Trends in Biotechnology and Plant Sciences
https://doi.org/10.62460/TBPS/2023.008
Volume 1, No. 1, 7-15

Review Article	Article History (23-002)	Received: 20 July 23	Revised: 13 Aug 23	Accepted: 28 Aug 23	Published: 7 Sep 23

HIGH THROUGHPUT PHENOTYPING: A REVOLUTIONARY APPROACH TO COMBAT SALINITY STRESS IN COTTON

Muhammad Nouman Khalid[1*], Ifrah Amjad[1], Iqra Fatima[2], Ali Ammar[1] and Zaid Rasheed[1]

[1]Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan
[2]Department of Botany, University of Agriculture Faisalabad, Pakistan

Corresponding author: noumankhalidpbg@gmail.com

ABSTRACT

This review paper comprehensively explores the potential of high throughput phenotyping (HTP) in assessing and combating salinity stress in cotton production. Salinity stress presents a significant challenge for global cotton crop, impacting the plant's physiological and molecular functions. HTP emerges as a potentially transformative tool for understanding and addressing this issue, employing advanced imaging techniques, spectral reflectance, and molecular technologies to rapidly and accurately measure plant traits. While HTP holds considerable promise, its implementation is not without challenges. The review dissects these barriers, ranging from technical hurdles associated with data acquisition, storage, and analysis to economic considerations involving equipment and maintenance costs. Notably, advancements in machine learning and artificial intelligence (AI) offer promising solutions to many of these challenges, with proven applications in image analysis, trait prediction and data integration. The review showcases the successful application of HTP across various global case studies, demonstrating its potential to revolutionize plant research and breeding. Further, the integration of AI and machine learning is poised to significantly enhance the capabilities of HTP, ushering in a new era of data-driven, efficient plant phenotyping. The paper concludes with a set of research and policy recommendations to optimize the use of HTP for salinity stress in cotton. These include promoting the integration of genomics and phenomics, improving image analysis algorithms, developing predictive models, and standardizing phenotypic data. At the policy level, the authors call for investments in HTP infrastructure, increased collaboration and data sharing, capacity building, and the incorporation of HTP into breeding programs. This review illustrates the immense potential of HTP to revolutionize our approach to salinity stress in cotton, ultimately contributing to more sustainable and resilient cotton production.

Keywords: Salinity stress, Molecular responses, Phenotyping, Cotton

Citation: Khalid MN, Amjad I, Fatima I, Ammar A and Rasheed Z, 2023. High throughput phenotyping: a revolutionary approach to combat salinity stress in cotton. Trends Biotech Plant Sci 1(1): 7-15. https://doi.org/10.62460/TBPS/2023.008

1. INTRODUCTION

Cotton, a major cash crop, holds paramount importance in the global textile industry. As the backbone of numerous economies worldwide, its efficient and sustainable production is highly significant. However, one of the key barriersto cotton productivity is salinity stress. Salinity stress, in agricultural terms, signifies the adverse conditions produced by high salt concentration in the soil, particularly of sodium chloride. It affects nearly 20% of global agricultural land and approximately one-third of irrigated land (Maryum et al. 2022).

1.1. Background and Importance of Salinity Stress in Cotton

Salinity stress can profoundly impact the growth, yield, and fiber quality of cotton crops. It causes physiological drought, nutritional imbalance and ion toxicity, resulting in yield reduction or even total crop failure in extreme cases. With the rising global temperatures and sea levels, soil salinization is predicted to expand, making it an urgent area for research and mitigation strategies (Araus and Cairns, 2014).

In terms of physiology, salinity stress disrupts the ionic and osmotic equilibrium of plant cells. High sodium concentration in the soil solution causes osmotic stress, making it difficult for the plant to take up water. Sodium ions can also enter the transpiration stream, leading to ion toxicity in the leaves. Additionally, salinity interferes with the uptake of essential nutrients like potassium, calcium and magnesium, leading to nutritional imbalances.

