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Genome Engineering for Crop Improvement

Concepts and Strategies in Plant Sciences

Series Editor

Chittaranjan Kole, Raja Ramanna Fellow, Government of India,
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Bidyut Kumar Sarmah · Basanta Kumar Borah
Editors

Genome Engineering for Crop Improvement

Editors
Bidyut Kumar Sarmah
ICAR-National Professor Programme
(Norman Borlaug Chair) and DBT-North
East Centre for Agricultural Biotechnology
Assam Agricultural University
Jorhat, Assam, India

Basanta Kumar Borah
Department of Agricultural Biotechnology
and ICAR-National Professor Programme
(Norman Borlaug Chair)
Assam Agricultural University
Jorhat, Assam, India

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Preface

The book *Genome Engineering for Crop Improvement* presents the application of non-conventional biotechnological approaches for the improvement of crop plants by modifying their genomes. It is a collaboration focussed on confronting the prime challenges in agriculture using genome engineering. The ten chapters in the book have been written with a vision to maximize the understanding of novel approaches used in the modification of plant genomes in order to address the most harrowing biotic and abiotic threats to modern agriculture.

Chapter 1 illustrates photo-assimilation as key to plant yield and productivity. The significance of photo-assimilation processes or primary carbon metabolism in source and sink organs, with special emphasis on starch metabolism, is discussed. In this regard, it is possible to edit genomes for synergistic enhancement of source and sink processes towards maximizing crop productivity.

A major goal of modern agriculture is manifestation of disease resistance. Resistance to bacterial, viral or fungal diseases was traditionally attained principally by chemical applications ensuing both human and environmental risks. With the advent of genome engineering tools, RNA silencing (RNA interference, RNAi) offers a safer alternative to precisely generate desired modifications. The basics of RNA interference pathways, its status vis-à-vis conventional insertion mutagenesis and generation of stably inherited phenotypes with special emphasis on wheat functional genomics is discussed in Chap. 2 of the book. Thereafter, we have highlighted RNA interference for conferring virus resistance in rice in Chap. 3. The chapter enumerates the progress of RNAi technology against ten of sixteen viruses known to infect rice plants. In Chap. 4, deployment of RNA silencing for control of fungal disease in plants with emphasis on host-induced gene silencing (HIGS) has been discussed.

Viruses are one of the most potent threats to crop productivity. We have specially dedicated Chap. 5 to enlighten the readers on the intricate mechanisms that make a plant resistant or vulnerable to viral attacks. These mechanisms provide hints to develop antiviral resistance in hosts. The chapter explains about engineering gene silencing-mediated resistance against plant viruses which may be achieved transcriptionally or post-transcriptionally. In addition, an epigenetic perspective has also been detailed. Other novel transgenic approaches like genome

editing, protein/peptide-mediated virus resistance including plantibodies and aptamers, etc., have been covered as well in the chapter.

The precise modern genome editing tool that emerged as a result of studying the bacterial immune response against viruses was the CRISPR-Cas machinery. Chapter 6 deals with the evolution and emergence of CRISPR-Cas technology as one of the most useful genome engineering tools. The technology has been utilized in the management of abiotic and biotic stress in plants which has been discussed in Chap. 7. The chapter presents the current regulations, future prospects and the usability of the machinery with regard to developing biotic and abiotic stress resistance in major crop plants.

Food security for the ever-growing population is the prime goal of today's agriculture. Rice and wheat still stand as the main food crops, and therefore, the challenges in their production have a direct bearing on global food security. Genome engineering is being widely applied for their improvement. We have accounted the biotic stresses in rice to be from major bacterial, viral, fungal and animate (insect and nematodes) sources. The status of genetic engineering in the development of resistance to such pests and pathogens has been elaborated in Chap. 8. So far as wheat is concerned, a major challenge in production is rust. Till now, resistance to rust has largely been achieved by identification of new sources of resistance from cultivated wheat and related wild species, mapping the traits and their transfer to popular wheat cultivars. Chapter 9 explores the recent advances in genomics and marker technologies and the possibility of conferring rust resistance to commercial wheat cultivars in a quick and precise manner using the technologies.

Besides modifying the genome using transgenesis, CRISPR-Cas and RNAi technologies, the book also highlights the potential within the gene pool of crops for traits such as stress-tolerance, disease resistance. Chapter 10 reviews the potential of cisgenesis, its applications, limitations, regulatory concerns and strategies to maximize its applicability in the improvement of crops. Cisgenesis is a promising technology, in this regard, if a desired gene is available within the gene pool of a crop.

In summary, the book aims to illuminate the potential, challenges and prospects of genome engineering in the improvement of major cultivated crops, bearing in mind the global goals for agriculture and food security. The editors wish to thank each and every contributor who have graciously accepted the invitation and contributed to this book. The editors also thank immensely Springer Nature for publishing this book.

Jorhat, India

Bidyut Kumar Sarmah
Basanta Kumar Borah

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Contributors

Sumita Acharjee Department of Agricultural Biotechnology and ICAR-National Professor Programme (Norman Borlaug Chair), Assam Agricultural University, Jorhat, India

Kirti Arora ICAR-National Institute for Plant Biotechnology, New Delhi, India

Anirban Bhar Division of Plant Biology, Bose Institute Centenary Campus, CIT Scheme, VII-M, Kankurgachi, Kolkata, West Bengal, India;
Department of Botany, Ramakrishna Mission Vivekananda Centenary College, Rahara, Kolkata, India

Basanta Kumar Borah Department of Agricultural Biotechnology and ICAR-National Professor Programme (Norman Borlaug Chair), Assam Agricultural University, Jorhat, India

Sambhavana Chauhan Department of Genetics, University of Delhi South Campus, New Delhi, India

Sampa Das Division of Plant Biology, Bose Institute Centenary Campus, CIT Scheme, VII-M, Kankurgachi, Kolkata, West Bengal, India

