

Jitendra Kumar · Aditya Pratap
Shiv Kumar *Editors*

Phenomics in Crop Plants: Trends, Options and Limitations

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Foreword



Growing world population is expected to cause a “perfect storm” of food, energy and water shortages by 2030 as demand for food and energy will jump by 50 % and for fresh water by 30 %, as the population tops at 8.3 billion. The overarching challenge before the policy makers and agricultural scientists is how to ensure food and nutrition security for an ever-increasing population from limited and fast depleting resources under climate change scenario, especially in countries like India where sizeable population is still suffering from the triple burden of malnutrition. To meet the future demand of agricultural production, we need to develop more productive and nutritious varieties of agricultural crops which incorporate both high intrinsic yield potential and resilience under climatic stresses. This requires discovery and deployment of superior but complex traits from the vast germplasm resources being held in various gene banks to agronomically superior varieties efficiently and precisely.

Traits of breeders’ interest such as grain yield, plant growth and resistance to biotic and abiotic stresses are complex as these are controlled by many genes of minor effects and highly influenced by environmental factors and their multi-dimensional interactions. In the past, plant breeders were successful in selecting desirable varieties empirically on the basis of visual observations, more so for qualitative traits; but empirical selection remains elusive and less effective for traits essential for meeting the current challenges such as underground, physiological and biochemical traits. Recent advances in genomics have created enormous genomic resources in several crop species which have the potential to increase harvestable yield

manifolds. However, available gene sequences and molecular markers could not be mainstreamed in crop improvement programs mainly due to the lack of precise phenotyping data. Therefore, it is imperative to phenotype the available germplasm precisely and efficiently in various crop species.

The current knowledge and voluminous information generated on phenotyping tools and techniques available in literature need to be consolidated so that researchers and scholars have access to such vast knowledge at one place. The present book, *Phenomics of Crop Plants: Trends, Options and Limitations*, which is a meticulously edited volume, is an attempt in this direction to bring together information on precision phenotyping under controlled versus natural environments, digital and image based phenotyping, phenomics of biotic and abiotic stresses and functional traits, and precision nutrient management. This book also covers experimental designs and biometrical approaches suitable for precision phenotyping of complex traits, and how phenomics can help to harness potentiality of genomics. Various chapters in this book have been contributed by renowned scientists whose research contributions are acknowledged globally. I am quite hopeful that the information contained in this book will boost research efforts of plant scientists to bring about a major breakthrough in agricultural production and will serve as a resource material for those who are involved in teaching and research in agricultural crops. I congratulate the editors Jitendra Kumar, Aditya Pratap and Shiv Kumar for bringing out this book timely on such an important and emerging aspect and hope that it would be widely read by scholars and researchers.

Secretary, DARE and Director General, ICAR
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S. Ayyappan

Preface

It has been estimated that agricultural production must be doubled by 2050 in order to meet the predicted demand of growing world population. Achieving this goal poses a serious challenge to plant breeders as the current agricultural production growth rate of 1.3 % per annum is below the population growth rate. In the recent past, research advances have been made in the development of genomic tools and techniques which have the potential to increase the rate of genetic improvement. The whole genome studies have the potential to greatly facilitate genetic dissection of complex traits such as yield and stress tolerance by using technological advances in genotyping and sequencing. However, successful application of genomics towards the genetic improvement of crop plants depends upon our ability of precision phenotyping of these complex traits. Low cost and high-throughput genotyping has paved the way for the development of large mapping populations and diversity panels of thousands of recombinant inbred lines. These genetic resources require precise phenotyping. Marker-assisted recurrent selection (MARS) and genome-wide selection require phenotypic data, although conceptually selections are made on the basis of genetic information. A single phenotyping cycle is used to identify markers for subsequent selection through generations. In transgenic studies also, phenotyping is necessary for identification of promising events. Molecular breeding populations sometimes include up to 5,000 lines and their accurate characterization simultaneously is a challenging task. Also phenotyping of such complex traits are labor intensive, and many other interesting traits involved in biological processes are currently not suitable for genetic mapping due to the lack of approach to efficient and reliable measurement. The success in development of improved varieties relies on the ability to identify the best genetic variation for advancement. Because breeding is essentially a numbers game, more crosses and environments are required to identify superior variation with greater probability. Therefore, plant breeders want to phenotype a large number of lines rapidly and accurately to identify the best progeny. Advances in phenotyping are essential to capitalize on the developments in conventional, molecular, and transgenic breeding and ensure genetic improvement of crops for future food security.