1.2. Significance of High Throughput Phenotyping

Addressing the problem of salinity stress necessitates the development of salt-tolerant cotton varieties. A key step in this process is the identification of genetic traits associated with salt tolerance. This is where the significance of phenotyping comes into the picture. Phenotyping, the assessment of the physical and biochemical traits of an organism, provides crucial links between genotype and phenotype, aiding plant breeders in their selection processes (Yang et al. 2013).

Traditional phenotyping methods, however, are laborious and time-consuming. They often struggle to keep pace with the rapid advancements in genomics, leading to a phenotyping bottleneck. This limitation led to the emergence of high throughput phenotyping (HTP), a cutting-edge technology that integrates automation, remote sensing, and advanced data analysis techniques (Esmaeili et al. 2021). HTP can rapidly phenotype a large number of plants, providing detailed data about multiple plant traits, including those associated with salinity tolerance. By using non-destructive methods, it can capture dynamic changes in plant traits over time, offering a more nuanced understanding of plant responses to salinity stress. Hence, HTP serves as a critical tool for breeders to speed up the development of salt-tolerant cotton varieties (Khan et al. 2023).

1.3. Objective and Scope of the Review

This review aims to provide a comprehensive overview of high throughput phenotyping for understanding and mitigating salinity stress in cotton. We will delve into the different HTP techniques and their applications in the context of salinity stress. Furthermore, we will discuss the integration of HTP data with genomics and machine learning tools for a more holistic understanding of salinity tolerance in cotton. We will also present a range of case studies where HTP has been successfully utilized in breeding salinity-tolerant cotton varieties. The challenges and future prospects of employing HTP in cotton breeding programs will be critically evaluated. Through this review, we aim to highlight the revolutionary potential of HTP in revolutionizing the battle against salinity stress in cotton and hope to guide future research in this crucial area.

2. Salinity Stress: Understanding the Mechanism

2.1. Defining Salinity Stress

Salinity stress in plants is a response to excessive salt levels in the soil, often resulting from high concentrations of sodium chloride. This condition can harm many crops, including cotton, which generally thrives in soils with moderate to low salinity. Salinity in soils can result from natural processes, like mineral weathering and sea spray, or from anthropogenic activities, such as irrigation with saline water and poor agricultural practices (Khan et al. 2023).

Salinity stress can be classified into two types: primary and secondary salinity. Primary salinity occurs naturally, typically in areas with high evaporation rates, low rainfall, or near coastal regions. Secondary salinity, on the other hand, is a result of human activities that alter the water balance in the soil, such as inappropriate irrigation practices (Furbank and Tester, 2011).

2.2. Impact of Salinity Stress on Cotton Production

High soil salinity can profoundly impact cotton production, leading to reduced crop yields and poor fiber quality. When exposed to high salinity, cotton plants exhibit stunted growth, wilting, leaf chlorosis, and in severe cases, plant death. The harmful effects of salinity stress can be seen at all stages of cotton growth, from germination to fiber development (Li, 2020).

Salinity stress restricts the uptake of water and essential nutrients by increasing the soil osmotic potential, resulting in physiological drought. High sodium concentrations can also disrupt the uptake of other cations, such as potassium and calcium, leading to nutritional imbalances. Furthermore, when sodium and chloride ions reach toxic levels in plant tissues, they can cause cellular damage and impede metabolic processes (Smith et al. 2021).

2.3. Physiological and Molecular Responses of Cotton to Salinity

Cotton plants, like other crops, have evolved physiological and molecular mechanisms to cope with salinity stress. On a physiological level, cotton plants can regulate their water potential by accumulating solutes, such as proline and glycine betaine, to maintain water uptake in saline conditions. They can also compartmentalize or exclude sodium ions to protect their cells from salt damage (Li et al. 2021).

On a molecular level, cotton plants modulate the expression of numerous genes in response to salinity stress. These include genes involved in ion transport, osmotic adjustment, signal transduction, and stress response. For instance, the overexpression of GhSOS1, a sodium/proton antiporter gene in cotton, has been shown to enhance salt tolerance by maintaining ion homeostasis. In addition to these responses, the antioxidant defense system of cotton plays a significant role in mitigating the oxidative damage caused by salinity stress. This system includes enzymes like superoxide dismutase, catalase, and peroxidases, which neutralize reactive oxygen species produced under stress conditions (Rabara et al. 2014).