Indranil Dasgupta Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi, India

Moloya Gohain DBT-North East Centre for Agricultural Biotechnology, Assam Agricultural University, Jorhat, India

Dinesh Gupta Bioinformatics Group, ICGEB, New Delhi, India

Seon-Kap Hwang Institute of Biological Chemistry, Washington State University, Pullman, WA, USA

A. Abdul Kader Jailani Division of Plant Pathology, IARI, New Delhi, India

Akansha Jain Division of Plant Biology, Bose Institute Centenary Campus, CIT Scheme, VII-M, Kankurgachi, Kolkata, West Bengal, India

Suhas G. Karkute ICAR-National Institute for Plant Biotechnology, New Delhi, India

Kanti Kiran ICAR-National Institute for Plant Biotechnology, New Delhi, India

Kaan Koper Institute of Biological Chemistry, Washington State University, Pullman, WA, USA

Gaurav Kumar Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi, India

Charu Lata National Institute of Science Communication and Information Resources, New Delhi, India

Rohit Mago Agriculture and Food, Commonwealth Scientific and Industrial Research Organisation (CSIRO), Canberra, ACT, Australia

Tapan Kumar Mondal ICAR-National Institute for Plant Biotechnology, New Delhi, India

Sunil Kumar Mukherjee Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Thomas W. Okita Institute of Biological Chemistry, Washington State University, Pullman, WA, USA

Manchikatla V. Rajam Department of Genetics, University of Delhi South Campus, New Delhi, India

Anirban Roy Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Bidyut Kumar Sarmah ICAR-National Professor Programme (Norman Borlaug Chair) and DBT-North East Centre for Agricultural Biotechnology, Assam Agricultural University, Jorhat, India

Priyabrata Sen Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat, India

Aditi Singh Bioinformatics Group, ICGEB, New Delhi, India

Salvinder Singh Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat, Assam, India

Amolkumar U. Solanke ICAR-National Institute for Plant Biotechnology, New Delhi, India

Ram Sevak Singh Tomar ICAR-National Institute for Plant Biotechnology, New Delhi, India

Andreas E. Voloudakis Laboratory of Plant Breeding and Biometry, Department of Crop Science, Agricultural University of Athens, Athens, Greece

Abbreviations

3PGA	3-Phosphoglyceric acid
6PGDH	6-phosphogluconate dehydrogenase
ABA	Absicisic acid
ACL	Acyl carrier protein
AFLP	Amplified Fragment Length Polymorphism
AGO	Argonaute
AGO1	Argonaute 1
AGPase	ADP-glucose pyrophosphorylase
AgRenSeq	Association Genetics with R gene enrichment sequencing
AMCV	*Artichoke mottled crinkle virus*
amiRNA	Artificial miRNA
amiRNAs	Artificial microRNAs
AMT	Aminotransferase
APHIS	Animal and Plant Health Inspection Service
atasiRNAs	Artificial tasiRNAs
ATP	Adenosine triphosphate
Avr	Avirulence
BAC	Bacterial Artificial Chromosome
BaMMV	Barley mild mosaic virus
BCAT	Branched-Chain Amino Acid Aminotransferase
BDL	Baodali
BeYDV	Bean yellow dwarf virus
BIN2	Brassinosteroid Insensitive 2
bp	Base pair
BPH	Brown Planthopper
BR	Brassinosteroid
BSA	Bulked Segregant Analysis
BT1	Plastidial ADP-glucose transporter
b-ZIP	Basic leucine zipper
CaMV	Cauliflower mosaic virus
Cas	CRISPR associated protein
Cas 9	CRISPR associated protein 9

CBL	Calcineurin B-like proteins
CCP	Capsid core protein
CE	Capping Enzyme
CI	Cytoplasmic Inclusion
CK	Cytokinin
CLCuMuV	Cotton leaf curl Multan virus
CMV	Cucumber Mosaic Virus
COMT	Caffeoyl-CoA 3-O-methyltransferase
CP	Coat Protein
CPMR	Coat Protein-Mediated Resistance
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CRISPR/Cas9	Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9
CS	Capsaicinoid synthase
CUL	Cullin-RING Ligases
DArT	Diversity Arrays Technology
DArtSeq	Diversity Arrays Technology Sequencing
DCL	Dicer like
DCL 1	Dicer like 1
DEFp	Polyprotein
DEP1	Dense and erect panicle 1
dsRNA	Double stranded RNA
DST	Drought and salt tolerance
ECIP1	Ethylene insensitive 2 (EIN2) interacting protein 1
eGMO	Epigenetically modified crops
eIF	Eukaryotic (translation) initiation factor
eIF4E	Eukaryotic translation initiation factor 4E
EIN2	Ethylene-insensitive protein 2
EMS	Ethyl Methanesulfonate
EST	Expressed Sequenced Tag
ETI	Effector-Triggered Plant Immunity
Exp2	Expansin gene
Fab	Fragment antigen binding
FaTA	acylACP thioesterase
FBPA	Fructose 1,6-bisphosphate aldolase
FDA	US Food and Drug Administration
FSANZ	Food Standards Australia New Zealand
GBSS	Granule-Bound Starch Synthase
GDC-H	Glycine decarboxylase-H protein
GE	Genome editing
GES	Genome Editing Systems
GFP	Green fluorescent protein
Glc1P	Glucose-1-phosphate
GlcDH	Glycolate dehydrogenase
GLH	Green leafhopper

