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Epigenetics in Plants of Agronomic Importance: Fundamentals and Applications

Transcriptional Regulation and
Chromatin Remodelling in Plants

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ISBN 978-3-319-07970-7 ISBN 978-3-319-07971-4 (eBook)
DOI 10.1007/978-3-319-07971-4
Springer Cham Heidelberg New York Dordrecht London

Library of Congress Control Number: 2014942693

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Printed on acid-free paper

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Preface

The first Agricultural Revolution, that is, the initial transition from hunting and gathering to settled agriculture, is considered by many to have begun around 12,000 years ago. Since then, humans have domesticated hundreds of plant species and it is considered that the evolution of crop plants took place as human behavioral ecology changed from food gathering to farming. Domestication of wild species of plants comprises a variety of evolutionary changes (phenotypic and genetic divergence amongst domesticated populations) that may diminish the fitness of a plant in the wild but increase it under human exploitation. Since then, the selection of populations with desirable alleles, the meticulous breeding of high yielding genotypes, ease of farming and quality, and numerous technological advances have allowed crop production to increase and in this way supply the nutritional requirements of an ever-increasing human population.

During the last decades, and in particular as a part of the Green Revolution, modern breeding methods, novel research, development, and technology transfer initiatives have increased dramatically agriculture production worldwide. Many beneficial traits in crop species include, for example, increased yield, enhanced abiotic/biotic stress tolerance, improved nutritional quality, delayed ripening, increased post-harvest quality, delayed senescence, etc. However, it is now patent that if agriculture is to support human population for years to come, additional sustainable strategies for crop production must be developed (e.g., exploiting the positive associations with soil organisms while avoiding the negative ones), in concert with a profound understanding of the relationship between crop genotype and environment. Thus, it is opportune to evaluate the mechanisms that plants may have evolved to adapt to sudden changes in the environment. Furthermore, we need to comprehend the mechanisms by which (epi)genetic variation may modify plant gene regulation and phenotype, and we should concentrate on how the (epi)genome acts as a potent new source of diversity for agronomical important traits and its potential for exploitation in crop improvement programs. Epigenetic phenomena influence gene expression at the chromatin structure and organization level thereby modulating the access of regulatory complexes to the genome. Current research on epigenetic mechanisms suggests they are involved in almost every aspect of plant

life including agronomically important traits such as flowering time, fruit development, responses to environmental factors, and plant immunity. Hence, epigenetics is emerging as a very important field in plant genetic improvement. Although fundamental epigenetic mechanisms in crops are beginning to be elucidated, we anticipate they will be extensively employed in the future for crop improvement.

The idea of publishing this book has arisen from the fact that Epigenetics has become an important player in the study of gene regulation not only in mammals but also in plants. The inception of research in epigenetics came from the desire to understand how it affects plant development and behavior. Plants are vital in our life because they provide us with oxygen, food, clothing, and medicines. This book gives us comprehensive knowledge about the fundamentals and applications of epigenetics in plants of agronomic importance. A total of eight chapters describe the importance of epigenetics in agriculture and highlight the applications of this field in crop plants, such as coffee, maize, tomato, wheat, sugarbeet, beans, and others. Topics cover from general mechanisms of epigenetic regulation, such as DNA methylation and posttranslational modifications of histones, to the smallest player with the biggest role in gene regulation, small RNAs. We believe the information contained in this book will enhance the knowledge to develop, in the future, novel approaches to manipulate and selectively activate and/or inhibit proteins and metabolic pathways to counter plant pathogens, to better cope with environmental stresses and to increase crop productivity. In the foreseeable future there would be a strong presence of epigenetics in food production, plant fitness, and crop improvement. We hope that readers of this book will find a first glance of the many contributions the field of epigenetics may bring to the table in order to help cover the food demand in the world. Finally, we would like to thank all colleagues who agreed to provide outstanding chapter contributions.

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Chapter 1

The Role of Germinally Inherited Epialleles in Plant Breeding

Megan House and Lewis Lukens

Abstract Plant breeding focuses on repeated selection of individuals with desired traits from phenotypically variable populations. Breeders may be able to explain the broad sense heritability for a trait, the proportion of the total trait variance between genetically distinct lines compared to within a line, or the narrow sense heritability, the proportion of the trait variation that is due to the additive effects of genes. However, breeders rarely know the underlying causes of the observed genetic variation. In this chapter, we take a trait-focused approach to review the degree to which plant variation is due to epigenetic variation and to what degree epigenetic factors are suitable for selection in plant breeding. We suggest that the amount of trait variation that is due to heritable differences in chromatin states is far lower than variation due to changes in the primary sequence of DNA. In addition, epigenetic states are often unstable, and selection on only a small number of epigenetic states could lead to consistent plant improvement.