Understanding these complex responses to salinity stress is crucial for developing effective strategies to improve cotton's salt tolerance. High throughput phenotyping, combined with advanced genomics tools, offers a promising avenue to unravel these mechanisms and accelerate the breeding of salt-tolerant cotton varieties.

3. High Throughput Phenotyping: An Overview

3.1. Definition and Evolution

High throughput phenotyping (HTP) refers to the rapid, detailed, and automated measurement of phenotypic traits on a large scale. Traditional phenotyping, although informative, often proves laborious, subjective, and time-consuming. The advent of genomic tools and resources necessitated the development of phenotyping techniques that could match their pace and precision (Li et al. 2014).

Thus, high throughput phenotyping emerged as a response to overcome these limitations, increasing the speed, accuracy, and scale of phenotypic data collection. With the integration of advanced imaging techniques, automation, and machine learning, HTP has evolved into a powerful tool in plant breeding and genetics.

3.2. Techniques and Technologies Employed

Several techniques and technologies are employed in high throughput phenotyping, broadly categorized into image-based and sensor-based techniques. Image-based HTP utilizes various imaging modalities such as RGB (red, green, blue), hyperspectral, thermal, fluorescence, and 3D imaging to capture detailed phenotypic data. For instance, RGB imaging is commonly used for measuring growth and morphological traits, while hyperspectral imaging provides insights into biochemical traits. Thermal imaging can assess plant water status, and 3D imaging enables the quantification of plant architecture. Sensor-based HTP, on the other hand, uses a variety of sensors to measure specific plant traits. These include spectroradiometers for spectral reflectance, capacitance sensors for leaf area, porometers for stomatal conductance, and more.

High throughput phenotyping can be performed at different scales, from the laboratory to the field. Lab-based HTP systems offer high-level control over environmental conditions and can capture subtle phenotypic variations. Field-based HTP platforms, including ground vehicles and unmanned aerial vehicles (UAVs), enable phenotyping under real-world conditions, capturing the plant's response to its natural environment.

3.3. Scope and Limitations

High throughput phenotyping holds enormous potential in plant research and breeding. It can rapidly generate vast amounts of phenotypic data, enabling the identification of complex traits and the elucidation of genotype-phenotype relationships. This is particularly valuable in stress tolerance, where traits are often multigenic and influenced by environmental conditions. However, high throughput phenotyping is not without limitations. The large volumes of data generated pose a significant challenge in terms of storage, management, and analysis. This necessitates the development of advanced data analysis tools and computational infrastructure. Furthermore, while lab-based HTP offers precision and control, it may not accurately reflect the plant's performance in the field. Conversely, field-based HTP is subject to environmental variability, potentially adding noise to the data.

Despite these challenges, the development of more sophisticated imaging techniques, machine learning algorithms, and integrated phenomics platforms continues to expand the possibilities of HTP. It holds great promise for enhancing our understanding of plant biology, accelerating breeding programs, and ultimately, securing our food and fiber future.

4. The Nexus between High Throughput Phenotyping and Salinity Stress

4.1. Unravelling the Potential of High Throughput Phenotyping

High throughput phenotyping (HTP) can be a game-changer in understanding and combating salinity stress in cotton. Salinity tolerance is a complex trait controlled by multiple genes and influenced by environmental factors. Traditional phenotyping methods often fall short in capturing this complexity, leading to a disconnect between genotypic and phenotypic data (Cushman and Bohnert, 2000).

HTP with its ability to generate extensive, detailed, and dynamic phenotypic data, can bridge this gap. By capturing the plant's response to salinity stress at different stages of growth and under varying conditions, HTP provides a more holistic view of the plant's adaptive mechanisms (Li et al. 2014). Moreover, HTP data can be integrated with genotypic data to perform Genome-Wide Association Studies (GWAS) and Quantitative Trait Loci (QTL) mapping. These analyses can help identify the genes associated with salinity tolerance, aiding in the selection of salt-tolerant varieties (Hanjagi and Awaji, 2018).