glp	Glucagon-like peptide
GM	Genetically Modified
GMO	Genetically Modified Organism
GPT	Glucose-6-phosphate translocator
GRF4	Growth Regulating Factor 4
GS1	Glutamine Synthetase 1
GS2	Grain size on chromosome 2
GSK2	Glycogen synthase kinase 2
GTP	Glucose-6-phosphate translocator
GUS	Beta Glucuronidase
gusA	β-glucuronidase
GWAS	Genome-Wide Association Studies
HAP	Heme activator protein
HDA	Histone deacetylase
HDI	Histone deacetylase inhibitor
HDR	HomologyDirected Repair
HIGS	Host induced gene silencing
HMW	High Molecular Weight
HMW-GS	High-Molecular-Weight Glutenin Subunit
hpRNAs	Hairpin RNAs
HR	Hypersensitive response
IAA	Indole-3-acetic acid
ICP	Inner core protein
IM	Intracellular Movement
iPB	*In planta* particle bombardment
IR	Intergenic Region
IRs	Inverted Repeats
IRM	Insect Resistance Management
ISAAA	International Service for the Acquisition of Agri-biotech Applications
JA	Jasmonic acid
JAZ2	Jasmonatezim domain protein 2
JDL	Judali
KAS	Ketoacyl-ACP synthase
KASP	Kompetitive Allele Specific PCR
KO	Knock-out
LMV	Lettuce mosaic virus
LP	Larger panicle
LS	ADP-glucose pyrophosphorylase large subunit
MADS-box	Minichromosome Maintenance Factor 1, Agamous, Deficiens, and Serum Response Factor-box
MAP	Microtubule associated protein
MAPK	Mitogen-activated protein kinase
MAPKK	Mitogen-activated protein kinase kinase
MARPLE	Mobile And Real-time PLant disease

MCC	Minor Core Capsid
MIGS	MiRNA induced gene silencing
miRNA	Micro RNA
MMEJ	Micro-Homology-Mediated End-Joining
MOC	Major Outer Capsid
MP	Movement Protein
MR	Moderate Resistance
MTP	Minimal Tiling Path
NABP	Nucleic Acid Binding Protein
NAC	NAM, ATAF1, 2 and CUC2
NAM	No apical meristem
NCP	Nucleocapsid protein
NGFR	Nerve growth factor receptor
NGS	Next Generation Sequencing
NHEJ	Non-homologous end joining
NHR	Nonhost resistance
NIa-pro	Nuclear inclusion protein
NIPB	National Institute for Plant Biotechnology
NMD	Nonsense mediated decay
NPBT	New Plant Breeding Techniques
NPQ	Non-photochemical quenching
NPR1	Pathogenesis-related gene 1
NR	No Resistance
NSP	Nucleocapsid structural protein
nt	Nucleotide
NTP	Nucleotide triphosphate binding protein
NTT1	Adenylate translocator
OCP	Outer core protein
ODM	Oligonucleotide-Directed Mutagenesis
PacBio	Pacific Biosciences
PAL	Phenylalanine ammonia lyase
PAM	Protospacer-associated motif
PAMPs	PATHOGEN associated molecular patterns
PBZ	Probenazole
PCD	Programmed Cell Death
PCI	Participatory Crop Improvement
P-DNA	Plant-derived transfer DNA
PDR	Pathogen Derived Resistance
PDS	Phytoene Desaturase
PEBP	Phosphatidylethanolamine-binding protein
PEBV	Pea early browning virus
PEPC	Phosphoenolpyruvate carboxylase
PG	Polygalacturonase
PGIP	PG-inhibiting proteins
phasiRNAs	Phased pattern

Pho1	Plastidial α-glucan phosphorylase
Pho2	Cytosolic α-glucan phosphorylase
Pi	Inorganic phosphate
PIP	Plant-Incorporated Protectants
PoPMV	*Poplar mosaic virus*
PPB	Participatory plant breeding
PPi	Pyrophosphate
PPKL1	Serine/threonine protein phosphatase
PPO	Polyphenol Oxidase
PPV	Plum pox virus
PR	Pathogenesis-related
pri-siRNA	Primary siRNA
PTGS	Post-Transcriptional Gene Silencing
PTI	Pathogen or Pattern triggered immunity
PTST1	Protein Targeting to Starch
PVX	*Potato virus X*
PYL	Pyrabactin resistance 1-like
QTL	Quantitative Trait Loci
R	Resistance gene
RAPD	Random amplified polymorphic DNA
RBSDV	Rice black-streaked dwarf virus
RDD	Rice Dwarf Disease
RdDM	RNA dependent DNA methylation
RDM	RNA-Dependent Methylation
RDRs	RNA dependent RNA polymerases
RDV	Rice Dwarf Virus
RGA	Plant resistance gene analogs
RGDD	Rice gall dwarf disease
RGDV	Rice gall dwarf virus
RGEN RNPs	RNA-guided engineered nucleases RNPs
RGSV	Rice Grassy Stunt Virus
Ries-keFeS	Rieske protein
RISC	RNA induced silencing complex
RNAi	RNA interference
RNMV	Rice Necrosis Mosaic virus
RNP	Ribonucleoproteins
ROS	Reactive Oxygen Species
RQC	RNA quality control
RRSV	Rice ragged stunt virus
RSV	Rice Stripe Virus
RT/RNase H	Reverse transcriptase/ribonuclease H
RTBV	Rice tungro bacilliform virus
RTD	Rice tungro disease
RTL	RNAse III like
RTSV	Rice Tungro Spherical Virus

