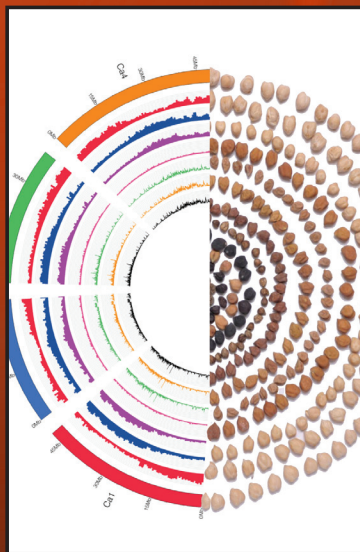


# CA **T**ranslational GT **G**enomics for TG **C**rop Breeding

**Volume II: Abiotic Stress, Yield and Quality**

**Editors: Rajeev K. Varshney  
Roberto Tuberosa**



**WILEY** Blackwell



**Translational Genomics for Crop Breeding,  
Volume II: Abiotic Stress, Yield and Quality**



# **Translational Genomics for Crop Breeding, Volume II: Abiotic Stress, Yield and Quality**

Edited by

Rajeev K. Varshney

Roberto Tuberosa

**WILEY** Blackwell

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## Foreword

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ICRISAT's mission is to reduce poverty, hunger, malnutrition, and environmental degradation in the dryland tropics. To accomplish this, we need to address the challenges presented by population explosion and climate change and their impacts on agriculture.

Today about 70% of the food-insecure population lives in developing countries, mostly as small-scale and subsistence farmers, and their population growth is expected to triple by 2100. They eke out a living and feed themselves from food crops cultivated in degraded lands in an unequivocally warmer climate system.

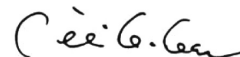
To achieve global food security, the development of crop varieties that produce high yields in harsh climatic conditions will be a key strategy. Although several desirable traits have been developed in crop species through integrated breeding practices, self-sufficiency in food grains and legumes *per se* has remained an elusive dream for poor people in the developing world.

The knowledge generated through advances in genomics during the past two decades has enormous potential in advancing the quest for abiotic stress-tolerant crops in the arid and semi-arid regions of the world. Moreover, DNA-based marker technologies have increased the precision in marker-assisted selection (MAS), thereby reducing the time for improving traits of interest. Genome sequence information for important crops like rice, sorghum, maize, soybean,

chickpea, pigeonpea, tomato, and so on is now available to enable greater understanding of traits through comparative studies. It is important to further translate available genome information in crop breeding so that farming communities will be benefitted sooner than later.

*Translational Genomics for Crop Breeding: Abiotic Stress, Yield and Quality*, edited by Dr. Rajeev K. Varshney, our own ICRISAT scientist, and Professor Roberto Tuberosa from the University of Bologna, Italy, provides a concrete step in this direction. It is the second of two volumes where the eminent editors have carefully selected authors who are experts in translational genomics in crop breeding for different traits in different crop species to write the various chapters of this publication. These chapters provide examples of translational genomics for enhancing tolerance to abiotic stresses and quality traits in a number of crops. I believe that such a book is very timely, informative, and of such quality that it will fill the gap that exists presently between genome science and crop breeding.

Through this publication, we hope the elusive dream of poor people in the developing world will sooner become a reality.



Hyderabad  
Date: June 10, 2013

William D. Dar  
Director General,  
ICRISAT



---

# Preface

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Ever since the discovery about four decades ago of the first molecular tools to investigate DNA structure and function, the science of genomics has contributed tremendously to investigate the genetic and functional basis of plant phenotypes and how this variability affects crop productivity. More recently, further advances in high-throughput genotyping technologies and genome sequencing have accelerated gene discovery and allele mining and the application of this knowledge to crop improvement. This aspect of translational genomics has also been referred to as genomics-assisted breeding (GAB). Due to the continuous and coordinated efforts of the global scientific community involved in crop improvement, in a number of cases GAB has become an integral part of crop-breeding programs.

Although substantial efforts have been made in the past century to mitigate the negative effects of abiotic stresses on crop productivity and improve the nutritional quality of crops, the progress achieved through conventional breeding approaches has been limited and will be insufficient to meet the ever-growing needs of humankind in the next decades. Within this daunting scenario, the advances in genomics during the past decades provide new avenues for enhancing our understanding of the genetic basis of complex traits while accelerating crop improvement. While a good number of success stories have been completed or are in progress for resistance to biotic stresses, GAB has limited examples of success stories for the improvement of resistance to abiotic stresses, quality, and

yield *per se*. In view of the fast-growing need for developing new cultivars with enhanced tolerance to abiotic stresses, better quality and/or higher yield, this volume compiles reviews from leading authorities in their own fields of expertise while describing the progress and presenting ideas for future applications.

The editors thank all the authors of the different chapters (Appendix I) for the skillful summaries of the research work in their area of expertise and in some cases for sharing unpublished results in order to make the articles as up to date as possible. We also appreciate their cooperation in meeting the deadlines and in revising their manuscripts. While editing this book, the editors also received strong support from several colleagues (Appendix II), who willingly reviewed the manuscripts. Their constructive and critical suggestions have been very useful for improving the quality of manuscripts.

We are also grateful to colleagues and staff from our respective laboratories, who have helped us complete the editing of this volume in parallel with their demanding responsibilities. In particular, Manish Roorkiwal, B. Manjula, Pawan Khera, and Mahendar Thudi helped RKV with the editorial work. RKV is thankful to his wife Monika for her constant encouragement and support, and to Prakhar (son) and Preksha (daughter) for their love and cooperation. Similarly, RT is thankful to his wife Kay for her patience and editorial contributions. RKV would also like to extend his sincerest thanks to Dr. William D. Dar, Director General, ICRISAT,

for his guidance and support in completing this book. The cooperation and help received from Justin Jeffryes, Anna Ehler, Kelvin Matthews, Erin Topp of Wiley Blackwell, and Shikha Sharma of Aptara Corp. during various stages of development and completion of this book are also gratefully acknowledged. RKV would also like to mention that the book was edited during the tenure of RKV as Director, Center of Excellence in Genomics (CEG), ICRISAT, Hyderabad (India), Theme Leader – Comparative and Applied Genomics (CAG), Generation Challenge Programme (GCP) and Adjunct positions at the University of Western Australia, Crops Research Institute of Guangdong Academy of Agricultural Sciences (GAAS), China and BGI-Hongkong Research Institute, China.