4.2. High Throughput Phenotyping for Salinity Tolerance: Current Insights

Several studies have showcased the utility of HTP in studying salinity tolerance in crops, including cotton. These studies employ various HTP techniques, such as RGB imaging, hyperspectral imaging, and thermal imaging, to measure traits indicative of salinity tolerance (Le Marié et al. 2014).

For instance, RGB imaging has been used to measure growth-related traits, such as plant height, leaf area, and biomass, which are typically reduced under salinity stress. Hyperspectral imaging, on the other hand, can measure biochemical traits like chlorophyll content, indicating the plant's photosynthetic efficiency under stress conditions. Thermal imaging can provide insights into the plant's water status, as salinity stress often induces physiological drought. Plants with better water management strategies, such as efficient stomatal control or osmotic adjustment, may show lower leaf temperatures, indicating better tolerance to salinity stress (Raza et al. 2022).

Apart from these, HTP platforms can also capture more nuanced traits, such as the plant's circadian rhythm, stress recovery pattern, and root architecture, shedding light on the subtler aspects of the plant's response to salinity stress. While these findings are promising, the application of HTP in cotton breeding for salinity tolerance is still in its infancy. More research is needed to standardize HTP protocols, identify reliable stress indicators, and validate the findings in diverse cotton genotypes and field conditions. Nonetheless, the integration of HTP with genomic tools and machine learning algorithms holds immense potential to accelerate the development of salt-tolerant cotton varieties, ensuring sustainable cotton production in a changing climate.

5. High Throughput Phenotyping Techniques for Salinity Stress

5.1. Imaging Techniques

Imaging techniques have gained prominence in high throughput phenotyping for their non-invasive, high-resolution, and dynamic trait capture capabilities. In the context of salinity stress, several imaging modalities have been employed. RGB imaging, which captures visible light like a standard camera, is commonly used to measure growth-related traits like plant height, leaf area, and biomass, which are generally reduced under salinity stress (Yang et al. 2021).

Hyperspectral imaging captures a wide range of wavelengths, providing a spectral signature that reflects the plant's biochemical composition. This can be used to measure traits like chlorophyll content and leaf water content, which may indicate the plant's photosynthetic efficiency and water status under stress conditions (Swathy et al.). Thermal imaging measures the plant's infrared radiation, providing an indicator of its water status. Under salinity stress, cotton plants may exhibit increased leaf temperatures due to reduced transpiration, making this a potential stress marker. Fluorescence imaging can be used to assess the plant's photosynthetic performance under salinity stress. Salinity stress often impairs photosystem II, leading to changes in chlorophyll fluorescence parameters, which can be detected using this technique.

5.2. Spectral Reflectance

Spectral reflectance, or the proportion of light reflected by an object across different wavelengths, is another valuable tool in high throughput phenotyping for salinity stress. By using spectroradiometers or hyperspectral cameras, one can measure the spectral reflectance of cotton plants, providing a wide array of information about their physiological and biochemical status. Certain reflectance indices, such as the Normalized Difference Vegetation Index (NDVI), have been widely used to estimate plant vigor and stress status. Under salinity stress, cotton plants may show reduced NDVI values due to impaired photosynthesis and growth (Gosa et al. 2019).

5.3. Molecular and Genomic Techniques

Alongside these phenotypic techniques, molecular and genomic techniques play a crucial role in unravelling the genetic basis of salinity tolerance. Genomic tools, such as whole-genome sequencing and genotyping by sequencing (GBS), can be used to generate high-density genetic maps and identify quantitative trait loci (QTL) associated with salinity tolerance. Transcriptomic techniques, like RNA-seq, can provide insights into the gene expression changes under salinity stress, revealing the molecular pathways involved in stress response (Tester and Langridge, 2010).

Furthermore, techniques like gene editing (CRISPR-Cas9) and transformation can be used to validate the function of candidate genes and generate transgenic lines with improved salinity tolerance. Integration of these molecular and genomic techniques with high throughput phenotyping can enhance our understanding of the genotype-phenotype relationship, accelerating the breeding of salt-tolerant cotton varieties.