RuBisCO	Ribulose-1,5-bisphosphate carboxylase/oxygenase
RYMV	Rice yellow mottle virus
SA	Salicylic acid
SAM	Shoot apical meristem
SAR	Systemic acquired resistance
SBE	Starch branching enzyme
SBPase	Sedoheptulose-1,7-bisphosphatase
SBPH	Small brown plant hopper
SCF	Skp1/Cullin1/F-box
scFv	Single chain variable fragment
sgRNA	Short guide RNA
shRNA	Short hairpin RNA
SIGS	Spray induced gene silencing
siRNAs	Small interfering RNAs
SMRT	Single molecule real-time
SNP	Single Nucleotide Polymorphisms
SP	Structural protein
sp.	Species
SR	Strong Resistance
SRBSDV	Southern rice black-streaked dwarf virus
sRNAs	Small RNAs
SS	ADP-glucose pyrophosphorylase small subunit
SSN	Sequence-Specific Nucleases
SSR	Simple sequence repeats
T6P	Trehalose-6-phosphate
TaeIF4E	Triticum aestivum Initiation Factor 4E
TaGLP4	Triticum aestivum Germ Like protein 4
TaGW2	Triticum aestivum Grain Weight 2
TALE	Transcriptional activator-like effectors
TALEN	Transcription activator-like effector nucleases
tasiRNAs	*Trans* acting siRNAs
TaVRN2	Triticum aestivum VERNALIZATION 2
TEV	Tobacco etch virus
TGS	Transcriptional gene silencing
TGW6	Thousand-Grain Weight 6
TILLING	Targeted Induced Local Lesions IN Genomes
TMT1	Tonoplast monosaccharide importer
TMV	Tobacco mosaic virus
TNFR	Tumor Necrosis Factor Receptor
TriMV	Triticum Mosaic Virus
TRV	Tobacco rattle virus
TSWV	Tomato spotted wilt virus
TuMV	Turnip mosaic virus
TYLCV	Tomato yellow leaf curl virus
Ub	Ubiquitin

UPS	Ubiquitin proteasome system
USDA	United States Department of Agriculture
UTR	Untranslated region
VA	Viroplasm associated
vasiRNA	Virus activated host siRNA
vc	Viral complementary
VCP	Viroplasm component protein
VIGS	Virus Induced Gene Silencing
VPg	Viral Genome Linked Protein
vsiRNAs	Virus derived small interfering RNAs
VSRs	Viral Suppressors of RNA silencing
VSS	Virus Silencing Suppressor
VWFC	von Willebrand factor type C
WSMV	Wheat Streak Mosaic Virus
WT	Wild type
WTP	Willingness-to-pay
WY3	Waiyin
ZFN	Zinc-Finger Nuclease

Chapter 1
Source-Sink Relationships and Its Effect on Plant Productivity: Manipulation of Primary Carbon and Starch Metabolism

Kaan Koper, Seon-Kap Hwang, Salvinder Singh, and Thomas W. Okita

Abstract The rate of photo-assimilation in source organs (source strength) and the rate of conversion of this photo-assimilate into end products in sink organs (sink strength) are the two key metabolic processes that determine plant productivity and yield. Enhancement of either the source or the sink processes alone will often have limited returns due to the feedback inhibition from the other process. Consequently, maximizing plant productivity requires synergistic improvement of both source and sink processes. In this chapter, we will talk about the advancements in improving plant productivity through the modification of primary carbon metabolism in source and sink organs, with special emphasis on starch metabolism. Furthermore, we will discuss the future directions for enhancing source and sink processes in crop species via the usage of modern genome editing techniques.

Keywords Source · Sink · Productivity · Yield · Primary carbon metabolism · Photosynthesis · Rubisco · Calvin-Benson cycle · Starch · AGPase · Pho1 · Genome editing · CRISPR · GMO

K. Koper · S.-K. Hwang · T. W. Okita (✉)
Institute of Biological Chemistry, Washington State University, Pullman, WA 99164, USA
e-mail: okita@wsu.edu

K. Koper
e-mail: koper@wisc.edu

S.-K. Hwang
e-mail: hwangs@wsu.edu

S. Singh
Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat, Assam 785013, India
e-mail: salvinder.singh@aau.ac.in

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1.1 Introduction

Source-sink interactions play a significant role in plant productivity. Plant productivity for food and animal feed needs to be significantly increased by 60–120% to meet the dietary needs of the burgeoning human population worldwide (Bodirsky et al. 2015; Nations 2015; O'Neill et al. 2010; Ort et al. 2015). Such efforts will be mitigated by the threat of global warming, simultaneous erosion of arable land, and higher demand for plant-based biofuels (Pimentel and Burgess 2013). Global temperatures are expected to continue to increase by a further 1.5°C between 2030 and 2052 (Masson-Delmotte et al. 2018), which will have a major impact on cereal production. For example, yield reductions in bread wheat (*Triticum aestivum*) are strongly associated with increases in temperatures beyond optimal growth cycle temperatures (17–25°C) and maximum day temperatures (up to 32°C) during grain filling. Temperatures beyond these ranges may elicit stress responses and hence result in further yield reductions (Boehlein et al. 2019; Cossani and Reynolds 2012; Farooq et al. 2011; Gol et al. 2017). For wheat, the production and/or uptake, transport, storage and remobilization of the critical metabolites required for optimal growth rates and yields are dynamic processes involving feedback and feedforward sink–source interactions that can be disrupted in plants under heat stress (Asseng et al. 2017; Hütsch et al. 2019; Kumar et al. 2017).

Crop yields are influenced by the plant's capacity to capture light, to assimilate carbon, and to allocate this carbon into sink organs such as tubers/fruits/seeds as well as by agronomic practices and the environment (Long et al. 2006b; Smith et al. 2018). Crop productivity cannot reach its maximum potential unless suboptimal agronomic practices, as well as carbon assimilation (source strength) and its reallocation in sink tissues and organs, are improved (Bihmidine et al. 2013; Long et al. 2006b; Smith et al. 2018). To ensure the flow of nutrients from source organs to sink organs where they are needed, the source-sink transport system must be tightly regulated (Joana Rodrigues 2019).

