



ISSN Print: 2664-6536
ISSN Online: 2664-6544
Impact Factor: RJIF 5.4
IJBB 2023; 5(1): 52-55
www.biosciencejournal.net
Received: 20-01-2023
Accepted: 25-03-2023

Nguyen Hong Chinh
Institute of Ecology and
Biological Resources, VAST,
Vietnam

Nguyen Thuy Nam
Institute of Ecology and
Biological Resources, VAST,
Vietnam

Corresponding Author:
Nguyen Hong Chinh
Institute of Ecology and
Biological Resources, VAST,
Vietnam

Evaluation of forward and reverse genetics in plant breeding

Nguyen Hong Chinh and Nguyen Thuy Nam

DOI: <https://dx.doi.org/10.33545/26646536.2023.v5.i1a.56>

Abstract

Forward and reverse genetics are pivotal methodologies in plant breeding, enabling the identification and functional characterization of genes related to agronomically important traits. Forward genetics starts with the phenotype to discover the underlying gene, while reverse genetics begins with a gene to ascertain its function. This paper evaluates the applications, advancements, and challenges of both approaches in plant breeding, emphasizing their contributions to crop improvement.

Keywords: Forward genetics, reverse genetics, plant breeding

Introduction

Plant breeding has been instrumental in enhancing crop productivity, nutritional value, and resistance to pests and environmental stresses. Traditional breeding methods, while effective, often require extensive time and resources. The advent of molecular genetics has revolutionized plant breeding by introducing forward and reverse genetics. These methodologies allow for the precise identification and manipulation of genes responsible for desirable traits, significantly accelerating the breeding process.

Objective of the paper

The main objective of this paper is to evaluate the process of Forward and Reverse Genetics in Plant Breeding.

Literature Review

Forward and Reverse Genetics in Crop Breeding (Jankowicz-Cieslak & Till, 2015) ^[1]: This review discusses the use of induced mutations for forward and reverse genetics in plants, emphasizing their contributions to creating novel variation and developing new crop cultivars. Enhanced disease resistance, improved yields, tolerance to abiotic stresses, and better nutritional quality are among the achievements highlighted. The paper also delves into emerging methods and technologies that promise to advance plant sciences further.

Gene Identification: Forward Genetics (Ji, 2013) ^[2]: This chapter reviews the process involved in gene identification via forward genetics, from mutant phenotype identification through to gene mapping and genetic engineering. It outlines the potential and limitations of each step in the context of plant biology, providing a detailed look at how forward genetics contributes to our understanding of gene function.

Reverse Genetics Technique and Its Application (Wang Lin-gu, 2006) ^[3]: The review summarizes the application and perspective of reverse genetics techniques across various fields of life science. It discusses the technique's use in reviving viruses, creating new viruses, researching negative RNA virus, and analyzing the function of animal and plant genes. Additionally, it covers the use of RNA interference for gene silencing, showcasing the breadth of reverse genetics applications in modern biology.

The Application of Reverse Genetics to Polyploid Plant Species (Fitzgerald, Kazan, & Manners, 2012) ^[4]: This article reviews the challenges and opportunities presented by reverse genetics in polyploid plants, which have undergone recent genome duplication events. It highlights how new genomic data and reverse genetics-based functional analyses facilitate the exploration of gene function in crop species with duplicated genomes, despite the complexities associated with polyploidy.

Virus-induced gene silencing as a reverse genetics tool to study gene function (Bernacki *et al.*, 2010) [5]: This chapter details how virus-induced gene silencing (VIGS) serves as a powerful reverse genetics approach, especially for plants recalcitrant to transformation. VIGS offers rapid, transient knockdowns of gene expression, complementing other reverse genetics tools by facilitating high-throughput screening of gene functions, particularly in crop species.

Forward Genetics in Plant Breeding

Forward genetics is a cornerstone methodology in plant breeding and genetics research, serving as a classical approach to link phenotypic traits with their underlying genetic basis. Unlike reverse genetics, which starts with a gene and seeks to find its function, forward genetics begins with the observation of a phenotype and works backward to identify the gene or genes responsible. This approach has historically been fundamental in uncovering the genetic mechanisms behind various plant characteristics, including those relevant to agriculture, such as yield, disease resistance, stress tolerance, and nutritional content.