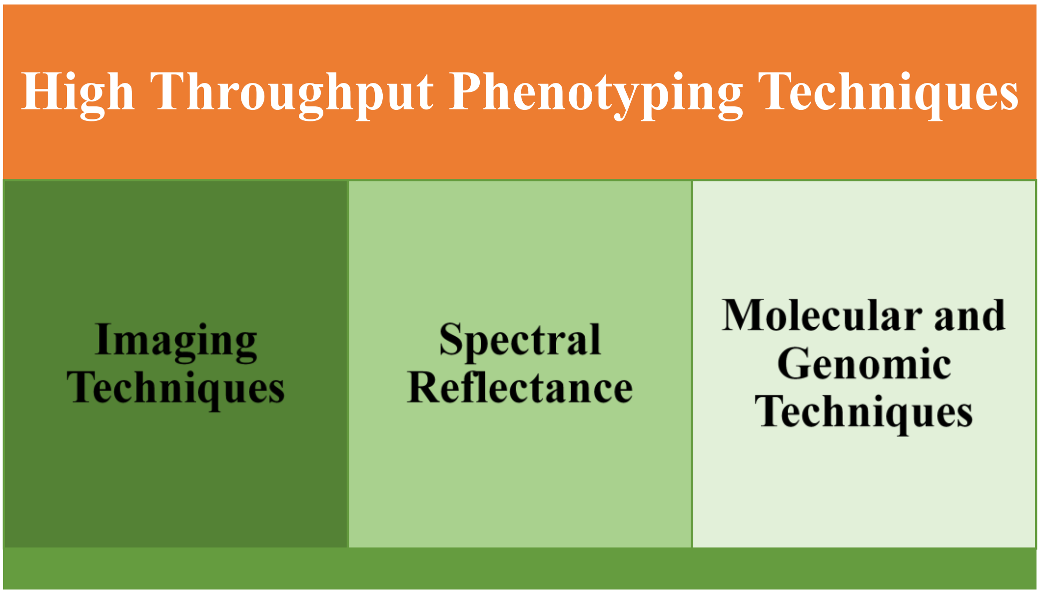

Fig. 1: Different high throughput phenotyping techniques for salinity stress in plants.

6. Case Studies: Successful Application of High Throughput Phenotyping

6.1. Global Case Studies

The application of high throughput phenotyping (HTP) has been successful in various crop studies worldwide, demonstrating the power of this approach in understanding and enhancing plant performance under environmental stress. A study of wheat in Australia employed HTP in conjunction with a controlled-environment facility to identify salinity tolerance traits at the seedling stage. Using imaging techniques, the research observed growth responses to salinity stress in different wheat varieties, allowing the selection of salt-tolerant phenotypes for further breeding (Muthuramalingam et al. 2022).

In the US, a maize study utilized HTP and hyperspectral imaging to track the spectral signatures of various maize genotypes subjected to drought and salinity stress. The high-resolution data allowed the researchers to identify key spectral indices associated with stress tolerance, thereby providing a fast, non-destructive method to screen for stress-resilient genotypes. In China, rice researchers have used HTP with digital and thermal imaging to assess the impact of salinity stress on seedling stage growth. The phenotypic data, combined with genotypic information, helped the researchers identify QTLs associated with salinity tolerance, accelerating the breeding of salt-tolerant rice varieties (Shelden and Roessner, 2013).

6.2. Future Prospects

Looking forward, HTP holds immense potential for advancing plant research and breeding. However, its broader implementation and impact require addressing some challenges.

Firstly, the data management and analysis are a significant bottleneck, given the sheer volume of data generated by HTP. Future work should focus on the development of efficient data management tools and machine learning algorithms to extract meaningful insights from this data. Secondly, there is a need for more integrated HTP platforms that can capture a comprehensive range of traits under diverse conditions. Combining different imaging modalities, sensor types, and scales (from the lab to the field) will enable a more holistic understanding of plant responses to environmental stress. Thirdly, a more robust integration of phenotypic and genotypic data is needed. With the advent of genomic tools and resources, the bottleneck in plant breeding has shifted from genotyping to phenotyping. HTP can fill this gap, but there is a need for more sophisticated statistical and bioinformatics tools to bridge the genotype-phenotype divide. Lastly, the application of HTP in breeding programs is still limited. Future efforts should aim to validate the predictive power of HTP in breeding contexts, demonstrating its utility in selecting superior genotypes and predicting their performance in the field.