The balance between source and sink dynamics becomes evident when one of the processes is disturbed. Improving light quality and intensity and increasing CO_2 concentration and photoperiod lead to better carbon fixation, and, in turn, enhanced plant growth and yield (Kirschbaum 2011; Long et al. 2006a; Watson et al. 2018; Yang et al. 2017; Yao et al. 2017). Photosynthesis in source organs is also stimulated by increased sink demand. However, sugars start to build up in source organs when the CO_2 assimilation rate exceeds the demand by sink organs (Ainsworth and Long 2005; McCormick et al. 2006), which leads to the downregulation of photosynthesis-related genes and photosynthetic rate (Ainsworth and Bush 2011; Chang and Zhu 2017). Thus, overall, there is a high positive correlation between source strength (carbon assimilation and export) and sink strength (end-product utilization) (Muller et al. 2011).

It is evident from the existing literature that both source and sink processes co-limit whole plant carbon fluxes, and neither should be considered in isolation (Körner 2015; Ludewig and Sonnewald 2016). This view is further supported by metabolic control

analysis of net carbon flux in potato (Sweetlove et al. 1998) and soybean (Farrar and Jones 2000). As such, it is evident that the greatest impact on yield will be achieved via the simultaneous manipulation of both source and sink (Reynolds et al. 2012). From an engineering perspective, it is clear that increases in just the source organs will lead to a sink bottleneck and vice versa. For example, by increasing photo-assimilate production, the conversion of these photo-assimilates into sink organ biomass will become more strongly limited by the capacity of the sink to take up and utilize the photo-assimilates (Sweetlove et al. 2017). This has been convincingly demonstrated by transgenic potato lines where maximum plant productivity was only attained when the source and sink strength were simultaneously enhanced. Sink strength of potato tubers was increased by the simultaneous overexpression of the plastidial glucose-6-phosphate and adenylate transporters while the source strength of leaf mesophyll tissue was enhanced by downregulating the leaf ADP-glucose pyrophosphorylase while overexpressing a cytosolic pyrophosphatase (Jonik et al. 2012). These manipulations resulted in enhanced sucrose export and source activity resulting in a doubling of starch yield in potato tubers.

1.2 Manipulation of Source for Enhanced Photosynthesis

The long-standing interest in source-sink interaction arises from the potential of manipulating it for greater yields. Many manipulations have been made in modifying the activities of enzymes and transporters related to source capacity, of long and short distance transport resistance, and of photosynthesis to increase crop yield (Ludewig and Sonnewald 2016; Sweetlove et al. 2017).

1.2.1 Engineering Rubisco Enzyme

Ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) is the primary enzyme in the C_3 photosynthetic pathway. This carboxylating enzyme reacts not only with CO_2 but also with O_2, leading to photorespiration which wastes assimilated carbon (Erb and Zarzycki 2018). Under current atmospheric conditions, O_2 inhibits photosynthesis in C_3 plants by as much as 40%. Under stress conditions, such as high temperature and drought, the suppression further increases through the decline of intercellular CO_2 concentration due to closure of leaf stomata (Orr et al. 2017). Rubisco shows inefficiencies because of the slow CO_2-fixation rate and relatively poor specificity for CO_2 over O_2. In view of these characteristics, Rubisco becomes a key engineering target to improve photosynthesis in crop plants (Carmo-Silva et al. 2015; Sharwood 2017; Whitney et al. 2011a). Whitney et al. (2011b) discovered that a single amino acid mutation acted as a catalytic "switch" converting *Flaveria* Rubisco from a "C_3 style" enzyme to a "C_4 style" and vice versa (Whitney et al. 2011b).

A significant technical barrier in engineering Rubisco is that it is composed of multiple large and small subunit polypeptides encoded by the plastid and nuclear genome (Maliga and Bock 2011). Initially, research has focused on manipulating the chloroplast-encoded large subunit, which contains a catalytic site, although recent reports have highlighted the impact on catalysis by the small subunit (Atkinson et al. 2017; Ishikawa et al. 2011; Laterre et al. 2017; Morita et al. 2014). Engineering of the nucleus-encoded small subunit gene family (RbcS) is less technically challenging as nuclear transformation is already established for may plant species compared to those fewer amenable to chloroplast transformation (Bock 2015; Maliga and Bock 2011). Introduction of more efficient, foreign Rubisco proteins have not been successful to date because of complicated assembly requirements of the enzyme in the chloroplast, although important advancements have been made in co-engineering by introducing Rubisco alongside assembly chaperones (Bracher et al. 2017; Whitney et al. 2015). The Rubisco from cyanobacteria *Synechococcus elongatus* could support tobacco growth but only at elevated CO_2 (Lin et al. 2014; Occhialini et al. 2016). However, when the cyanobacterial carbon-concentrating mechanism was also introduced together with the cyanobacterial Rubisco, the tobacco plants were capable of surviving at ambient CO_2.

1.2.2 Engineering the Calvin-Benson Cycle and Photorespiratory Pathway

Several enzymes of the Calvin-Benson cycle are potential targets to enhance CO_2 fixation in plants (Ainsworth et al. 2012; Feng et al. 2007; Raines 2003, 2011; Singh et al. 2014). Overexpression of sedoheptulose-1,7-bisphosphatase (SBPase) improved growth rates of tobacco and rice (Feng et al. 2007; Lefebvre et al. 2005; Rosenthal et al. 2011). Recently, Simkin et al. (2015, 2017) demonstrated improved photosynthesis and increased biomass in *Arabidopsis* by simultaneously manipulating three genes: SBPase, fructose-1,6-bisphosphate aldolase (FBPA) and glycine decarboxylase-H protein (GDC-H), the latter a component of the photorespiratory pathway (Simkin et al. 2015, 2017). Quantum efficiency of photosystem II and the CO_2 fixation rate were significatly increased in these lines. Moreover, the co-expression of GDC-H with SBPase and FBPA resulted in a cumulative positive impact on leaf area and biomass (Simkin et al. 2017). In addition to the Calvin-Benson cycle enzymes, overexpression of membrane transporters in crop species such as the *IctB* gene (Long et al. 2016), which encodes an inorganic carbon transporter B, is a viable approach as evidenced by the studies in soybean (Hay et al. 2017) and rice (Gong et al. 2015).