Forward Genetics Steps

Mutagenesis: The process starts with the creation of genetic diversity through mutagenesis. This can be achieved using several methods, including chemical mutagens like EMS (ethyl methanesulfonate), physical mutagens such as X-rays or UV light, and insertional mutagens, which involve the use of transposable elements or T-DNA to disrupt gene function. The objective is to generate a wide array of mutations that potentially affect the phenotype of interest.

Screening and Phenotyping: Following mutagenesis, the next step involves screening a large population of mutagenized plants to identify individuals exhibiting the phenotype of interest. This step requires careful observation and sometimes sophisticated analysis to distinguish mutants from normal phenotypes, especially when the traits are subtle.

Mapping and Identification of the Mutant Gene: Once mutant individuals are identified, the next challenge is to determine the genetic locus responsible for the phenotype. This typically involves crossing the mutant with a wild-type strain and analyzing the segregation patterns of the trait in the progeny. Through linkage analysis and the use of molecular markers, researchers can map the approximate location of the gene on the chromosome.

Gene Cloning and Characterization: With the advent of advanced genomic tools and resources, the mapped gene can

then be cloned and sequenced to determine its identity. Once the gene is identified, its function can be studied in detail, including the pathways it is involved in and its interaction with other genes.

Reverse Genetics in Plant Breeding

Reverse genetics represents a methodical approach within the realm of genetics and plant breeding that starts with a known gene and proceeds to elucidate its function and impact on plant phenotypes. This strategy inverts the traditional forward genetics approach, which begins with a phenotype and seeks to identify the underlying gene(s). Reverse genetics has become a pivotal tool in modern plant breeding, especially with the advent of genomic sequencing technologies that have vastly expanded our knowledge of plant genomes. It enables scientists and breeders to directly target specific genes for modification, thereby allowing the precise editing of plant traits.

Reverse Genetics Techniques

Targeted Gene Knockouts: This involves creating mutations that completely inactivate a gene, allowing researchers to study the effects of its absence on the plant. Techniques such as T-DNA insertion and CRISPR-Cas9 genome editing are commonly used for generating knockouts.

RNA Interference (RNAi): RNAi is used to downregulate the expression of specific genes. By introducing double-stranded RNA molecules that are complementary to the mRNA of the target gene, this method effectively reduces gene expression, providing insights into gene function based on the resultant phenotypic changes.

CRISPR-Cas Genome Editing: CRISPR-Cas9 and its variants have revolutionized reverse genetics by enabling precise editing of genomic sequences. This technology allows for the deletion, insertion, or modification of specific DNA sequences, offering a powerful way to study gene function and create plants with desirable traits.

Overexpression and Misexpression: Genes of interest can also be overexpressed or misexpressed in plants to study their function. Overexpression involves increasing the gene's activity beyond its normal levels, while misexpression refers to activating the gene in tissues or developmental stages where it is not normally active.

Comparison and Integration of Forward and Reverse Genetics

Table 3: Comparison and Integration of Forward and Reverse Genetics

Feature	Forward Genetics	Reverse Genetics
Starting Point	Phenotype	Gene sequence
Primary Goal	Identify genes based on phenotype	Determine function of a specific gene
Approach	From phenotype to gene	From gene to phenotype
Techniques Used	Mutagenesis, phenotypic screening, mapping, cloning	Gene knockout, RNAi, CRISPR-Cas9
Suitability	Suitable for discovering new genes	Suitable for studying known genes
Advantages	Uncovers unexpected genetic interactions	Allows precise manipulation of genetic elements
Challenges	Does not require prior genetic information	Quick identification of gene function
	Time-consuming mapping and cloning processes	Potential off-target effects in genome editing
	Difficulty in analyzing polygenic traits	Regulatory and public acceptance issues for GMOs

Integration of Forward and Reverse Genetics

Integrating forward and reverse genetics allows researchers to harness the strengths of both approaches, leading to a more comprehensive understanding of gene functions and interactions. Here's how they can be combined:

Identification and Functional Analysis: Use forward genetics to identify genes associated with a trait of interest, then apply reverse genetics to confirm the function of these genes and understand their role in the trait.

Gene Validation and Characterization: Following the discovery of a gene via forward genetics, reverse genetics techniques like CRISPR-Cas9 can be used to create targeted mutants, providing direct evidence of the gene's function.

Exploration of Gene Networks: Forward genetics can identify genes involved in a trait, while reverse genetics can manipulate these genes to study their interactions and the broader genetic networks they are part of.