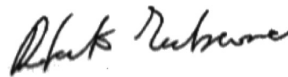
We hope that this book will be helpful and useful as a ready guide to students, young researchers, crop specialists, breeders, and pol-

icy makers for contributing to the development of new cultivars more resilient to abiotic stresses and with a better nutritional quality. Lastly, we would appreciate if the readers can point out any errors and give comments/suggestions, as this would be useful for the future, revised and updated editions.



Hyderabad, India  
June 09, 2013

(Rajeev K. Varshney)



Bologna, Italy  
June 09, 2013

(Roberto Tuberosa)

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## **Chapter 1**

# **Translational Genomics for Crop Breeding: Abiotic Stress Tolerance, Yield, and Quality, An Introduction**

*Rajeev K. Varshney and Roberto Tuberosa*

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### **Abstract**

In the context of global climate change and population explosion, feeding the world's population and addressing the issues of malnutrition, especially in developing countries, are daunting tasks before the global scientific community. The yield gains achieved through conventional breeding are not very promising, as several abiotic stresses such as drought, salinity, cold, flooding, submergence, and mineral toxicity have been leading to significant yield losses and reducing the quality of produce. In recent years, advances in genomics research and next generation sequencing (NGS) technologies have largely facilitated understanding and identifying gene networks that are involved in controlling genetic variation for agronomically valuable traits in elite breeding populations. The availability of genome-sequence information, transcriptomic resources, molecular markers, and genetic maps for major crops such as rice, maize, and sorghum have enabled adoption of genomics-assisted breeding (GAB) approaches, including marker-assisted backcrossing (MABC) and marker-assisted recurrent selection (MARS). Nevertheless, during the last decade significant genomic resources were also developed in less-studied crops and efforts are underway in deploying these genomic tools in breeding. Furthermore, the new bioinformatics approaches and decision-support tools developed are able to enhance the precision in selection and complement the success of GAB approaches.

This volume essentially focuses on the research on abiotic stress tolerance and the quality enhancement of agricultural produce. Further, this introductory chapter summarizes the key success stories and lessons learned in the field of genomics tools for crop improvement. In addition, this chapter also emphasizes the essence of deploying genome-wide association mapping and nested association mapping (NAM), as well as genomic selection (GS) approaches for crop improvements, in the context of the availability of a plethora of low-cost and high-throughput sequencing technologies.

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*Translational Genomics for Crop Breeding, Volume II: Abiotic Stress, Yield and Quality.*

Edited by Rajeev K. Varshney and Roberto Tuberosa.

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## Introduction

Despite continuous efforts to improve agricultural crops, changes in climate in the past two decades have had a tremendous influence on crop production and productivity. Further, climate changes will have a significant impact on the food security of humankind, especially in developing countries (Lake et al. 2012). It is expected that global temperatures will increase about 3°C by 2100 (Schneider et al. 2007), a change that would drastically curtail global crop production. Additionally, other critical abiotic stresses such as drought, salinity, cold, flooding, submergence, mineral toxicity, and so forth hamper the growth, development, yield, and seed quality of crops. In fact, these abiotic stresses represent the main cause of crop failure worldwide, curtailing average yields of all major crops by more than 50%. Furthermore, the quantitative inheritance and low heritability of resistance to these stresses, coupled with a strong Genotype x Environment x Management interaction of the yield response of crops to abiotic stresses, greatly limit a more accurate dissection and manipulation of such response. Since the world population is increasing at an alarming rate, minimizing these losses is also a major concern for all nations, particularly those with a strong increase in food demand. Besides increasing the production potential, the nutritional quality of the produce needs to be improved to avoid the malnutrition that billions are already facing, particularly in developing countries (Müller and Krawinkel 2005; Bouis et al. 2010).

In the context of rapidly growing demand for the staples of our sustenance, conventional breeding programs are struggling to achieve the yield gain required to adequately meet the burgeoning demand for food and plant-derived products (Tester and Langridge 2010). Accordingly, genomics-assisted breeding (GAB, Varshney et al. 2005) approaches are increasingly being adopted to improve the accuracy and effectiveness of selection while allowing for the dissec-

tion of the traits controlling the adaptive response of crops to unfavorable conditions. While availability of molecular markers and genetic maps are the prerequisites for GAB, several orphan crops, neglected at the global level but important for food security at the regional level, until recently lacked the genomic resources and platforms to implement GAB. However, in recent years significant progress has been achieved in the development of genomic resources in a number of orphan crops that have thus become genomic resource-rich crops (Varshney et al. 2009, 2010). As a result, GAB activities including trait mapping and marker-assisted selection (MAS) methods, such as marker-assisted backcrossing (MABC) and marker-assisted recurrent selection (MARS), are increasingly being adopted in breeding programs for major crops and have begun to be deployed in less-studied crops as well (Kulwal et al. 2012; Varshney et al. 2012).

Volume I of this book series presents reviews of genomics applications in crop breeding for biotic stress resistance, while this volume (Volume II) focuses on research endeavors in abiotic stress tolerance and the enhancement of the quality of agricultural produce. Four chapters in Volume II deal with ongoing research on tolerance to submergence, salinity, drought, and phosphorus (P) deficiency in rice. Another chapter discusses work on cloning and molecular breeding work for aluminum toxicity in sorghum. Research on freezing tolerance, an important trait in the Western world, in wheat and barley is summarized in another chapter. One chapter is focused on molecular breeding efforts for stay-green tolerance, an important drought tolerance trait in sorghum. Four chapters in the volume are devoted to quality improvement traits in rice, maize, peanut, and tomato. In the last chapter, authors discuss advances in sugarcane genomics and its applications for enhancing yields and ongoing efforts in genomic selection (GS). Some highlights of these chapters have been summarized in this introductory chapter.

## Enhancing Tolerance to Abiotic Stresses in Rice

Submergence stress affects more than 15 Mha in lowland rice-growing areas of South and Southeast Asia. Chapter 2 provides insights into the ongoing efforts at the International Rice Research Institute (IRRI), Manila, Philippines, to improve submergence tolerance in rice. Following the identification of *Sub1* (*Submergence1*) locus and three ethylene responsive factors (ERFs) in rice, Septiningsih and colleagues report the development of eight *Sub1* varieties by the IRRI, six of which are already widely grown in several countries.