In recent years, there has been increased interest in development of high-throughput phenotyping tools and techniques for screening of agronomic, physiological, and biochemical traits expressing especially under biotic and

abiotic stresses. These techniques have become much more advanced and have now entered the era of high-throughput field phenotyping. Several phenotyping platforms have been developed around the world, which are fully automated facilities in greenhouses or growth chambers with robotics, precise environmental control, and remote sensing techniques to assess plant growth and performance. Consequently, voluminous literature has been generated on different aspects of phenotyping which is scattered in numerous journals and books. However, no single publication is available to provide a comprehensive insight into this literature with a focus on phenomics of crop plants. This book, *Phenomics of Crop Plants: Trends, Options and Limitations*, is an attempt in this direction to bring together various high throughput, advanced phenotyping tools, techniques and platforms for directed genetic improvement in crop plants.

The present book comprises 19 chapters contributed by renowned scientists in their fields of expertise. The first chapter presents an overview on the recent developments in phenotyping. The second chapter deals with traits that require precise phenotyping. Chapter 3 discusses various issues related to phenotyping under controlled and natural environments while the subsequent three chapters (Chaps. 4, 5, and 6) deal with the imaging tools in phenotyping agronomic and physiological traits in crop plants. Chapters 7, 8, and 9 focus on phenotyping tools available for heat and drought related traits and soil problems. Chapter 10 deals with screening methods for diseases and possibility of using the recent developments in the field of phenomics. The subsequent three chapters (Chaps. 11, 12, and 13) discuss the advances in phenotyping of functional traits, role of fluorescence approaches for understanding the functional traits of photosynthesis and use of NMR in identification of subcellular structural and metabolic challenges. The next two chapters are on precision nutrient management and identification of nutritional and anti-nutritional factors of seeds (Chaps. 14 and 15). The subsequent two chapters (Chaps. 16 and 17) discuss the role of experimental designs for precision phenotyping and use of biometrical approaches in data analysis of the complex traits. As vast amount of genomic resources are now available in several crop plants, precision phenotyping can harness the potentiality of these genomic resources for accelerating the genetic improvement through mainstreaming them in the ongoing breeding programs. Therefore, the next two chapters (Chaps. 18 and 19) deal with how the available genomic resources can be utilized in a better way by using the available phenomics platforms worldwide for precise phenotyping of agronomic and physiological traits. Each chapter of this book has focused on the current trends, available options for phenotyping the target traits and limitations in their use for phenomics of crop plants.

The review of entire published work was neither possible in a single volume nor was the aim of this book. However, the contributors of individual chapters have provided exhaustive list of references on significant work done so far on different aspects of phenomics. Keeping in view the scope of the book, a little overlap in the subject is possible albeit all chapters have been dealt in depth by various experts. We are extremely grateful to all the authors

who despite being busy with their research and academics completed their chapters with a professional approach and great care.

We are highly indebted to Dr. S. Ayyappan, Secretary, Department of Agricultural Research and Education (DARE), Government of India, and Director General, Indian Council of Agricultural research (ICAR); and Dr. Mahmoud Solh, Director General, International Centre for Agricultural Research in the Dry Areas (ICARDA) for encouragement and inspiration in bringing out this publication. We are also thankful to Prof. Swapan Datta, Deputy Director General (Crop Science), ICAR; Dr. Maarten van Ginkel, Deputy Director General (Research), ICARDA; Dr. Michael Baum, Director of BIGM, ICARDA and Dr. B. B. Singh, Assistant Director General (Oilseed and Pulses), ICAR, for providing support and state-of-the-art facilities to carry out research on pulses. Dr. N. P. Singh, the present Director and Dr. N. Nadarajan, Ex-Director of IIPR, Dr. S. K. Chaturvedi, Head, Crop Improvement Division, IIPR, have been the source of encouragement for the present endeavor. Several people have rendered invaluable help in bringing this publication to life and they deserve our heartfelt appreciation and gratitude: Dr. Sanjeev Gupta, Project Coordinator, MULLaRP, IIPR, for technical comments and scientists of Crop Improvement Division, IIPR, for their valuable technical inputs during the course of editing the chapters; Mr. Ramesh Chandra, Senior Technical Assistant; Mr. Rohit Kant, Miss Nupur Malviya and Rakhi Tomar, Senior Research Fellows, for helping in compilation of references and voluminous correspondence, and Springer International for bringing the book through printing process with a thorough professional approach. Last but not least, our kids Neha, Gun and Puranjay and our better halves, Mrs. Renu Rani, Dr. Rakhi Gupta and Dr. Pankaj Rani Agrawal, deserve special thanks for their unstinting help, patience and emotional support during the course of this book.

