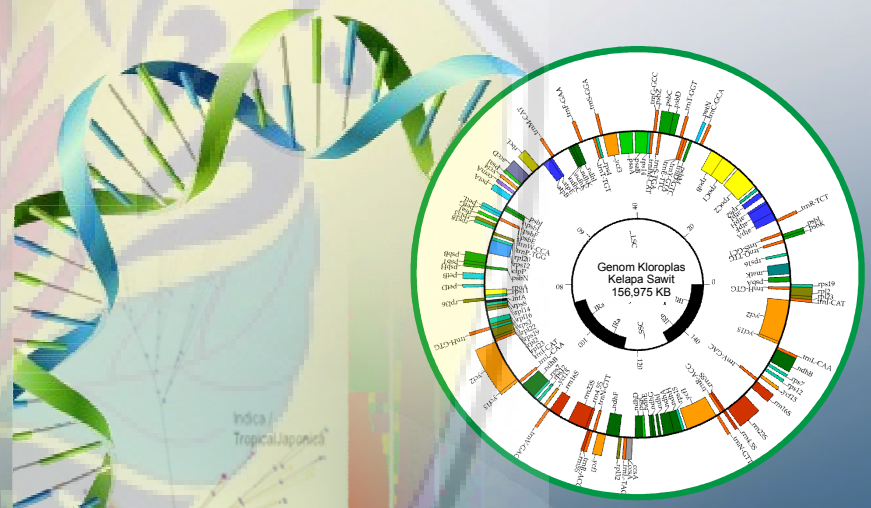

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PRE-BREEDING AND GENE DISCOVERY FOR FOOD AND RENEWABLE ENERGY SECURITY

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FOREWORD

Agricultural development in Indonesia is still faced with many challenges: most farmers are poorly resourced with land-ownership less than 0.5 ha and lives in poverty at rural areas; the production of some basic food sources barely keep pace with the rapid population growth; the unpredictable climate change threatens the effort to increase food production and might endanger food security. Fortunately, Indonesia is rich in biodiversity, which can be exploited to fulfill its necessity.

For the next 30 years, Indonesia gradually focuses its agricultural development toward a sustainable bio-industry agriculture system which treats agricultural land and other production factors as an industrial unit to produce main products for food security and other products for energy security and industry based on zero-waste principle, i.e. reduce, reuse and recycle. As a tropical country, Indonesia has the advantage of having high opportunity to harvest solar energy and transform it to biomass as the basis of bio-industry agriculture.

In order to face the above challenges and make use of those advantages, we need to optimally and sustainably use our rich genetic resources through pre-breeding and gene discovery. Our experties in this area are limited, not to mention the multidisciplinary nature of this subject. This calls for international collaboration among scientists of different background.

As the largest research organization in the country, particularly in agriculture and related sciences, Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, is now entering the second phase of its development by strengthening international networks and applications of advance sciences, such as biotechnology, bioinformatics, and bioprocesses. The shift in our prioritizing is reflected in recent investment, particularly in human resources and facilities, and expressed in our tagline: *Science*, *Innovation*, *Networks*. We took this big step in order to set the foundation for our research system to meet the challenges of agriculture in the coming years.

It is my sincere hope that this book will provide valuable information for agricultural scientists, plant breeders, gene bank managers, and research managers as well as policy makers in expanding their horizon in agriculture development, and trigger new bright ideas.

Dr. Muhammad Syakir
Director General of IAARD

PREFACE

This book contains selected papers presented at the International Conference on Pre-breeding and Gene Discovery for Food and Renewable Energy Security (ICPGD), held in Bogor, Indonesia, August 13–15, 2014, at the occasion of the 40th commemoration of the Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture. It consists of four chapters providing current status of pre-breeding and gene discovery in plant and microbes in Indonesia and participating countries in the ICPGD 2014.

Chapter 1 provides a policy and supporting activities to pre-breeding and gene discoveries, which include genetic resources management, public-private partnership, and program on genetic resources utilization, in particular, through the application of advance techniques. Chapter 2 consists of six papers related to pre-breeding and gene discoveries in plant. The content of this chapter is primarily on the emerging concept and understanding of advanced technology of pre-breeding and gene discovery in plants, such as the progress of genome sequences and molecular markers development and their application in plant breeding program, and strategies for gene discoveries which are important for sustainable agriculture. Chapter 3 consists of six papers related to gene discoveries in microbes, primarily addressed current status of gene discovery in microbes in Indonesia, strategies of gene discovery for renewable energy development and crops productivity, and environmental metagenomic and microbial genomics. In addition, Annex contains selected abstract of papers presented at the conference in order to enrich readers' information on the topics.