More than 444 Mha of global rice-growing area is affected by soil salinization (FAO, 2010). Soil salinization is a major problem in coastal areas of the regions where rice-based farming predominates. Reportedly rice yields are reduced by up to 50% when grown under moderate (6 dS/m) salinity levels (Ren et al. 2005). The losses due to soil salinization can be overcome by soil reclamation or by improving salinity tolerance in the crops. Efforts toward understanding the genetic basis of the trait for crop improvement has revealed that several genes are independently involved in salinity tolerance at different stages of crop cycles. In Chapter 3, Platten and colleagues provide an overview of genomics applications in enhancing salt tolerance in rice.

Drought is the major limiting factor to crop production, and cereals especially experience various kinds of drought stresses, depending on the timing and intensity of the water stress relative to the reproductive stage of the crop. In the case of rice, in Asia about 34 Mha of rain-fed lowland rice and 8 Mha of upland rice (Huke and Huke 1997) are frequently subjected to drought stress. Progress in developing high-yielding, drought-tolerant rice cultivars by conventional breeding has been slow, largely because of difficulties in precisely defining the target environment, complex interactions of drought tolerance with environments and management practices, and lack of appropriate screening methodology.

However, during the past decade the availability of large-scale genomic resources and genome sequences have enabled the adoption of various GAB approaches in rice (see Collard et al. 2008). These efforts are summarized in Chapter 4 by Kumar and colleagues.

Sixteen essential elements are required for rice during the crop cycle. The major nutrients such as nitrogen (N), phosphorus (P), and potassium (K) are largely supplied as chemical fertilizers. The excess application of P, owing to its insoluble nature, leads to deficiencies of copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn). Additionally, erosion of P-enriched soils enhances eutrophication in fresh water (Wolf 1996). Most of the rain-fed rice grown in Asia and Africa is cultivated on problematic soils, especially when P becomes unavailable to the crop as it adheres to soil particles. Hence, in this context, development of crops with enhanced efficiency of P utilization and production of higher biomass is essential. Chapter 5 by Heuer and colleagues essentially discusses the issues related to P deficiency in rice production. Further, the authors also highlight the need for the adoption of molecular breeding approaches and summarize the molecular breeding efforts for enhancing P utilization efficiency in rice.

## Enhancing Tolerance to Abiotic Stresses in Wheat and Barley

Freezing/cold tolerance in crop plants is most important in the context of global climate change. Freezing tolerance is important in temperate cereals such as wheat (*Triticum* spp.), barley (*Hordeum vulgare*), and rye (*Secale cereale*). Long exposures of winter wheat and barley varieties to non-freezing cold temperatures (Dhillon et al. 2010) will accelerate flowering time (vernalization) and improve freezing tolerance (cold acclimation). In the case of wheat, Kobayashi et al. (2005) reported that *Vrn-Fr1* controls both frost tolerance and vernalization. Chapter 6, by Gabor and colleagues, reports on the developmental plasticity of these *Triticeae* crops

upon onset of the cold stress. This chapter also summarizes the accumulated knowledge of the past 20 years in the area of genetics and genomics of the mechanism of freezing tolerance and the genomic tools available for enhancing freezing tolerance in the *Triticeae* crops.

### **Enhancing Tolerance to Abiotic Stresses in Sorghum**

Aluminum (Al) is the third most abundant element on earth after oxygen and silicon (Ma et al. 2001). It is a light metal that makes up 7% of the earth's crust. Half the arable soils across the globe and especially those in Africa, Asia, and South America are affected by aluminum toxicity (<http://www.news.cornell.edu/stories/Aug07/SoilsKochian.kr.html>). Chapter 7 by Magalhaes and colleagues deals with the existing diversity with respect to aluminum tolerance in sorghum and maize germplasm accessions as well as the molecular, physiological, and genetic basis of Al tolerance in both crops. The authors provide insights into the structure and functional analysis of membrane transporters such as Al-activated malate transporter (ALMT1) and multidrug and toxic compound efflux (MATE) involved in Al tolerance.

Furthermore, 20 to 30% of production losses in sorghum are due to lodging. Stay-green trait, however, has been an indirect selection criterion used by breeders for enhancing lodging resistance. Stay green is associated with increased grain yield and grain size in the sorghum crop under terminal drought, a common occurrence in arid and semiarid regions across the globe (Jordan et al. 2012). Among several genotypes identified with the stay-green trait, BTX645 has been a useful resource in developing commercial hybrids (Harris et al. 2007). Four major quantitative trait loci (QTLs) and several minor QTLs can enhance stay-green traits and several efforts are underway to introgress these QTLs into various genetic backgrounds. However, Vadez and colleagues in Chapter 8 report that this undertaking has been quite challenging owing to limited

polymorphism among the parental lines for this trait. The physiological, genetic, and molecular breeding aspects for the stay-green trait are discussed at length in this chapter.

### **Improving Quality and Yield Through Molecular Breeding in Rice, Maize, Peanut, and Sugarcane**

Besides increasing production and productivity, agricultural produce also should fulfill the requirement of consumer acceptance in terms of quality, in order to fetch a good market price. Hence, grain quality improvement also forms the major concern for cereal breeders. In Chapter 9, Hori and Yano describe rice grain quality traits in terms of physical and cooking qualities that are of interest to the consumer. In fact, grain quality is influenced by climate changes, such as high temperatures at the grain ripening stage, and grain components such as amylose, amylopectin, and proteins are greatly affected by such changes. This chapter summarizes the efforts to understand the genetics of grain quality traits and the multiple genes/QTL contributing to grain quality. The chapter also emphasizes the need for developing novel quality evaluation instruments/approaches, such as TILLING (Target Induced Local Lesion IN Genome) that can enhance the qualities related to the cooking and eating of *japonica* rice.

About 190 million children under the age of five years are suffering from malnutrition, especially in the underdeveloped and developing countries in Asia and sub-Saharan Africa (WHO, 2009). Malnutrition can be overcome by supplementing dietary requirements with micronutrients or through promotion of dietary diversification. However, these strategies have been only partially adopted and appear not to have improved the nutrient deficiencies in South African children since 1994 (Labadarios et al. 2005). Nevertheless “biofortification” – breeding staple crops with increased nutritional value – has emerged as a potential long-term strategy to improve nutritional security. Babu and



colleagues in Chapter 10 provide a comprehensive overview on biofortification in maize and highlight two specific cases of genetic improvement in maize that resulted in high nutritional value, particularly with respect to essential amino acid content in the endosperm. Besides emphasizing the molecular marker-assisted QPM (quality protein maize) breeding, the chapter also throws light on its impact in the developing world. Furthermore, the essence of provitamin A, Fe, and Zn, and low-phytate content, and the possibilities for genetically engineered, high-lysine maize are elegantly discussed in the chapter.