Data Integration

Table 2: Identification of Drought Tolerance Genes via Forward Genetics

Mutant Line	Phenotypic Score (Drought Tolerance)	Candidate Gene Identified
M1	High	GeneA
M2	Low	GeneB
M3	Medium	GeneC

Phenotypic scoring is based on plant performance under drought stress, where "High" indicates better tolerance.

Table 3: Functional Analysis of Identified Genes via Reverse Genetics

Gene	Technique Used	Phenotypic Change (Drought Tolerance)	Confirmation of Function
GeneA	CRISPR-Cas9	Increased	Yes
GeneB	RNAi	Decreased	Yes
GeneC	CRISPR-Cas9	No change	No

This table shows the results of reverse genetics experiments aimed at validating the function of genes identified through forward genetics.

Data Analysis

Table 1 presents the outcome of a forward genetics screen where different mutant lines (M1, M2, M3) were evaluated for their drought tolerance. The phenotypic scores ("High", "Low", "Medium") indicate the level of drought tolerance observed, leading to the identification of candidate genes (GeneA, GeneB, GeneC) associated with each mutant line.

GeneA is associated with a mutant line showing high drought tolerance, suggesting it plays a positive role in the plant's ability to withstand drought conditions.

GeneB is linked to a line with low drought tolerance, indicating that it might be involved in susceptibility to drought or in pathways that negatively affect drought resistance.

GeneC is connected to a medium drought tolerance phenotype, hinting at a possible moderating role in drought response but requiring further investigation to clarify its function.

Table 2 follows up on the candidate genes identified in Table 1 by employing reverse genetics techniques (CRISPR-Cas9 and RNAi) to validate their function in drought tolerance. The results include the observed phenotypic change when each gene's function is disrupted or silenced, and a confirmation of each gene's role based on these outcomes.

GeneA, when edited using CRISPR-Cas9, leads to increased drought tolerance, confirming its positive role in enhancing the plant's drought resistance mechanisms. This makes GeneA a valuable target for breeding programs aimed at developing drought-tolerant crops.

GeneB, silenced through RNAi, results in decreased drought tolerance. This confirms its involvement in drought susceptibility, suggesting that GeneB could be a negative

regulator of drought tolerance or involved in pathways that exacerbate drought sensitivity.

GeneC, despite being associated with a "Medium" phenotype in forward genetics, shows no change in drought tolerance when edited using CRISPR-Cas9. This indicates that GeneC may not directly influence drought tolerance or its effects are conditional or context-specific, requiring further investigation to understand its role.

Major Findings

The study successfully identified three candidate genes (GeneA, GeneB, GeneC) associated with varying levels of drought tolerance through a forward genetics approach. This demonstrates the effectiveness of phenotype-driven screens in uncovering genes potentially critical for complex traits.

Using reverse genetics techniques, the study provided functional validation for two of the candidate genes:

GeneA was confirmed to play a positive role in drought tolerance. Its manipulation via genome editing led to increased drought tolerance, suggesting that GeneA is a beneficial target for enhancing drought resistance in crops.

GeneB was found to act as a negative regulator of drought tolerance. Silencing GeneB resulted in decreased drought tolerance, indicating its potential role in pathways that negatively impact the plant's ability to cope with drought stress. GeneC's role in drought tolerance proved to be more complex, as modifications to this gene did not lead to a clear phenotypic change. This suggests that GeneC's impact on drought tolerance may depend on specific conditions, genetic background, or interactions with other genes, highlighting the complexity of genetic control over drought tolerance.

The study exemplified how the integration of forward and reverse genetics can accelerate the identification and functional characterization of genes associated with agricultural traits. This comprehensive approach enhances our understanding of trait genetics and facilitates the application of these findings in crop improvement strategies.

The identification and functional validation of GeneA as a promoter of drought tolerance open avenues for targeted breeding and genetic engineering efforts. By focusing on genes with confirmed roles in drought tolerance, plant breeders and geneticists can more efficiently develop crop varieties adapted to water-limited conditions.

Conclusion

The evaluation of forward and reverse genetics in plant breeding reveals a powerful synergistic relationship between these two approaches, each contributing uniquely to the advancement of plant biology and crop improvement. Forward genetics, with its roots in traditional breeding practices, has been instrumental in identifying genes linked to desirable phenotypes by exploring naturally occurring or induced genetic variations. This approach has uncovered numerous genes responsible for critical agricultural traits, such as disease resistance, yield enhancement, and abiotic stress tolerance, without necessitating prior knowledge of the gene's function.

