A concise operating manual for

INTELLIGENT CROP BREEDING

Shoaib Ur Rehman
Jin-Ying Gou
Weilong Guo

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Authors

Dr. Shoaib Ur Rehman
Institute of Plant Breeding and Biotechnology, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan

Dr. Jin-Ying Gou
Wheat Genetics and Genomics Centre, China Agricultural University, Beijing, China

Dr. Weilong Guo
Wheat Genetics and Genomics Centre, China Agricultural University, Beijing, China

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Preface

Agricultural productivity is one of the defining challenges at present. The demand for climate smart crops has never been greater in the face of climate change and population surge. This requires redesigning of agricultural science beyond conventional procedures and protocols to embrace a new era of precision agriculture.

Interdisciplinary convergence of technology, life and data science is the need to this era. Prevailing tools for gene sequencing permit us to explore blueprints of plant genetic background with high precision. This volume "A Concise Operating Manual for Intelligent Crop Breeding" is a result of a teamwork with international researchers who are enthusiastically shaping this new frontier. Our aim is to offer a comprehensive and practical guide that bridges the gap between academic theory and laboratory application. We anticipate that the effective application of this approach hinges on a solid base in basic laboratory practices. Without high-quality of molecular samples and careful preparation of molecular biology experimentation, even the state-of-the-art techniques will produce erratic outcomes.

We provide robust protocols for high quality isolation of nucleic acids which is a prerequisite of high throughput genotyping. More focus is given to Cleaved Amplified Polymorphic Sequences (CAPS), derived CAPS (dCAPS) and Kompetitive Allele Specific PCR (KASP) assays. The techniques are reliable to genotype breeding populations for SNP-based markers making the backbone of modern crop breeding.

"A Concise Operating Manual for Intelligent Crop Breeding" is hence designed as basic resource for a broad researcher- from postgraduates embarking on their careers to veteran molecular plant breeders and researchers looking to integrate these techniques into their work.

Dr. Shoaib Ur Rehman
Date: 30-June-2026

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Chapter 01

SNP Genotyping in Plant Breeding

1.1 Introduction to Genotyping

Meeting the needs of the ever-increasing global population requires a broader understanding of the diversity and distribution of plant species. Extreme weather conditions and climate change have been identified as significant contributors to agricultural losses and crop deterioration (Backlund et al. 2008). Although significant improvements occurred during the last century in agricultural production (Bohra et al. 2014), further advancements are required to tackle new challenges. Today, agricultural fields still require new crop varieties due to ongoing cultural and social changes. Plant producers must meet market demands, customers preferences, and agronomic challenges. Significant improvements made so far have been attained by traditional breeding techniques. In contrast, biotechnology plays a crucial role in shaping future agricultural prospects and is a prerequisite for accelerating crop development (Lucht 2015). The research and practical application of genetic markers in agricultural biotechnology hold significant promise for future crop breeding (Lateef 2015). DNA markers associated with yield are commonly used in cultivar development for various crops, including maize (*Zea mays*) (Ribaut and Ragot 2007), rice (*Oryza sativa*) (Jena and Mackill 2008), tomato (*Lycopersicon esculentum*) (Tiwari et al. 2022), and wheat (*Triticum aestivum*) (Song et al. 2023).

A naturally occurring genetic variation at a single base site/position in a DNA sequence is referred to as a single nucleotide polymorphism (abbreviated as SNP). Generally, SNPs involve a change from one nucleotide base to another through transversions or transitions. However, single-base Insertion/Deletion (InDels) are also classified as SNPs. Two-nucleotide alterations and small InDels (spanning a few nucleotides) are frequently grouped with SNPs in genotyping pipelines. Plant scientists investigate whether and how SNPs influence phenotypic traits. These genetic variations at specific positions serves as effective molecular markers in both animal (Johnsson 2023) and plant species (Bohra et al. 2014; Ribaut and Ragot 2007; Song et al. 2023; Tiwari et al. 2022). The distribution pattern of these genetic variations differs across species, and even the genetic loci contributing to disease

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