Modifying the effects of photorespiration has also drawn considerable attention. Interestingly, both enhancing and bypassing photorespiration were able to enhance carbon assimilation and growth in *Arabidopsis* (Betti et al. 2016; Timm et al. 2015). A viable approach has been to avert CO_2 and energy costs of photorespiration by

introducing synthetic "photorespiratory bypass" pathways in the chloroplast that direct CO_2 release in the proximity of Rubisco (Kebeish et al. 2007; Maier et al. 2012). Nölke et al. (2014) demonstrated that the productivity and yields of potato (*Solanum tuberosum*) are increased by enhancing photosynthetic carbon fixation via expression of a polyprotein (DEFp) comprising of all three subunits (D, E, and F) of the bacterial *glycolate dehydrogenase* (*GlcDH*). Transgenic plants accumulated DEFp in the plastids, and the recombinant protein was active *in planta*, reducing photorespiration, and improving CO_2 uptake with a significant impact on carbon metabolism (Nölke et al. 2014).

The yield potential of rice (*Oryza sativa* L.) is limited by source capacity to fill a large number of grain 'sinks' produced in modern varieties. One solution to this problem is to introduce a more efficient, higher capacity photosynthetic mechanism to rice viz. the C-4 photosynthetic pathway (Furbank 2016). The C_4 rice project is one of the most ambitious of such approaches (https://c4rice.org).

1.2.3 Engineering Light-Use Efficiency in Plants

Major losses of energy can occur during the conversion of absorbed light energy into photochemical reactions (Stitt 2013). Conceptual analysis (Murchie and Niyogi 2011) suggested that this is an area where substantial improvement can be made. Kromdijk et al. (2016) showed that modification of the key components of the xanthophyll cycle, as well as the PsbS subunit of photosystem II, accelerated the relaxation of non-photochemical quenching (NPQ), thus enabling the tobacco plant to use light for photosynthesis as opposed for heat dissipation. This resulted in an increased rate of biomass production by as much as 20% in glasshouse-grown plants and about 15% in field-grown plants. The conservation of NPQ across plants suggests that this is a viable approach to improve the growth of other crops.

A more novel approach to enhance light energy capture is by incorporating the bacterial chlorophylls found in many anoxygenic photosynthetic organisms into photosystem I or II in plants with the aim of extending the light absorption spectrum to the far red region (Blankenship et al. 2011). Alternatively, increasing the chloroplastic electron transport rates in *Arabidopsis* by overexpressing the Ries-keFeS protein also achieves increased biomass and seed yield (Simkin et al. 2017).

1.2.4 Engineering Enzyme Activities in Source and Sink Organs

The assimilation and metabolism of nitrogen have the potential to influence source/sink activity and, correspondingly, the final yield (Good et al. 2004; Yamaya et al. 2002). Overexpression of the cytosolic *glutamine synthetase 1* (*GS1*) gene can

increase nitrogen use efficiency and crop productivity in different species (Thomsen et al. 2014). Although the enhancement differed among species and growth conditions, overexpression of a plant-specific *Dof1* transcription factor led to the upregulation of multiple genes involved in carbon skeleton production including that for phosphoenolpyruvate carboxylase (PEPC), which improves N assimilation and growth especially under low N supply (Thomsen et al. 2014; Yanagisawa et al. 2004). A large number of genes/loci selected during rice breeding have been detected through analyses of genome information, many of which are related to N metabolism (Xie et al. 2015). Finally, manipulation of malate dehydrogenase and succinate dehydrogenase, two enzymes of the tricarboxylic acid cycle, produced source-mediated increases in growth and yield in tomato (Araújo et al. 2011; Nunes-Nesi et al. 2005).

Many efforts to enhance sink strength has been carried out in potato and tomato using the class I patatin promoters, which confer sink-specific expression in these species (Jefferson and Bevan 1987; Rocha-Sosa et al. 1989). Transgenic potato overexpressing the plastidial transporters, glucose-6-phosphate translocator (GPT) and the adenylate translocator (NTT1) (Zhang et al. 2008), and sucrose synthase elevated tuber starch and plant dry weight. Conversely, downregulation of the plastidial adenylate kinase and uridine monophosphate synthase, which led to increased levels of adenylates and uridinylates, resulted in increased starch levels and yield (Baroja-Fernández et al. 2009; Geigenberger et al. 2005; Regierer et al. 2002). In *Arabidopsis*, overexpression of the tonoplast monosaccharide importer TMT1 altered cellular sugar sensing and increased biomass production (Wingenter et al. 2010).

Sink activity has been shown to play a significant contribution to grain yields in the major cereal crops (McCormick et al. 2008; Slewinski 2012; Smidansky et al. 2002). In maize, the sucrose concentration of developing ears and the final yield have been significantly increased by reducing the sugar signal trehalose-6-phosphate (T6P) (Nuccio et al. 2015). Griffiths et al. (2016) showed that "chemical intervention" method can be used for exploiting T6P signaling. Although T6P is plant-impermeable, the generation of plant-permeable T6P precursors that released T6P in a light-activated manner increased grain yield and recovery from stress (Griffiths et al. 2016).

1.3 Manipulation of Starch Biosynthesis for Enhancing Sink Strength

Starch biosynthesis is the most important metabolic pathway directly influencing sink strength in plants. ADP-glucose pyrophosphorylase (AGPase) is an enzyme controlling the rate-limiting step in starch biosynthesis, and thus even slight changes in the enzymatic properties of this enzyme can significantly affect starch production. In addition to AGPase, the plastidial starch phosphorylase (Pho1) can also generate a complementary carbon flux into starch. In this section, we will discuss the roles

of AGPase and Pho1 in starch biosynthesis in both source and sink tissues, and the engineering of these pathways to increase plant productivity and yield.