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Dr. Jitendra Kumar, born in 1973, is presently working as a Senior Scientist in the Division of Crop Improvement at Indian Institute of Pulses Research, Kanpur. He has an excellent research career throughout. He secured Gold Medal during masters' programme and pursued his Ph.D. in Genetics and Plant Breeding from G. B. Pant University of Agriculture and Technology, Pantnagar, India. He was awarded CSIR-Research Associateship during 2003–2005 for postdoctoral studies at the Institute of Integrative Medicine, Jammu (India). He has more than 14 years of research experience in genetic improvement using both conventional and molecular marker-assisted breeding approaches on various crops including sunflower, medicinal and aromatic, cereal and pulse crops. He has done work on development of SSR markers, identification of QTLs for preharvest sprouting and high grain protein content and marker-assisted breeding in wheat for pyramiding the preharvest sprouting tolerance and high grain protein content and leaf rust resistance and developed a number of lines. During this period, he undertook study tours of several countries including Austria, Syria, Bangladesh, Nepal, Lebanon and Canada. His research interests include conventional and molecular breeding, QTL analysis and marker-assisted selection for crop improvement. He has more than 100 publications including research and review articles in reputed national and international journals, book chapters, meeting reports, popular articles, and bulletins. He has also co-edited three books including *Biology and Breeding of Food Legumes* published by CABI, Oxfordshire, *Alien Gene Transfer in Crop Plants: Innovations, Methods and Risk Assessment* and *Alien Gene Transfer in Crop Plants: Achievement and Impacts* both published by Springer, New York, USA. He has developed high-yielding varieties (IPL 316 and IPL 526) of lentil and several others are in the pipeline. His current priorities include involvement of molecular marker technology in conventional lentil breeding programme for making genetic improvement towards biotic and abiotic stresses.

Dr. Aditya Pratap, born on October 18, 1976, is currently working as a Senior Scientist (Plant Breeding) in the Crop Improvement Division, Indian Institute of Pulses Research, Kanpur. He obtained his Master's and Ph.D. degrees in Plant Breeding and Genetics from CSK Himachal Pradesh Agricultural University, Palampur, India, in 1999 and 2003. Holding a brilliant academic and service record, he has been associated with crop research since last 10 years. He has worked on genetic improvement of crop plants including wheat,

triticale, rapeseed-mustard, chickpea and *Vigna* species and has been instrumental in the development of haploidy breeding protocol in cereals through chromosome elimination technique. He has been associated with the development and release of five crop varieties including two in rapeseed-mustard (RSPT-2 and RSPR03), two in green gram (IPM 02-14 and IPM 02-3) and one in facultative winter wheat (DH 114). He has developed two extra early mungbean genotypes (IPM 205-7 and IPM 409-4 (48 days maturity)) besides being instrumental in establishing prebreeding garden of rapeseed-mustard at SKUAST-Jammu and of pulses at IIPR, Kanpur. Presently, he is working on genetic improvement of green gram (*Vigna radiata*) through distant hybridization aided by conventional and biotechnological tools. His research interests include distant hybridization, doubled haploidy breeding, plant tissue culture, and molecular breeding. To his credit, he has about 120 publications which include research papers published in high-impact journals as well as reviews/chapters for best international publishers including Springer, Academic Press and CRC. He has published four books entitled, *Haploidy Breeding in Triticale and Triticale X Wheat Hybrids: Comparison of Anther Culture and Chromosome Elimination Techniques* by Lambert Academic Publishing, Germany; *Biology and Breeding of Food Legumes* published by CABI, Oxfordshire; *Alien Gene Transfer in Crop Plants: Innovations, Methods and Risk Assessment* and *Alien Gene Transfer in Crop Plants: Achievements and Impacts*, both published by Springer, New York. He is also a recipient of the prestigious Norman E. Borlaug International Agricultural Science and Technology Fellowship. He is an acknowledged speaker and has several awards to his credit.

Dr. Shiv Kumar is Food Legumes Coordinator and works as Lentil and Grasspea Breeder at the International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat platform, Morocco. Before joining the present position, he served the Indian Council of Agricultural Research as a Plant Breeder for 18 years. He also served the International Crops Research Institute for Semi-Arid Tropics as Post Doctoral Fellow and worked on basic studies in chickpea breeding and genetics between 1991 and 1993. His post-doctoral work has led to identification of extra early photo-thermo insensitive genotypes in chickpea which have been used as donors across the globe. Dr. Kumar has over 25 years of research experience on basic and applied aspects of breeding rice and pulses including chickpea, grasspea, *Vigna* crops and lentil. He has been associated in the development of 28 varieties of pulse crops and one variety of rice. He also identified useful new germplasm for use in breeding program of rice, lentil, chickpea, grasspea, mungbean and urdbean. He has to his credit more than 300 articles including 110 research papers in refereed journals, 52 book chapters, 6 books, 7 technical bulletins and 2 training manuals. He also received a number of academic distinctions and awards including Rockefeller Fellowship, Best Scientist Award from IIPR for the years 2005 and 2008, and Best Research Team Award from MULLaRP of ICAR in 2008. His research interests include pre-breeding activities, genetic enhancement through conventional and marker-assisted breeding and biometrical genetics.