Keywords Epigenetics • Plant breeding • Epialleles • Epimutagens • Trait variation

1.1 Introduction

The term “epigenetics” has a number of definitions. Waddington (1942) used the term to explain how one genome gives rise to multiple cell lineages that follow diverse developmental trajectories. In other words, epigenetics referred to mechanisms that enable the developmentally appropriate expression of genes. In Waddington’s conception, epigenetic information laid down in development is erased during gametogenesis, consistent with the Mendelian principle that genes

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passed across generations are unaltered by developmental or environmental stimuli. More recently, epigenetics can refer to meiotically heritable changes in gene function that are not due to differences in nucleotide sequence, and we use this definition in our work. Here, an organism imposes chemical changes to DNA or chromatin within a germ cell, and these changes are transmitted to the subsequent generation. Epimutation is a process that generates an epiallele, and the term epiallele refers to a gene with distinct biochemical modifications. Thus, a trait that both varies within a population because of polymorphic nucleotide sequence (s) and is correlated between parent/offspring pairs because of shared nucleotide sequence(s) exhibits genetic inheritance. A trait that both varies within a population because of variable chromatin structures and is correlated between parent/offspring pairs because of these structures exhibits complete epigenetic inheritance.

In this chapter, we first review how the inheritance of variable chromatin states—induced chemically, genetically, or by the environment—can contribute to phenotypic variation. We then address the stability of epialleles across generations. Finally, we highlight the role of epigenetic variation in plant breeding.

1.2 Meiotically Inherited Epigenetic Differences Can Cause Phenotypic Variation

Many epialleles characterized to date are marked by DNA methylation differences. Treatment of plants with DNA methylation inhibitors, such as 5-azacytidine (5azaC) and 5-azadeoxycytidine (azadC), can induce heritable, phenotypic changes (Fig. 1.1, Table 1.1). For example, Fieldes (1994) induced heritable phenotypic changes in flax by 5azaC treatment. Relative to untreated plants, plants growing from treated seeds were often shorter, had fewer leaves on the main stem, and had a reduced flowering times (Fieldes 1994; Amyot 1997; House 2010). From first generation progeny of treated plants, Fieldes et al. selected six lines of flax that were short and early-flowering (Fieldes 1994). Flax is self-pollinating, and these traits were stably transmitted to the next generation. Flowering time variation in populations derived from crossing the early-flowering line to the wild type indicated that at least three independent epialleles contribute to early flowering (Fieldes and Amyot 1999). In Triticale, a wheat x rye hybrid plant, plants from seeds treated with 5azaC have a number of heritable, phenotypic differences relative to plants from untreated seeds (Heslop-Harrison 1990). The 5azaC treatment resulted in plants that are taller than controls, have increased tillering and an increased time to maturity, and these novel traits persisted through two subsequent generations, at which point the study concluded (Heslop-Harrison 1990). Akimoto et al. (2007) noted that two plants grown from a population of 100 rice seeds (*Oryza sativa* spp. *japonica*, ‘Yamada-nishiki’) treated with azadC differed from plants grown from untreated seeds. Most remarkable was a line that was dwarf and flowered 10–14 days early. Similar, chemically induced heritable variation has been observed in