Peanut is the most important food legume and oilseed crop cultivated in arid and semi-arid regions of the world. About 45-51% of the dry weight of peanut seeds is oil (Chamberlin et al. 2011). Among fatty acids, oleic and linoleic acids are major fatty acids that determine the oil quality; hence the ratio of the two, the O/L ratio, is critical. In Chapter 11, molecular breeding efforts aimed at improving the oil quality in peanut, undertaken at the Kazusa DNA Research Institute and the Chiba Prefectural Agriculture and Forest Center, both located in Japan, is discussed by Kolikonda and colleagues. This chapter also provides the cost comparisons (costs involved) of conventional and molecular breeding programs.

The cultivated tomato, the most popular vegetable crop in the world, is an important model system for genetics and genomics studies. Marker-assisted selection has been employed extensively in tomato breeding for improving many simple traits. Kinkade and Foolad in Chapter 12 look for QTL analysis approaches and focus on the use of new “omics” technology and its potential use for improving fruit quality in tomato breeding. Progress on reverse genetics approaches, such as TILLING and the bioinformatic workflows to handle high-throughput identification of mutations in candidate genes are discussed.

In sugarcane, up until two decades ago most of the breeding efforts for improvement were

purely traditional. Chapter 13, by Gouy and colleagues, highlights recent advances in genomics and its applications for enhancing sugar yields. The chapter also highlights the ongoing efforts on genomic selection (GS) for enhancing yield gains in sugarcane.

## Summary and Outlook

This volume presents a number of comprehensive and informative articles written by eminent scientists in the area of crop genomics and molecular breeding. It is important to mention here that the traits and crops discussed in this volume provide just some examples on how genomics can help facilitate the enhancement of tolerance to abiotic stresses and quality in crops.

Volume I of this series offers comprehensive reviews of biotic stress tolerance in a range of crops. As compared to the selected examples of GAB for biotic stress tolerance, it is clear that although rice has made significant progress in GAB for abiotic stress tolerance, most success stories of GAB are related to biotic stresses. This may be attributed to the partially qualitative inheritance and higher heritability of disease resistance as compared to abiotic stress tolerance. Similar to submergence tolerance, if the QTLs contribute higher phenotypic variance, GAB approaches such as MAS and MABC can be deployed in breeding programs. However, in the case of tolerance to abiotic stresses and yield, where several and small-effect QTLs are involved, simple molecular breeding approaches such as MABC and MAS are not as effective. In those cases, MARS (Bernardo and Charcosset 2006) and GS (Heffner et al. 2009, 2010, 2011; Heslot et al. 2012; Nakaya and Isobe 2012) are expected to be the most promising approaches. In this context, and in addition to biparental linkage mapping, mapping approaches such as genome-wide association studies (GWAS; Huang et al. 2010; Zhao et al. 2011; Li et al. 2012; Pasam et al. 2012) and nested association mapping (NAM; Hung et al. 2011; Kump et al. 2011; Cook et al. 2012) can be

implemented due to the availability of low-cost, high-throughput sequencing technologies. While GAB approaches are routinely deployed in the private sector and in developed countries, availability of breeder-friendly decision support tools is required for enhanced adoption of GAB in developing countries. In this context, some tools like integrated system for marker-assisted breeding (ISMAB) (<https://www.integratedbreeding.net/ib-tools/breeding-decision/marker-assisted-back-crossing-tool>), OptiMAS (<https://www.integratedbreeding.net/node/1407>), GS modules (Pérez-Rodríguez et al. 2012; de Los Campos et al. 2013) and platforms like Integrated Breeding Platform (IBP) (<https://www.integratedbreeding.net/>) are being developed.

We hope that these two volumes will allow graduate students and young scientists to better appreciate the potential of GAB and will encourage them to pursue careers in this exciting area of crop improvement. In addition, GAB practitioners as well as policy makers should be able to use these volumes for developing the road map for the improvement of target crops in their respective geographical areas.

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## Chapter 2

# Applying Genomics Tools for Breeding Submergence Tolerance in Rice

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### Abstract

Flooding stress is one of the most important abiotic stresses constraining rice production, especially in rain-fed lowland areas. The effect of this stress has intensified in past decades and is predicted to increase in the years to come as a result of global climate change. At the International Rice Research Institute (IRRI), breeding for tolerance to submergence imposed by flash flooding during the vegetative stage has been one of the institute's priority objectives for more than three decades. Several tolerant breeding lines have been developed through conventional breeding; however none of those early varieties has been widely accepted by farmers. An important breakthrough was the identification of the major quantitative trait locus (QTL) *SUB1* in the mid-1990s, which led to the identification of three ethylene responsive factors (ERFs), of which *SUB1A* is the primary contributor for tolerance. These findings and the available molecular marker technology have enabled breeders to develop submergence-tolerant varieties through a fast-track marker-assisted backcrossing (MABC) strategy to introgress *SUB1* using mega varieties as recurrent parents. Currently eight *Sub1* varieties have been developed in IRRI, six of which have been released in several countries. The success of *Sub1* varieties has inspired the development of new breeding products for other stresses using a similar strategy, such as tolerance to anaerobic conditions during germination and stagnant flooding. Recent advances in genomics have tremendously increased the efficiency of marker-assisted breeding, bringing us to the point where rice varieties resilient to multiple stresses can be developed to meet future challenges facing rice production, most of which have intensified with global climate changes.

### Introduction

Flooding stress is a widespread problem that adversely affects farmers in rice-growing areas, especially in the flood-prone, rain-fed lowlands of South and Southeast Asia and West Africa.

More than 20 million ha of rain-fed lowlands are flood prone, with conditions ranging from flash flood to deepwater, which can be up to several meters and last for months (Mackill et al. 2010). Over thousands of years, rice has developed adaptive mechanisms to grow well in

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flooded ecosystems, as we can see in today's lowland rice (Pampolino et al. 2008); however, too much water at any stage of development could lead to serious injury or total crop loss. Recently weather patterns have become increasingly irregular as a result of climate change (Wassmann et al. 2009; Jagadish et al. 2012), and unexpected heavy rains can inundate rice fields along riverbanks and in low-lying areas and damage crop production. In recent years, destructive typhoons and heavy rains have caused huge damage in Bangladesh, Cambodia, Myanmar, the Philippines, Laos, Vietnam, Pakistan, and, most recently, Thailand, causing these countries to lose millions of tons of rice.