Jitendra Kumar, Aditya Pratap, and Shiv Kumar

Abstract

Precise and accurate measurement of traits plays an important role in the genetic improvement of crop plants. Therefore, a lot of development has taken place in the area of phenomics in the recent past. Both forward and reverse phenomics have been evolved, which can help in identification of either the best genotype having the desirable traits or mechanism and genes that make a genotype the best. This includes development of high throughput non-invasive imaging technologies including colour imaging for biomass, plant structure, phenology and leaf health (chlorosis, necrosis); near infrared imaging for measuring tissue and soil water contents; far infrared imaging for canopy/leaf temperature; fluorescence imaging for physiological state of photosynthetic machinery; and automated weighing and watering for water usage imposing drought/salinity. These phenomics tools and techniques are paving the way in harnessing the potentiality of genomic resources in genetic improvement of crop plants. These techniques have become much more advanced and have now entered the era of high throughput integrated phenotyping platforms to provide a solution to genomics-enabled improvement and address our need of precise and efficient phenotyping of crop plants.

Keywords

Phenomics • Forward • Reverse • Phenome • Phenes • Genomics • Genes

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1.1 Introduction

Worldwide demand for crops is increasing rapidly due to rising global population, rising demand for biofuel and feed stocks and changing food preferences. Meeting future demand of agricultural production poses the greatest challenge to agricultural scientists and policy makers

(Bruinsma 2003) because demand for cereals, biofuels and feed stocks has already surpassed the current supply and is expected to rise further in the near future (Furbank et al. 2009; Sticklen 2007). Therefore, there is a competition among crops for arable land in order to increase their production. Rising global mean temperature by 0.8 °C since the 1850s, which is expected to increase further by 1.8–4.0 °C by the end of this century, will have further impact on agricultural production due to changing climate (Solomon et al. 2007) and prevalence of abiotic stresses with more intensity and frequencies (Tester and Langridge 2010). It has been estimated that in future average crop yields may decline across Africa and South Asia by 8 % by the 2050s (Knox et al. 2012). These declines in yields have been predicted about 17 % in wheat, 5–16 % in maize, 11–15 % in sorghum, and 10 % in millet across above regions under regimes of climate change (Wheeler and von Braun 2013). Therefore, development of ‘climate-smart’ germplasm would be a priority to tackle these future challenges of climate change (Ziska and Bunce 2007; Leakey et al. 2009).

The use of conventional plant breeding methods has made substantial gain in crop yield worldwide. However, researchers are now observing that current breeding methods will not be sufficient to meet the projected future demand of foods (Furbank et al. 2009; Tester and Langridge 2010; Sticklen 2007). Therefore, this has shifted our focus towards the use of genomics and gene technology advances for assisting the current breeding programs in order to increase grain yields. These developments are being utilized in trait discovery, genetic dissection of complex traits and discovery of associated genes and their deployment in varieties. This has resulted so far in more than 5,000 publications on mapping of quantitative trait loci (QTL) and their isolation during the past years (Zamir 2013). In spite of these efforts, the identified QTLs/genes could not be deployed in mainstream breeding programs because identification of most of these QTLs/genes was not based on the precise and accurate phenotyping data of targeted traits. Hence, association of these QTL/genes with the

phenotype in a ‘real world’ environment remains elusive as many false positive QTL have been reported earlier.

Although a large collection of germplasm of different crop species are available worldwide, phenotypic descriptions of these genome wide knockout collections are still limited. As a result, it restricted the use of genomic resources for identifying the allelic variation for a promising candidate gene in natural germplasm collection (see Miyao et al. 2007). The poor utilization of genomic resources could also be due to the lack of analysis of invisible traits and sometimes complex phenotypic effects of genetic modification. Therefore, identification of a candidate germplasm that carries genes for targeted traits is only possible when we will have the precise and accurate phenotyping profile of the germplasm. Phenotyping of valuable agricultural traits such as grain yield, abiotic stress tolerance, and nutritional quality is widely recognized as the most laborious and technically challenging because replicated trials are necessary across multiple environments over a number of seasons. Some of the current phenotyping tools also require destructive harvesting at fixed time intervals or at a particular phenological stage and are slow and costly. These bottlenecks in field phenotyping have driven intense interest over the past decade and hence efforts have been made on development of new high throughput phenotyping tools and techniques such noninvasive imaging, spectroscopy, image analysis, robotics and high-performance computing for phenotyping. These tools can not only be used in laboratories but also in field leading to high-throughput analysis of phenotypes in natural conditions as well as under controlled-environment conditions. Now, field evaluation of plant performance is much faster, and facilitates a more dynamic, whole-of-lifecycle measurement less dependent on periodic destructive assays. The dedicated high throughput controlled-environment facilities have also improved the precision in recording the data and reduce the need for replication in the field. Thus these advances have revolutionized the field of the accurate and precise phenotyping for

important traits and bring us to the age of ‘phenomics’ and overview of these developments have been presented in this chapter.