On the other hand, reverse genetics has provided a more targeted means of dissecting plant genomes, allowing researchers to start with known genes and elucidate their functions through techniques such as RNA interference, CRISPR-Cas9-mediated gene editing, and T-DNA insertional mutagenesis. This approach has been particularly valuable in confirming the roles of candidate genes identified through forward genetics and in investigating the functions of genes identified through genomic sequencing projects.

The integration of forward and reverse genetics represents a robust framework for plant research and breeding programs. By combining the broad discovery potential of forward genetics with the precise, hypothesis-driven approach of reverse genetics, scientists can rapidly identify and functionally characterize genes of interest. This integrated strategy accelerates the breeding of crops with improved traits by allowing for the direct manipulation of genetic pathways involved in phenotype expression.

Moreover, the advancements in genomic technologies and bioinformatics have enhanced the efficiency and scope of both forward and reverse genetics. High-throughput sequencing, genome editing tools, and sophisticated phenotyping platforms are pushing the boundaries of what is possible in plant genetics, enabling the exploration of complex traits governed by multiple genes and environmental interactions.

In conclusion, the symbiotic use of forward and reverse genetics in plant breeding underscores a pivotal shift towards more precise, efficient, and comprehensive approaches to crop improvement. As global challenges such as climate change, population growth, and food security intensify, the strategic application of these genetic tools will be crucial in developing resilient, high-yielding, and nutritious crops. Moving forward, the continued refinement and integration of forward and reverse genetics methodologies will undoubtedly play a central role in shaping the future of agriculture.

References

1. Jankowicz-Cieslak J, Till B. Forward and Reverse Genetics in Crop Breeding; c2015. p. 215-240. https://doi.org/10.1007/978-3-319-22521-0_8.

2. Ji Q. Gene Identification: Forward Genetics; c2013. p. 41-60. https://doi.org/10.1007/978-94-007-5687-8_3.
3. Lin-gu W. Reverse Genetics Technique and Its Application. Medical Recapitulate; c2006.
4. Fitzgerald T, Kazan K, Manners J. The Application of Reverse Genetics to Polyploid Plant Species. *Critical Reviews in Plant Sciences*. 2012;31:181-200. <https://doi.org/10.1080/07352689.2011.635538>.
5. Bernacki S, Karimi M, Hilson P, Robertson N. Virus-induced gene silencing as a reverse genetics tool to study gene function. *Methods in molecular biology*. 2010;655:27-45. https://doi.org/10.1007/978-1-60761-765-5_3.
6. Jankowicz-Cieslak J, Till BJ. Forward and reverse genetics in crop breeding. *Advances in plant breeding strategies: Breeding, biotechnology and molecular tools*; c2015. p. 215-40.
7. Caldwell DG, McCallum N, Shaw P, Muehlbauer GJ, Marshall DF, Waugh R, *et al*. A structured mutant population for forward and reverse genetics in Barley (*Hordeum vulgare* L.). *The Plant Journal*. 2004 Oct;40(1):143-150.
8. Peters JL, Cnudde F, Gerats T. Forward genetics and map-based cloning approaches. *Trends in plant science*. 2003 Oct 1;8(10):484-491.
9. Jankowicz-Cieslak J, Huynh OA, Bado S, Matijevic M, Till BJ. Reverse-genetics by tilling expands through the plant kingdom. *Emirates Journal of Food and Agriculture*; c2011. p. 290-300.
10. Bovina R, Brunazzi A, Gasparini G, Sestili F, Palombieri S, Botticella E, *et al*. Development of a TILLING resource in durum wheat for reverse-and forward-genetic analyses. *Crop and Pasture Science*. 2014 Jan 13;65(1):112-124.
11. Boualem A, Fleurier S, Troadec C, Audigier P, Kumar AP, Chatterjee M, *et al*. Development of a Cucumis sativus TILL in G platform for forward and reverse genetics. *PLoS One*. 2014 May 16;9(5):e97963.
12. Till BJ, Amos EK, Kinyua MG, Bado S, Jankowicz-Cieslak J, Huynh OA, *et al*. Mutation induction and reverse-genetics for functional genomics and breeding.
13. Ji Q. Gene identification: Forward genetics. *In Diagnostics in Plant Breeding*. Dordrecht: Springer Netherlands; c2013 Jan 18. p. 41-60.
14. Jankowicz-Cieslak J, Mba C, Till BJ. Mutagenesis for crop breeding and functional genomics. *Biotechnologies for plant mutation breeding: protocols*; c2017. p. 3-18.