In conclusion, while HTP is a powerful tool in plant research, its true potential lies in its application in plant breeding. By enabling the rapid, detailed, and accurate assessment of plant traits, HTP can revolutionize the way we breed crops, accelerating the development of varieties that are resilient to environmental stress and capable of meeting our future food and fiber needs.

7. Challenges and Opportunities in Implementing High Throughput Phenotyping

7.1. Technical Challenges

High Throughput Phenotyping (HTP) techniques present several technical challenges that can limit their application. The first hurdle is data acquisition. Gathering high-quality, consistent data is critical to successful phenotyping, and this can be hindered by technical issues such as equipment malfunctions, poor calibration, or incorrect data processing. The second challenge is the sheer volume of data. HTP technologies can generate vast amounts of data that require robust storage, management, and processing infrastructures. Analysing this data requires advanced data analysis tools and algorithms, and this can be a significant challenge given the multidimensional and time-series nature of the data (Altaf et al. 2023).

Finally, linking phenotypic data with genetic data is another technical challenge. Unravelling the complex relationships between genotype and phenotype requires sophisticated bioinformatic and statistical tools and expertise in genetics, molecular biology, and computer science.

7.2. Economic Considerations

Economic constraints are a significant challenge in the implementation of HTP. The initial investment for HTP equipment can be high, and this may be prohibitive for some research institutions, especially those in developing countries. Furthermore, the maintenance of these systems and the cost of data storage and processing can add to the financial burden. Additionally, the cost-effectiveness of HTP in breeding programs is still uncertain. While HTP can potentially accelerate breeding cycles and improve selection accuracy, these benefits need to be weighed against the costs associated with the technology (Shelake et al. 2022).

7.3. Opportunities for Future Research

Despite these challenges, the implementation of HTP presents several exciting opportunities for future research. Firstly, the integration of HTP with other emerging technologies, such as genomics, machine learning, and artificial intelligence, can revolutionize our understanding of plant biology and improve crop breeding. The use of HTP in conjunction with genomics can help decipher the genetic basis of complex traits and accelerate the development of crop varieties with improved stress tolerance, yield, and quality. Machine learning and AI can aid in the analysis of HTP data, enabling the identification of novel stress indicators and predictive models for crop performance. Secondly, HTP can provide novel insights into plant responses to environmental stress. By capturing dynamic, spatial, and temporal trait data, HTP can shed light on thess plants's mechanisms used to cope with stress and the factors influencing these responses. Lastly, the application of HTP in breeding programs presents a significant opportunity. By enabling the rapid, detailed, and accurate assessment of plant traits, HTP can revolutionize the way we breed crops, leading to the development of varieties that are resilient to environmental stress and capable of meeting our future food and fiber needs (Hassan et al. 2022).

In conclusion, while implementing HTP presents several challenges, it also opens up many opportunities for advancing plant research and breeding. By addressing these challenges and capitalizing on these opportunities, we can harness the full potential of HTP in our quest for sustainable and resilient agricultural systems.

8. Role of Machine Learning and Artificial Intelligence in High Throughput Phenotyping

8.1. Current Applications

Machine Learning (ML) and Artificial Intelligence (AI) have emerged as pivotal tools in the application and advancement of High Throughput Phenotyping (HTP). Currently, ML algorithms are extensively used for image analysis in HTP, such as image segmentation, feature extraction, and trait measurement (Zafar et al. 2020). For instance, Convolutional Neural Networks (CNNs), a type of deep learning algorithm, have been successfully applied to analyze RGB, hyperspectral, and thermal images, providing accurate measurements of plant traits like leaf area, chlorophyll content, and leaf temperature (Razzaq et al. 2021). In addition to image analysis, ML and AI have been applied to predict plant traits based on image data. Regression algorithms, such as Support Vector Machines (SVMs) or Random Forests, can be trained on phenotypic and image data to predict traits of interest, enabling the indirect measurement of hard-to-measure traits. Finally, ML and AI can aid in the integration and interpretation of multi-modal and multi-scale phenotypic data. Clustering algorithms can identify patterns and associations in the data (Zafar et al. 2022), revealing trait combinations that contribute to stress tolerance, while classification algorithms can distinguish between different stress types or stress levels based on phenotypic signatures (Razzaq et al. 2020).