The most common flooding stress is the "flash flooding" that occurs during the monsoon season with different intensities and durations. During these episodes, water completely submerges the established rice crop for a short period from several days up to two weeks. If rice plants remain submerged for more than five days, they start to die and do not recover after the water recedes. The severity of the stress and the number of days that rice plants can survive underwater depends on environmental conditions, such as temperature, water turbidity, solar radiation, and soil fertility (Setter et al. 1997; Das et al. 2009). In flash flood-prone regions, farmers usually cultivate landraces that are tall and moderately tolerant of submergence but that have low yield. In some other areas where high-yielding but submergence-intolerant rice varieties have been cultivated, farmers often suffer from crop losses or significant yield reduction caused by flash-flood episodes.

It is not uncommon for floodwaters to stay in the field from two weeks to several months in some low-lying rice areas. In this situation, the water level is generally 20 to 50 cm in depth, which is referred to as stagnant flooding (Septingsih et al. 2009; Mackill et al. 2010; Singh et al. 2011). Even though plants are not completely submerged under these conditions, grain production is greatly reduced due to poor tillering and

greater susceptibility to lodging (Tuong et al. 2000; Singh et al. 2011). In some areas, stagnant flooding can also immediately follow a flash-flood period. In this situation, farmers usually rely on landraces that can cope with both stresses, although these tend to be very low yielding. In areas where progressive flood waters reach a depth of several meters for several months, farmers usually cultivate "floating rice" or "deepwater rice" that shows rapid internode elongation with the rising floodwater, maintaining the uppermost leaves and panicles above the surface (Catling 1992). Deepwater rice generally has poor yield due to excessive vegetative growth.

Flash floods can also cause excessive damage during germination and early seedling growth, resulting in poor crop establishment in direct-seeded rice areas. This can occur in both irrigated areas when the land is not level and in flood-prone rain-fed ecosystems when rainfall occurs within a few days following seeding (Ismail et al. 2009; Angaji et al. 2010). Most rice varieties are unable to vigorously germinate, elongate, and survive under complete submergence and, as a consequence, seedling establishment is very poor or completely absent in fields that are flooded. Poor seedling germination is also a problem if fields are not properly leveled, resulting in the formation of puddles in which seedlings are submerged, and in fields with drainage problems, if heavy rains occur directly after seeding. The hazards of flooding during germination can prevent farmers from adopting direct-seeded rice technology or force them to discontinue this practice (Konchan and Kono 1996). Farmers usually wait for the "right" time to sow or broadcast their seeds; however, if an unexpected flood destroys their crops, they reseed or transplant their fields. Apart from being costly and labor demanding, the delay caused by re-planting and thereby late harvest of the first crop can delay the planting of the following season's crop.

Efforts to identify submergence-tolerant rice genotypes were initiated at the International Rice

Research Institute (IRRI) during the 1970s (Vergara and Mazaredo 1975). Among other varieties, FR13A, which was derived from pure-line selection of the landrace “Dhalputtia” originating from Orissa, India, was identified as the most tolerant cultivar (HilleRisLambers and Vergara 1982) and subsequently used extensively as a tolerant donor in breeding programs. Several breeding lines tolerant of submergence have been developed, including IR49830-7-1-2-1, a highly tolerant and high-yielding indica-type line (Mackill et al. 1993), which was released in Cambodia under the name “Popoul” in 1999. However, despite its tolerance of submergence, this variety was not widely adopted by farmers because it lacked several key traits, such as locally preferred grain quality (Neeraja et al. 2007).

A major landmark in the history of submergence-tolerance breeding was the identification of the major quantitative trait locus (QTL) *Submergence 1* (*SUB1*) that controls this trait (Xu and Mackill 1996). This early work ultimately led to the cloning of the *SUB1* region in FR13A and subsequently to the identification of the ethylene-responsive factor (ERF) gene *SUB1A-1* which is necessary and sufficient for submergence tolerance (Xu et al. 2006). Based on the sequence information, *SUB1*-specific molecular markers were developed that facilitated a precise marker-assisted backcrossing (MABC) system that is now successfully being deployed to introgress the *SUB1* QTL into widely grown “mega-varieties” in South and Southeast Asia, as well as in Africa (Neeraja et al. 2007; Septiningsih et al. 2009; Bailey-Serres et al. 2010; Manzanilla et al. 2011; Mackill et al. 2012). As a result of repeated backcrossing to the respective recipient parent, the improved submergence-tolerant varieties carry the FR13A *SUB1* locus but are otherwise identical to the original variety. Most importantly, grain quality and other locally preferred traits are unaltered in these new varieties, which enhance adoption by farmers and variety release in the target coun-

tries. These new varieties are called “Sub1” or “Scuba” rice.

Breeding direct-seeded rice varieties, which requires tolerance of flooding during germination, also began in the past. However, success was very limited mainly due to the lack of tolerant donor varieties, and the complexity of the trait (Yamauchi et al. 1993; Yamauchi and Winn 1996; Biswas and Yamauchi 1997). More recently, after screening thousands of rice accessions, several landraces have been identified that are tolerant of submergence during germination (also referred to as anaerobic germination, AG) (Angaji et al. 2010). The analysis of mapping populations derived from tolerant donor parents has led to the identification of promising QTLs that might be useful for direct-seeded systems, as described in more detail below. As was the case for submergence and AG tolerance, efforts to develop varieties for stagnant flooding and deepwater conditions were made over several decades at IRRI (HilleRisLambers and Seshu 1982). More recently, a concerted effort has started to combine tolerance of stagnant flooding with submergence tolerance (based on *SUB1*). This is particularly important for short rice varieties, for example, Swarna, which was the first mega-variety introgressed with *SUB1* (Swarna-Sub1). One variety that combines tolerance of submergence and stagnant flooding has been developed by conventional breeding based on phenotype selection (IRRI 119), which was released in the Philippines as PSB Rc68 (Septiningsih et al. 2009; Mackill et al. 2010). More recently, breeding lines that are high yielding and perform well under stagnant flooding have also been developed through conventional breeding (Mackill et al. 2010).