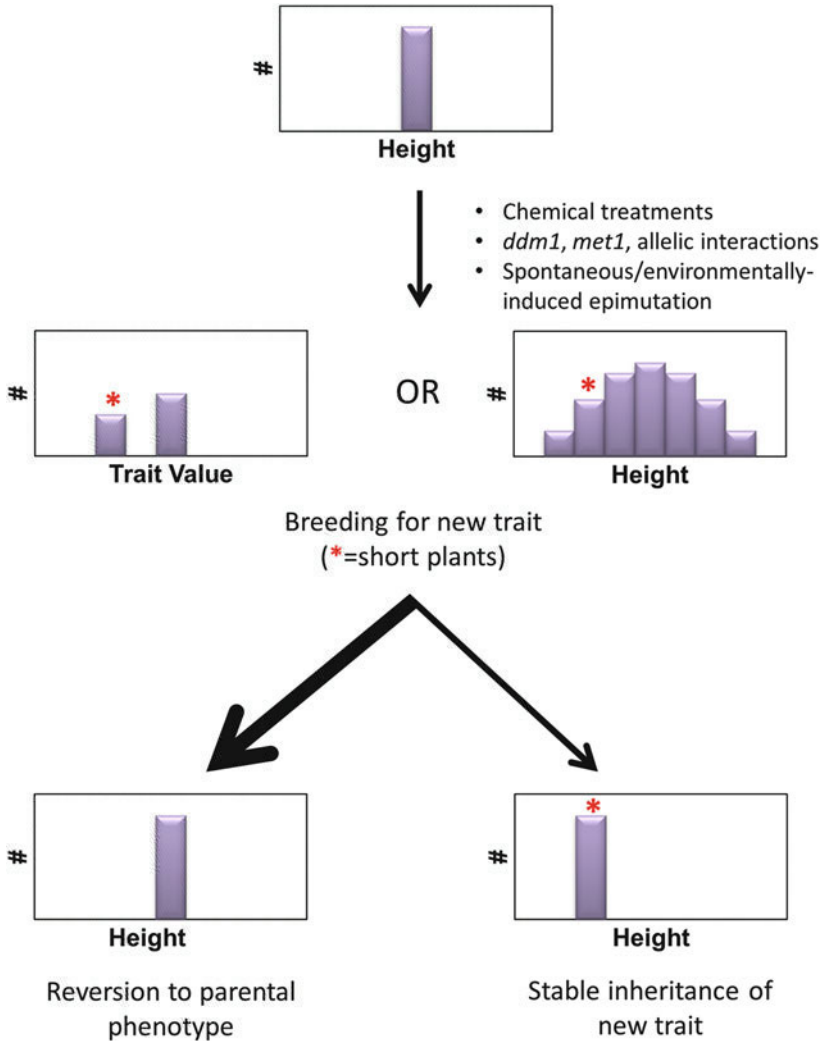


Fig. 1.1 Epimutations induced by several phenomena can generate phenotypic novelty that is in some cases stably inherited. Within the histograms above, the X axis represents a trait value for a plant, for example plant height. The Y axis represents the number of individuals within a population with that trait value. New discrete or continuous trait values arise because of epimutation. The *asterisk* represents a new, favorable trait value. The *arrows* represent the relative frequency of outcomes of selection for the *asterisk* plants. On the *left*, selection was not successful. The trait has reverted to its ancestral value. On the *right*, selection successfully shifted the trait value of the population

other crops, including Brassica (altered leaf morphology, reduced number of anthers, altered phyllotaxy, deformed flowers and change in the time to flowering) (King 1995), rice (dwarfism and delayed ear emergence) (Sano et al. 1990), and

Table 1.1 Examples of epialleles described in this chapter

Species	Locus	Nature of change	Trait affected	References
<i>L. vulgaris</i>	<i>Lcyc</i>	Spontaneous	Floral architecture	Gustafsson (1979), Cubas et al. (1999)
Tomato	<i>Cnr</i>	Spontaneous	Skin pigmentation and fruit ripening	Thompson et al. (1999), Manning et al. (2006)
<i>Zea mays</i>	<i>B1</i>	Spontaneous (Paramutation)	Pigmentation	Coe (1966), Patterson et al. (1993, 1995), Stam et al. (2002)
Flax	?	Induced (5azaC)	Height, flowering time and leaf number	Fieldes (1994), Fieldes et al. (2005)
Rice	?	Induced (azadC)	Height and pathogen resistance	Akimoto et al. (2007)
Triticale	?	Induced (5azaC)	Height, tillering and flowering time	Heslop-Harrison (1990)
Maize	<i>Spm</i>	Spontaneous	Anthocyanin production	McClintock (1957, 1965), Peterson (1966), Fowler and Peterson (1978), Banks et al. (1988)
Arabidopsis	<i>SUP</i>	Mutagen	Floral morphology	Jacobsen and Meyerowitz (1997), Ito et al. (2003)
Arabidopsis	<i>FWA</i>	Mutagen	Flowering time	Soppe et al. (2000)

Melandrium (appearance of bisexual flowers on a normally dioecious plant) (Janoušek et al. 1996).

The concept that all variation generated by DNA demethylating agents is caused by epigenetic variation is attractive, but some heritable traits among treated populations may have a genetic basis. 5azaC and azadC demethylating agents can act as weak mutagens (Zimmermann and Scheel 1984). In addition, demethylation can activate quiescent transposons leading to transposon mutagenesis (Scortecci et al. 1997).