1.3.1 AGPase and Its Critical Role in Starch Biosynthesis

AGPase belongs to the nucleotidyltransferase (adenylyltransferase) family and catalyzes the reaction;

$$\text{Glc} - 1 - \text{phosphate} + \text{ATP} \Leftrightarrow \text{ADP} - \text{glucose} + \text{PPi}$$

Plant AGPases is composed of two small and two large subunits (SS and LS) that co-assemble to form a heterotetrameric enzyme (Ballicora et al. 2003, 2004; Iglesias et al. 1993, 1994; Iglesias and Preiss 1992; Okita et al. 1990; Preiss 1984; Preiss and Romeo 1994; Preiss and Sivak 1998). The AGPase LS mainly plays a regulatory role with varying catalytic capacity depending on the isoform while the SS is both catalytic and regulatory (Cross et al. 2004; Hwang et al. 2005). In plants, the AGPase reaction constitutes the first committed step of starch synthesis, as the ADP-glucose produced by AGPase is used by starch synthases for elongating α-1,4-glucosidic chains (Ballicora et al. 2004; Iglesias and Preiss 1992; Preiss and Sivak 1998; Stark et al. 1992). Predictably, the catalytic activity of plant AGPases and, in turn, the carbon flux into starch, are tightly regulated by the energy state and metabolic needs of the cell through the use of allosteric effectors, redox state, and its expression level (Ballicora et al. 2004; Ohdan et al. 2005; Tiessen et al. 2002).

The plant AGPase responds strongly to two allosteric regulators; activator 3-phosphoglycerate (3PGA) and inhibitor inorganic phosphate (Pi) (Ballicora et al. 2004; Sowokinos 1981; Sowokinos and Preiss 1982; Tuncel and Okita 2013). In source tissues, the 3PGA/Pi ratio controls AGPase activity and, in turn, carbon allocation between sucrose and transitory starch synthesis. Moreover, the allosteric regulation of AGPase in sink tissues allows starch synthesis to be in sync with the influx of sucrose from source organs (Ballicora et al. 2004; Tuncel and Okita 2013).

In addition to allosteric regulation, AGPases from many different species and tissues are regulated by the redox state (Ballicora et al. 2000; Fu et al. 1998; Tuncel et al. 2014). Under oxidizing conditions, AGPase activity is low due to the formation of one or more disulfide bridges, which lower catalytic activity. While under reducing conditions, disulfide bonds are disrupted and enzyme activity increases (Ballicora et al. 2000; Fu et al. 1998; Tuncel et al. 2014). In photosynthetic tissues, the redox signal that activates AGPase is generated in the light and by the increases in sugar levels (Geigenberger 2011; Hendriks et al. 2003; Kolbe et al. 2005; Michalska et al. 2009). Whereas in non-photosynthetic tissues, the redox signal is generated in response to the influx of sucrose (Geigenberger and Stitt 2000; Tiessen et al. 2002) as well as by the increases in sugar levels (Tiessen et al. 2003). Redox activation of AGPase can override constraints generated by allosteric regulation, allowing the enzyme activity to increase in response to specific external stimuli even under

allosterically unfavorable conditions (Geigenberger 2011; Tiessen et al. 2002; Tuncel et al. 2014). This allows the plant to fine-tune the flux into starch biosynthesis based on its needs.

The final level of control of AGPase activity is at the protein expression level. In most plant species examined, the large subunit is encoded by multiple genes while the small subunit is encoded usually by one or two genes (Crevillen et al. 2003; Georgelis et al. 2007). The expression of the AGPase large subunits is regulated both temporally and spatially (Crevillén et al. 2005; Geigenberger 2011; Ohdan et al. 2005; Tetlow et al. 2004). As AGPase hetero-tetramers formed with different large subunit isoforms exhibit different kinetic and allosteric properties (Crevillen et al. 2003; Geigenberger 2011; Tetlow et al. 2004), regulating the protein expression at the subunit level allows the formation of enzymes best suited for the cellular needs. AGPase expression is also affected by metabolites, where increasing sugar levels elevate expression (Müller-Röber et al. 1990; Sokolov et al. 1998), whereas increases in nitrate or phosphate decrease expression (Nielsen et al. 1998; Scheible et al. 1997).

1.3.2 Attempts to Increase Plant Productivity in Various Species Through the Alterations of the AGPase Pathway

Under conditions where photosynthesis is not limited by temperature, light, or availability of captured CO_2, the main factor that governs plant productivity and yield is the utilization of photo-assimilate into end-products (Chen et al. 1994; Hocking and Meyer 1991; Pammenter et al. 1993; Pieters et al. 2001; Rowland-Bamford et al. 1990; Stitt and Quick 1989; Sun et al. 1999a). Overall, the capacity of a plant to convert photo-assimilates into end-products is determined by the collective ability of the organism to transport photo-assimilates from source to sink organs and convert them into “storable” end-products, such as starch, oils, and proteins (Herbers and Sonnewald 1998).

Due to its critical role in initiating carbon flux into starch biosynthesis, AGPase has been extensively targeted at multiple levels to enhance starch biosynthesis and the efficiency of photo-assimilate utilization. Two main approaches have been employed to increase plant productivity through the AGPase pathway. First, increasing the overall enzymatic activity through increased AGPase abundance either by overexpression or by increasing the steady-state levels of the active enzyme by improving its heat stability (Boehlein et al. 2008; Greene and Hannah 1998). Second, increasing the overall enzymatic activity by introducing AGPases with better kinetic or allosteric properties (up-regulatory mutants). Below, we describe specific biotechnological approaches aimed to improve plant productivity through modification of the AGPase pathway.