This chapter will review the progress made in applying genomics tools to unravel the molecular and physiological basis of different types of submergence tolerance, as well as efforts to use this information to develop improved rice varieties to meet the challenges of the future.

## Applying Genomics Tools for Molecular Studies and Breeding

### Identification of the QTLs and Genes underlying Tolerance

Genetic dissection of quantitative traits using QTL mapping tools became feasible with the availability of DNA markers about two decades ago. Even though varietal improvement can be achieved using conventional breeding, QTL mapping has tremendous potential to breed varieties in a more effective and efficient way. Once a major QTL for an important trait has been mapped and the markers closely linked to the locus have been identified, the QTL can be used as a target in marker-assisted breeding to rapidly breed an improved variety. Furthermore, once the gene(s) underlying the QTL has(have) been cloned, markers can be developed from the target gene(s) for even more precise marker-assisted breeding. The cloned gene(s) and near-isogenic lines (NILs) that differ only at the locus of interest also provide a starting point for detailed study of molecular, physiological, and developmental mechanisms underlying the trait of interest.

#### *Tolerance to Flash Flood during the Vegetative Stage*

FR13A was identified as one of the best submergence-tolerant donors and was used extensively by breeders in the 1970s; however, very little was known about the genetic basis of the tolerance possessed by this variety. Independent studies using the submergence-tolerant variety FR13A identified the major *SUB1* QTL and several minor QTLs (Xu and Mackill 1996; Nandi et al. 1997; Toojinda et al. 2003). It was found that *SUB1* alone contributes 69% of the phenotypic variance (Xu and Mackill 1996) and could provide tolerance to complete submergence for up to two weeks. *SUB1* was then fine-mapped to a region of 0.06 cM using an F<sub>2</sub> segregating population of 2950 individuals

(Xu et al. 2000), and the underlying genes were finally cloned as a cluster of three ethylene-responsive factor (ERF) genes, *SUB1A*, *SUB1B*, and *SUB1C* (Xu et al. 2006). It was demonstrated through gene transformation that *SUB1A* was the main contributor for tolerance (Xu et al. 2006); and this finding has been confirmed through a progeny test of recombinants identified within the *SUB1* cluster in several thousand individuals in segregating populations (Septiningsih et al. 2009). It was also shown that *SUB1A* gene expression, which subsequently determines the amount of tolerance, is dosage-dependent. This suggests that, for improvement in hybrid rice, both parents should carry the *SUB1A* gene for maximal effect (Septiningsih et al. 2009). In addition, allelic survey studies showed that, in some cases, the expression of *SUB1A* is a more reliable parameter to use instead of the original allelic determination, that is, the *SUB1A-1*-tolerant allele and *SUB1A-2*-intolerant allele (Singh et al. 2010; Septiningsih et al. 2012).

#### *Tolerance to Anaerobic Conditions during Germination*

Tolerance of flooding during seed germination, referred to as anaerobic germination (AG), is one of the most important traits necessary to ensure good seedling establishment in direct-seeded rice in both rain-fed flood-prone and irrigated ecosystems. Several QTLs for AG tolerance were reported on chromosomes 1, 2, 5, and 7 (Jiang et al. 2004, 2006). Our group at IRRI identified several promising QTLs derived from a tolerant donor from Myanmar, Khao Hlan On (Angaji et al. 2010). The QTL with the largest effect was detected on the long arm of chromosome 9 (*qAG-9-2* or *AG1*), having a logarithm of odds (LOD) score of 20.3 and explaining 33.5% of the variation for this trait. Fine mapping of this QTL in the background of IR64 narrowed the locus down to a 58-kb region based on the Nipponbare sequence. Several candidate genes have been identified, and gene validation and



characterization are under way (unpublished data). This QTL is a promising target for marker-assisted selection for direct-seeded rice varieties. Another major QTL was detected on the short arm of chromosome 7 (referred to as *qAG7.1* or *AG2*), derived from the tolerant donor variety Ma-Zhan Red (Septiningsih et al. 2013). NIL development for fine mapping and varietal improvement for this QTL are under way. This QTL can be combined with *AG1* to confer higher tolerance, given that the effect of the two QTLs proved to be additive or synergistic. Studies using other unrelated sources of tolerance are under way to identify additional QTLs involving different tolerance mechanisms.

#### *Deepwater Rice*

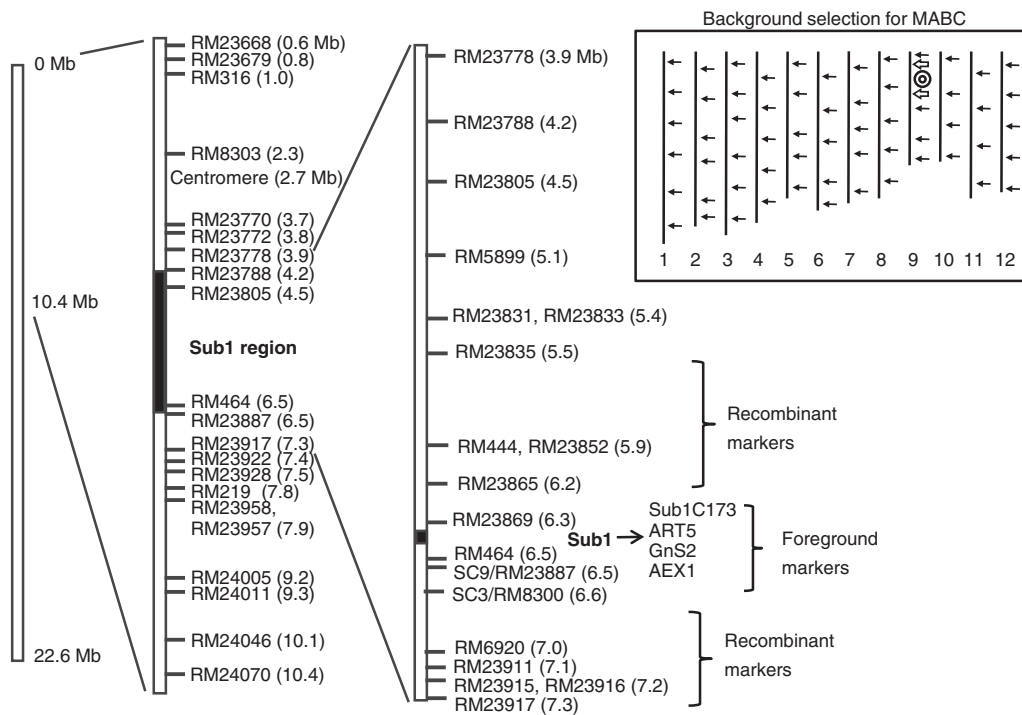
The most important trait for the survival of deepwater rice is rapid underwater internode elongation, to ensure that upper leaves maintain efficient photosynthesis (Catling 1992; Vergara et al. 1976). A number of studies have identified QTLs for deepwater traits, such as internode elongation and number of elongated internodes (Sripongpangkul et al. 2000; Nemoto et al. 2004; Hattori et al. 2007; Kawano et al. 2008). Even though different donors were used in studies on deepwater rice, QTLs on chromosomes 1, 3, and 12 were repeatedly detected in different mapping populations. Through NIL evaluation, it was confirmed that the QTL on chromosome 12 contributed the most rapid internode elongation in deepwater stress conditions (Hattori et al. 2008). By positional cloning, Hattori and colleagues (2009) identified the genes within this QTL: *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*). Like the *SUB1*s, these are ERF genes of subgroup VII, possessing a single AP2 DNA-binding domain. Both *SK1* and *SK2* are missing in the elite recurrent parent genome (Hattori et al. 2009). The presence of genes conferring abiotic stress tolerance have recently been found in donor landraces but not in the reference Nipponbare genome, as in the case of the donors for tolerance of submer-