1.2 Origin of Plant Phenotyping

Plant phenotyping has been a part of crop and variety selection since the time of human civilization when humans selected the best individuals of a crop species for domestication (Diamond 1997). Subsequently it has become common practice in plant breeding for selecting the best genotype after studying phenotypic expression in different environmental conditions and also using them in hybridization programs in order to develop new improved genotypes (Pearson et al. 2008; Fisher 1925; Annicchiarico 2002). Ecologists used phenotyping to study phenotypic plasticity of genotypes during the middle of the twentieth century and suggested the role of the genotype and environmental conditions in the expression of plant phenotypes under which it develops (Suzuki et al. 1981). Subsequently, developments in ecology in relation to phenotyping are the trait-based approaches, in which phenotypic characteristics of a wider range of different species are evaluated either in the field (Reich et al. 1992) or under laboratory conditions (Grime and Hunt 1975; Poorter et al. 1990). They were used to derive different strategies by which the ecological niche of species could be described (Grime 1979) and to analyze the interdependence of various traits (Wright et al. 2004).

1.3 Phenomics

The word ‘phenome’ refers to the phenotype as a whole (Soul 1967) i.e., expression of genome for a trait in a given environment while in phenomics we get high-dimensional phenotypic data on an organism at large scale. Actually phenomics is used as analogy to genomics. However it differs from genomics. In genomics, complete characterization of a genome is possible while in phenomics, complete characterization of

phenome is difficult due to the change in the phenotypic expression of traits over the environmental conditions (Houle et al. 2010).

1.4 Phenotype vs Phenomics

Phenotype of a plant can be described on the basis of morphological, biochemical, physiological and molecular characteristics. Different parameters are measured to describe these characteristics. Johanssen (1911) has coined the terms ‘genotype’ and ‘phenotype’. He demonstrated substantial variation in quantitative traits to which he called ‘phenotypical’ in genetically-identical material and thus proved that variation in a given observed traits is not controlled entirely by genetics. Therefore, use of statistical analysis has been suggested for identifying the differences among genotypes because phenotypic variation within a genotype can obscure phenotypic differences among genotypes. This leads to origin of pheno- word. After 1950, ‘phenotyping’ as a noun, ‘to phenotype’ as a verb and ‘phenome’ as the collective noun were introduced, which have been accepted scientifically and are being utilized commonly in literature.

1.5 Forward and Reverse Plant Phenomics

Plant phenomics is the study of plant growth, performance and composition. Figure 1.1 showed the use of forward and reverse phenomics in genetic improvement. Forward phenomics uses phenotyping tools to discriminate the useful germplasm having desirable traits among a collection of germplasm. This leads to identification of the ‘best of the best’ germplasm line or plant variety. Use of high-throughput, fully automated and low resolution followed by higher-resolution screening methods have accelerated plant breeding cycle by screening a large number of plants at seedling stage. Thus interesting traits can be identified rapidly at early stage and there is no need to grow plants up to the

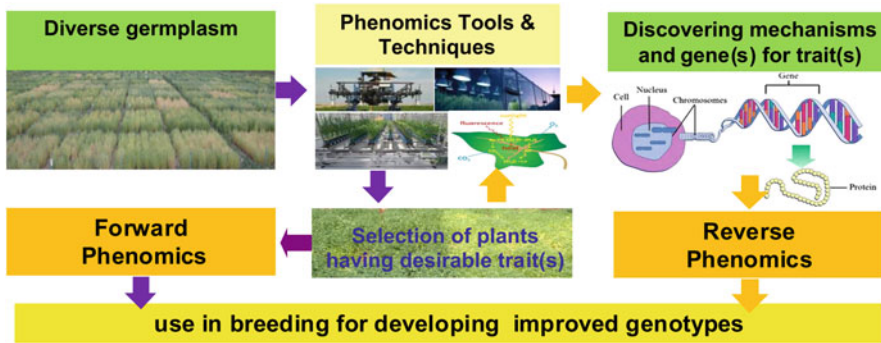


Fig. 1.1 Forward and reverse phenomics for genetic improvement in crop plants

maturity stage in field. Now it is possible in forward phenomics to screen thousands of plants in pots running along a conveyor belt, and travelling through a room containing automated imaging systems such as infra-red or 3D cameras. The pots are labelled with barcodes or radio tags, so that the system can identify which pots contain plants with interesting traits. The selected plants can then be grown up to produce seed for further analysis and breeding.

The reverse phenomics is used where the best of the best genotypes having desirable trait(s) is already known. Now through reverse phenomics, traits shown to be of value to reveal mechanistic understanding are dissected in details and subsequently the identified mechanisms are exploited in new approaches. Thus in reverse phenomics, we discover mechanisms which make ‘best’ varieties the best. This can involve reduction of a physiological trait to biochemical or biophysical processes and ultimately a gene or genes. For example, in case of drought tolerance, researchers try to work out the mechanisms underlying the drought tolerance and find out the gene or genes that are responsible for it. These genes are screened in germplasm or the gene can be bred into new varieties.