1.3.3 *Improving Yield Through Increasing AGPase Activity in Source Tissues*

In source tissues, increasing AGPase activity can improve productivity and yield through two mechanisms. First, improved AGPase activity increases the utilization of photo-assimilate into transitory starch when sucrose synthesis or transport to sink tissues are saturated. This would reduce feedback inhibition of photosynthesis by improving Pi recycling and allowing the plants to sustain higher photosynthetic efficiency under conditions where photosynthesis is not limited by environmental conditions (temperature, light, CO_2) (Pammenter et al. 1993; Sun et al. 1999a). Second, the larger amount of transitory starch accumulated can be broken down during the night and fuel vegetative and/or reproductive growth.

In *Arabidopsis* plants, the effect AGPase and transitory starch on photosynthesis, growth, and yield were evident for the AGPase SS (TL25) and LS (TL46) mutant lines (Sun et al. 1999b, 2002). These mutant plants showed reduced photosynthetic capacity and growth rate than the wildtype with transitory starch levels correlating with CO_2 assimilation and growth rates (Sun et al. 1999b, 2002). When the TL46 mutant line was complemented with the wildtype LS gene, transitory starch level, photosynthetic rates, and yield were restored to wildtype levels (Gibson et al. 2011; Obana et al. 2006). However, when the TL46 mutants were transformed with a mutant AGPase LS with upregulated allosteric properties (increased 3PGA and lower Pi sensitivity), they exhibited higher growth, yield, and photosynthetic capacity than wildtype (Gibson et al. 2011; Obana et al. 2006).

Different responses were readily evident for a maize line lacking the leaf AGPase SS (*agps-m1*) (Slewinski et al. 2008) and for a rice line lacking the AGPase LS (*apl1*) (Rösti et al. 2007). Under controlled growth conditions, both the maize (Slewinski et al. 2008) and the rice (Rösti et al. 2007) mutant lines grew similar to their wild types although transitory leaf starch accumulation was significantly reduced (Rösti et al. 2007; Slewinski et al. 2008). It is worth noting that the leaves of small grain cereals are naturally not strong leaf starch accumulators, but they instead store most of their nighttime carbon as sucrose or other soluble sugars (Hendry 1993; Huber 1981; Nakano et al. 1997; Ohashi et al. 2000). Investigation of soluble sugar levels in the leaves of starchless rice *apl1* mutants showed no significant day or night time difference between the mutant and wildtype (Rösti et al. 2007). The ability of small grain cereals to store nighttime carbon as soluble sugars most likely reduce the importance of transitory starch synthesis. Diminished importance of transitory leaf starch can also be observed for the starchless mutants of Arabidopsis (Lin et al. 1988), tobacco (*Nicotiana Sylvestris*) (Huber and Hanson 1992), and pea (Harrison et al. 1998) that grow like wildtype under extended photoperiods, but suffer if photoperiod is shortened.

Nevertheless, when the maize *agps-m1* was grown under field conditions, it showed lower overall yield and growth rate despite exhibiting CO_2 assimilation rates like wildtype (Schlosser et al. 2012). It is likely that the reduced carbon availability from starch degradation during the night impaired the growth of these plants

(Schlosser et al. 2012). Furthermore, transformation of rice plants with upregulated potato AGPase LS gene (*UpReg1*) (Gibson et al. 2011), or overexpression of the maize leaf AGPase LS and SS (Oiestad et al. 2016; Schlosser et al. 2014) demonstrated the ability to improve yields by 24–29% over wild type level through improved leaf starch synthesis. Overall, this shows an intrinsic capacity to increase yield through induction of leaf starch synthesis, even for species that do not primarily store significant levels of leaf starch.

1.3.4 Improving Yield Through Increasing AGPase Activity in Sink Tissues

An alternative approach to increase plant productivity and yield is to increase starch synthesis in sink tissues. This method has been a successful approach for many crop species partially because sink organs of starchy crops, such as cereal seeds and potato tubers, are economically valuable and readily harvested. In addition to their economic values, sink organs of plants generate the main pool for photo-assimilate deposition and constitute the bulk of the sink strength. Consequently, enhancing starch synthesis in sink organs increases the demand for carbon transported from the source organs. An increased sucrose consumption by the sink organs also alleviates the accumulation of sucrose in phloem or leaves, thereby minimizing the end-product inhibition of photosynthesis. Similar to source tissues, improved starch synthesis in sink tissues can be achieved by increasing overall AGPase activity.

The first study for increasing starch accumulation in sink tissues through enhancing AGPase activity was accomplished in potato tubers (Stark et al. 1992). Transformation of potato plant with a mutant *E. coli* AGPase gene (*glgC-16*) resulted in a 35–60% increase in tuber starch levels (Stark et al. 1992). However, when similar studies (Sweetlove and Burrell 1996) were repeated for a different potato cultivar, no apparent increases in starch levels were detected despite a fourfold increase in transgenic *glgC-16* AGPase activity versus the wildtype enzyme. Nevertheless, there was strong evidence for increased starch synthesis that was coupled with an increase in starch turnover (Sweetlove and Burrell 1996). In a comparable study (Ihemere et al. 2006), the expression of a modified *glgC* gene in cassava (*Manihot esculenta*) resulted in a 70% increase in AGPase activity and a 2.6-fold increase in tuberous root biomass.

The manipulation of AGPase activity in sink tissues has been successfully accomplished in maize, rice, and wheat. In addition to AGPase localized to plastids, cereal endosperm expresses a second cytosolic enzyme activity (Hannah and James 2008). This cytosolic isoform is responsible for the majority of the AGPase activity in endosperms of cereals maize (Denyer et al. 1996), rice (Sikka et al. 2001), wheat (Burton et al. 2002) and barley (Thorbjørnsen et al. 1996).

The capacity of this approach to increase seed weight was first demonstrated in maize (Giroux et al. 1996). Generation of a native LS *rev6* mutant that is less sensitive