Mutations within genes important for maintaining DNA methylation also act as epimutagens and generate heritable epialleles. For example, the *Arabidopsis thaliana* gene *DDM1* (*Deficient in DNA Methylation 1*) encodes an ATPase chromatin remodeler that is involved in the maintenance of DNA methylation in both CG and non-CG sequence contexts (Jeddeloh et al. 1999) and in the silencing of repeat elements such as transposons (Hirochika et al. 2000; Miura et al. 2001; Singer et al. 2001). Genomic DNA of the *ddm1* mutant is hypomethylated throughout the genome (Vongs et al. 1993). *ddm1* plants have weak phenotypes, but after several generations of selfing, novel traits including leaf structure, flowering time, flower structure, both increased and decreased apical dominance, and reduced internode length arise at high frequency within mutant lines (Kakutani

et al. 1996). Some epialleles that appear within the *ddm1* mutant background, such as the ball phenotype, are stably inherited (Kakutani et al. 1996; Soppe et al. 2000; Saze and Kakutani 2007). METHYLTRANSFERASE1 (MET1) is also required for propagating ^mCG methylation during DNA replication, and *Arabidopsis* (ecotype C24) *MET1* antisense lines show the heritable effects of aberrant DNA methylation patterns through the gradual loss of CG methylation (Finnegan et al. 1996). A number of traits arise in *met1* lines including reduced apical dominance, altered flowering time, altered floral morphology, decreased plant size, and altered leaf shape and size (Finnegan et al. 1996). As with *ddm1* mutants, floral traits persist in individuals without the silencing transgene (Finnegan et al. 1996).

As with chemical treatments, mutations that reduce DNA methylation can have secondary effects on DNA sequence through novel transpositions. The clam phenotype is a severe, heritable phenotype induced by *ddm1*. Plant growth is severely inhibited. Miura et al. (2001) discovered the trait was due to insertion of a CACTA family transposon released in the low methylation genome. Nonetheless, the rate of transposition is low in part because of RNA dependent DNA methylation (Miura et al. 2001; Singer et al. 2001; Teixeira et al. 2009). Transposons have unlikely generated the large amount of trait variation amongst mutant lines. For example, a locus controlling late flowering traits was genetically mapped to *FWA* (Soppe et al. 2000). *FWA* has tandem repeats within its promoter. While the repeats are methylated in diploid tissues of wild-type plants, exposure to *ddm1* has caused low DNA methylation levels and thus activated alleles to repress flowering (Soppe et al. 2000).

Some trait variation is also caused by allelic interactions between homologous alleles. Studies of maize pigmentation inheritance have revealed a number of these scenarios. Brink (1956) noted that the effect of R^r male gametes from R^rR^{st} plants with stippled aleurone differed from the effect of R^r male gametes from R^rR^r sibling plants. When crossed to a tester strain with colorless aleurone (rr), the latter gave the expected dark mottled kernels. However, the former produced testcross progeny with stippled seed (Brink 1956). Brink termed R alleles from the former cross as R' alleles. These R' epialleles are transmitted across generations. While kernels from $rr \times Rr$ test crosses show the expected dark mottling, kernels from the $rr \times R'r$ test crosses are weakly pigmented. The *booster1* (*b1*) locus in maize (Coe 1966) also regulates production of anthocyanin pigments. Plants homozygous for the *B-I* (*B-Intense*) allele at the *b1* gene have dark purple pigmentation and high levels of gene expression, whereas plants homozygous for the B' allele are lightly pigmented (Coe 1966) and have low levels of transcription at the *b1* gene (10- to 20-fold lower than *B-I* homozygotes) (Patterson et al. 1993). In heterozygotes that carry both the *B-I* allele and the B' allele, *B-I* is converted (paramutated) to B' with 100 % frequency (Coe 1966). The new B' allele is designated B' , and is able to paramutate a *B-I* allele to B' in the following generation (Coe 1966). A region of tandem repeats ~6 kb in length and ~100 kb upstream of the *b1* gene is crucial for the paramutagenicity and the paramutability of the B' and *B-I* alleles (Stam et al. 2002). Double stranded RNA is very likely the key factor that changes paramutable alleles to paramutagenic alleles (Alleman et al. 2006). RNA-dependent RNA polymerase,