gence, phosphorus deficiency, and deep water (Xu et al. 2006; Hattori et al. 2009; Heuer et al. 2009).

#### **Development of Sub1 Varieties**

Identification of the *SUB1* gene enabled marker-assisted selection (MAS) for submergence tolerance. The *SUB1* QTL has a large effect and the phenotypic difference between tolerant and susceptible types is consistent. A wealth of sequence polymorphisms in and around the gene cluster from both tolerant and susceptible parents provided useful markers for MAS (Xu et al. 2006; Neeraja et al. 2007; Septiningsih et al. 2009; Singh et al. 2010; Iftekharruddaula et al. 2011). Given the popularity of high-yielding varieties with grain quality attributes that lacked submergence tolerance, a large-scale MABC program was undertaken at IRRI using these popular varieties as recurrent parents. Instead of using the original donor FR13A, two FR13A-derived improved lines were used as donors: IR49830-7-1-2-2 (IR49830-7) and IR40931-33-1-3-2 (IR40931-33) (Mackill et al. 1993). This led to successful introgression of the *SUB1* locus into several popular varieties (mega-varieties) in India, Bangladesh, Indonesia, Laos, and the Philippines. By using precision MABC, the high yield and desirable grain and eating qualities of these mega-varieties were retained (Septiningsih et al. 2009; Singh et al. 2009).

Three levels of MABC were applied to ensure high precision in breeding and to reduce the time required for variety development: (1) foreground selection, in which markers tightly linked to *SUB1* are used to select for the locus; (2) recombinant selection, in which closely linked flanking markers are used to minimize the donor chromosomal segment containing *SUB1*; and (3) background selection, in which DNA markers are used to accelerate the recovery of the recurrent parent genome (Figure 2.1; Collard and Mackill 2008). The method of selecting recombinants on both sides of the target



**Fig. 2.1. Marker-assisted backcrossing (MABC) for *SUB1*.** A number of markers have been identified as useful foreground markers (used to retain the tolerant *SUB1* allele) and recombinant markers (flanking markers used to select for a small *SUB1* introgression). Once foreground and recombinant selection have reduced the population size, background selection is performed to eliminate donor introgressions across the rest of the genome and return to the recurrent parent genome (see inset). For a color version of this figure, please refer to the color plate.

locus in at least two backcross (BC) generations during MABC was first proposed by Young and Tanksley (1989) and applied in rice by Chen and colleagues (2000). Foreground and recombinant polymerase chain reaction (PCR)-based DNA markers, such as simple sequence repeat (SSR), cleaved amplified polymorphic sequences (CAPS), insertion/deletion (INDEL), and mismatched single nucleotide polymorphism (SNP) markers were generated to facilitate introgression of the *SUB1* allele from the FR13A-derived lines in the background of popular varieties through MABC (Neeraja et al. 2007; Septiningsih et al. 2009). The most common markers used for foreground selection are summarized in Table 2.1.

In the first stage, six varieties were enhanced with *SUB1* using MABC (Neeraja et al. 2007;

Septiningsih et al. 2009; Iftekharrudaula et al. 2011). In 2011, with the addition of Ciherang-Sub1 and PSB Rc18-Sub1, a total of eight Sub1 mega-varieties have now been developed (Table 2.2; Figure 2.2). Evaluation of these new cultivars has indicated that there is no negative effect of *SUB1* on other traits, and no linkage drag, especially as recombinant selection was performed. In many cases, the development of these new Sub1 varieties will make it easier to incorporate *SUB1* in the future because recombinant and background selection may not need to be as rigorous because the donor parents are highly adapted and possess many desirable agronomic characters. For example, a single backcross has been used for the development of Ciherang-Sub1 using IR64-Sub1 as the donor parent; this donor is closely related to the

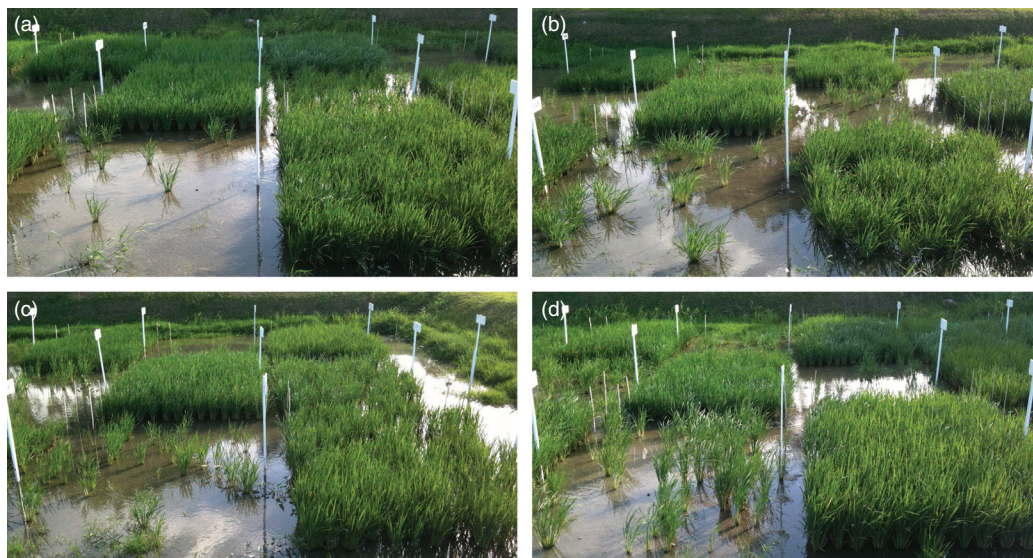
**Table 2.1.** The most common markers used in the development of Sub1 mega-varieties