8.2. Future Prospects

Looking forward, ML and AI have enormous potential to advance HTP and plant research. As the volume and complexity of HTP data increase, the need for robust, automated, and intelligent data analysis tools becomes more critical, making ML and AI indispensable. One promising avenue is the development of deep learning models for the analysis and interpretation of HTP data. Deep learning, a subfield of ML, can model high-level abstractions in data by using multiple layers of nonlinear transformations, making it particularly suited for complex and large-scale HTP data. Moreover, the integration of ML and AI with other emerging technologies can revolutionize HTP. For instance, the combination of ML/AI with genomics can facilitate the decoding of the genotype-phenotype relationship, accelerating the identification and breeding of stress-tolerant varieties. Meanwhile, the incorporation of ML/AI in automated phenotyping platforms can enable real-time, dynamic phenotyping, providing novel insights into plant growth and stress responses. In conclusion, ML and AI play a crucial role in HTP, aiding in data analysis, trait prediction, and data integration. By capitalizing on these technologies, we can harness the full potential of HTP, advancing our understanding of plant biology and improving our ability to breed resilient and productive crops.

9. Recommendations for Optimizing High Throughput Phenotyping for Salinity Stress in Cotton

9.1. Research Recommendations

Optimizing high throughput phenotyping (HTP) for salinity stress in cotton requires addressing several research gaps.

9.1.1. Integrating Genomics and Phenomics

High-throughput genomics is advancing at an unprecedented rate, and integrating these data with phenotypic data can accelerate the discovery of genes or quantitative trait loci (QTLs) associated with salinity tolerance. This requires more collaborative efforts between plant physiologists, breeders, and bioinformaticians.

9.1.2. Improving Image Analysis

Current image analysis algorithms are not perfect, and improvements are necessary to reduce errors and variability in trait measurement. More research is needed to develop robust, automated image analysis algorithms for different imaging modalities and traits.

9.1.3. Modelling Plant Responses to Salinity Stress

Developing predictive models for salinity tolerance based on phenotypic and genotypic data can guide breeding programs and help predict crop performance under different saline conditions. Machine learning and AI can be instrumental in this endeavour.

9.1.4. Standardizing Phenotypic Data

To facilitate data sharing and meta-analysis, there is a need for more standardized protocols for data collection, processing, and annotation. Efforts should be made to develop community-agreed metadata standards and ontologies for salinity stress phenotyping.