We wish to thank many parties that have contributed to the preparation of this book, particularly the Director General of IAARD and the Director of ICABIOGRAD that has provided us with tasks and resources for editing and printing. We wish all readers to enjoy and take advantages of this book.

Thank you.

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DISCOVERY OF SUCROSE METABOLIZING AND RELATED GENES TO ENHANCE SUGARCANE PRODUCTIVITY

Bambang Sugiharto

ABSTRACT

Phosynthetic carbon asimilation is a major determinant that limits growth and productivity in plants. Study on the activities of the carbon assimilating enzymes revealed that among the enzymes activity of sucrose-phosphate synthase (SPS, EC. 22.4.1.14) is fluctuated in parallel with sucrose content and growth of sugarcane. Cloning of the genes encoding for SPS found the presence of two cDNA clones, *SoSPS1* and *SoSPS2*, in sugarcane and the transcript of *SoSPS1* to be predominant in leaves, but that of *SoSPS2* to be distributed conservatively in all tissues. To increase sucrose accumulation, the *SoSPS1* gene was overexpressed in transgenic tomato and sugarcane. As the consequences, the activity of SPS and sucrose content was significantly increased in leaves of the transgenic plants, but not concomitant followed by significant increase of sucrose contents in the sink tissues of both transgenic plants. This discrepancy of sucrose accumulation might because have not accompanied by sucrose loading mechanism between leave as a source and sink tissue. Thus, the *SoSUT1*-cDNA encoding for sucrose transporter protein was cloned from sugarcane and double overexpression of *SoSPS1* and *SoSUT1* increased sharply sucrose content in sugarcane stem and fruit production in tomato. With regard to sucrose content, it is well reported that sucrose acts as a potent osmoprotectant that might induce drought stress tolerant. Whether the increased of sucrose content in transgenic sugarcane induce the drought tolerant is still remain to be elucidated. In addition, identification of a gene responsible for drought tolerance found a *SoDip22*-cDNA encoding for a hydrophilic protein with molecular mass of 15.9 kDa and the function might to adapt to drought stress. However, the drought stress tolerant sugarcane recently was achieved by genetic engineering of glycine betaine content.

Keywords: sucrose-phosphate synthase, sucrose transporter protein, sucrose, genetic engineering, sugarcane.

INTRODUCTION

Photosynthesis involves many processes, starting for harvesting the light energy from sun in photochemistry to CO_2 fixation and the subsequent carbon metabolism. In C4 plants like sugarcane, the major carbon assimilating enzymes are phosphoenolpyruvate carboxylase (PEPC), pyruvate orthophosphate dikinase (PPDK), and ribulose-1,5-bisphosphate carboxylase/ oxygenase (Rubisco). These enzymes can be considered as essensial limiting growth and productivity in sugarcane. However, carbon partitioning into sucrose, a major carbon mobile from source to sink tissue, plays a pivotal role in governing the plant productivity.

Sucrose and starch are primary products of the photosynthetic carbon assimilation in plants. The starch is synthesized in chloroplast and serves mainly as an intermediate deposit for photoassimilate, whereas sucrose is a carbon mobile synthesized in cytosol and plays a central role in exporting photoassimilates throughout the plant cells. Most of the photosynthetically assimilated carbon can be allocated, either to sucrose or to starch depending on several factors. This partitioning is an important to plant growth since sucrose synthesis is a prime determinant of carbon export from photosynthetically active cells (Gifford *et al.*, 1984).

Sucrose-phosphate synthase (SPS) is believed to be the key enzyme controlling biosynthesis of sucrose in plants (Huber and Huber, 1996). The SPS catalyzes a formation of sucrose-6 phosphate from fructose-6 phosphate and UDP-glucose in cytosol, then is converted into sucrose and is subjected for export from source to sink tissue. The activity of SPS can be a limiting factor for *de novo* sucrose synthesis in plants. Studies in sugarcane leave showed that SPS activity determines sucrose production and the growth rate (Sugiharto *et al*., 2005).

In most plants, sucrose synthesized in leaves is transported long distance in the vein to support the growth and development of sink tissue, such as roots, flowers, fruits, and seed. The transportation of sucrose is facilitated by sucrose-transporter proteins (SUT) which have an important role in symplasmic and apoplastic phloem sucrose loading mechanism. Many studies have indicated that reduction of the expression of sucrose transporter has deleterious effects on plant growth and development and enhancing the expression increased growth and development of plant (Kuhn and Grof, 2010).

GENETIC ENGINEERING OF SUCROSE METABOLIZING GENES

Recently, molecular tool have become available allowing for a more precise *in vivo* manipulation of postulated biochemical pathway. Genes encoding of the enzymes involved in biochemical pathway can be used either to inhibit or enhance of the enzymatic activities. Thus, it permits to change metabolism activity and increase growth and productivity of plants.