Primer	Sequence	Tm (°C)	Position	Type of marker	Accession	Expected size (bp)
RM8300 (SC3)F	GCTAGTGCAGGGTTGACACA	60	~300 kb upstream of <i>SUB1A</i>	SSR	NB <sup>a</sup>	200
RM8300 (SC3)R	CTCTGGCCGTTTCATGGTAT	60				
GnS2F	CTTCTTGCTCAACGACAACG	60	exon of <i>SUB1A</i>	CAPS ( <i>AluI/PvuII</i> )	Teqing	242
GnS2R	TCGATGGGGTCTTGATCTCT	60			26D17 <sup>b</sup> NB <sup>a</sup>	132 & 110 No product
AEX1F	AGGCGGAGCTACGAGTACCA	62	non-synonymous SNP for <i>SUB1A</i>	mismatch	26D17 <sup>b</sup>	231
AEX1R	GCAGAGCGGCTGCGA	62		specific for tolerance	Teqing NB <sup>a</sup>	No product No product
ART5F	CAGGGAAAGAGATGGTGGA	60	<i>SUB1C</i> promoter	15 bp insertion in NB/93-11	NB <sup>a</sup> /93-11	217
ART5R	TTGGCCCTAGGTTGTTTCAG	60			118k20 <sup>b</sup>	202
Sub1C173F	AACGCCAAGACCAACTTCC	60	exon of <i>SUB1C</i>	9 bp deletion in NB/93-11	NB <sup>a</sup> /93-11	164
Sub1C173R	AGGAGGCTGTCCATCAGGT	60			118k20 <sup>b</sup>	173

<sup>a</sup>Nipponbare<sup>b</sup>derived from IR40931-26**Table 2.2.** Current status of Sub1 lines developed through marker-assisted backcrossing (MABC)

Recurrent parent	Country of origin	Donor parent	Generation	Introgression size (Mb)	IRRI designation	Country where released	Year of release
Swarna	India	IR49830	BC <sub>3</sub> F <sub>2</sub>	2.3–3.4	IR05F102	India & Indonesia, Bangladesh, Nepal & Myanmar	2009 2010 2011
IR64	Philippines	IR40931	BC <sub>2</sub> F <sub>2</sub>	6.5–7.8	IR07F102	Philippines & Indonesia	2009
Samba Mahsuri	India	IR49830	BC <sub>2</sub> F <sub>2</sub>	6.5–9.2	IR07F101	Nepal, India	2011 2013
TDK1	Laos	IR40931	BC <sub>3</sub> F <sub>2</sub>	1.5–2.5	IR07F289	N/A <sup>a</sup>	
BR11	Bangladesh	IR40931	BC <sub>2</sub> F <sub>2</sub>	0.3–2.6	IR07F290	Bangladesh	2010
CR1009	India	IR40931	BC <sub>2</sub> F <sub>3</sub>	2.7–6.4	IR07F291	N/A <sup>b</sup>	
Ciherang	Indonesia	IR64-Sub1	BC <sub>1</sub> F <sub>2</sub>	6.5–7.8	IR09F436	Indonesia, Bangladesh	2012 2013
PSB Rc18	Philippines	IR64-Sub1	BC <sub>1</sub> F <sub>2</sub>	6.5–7.8	IR09F437	N/A <sup>c</sup>	

<sup>a</sup>Not applicable; under advanced evaluation in Lao PDR<sup>b</sup>Not applicable; released proposal submitted in India<sup>c</sup>Not applicable; under evaluation in the Philippines



**Fig. 2.2.** Photographs of Sub1 varieties compared with the original varieties (foreground) at 2 months after 16 days of submergence in an IRRI field trial. (a) Swarna vs Swarna-Sub1; (b) BR11 vs BR11-Sub1; (c) Samba Mahsuri vs Samba Mahsuri-Sub1; and (d) Ciherang vs Ciherang-Sub1. For a color version of this figure, please refer to the color plate.

recurrent parent Ciherang (i.e., Ciherang is derived from a cross with IR64).

## Performance of Sub1 Varieties

### *Performance of Sub1 Varieties under Controlled Flooding*

The aim of evaluating Sub1 varieties under controlled non-flooded conditions was to determine whether the incorporation of *SUB1* had any other effects on grain yield or grain quality of any particular popular variety used as a recipient. This question is important because submergence normally occurs once every 2-5 years, and any reduction in yield or changes in grain quality of these popular varieties will ultimately lead to their rejection by farmers and/or millers. A set of trials was conducted under controlled flooding in the field to determine the extent to which *SUB1* can enhance survival and yield in field conditions and whether it can work in several genetic backgrounds and under variable environments. These trials used the first set of three popular varieties introgressed with *SUB1*: Swarna, IR64, and Samba Mahsuri. When grown under con-

trolled non-flooded conditions, the pairs of NILs (pairs with and without *SUB1*) were almost identical in their growth, flowering and maturity duration, and yield, as well as in all aspects of grain quality. These findings suggest that *SUB1* does not have other effects on growth and yield in the absence of stress (Singh et al. 2009).

The same NILs were then evaluated under complete submergence for either 12 or 17 days in the field (Singh et al. 2009, 2011), where substantial differences were observed between the Sub1 lines and their recurrent parents. The survival of both groups of lines decreased following submergence, but the survival of the Sub1 lines was considerably higher. For instance, following 17 days of submergence, the survival of Samba Mahsuri and IR64 was only 7% and 11%, while that of Samba Mahsuri-Sub1 and IR64-Sub1 lines was 83% and 85%, respectively. This improvement in survival of the tolerant lines was associated with substantial suppression of shoot elongation and higher biomass, with more non-structural carbohydrates in the shoot, and chlorophyll retention in leaves after submergence, all of which were previously associated with