1.6 Genes and Phenotypes

To describe phenotype is more challenging than genotype because it changes over the environments. Therefore, the term ‘phenotype’

is not completely straight forward (Mahner and Kary 1997) and it varies among the various sub disciplines of biology. Ecologists traditionally define phenotype as trait when they refer to a phenotypic variable of a plant such as the specific leaf area (SLA). However, some ecologists also refer to traits in relation to characteristics of vegetation, such as the leaf area index (LAI). Like gene, ‘trait’ has been designated as ‘phene’. However it can be over simplification for a one-to-one relationship between gene and phene because one gene can have a range of pleiotropic effects and many genes can control a trait. The term ‘phenome’ is being utilized as a counterpart to ‘genome’. Thus as total constellation of all genes (alleles) present in an individual is known as genome. Therefore, similarly the phenome would be the aggregate of all the expressed traits of an individual. Actually, use of various terminology may overlap as they fulfill various and different needs for different niches of the scientific community. A clear and singular definition throughout the full domain of biology is desirable but probably unreachable (Mahner and Kary 1997).

1.7 Advances in Phenomics

Morphological, physiological and biochemical traits are important to breeders for making genetic improvement for yield, quality and tolerance to biotic and abiotic stresses. These traits have been discussed in details in Chap. 2.

Conventionally, phenotyping data on these traits are recorded either visually or manually, which is time-consuming and required a lot of efforts. This also increases chance of errors in measurement of traits. As a result, it increases chance to identify the false positive alleles, which leads to slow gain in genetic improvement. Therefore during the past few years, focus has been shifted on precise, accurate and rapid phenotyping of traits on a large scale. High-throughput phenotyping using non-invasive imaging technologies is a rapidly advancing field (www.plantphenomics.org.au; Furbank et al. 2009; Finkel 2009; Jansen et al. 2009; Berger et al. 2010). These techniques are based on colour imaging for biomass, plant structure, phenology and leaf health (chlorosis, necrosis), near infrared imaging for measuring tissue water content and soil water content, far infrared imaging for canopy/leaf temperature, fluorescence imaging for physiological state of photosynthetic machinery and automated weighing and watering for water usage imposing drought/salinity conditions. These advanced phenotyping techniques have been discussed in details earlier in a number of reviews (see Furbank and Teste 2011; Walter et al. 2012). The genotypes capable of maintaining stomatal conductance under salt induced osmotic stress have been selected successfully at the young seedling stage in wheat and barley using infrared thermography (Sirault et al. 2009). This technique has also been suggested to use for high-throughput seedling screening for drought tolerance in the vegetative stages of crop development and has great potential for low-cost, high-throughput field phenotyping. The genotypes having better photosynthetic capability and higher water use efficiency in field can be screened by measuring the canopy temperature using handheld hemipole based infrared thermometers (i.e. canopy temperature 'guns'). Chlorophyll fluorescence analysis has been used to test the maintenance of photosynthetic function under biotic and abiotic stresses leading to identification of resistance and susceptible genotypes. For this purpose, a commercial instrument namely pulse amplitude-modulated (PAM) or fluorometry has been

developed which is based on fluorescence parameter measured in stress (Baker 2008). It can be used on whole leaves or small plants. It used successfully for abiotic stresses screening in *Arabidopsis* and tobacco (*Nicotiana tabacum*) or seedlings of dicots such as canola (*Brassica napus*) or cotton (*Gossypium* ssp.) (Baker 2008; Woo et al. 2008). It can also be used to determine projected leaf area and hence the growth rate if measurements are taken regularly over time (Barbagallo et al. 2003). The chlorophyll fluorescence images of the affected area of the leaf allow the early detection of disease symptoms caused by the pathogens. These infected areas can be quantified leading to identification of the susceptible and resistant response to pathogen attack, at least in the case of mildew on barley leaves (Swarbrick et al. 2006; Chaerle et al. 2009). Leaf spectroscopy or hyperspectral reflectance spectroscopy using radiometric or, more recently, imaging sensors are another established optical techniques related to chlorophyll fluorescence, which have been developed to study the stress related phenomics (Jones and Vaughan 2010). However, its use in plant breeding is limited due to difficulties in interpreting canopy temperature data.