Genes encoding for SPS have been isolated from many plants, including from sugarcane (Sugiharto *et al*., 1997). We had identified the presence of two genes encoding for SPS protein, *SoSPS1* and *SoSPS2*, encoded for photosynthetic active SPS1 and constitutive SPS2 proteins, respectively. It was reported that overexpression of gene encoding for maize SPS elevated SPS activity and increased fruit sugar contents in transgenic tomato (Laporte *et al*., 1997). Moreover, the overexpression of *Arabidopsis* SPS gene elevated

sucrose pool in sink tissue and significantly increased stem height and dry biomass to the control in transgenic cotton (Park *et al*., 2008). We had also demonstrated that overexpression of sugarcane *SoSPS1* gene increased sugar content in transgenic tomato (Dewanti *et al*., unpublished results) and sugarcane (Miswar *et al*., 2007).

Sucrose synthesized in leaves as the source tissue is translocated to sink tissue and that the translocation is facilitated by SUT. There is a gene family encoding for SUT protein and among them SUT1 protein has a high affinity for sucrose translocation (Kuhn and Grof, 2010). Although many studies have indicated that reduction of expression of sucrose transporter genes have deleterious effects on plant growth and development, enhancing the expression of SUT1 protein increase sugar content in transgenic potato (Leggewie *et al*., 2003) and growth rate of pea cotyledon (Rosche *et al*., 2002). Thus, cloning of genes encoding for sucrose transporter were successfully conducted from sugarcane (Sugiharto, unpublished results) and that the overexpression of the gene increased sucrose content in transgenic sugarcane stem (Harjo, unpublished results).

The SPS is a key enzyme for sucrose synthesis in leave and the SUT is a responsible protein for the sucrose translocation from leave to the sink tissue. Thus, overexpression of the genes for SPS and SUT will enhance sucrose synthesis and translocation that lead to more significant sucrose content in sink tissue. We have found that double overexpression for the genes encoding for sugarcane SPS1 and SUT1 proteins significantly increase fruit production in transgenic tomato and sugar content in stem of transgenic sugarcane. This trait will be considered as an important target to increase growth and production in plants.

INTER-RELATION BETWEEN SUCROSE ACCUMULATION AND DROUGHT-TOLERANCE

Plants accumulate a set of proteins and low molecular weight compounds called compatible solutes under stress conditions. Compatible solutes are compounds that accumulate in stress-tolerant plant under water stress. They are water-soluble and do not disturb plant cell metabolism, such as sugars, amino acids, and polyols. They are involved in osmoregulation and stabilization of protein structure to maintain protein stability during water stress. Thus, introduction of the genes for biosynthesis of compatible solutes might useful to improve water tolerance of plants.

We have developed transgenic sugarcane either overexpression of the genes for sucrose biosynthesis or sucrose transporter. Overexpression of the

genes increased sucrose contents and that of double overexpression of the genes for sucrose synthesis and sucrose transporter increased more pronounced sucrose contents in stem of sugarcane. Thus, it is important to hypothesize that the increase of sucrose content due to the overexpression of those genes might lead to increase water tolerance in sugarcane.

Sucrose accumulation is a complex process and the protection against any stress caused by sucrose accumulation appears to use different mechanism to those used to protect from stress induced by water stress (Iskandar *et al.*, 2011). Molecular studies during water stress in sugarcane revealed that the expression of drought-inducible gene named *SoDip22* encoded for a small peptide is increased (Sugiharto *et al.*, 2002). This finding suggests that the protein functions to adapt to drought stress in leaves tissue, but detail study on this protein remains to be elucidated. Recently, drought-tolerant sugarcane has been developed by transformation of *betA* gene encoding for choline dehydrogenase from *Rhizobium meliloti*. The transformation resulted in increased glycine betaine contents that act as osmoprotectant and protect sugarcane against drought-stress. However, characterization of sucrose accumulation and water stress tolerance in sugarcane is still an important research topic to be elucidated in near future.

CONCLUDING REMARKS

Genetic engineering of sucrose metabolizing genes have achieved by cloning gene of SPS and SUT. Cloning have been done in the genes encoding for SPS present in two cDNA clones, *SoSPS1* and *SoSPS2* as well as *SUT1* in sugarcane and overexpressed. Overexpression of the genes for SPS and SUT will enhance sucrose synthesis and translocation in sink tissue, while the double overexpression for the genes significantly increase fruit production in transgenic tomato and sugar content in stem of transgenic sugarcane. The increase of sucrose content due to the overexpression of those genes might lead to increase water tolerance in sugarcane. A number of findings of molecular studies in respect to sucrose accumulation to protect water/drought stress were reported. However, characterization of sucrose accumulation and water stress tolerance in sugarcane need to be further elucidated in near future.

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