Digital imaging is one of the least complicated but useful methods for quantitatively determining the stress tolerance. It is popular approach for in situ crop phenotyping in controlled environment facilities. It uses to take the digital images of growth over a period of plant development and measures quantitative changes in images caused by the sum of stress response mechanisms. In addition to this, taking digital images in visible wavelength regions also give opportunity to identify color of the plants. As a result, it enables to quantify senescence arising due to nutrient deficiencies or toxicities, or pathogen infections. It has been used successfully to quantify toxicity of germanium (as a toxic analogue of boron) in a mapping population of barley (Schnurbusch et al. 2010) and identified a QTL at the same locus as previously identified for boron tolerance using a visual score of symptoms (Jefferies et al. 1999). The attempt was also made to measure the water use

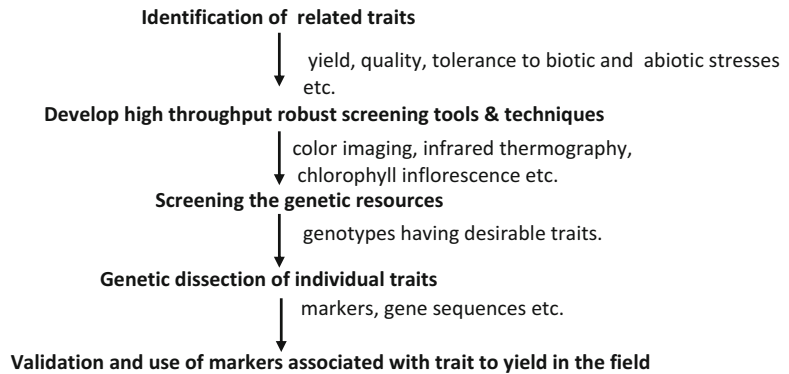
efficiency in plants (Harris et al. 2010). Use of non-destructive imaging using fluorescence and hyperspectral reflectance offers great promise in quantitative scoring of such adult plant resistance phenotypes. However use these techniques for screening biotic stresses is still limited.

1.7.1 Development Towards the Phenotyping Machines

ring the past one decade, vast amount of genomic resources have been developed and rapid development in genome sequencing has increased the genomic data bases such as, e.g. GABI DB or TAIR DB in model plant species and crop plants (Meinke et al. 1998; Riano-Pachon et al. 2009; Huala et al. 2001). High throughput genotyping platforms have increased the speed of genotype selection in breeding programs (Langridge and Fleury 2011). However phenotyping for complex traits related to anatomy, morphology, physiology and development is still less advanced, although high-throughput phenotyping techniques have increased our detection ability substantially at subcellular level for protein interactions or metabolism (Houle 2010; Kolukisaoglu and Thurow 2010). For plant breeders, screening component traits contributing to yield under field conditions at large scale is more important for making genetic improvement, but it is still lacking (Furbank and Tester 2011). However significant efforts have been made towards the development of automated phenotyping platforms during the past years (Granier et al. 2006; Jansen et al. 2009; Furbank and Tester 2011; Delseny et al. 2010; see Chap. 18) by taking advantages of throughput phenotyping facilities developed in the field of drug discovery, development, and animal behavior (Mayr and Bojanic 2009; Noldus et al. 2001). In brief, these platforms are equipped with sensor or image based systems under the controlled growth leading to establishment and implementation of the non-destructive imaging approaches for phenotyping (Furbank

and Tester 2011; Fiorani et al. 2012). In these platforms, we can measure the plant size and leaf area of large germplasm collections using 2D color images and dense canopy by using 3D image technology and magnetic resonance imaging (MRI) (Poorter et al. 1988; Dornbusch et al. 2012). The fluorescence and hyperspectral analysis allow evaluation of various plant traits in a fast and non-destructive manner to characterize the leaves and roots at physiological or biochemical level. However, only specific aspects of plant functioning can be evaluated in this way. An exciting new development is the robotised sensor-actor for destructive sampling of relevant plant parts has widen the phenotyping capabilities by automated measurement of cellular processes and/or gene expression at specific time points (Alenyà et al. 2012). Relevance of a laboratory and greenhouse phenotyping technique is actually tested in field because traits considered critical in the greenhouse may be less important in the field. For example, the canopy of a stand is more relevant than of a single plant under field conditions. Therefore, mobile platforms such as a tractor equipped with specific sensors enabled larger spatial flexibility have been developed for the mechanistic field phenotyping measurements with high accuracy and repeatability in given plots, while drones or airborne platforms can cover vast agricultural areas. Though multi- and hyperspectral technologies (Rascher and Pieruschka 2008; Comar et al. 2012) can be used to analyze physiological process, only few robust techniques such as the laser-induced fluorescence transient (LIFT) approach are available to estimate photosynthetic efficiency in the field (Pieruschka et al. 2010). Dedicated field sensors are already applied in precision agriculture for nutrient management (Scotford and Miller 2005) and may become important tools for sensing of plant disease in the near future (Mahlein et al. 2012). Establishment of wireless sensor networks enables continuous monitoring of the environment and crop properties and will provide valuable information for agricultural management (Ruiz-Garcia et al. 2009).

Fig. 1.2 Flow chart of application of non-destructive phenotyping in genetic dissection of trait



1.8 Harnessing the Potentiality of Genomics Through Phenomics

Vast amount of genomic resources are available in public domain but these could not be utilized with their potentially due to the lack of precise, accurate and high throughput phenotyping tools and techniques. Therefore, efforts have been made for the development of high throughput phenotyping tools and techniques for screening of morpho-physiological traits related to biotic and abiotic stresses. The genomic resources developed in a plant species can be linked with physiological and morphological data collected using current phenotyping approaches available at automated phenotyping platforms worldwide. These high throughput phenotyping tools collect the precise and accurate observations and allow analysis of data for understanding the whole phenome of the plant under a wide range of environmental conditions. Thus like genomic platforms, phenotyping platforms develop databases such as the plant meta-phenomics database (Poorter et al. 2010) or the Plant Trait database TRY (<http://www.try-db.org>, accessed September 2012) which bring together phenotypic responses to the environment for a wide range of plant traits and parameters. These phenotyping database along with available international genomic databases (TAIR, TIGR and NCBI, and with other ‘omics’ information such as metabolomic, proteomic and transcriptomic data) have now become important to understand the genetic architecture of complex traits.

Phenomics has not only allowed to dissect the complex traits through genomics but also helped to use genomic resources in discovering new genes/QTL, identification of function of a gene sequence and helped to increase the genetic gain for traits having low heritability (see chap. 17 for details). This understanding will allow us to simulate and predict plant properties in particular of complex traits such as yield or biomass, the most important challenge to address future needs of a growing human population. Both forward and reverse phenomics approaches can be used to harness the potentiality of genomic resources. The accurate, cost-effective, high-throughput phenotyping is pivotal to fine mapping of traits, regardless of the genetic approach for producing allelic recombination or assessing variation by re-sequencing technologies. Phenomics can be used in reverse genetic studies and can help to identify the function of a particular gene(s) in growth and development of crop plants and can be used to identify the allelic variation to target the associated genes (Fig. 1.2).

1.9 Conclusion

For making successful genetic improvement in crop plants, plant breeders first identify the desirable genotypes having target traits by screening a collection of germplasm accessions. These target traits then are combined together through hybridization. This cycle of selection-hybridization-selection has been implemented on the basis of visual observation since

domestication of crop plants. Though visual screening is easy and precise for qualitative and highly heritable traits, its use is less precise for quantitative traits and those traits, which are difficult to observe visually (physiological and biochemical traits). Moreover, vast amount of genomic resources have been developed in a number of crop species in the past. The available gene sequences and molecular markers could still not be associated with any traits due to the lack of phenotyping of germplasm collections. For utilizing these genomic resources and identification of desirable plants, the precise phenotyping of germplasm accessions for challenging traits is required in various crop species.

In the recent past, various techniques and methodologies have been developed for screening biotic, abiotic, physiological and biochemical traits in crop plants. These technologies have become very advanced in the era of digital science. These plant phenomics developments are actually helping to make simply plant physiology in 'new clothes'. Thus this trans-disciplinary approach promises significant new breakthroughs in plant science. Phenomics provides the opportunity to study previously unexplored areas of plant science, and it provides the opportunity to bring together genetics and physiology to reveal the molecular genetic basis of a wide range of previously intractable plant processes. The challenges ahead in plant-based agriculture will require the scale of quantum advances we have seen in information technology in the past 20 years and we need to build on these advances for security of global food, fiber and fuel.

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Abstract

The term plant phenotyping has been regenerated with the contribution of sensors, system technologies, and algorithms. This new plant describing concept allows multi-trait assessment with automatic measurements. Uniform structure, nondestructive measurements, precise results, and direct storage are the advantages of digital phenotyping. The hyper-spectral spectroradiometers and imaging technologies lead the way of new plant phenotyping applications. This high-throughput technique therefore requires lots of traditional and novel traits to present new characterization. Digital-based phenotyping in plants is new and still a developing area of research. The most often used traits of digital phenotyping are canopy temperature, chlorophyll fluorescence, stomatal conductance, chlorophyll content, leaf water potential, leaf area, fruit color, carbon isotope discrimination, light interception, senescence, and root traits which have been discussed in this chapter together with their advantages, limitations, and plant breeding potentials.

Keywords

Trait • Characterization • Phenomics • Phenotyping • Imaging • Plant breeding

2.1 Introduction

Plants are fundamental to life, providing the basic and immediate needs of humans for food and shelter. Domestication of plant species is an important step in the human history for food uses

and diversification. Three steps were proposed for plant domestication: (i) collecting seeds from their native habitat and planting them in areas where they were perhaps not adapted as well, (ii) inhibiting certain natural selection pressures by growing the plants in a field under cultivation, and (iii) applying artificial selection pressures by choosing characteristics that would not have necessarily been beneficial for the plant survival (Xu 2010). Selection pressure includes changes in allele frequency, gradations within and between

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