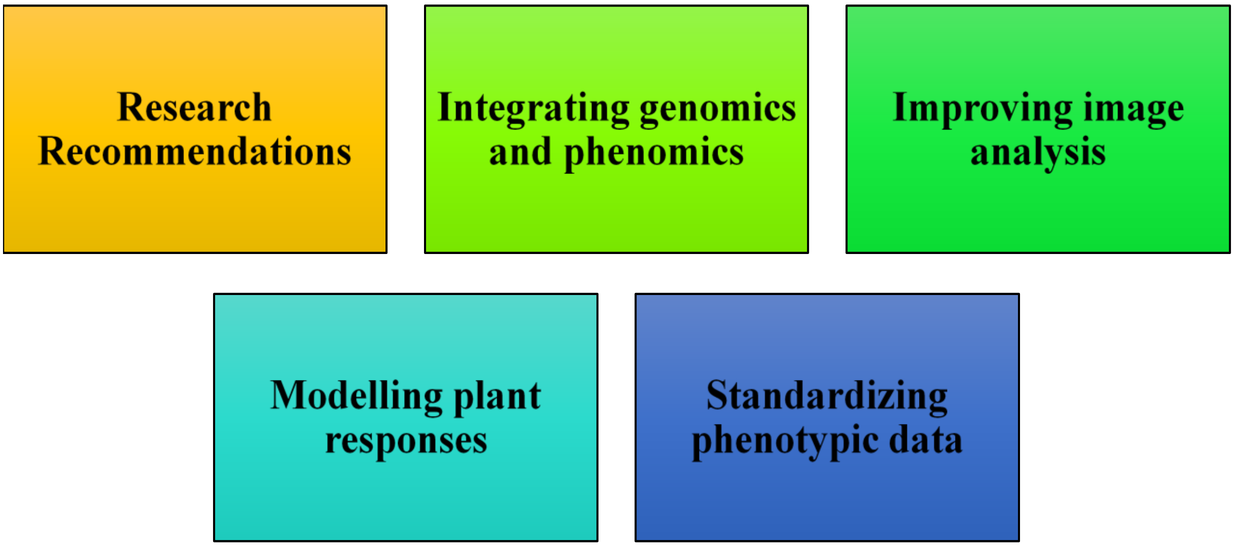

Fig. 2: Various recommendations for optimizing high throughput phenotyping for salinity stress in cotton

9.2. Policy Recommendations

At the policy level, several strategies can facilitate the optimization of HTP for salinity stress in cotton.

9.2.1. Investing in HTP Infrastructure

Governments and funding agencies should invest in HTP infrastructure, including high-resolution imaging systems, controlled-environment facilities, and data storage and processing capabilities. This investment should consider not only the initial equipment costs but also the costs of maintenance, training, and data management.

9.2.2. Promoting Collaboration and Data Sharing

Policies should encourage collaboration between researchers, institutions, and countries. This can facilitate the sharing of phenotypic data, research findings, and resources, accelerating the pace of research and application of HTP. Open-access data platforms can play a crucial role in this regard.

9.2.3. Training and Capacity Building

There is a need for more training programs in HTP, machine learning, and bioinformatics to build the necessary skills for the next generation of plant researchers. Funding agencies should prioritize grants and scholarships for training in these areas.

9.2.4. Incorporating HTP into Breeding Programs

While research on HTP is advancing rapidly, its incorporation into breeding programs is still limited. Policies should encourage the use of HTP in breeding programs, demonstrating its utility in selecting superior genotypes and predicting their performance in the field. In conclusion, optimizing HTP for salinity stress in cotton requires concerted efforts at the research and policy levels. By addressing these recommendations, we can harness the full potential of HTP, improving our understanding of salinity tolerance in cotton and accelerating the development of salt-tolerant cotton varieties.

10. Conclusion

10.1. Summary of Findings

Our review has explored the current state of high-throughput phenotyping (HTP) in the context of salinity stress in cotton, revealing its potential and challenges. We found that salinity stress presents a significant challenge to cotton production, impacting physiological and molecular functions within the cotton plant. In response, HTP is emerging as an invaluable tool for understanding and addressing salinity stress. Through advanced imaging, spectral reflectance, and molecular techniques, HTP allows for the comprehensive, accurate, and efficient measurement of plant traits. It promises to speed up the selection process and improve the accuracy of trait measurements, enabling the rapid development of salinity-tolerant cotton varieties.

Despite its potential, the implementation of HTP faces significant technical and economic challenges, including issues with data acquisition, storage, and analysis and the high cost of equipment and maintenance. However, the advent of machine learning and artificial intelligence presents promising solutions to these challenges, with applications in image analysis, trait prediction, and data integration. Additionally, the review highlighted the successful application of HTP in various case studies, demonstrating its efficacy and potential to revolutionize plant research and breeding. The incorporation of machine learning and artificial intelligence is poised to enhance the capabilities of HTP, enabling more sophisticated data analysis, trait prediction, and data integration.

10.2. Concluding Remarks

Salinity stress remains a significant challenge for cotton production. However, the advent of HTP, bolstered by machine learning and artificial intelligence, offers an unprecedented opportunity to advance our understanding of salinity tolerance and expedite the development of salt-tolerant cotton varieties. While significant challenges remain, including technical and economic barriers, our review highlights many opportunities for future research. By integrating genomics and phenomics, improving image analysis, developing predictive models, and standardizing phenotypic data, we can unlock the full potential of HTP. At the policy level, investing in HTP infrastructure, promoting collaboration and data sharing, building capacity, and incorporating HTP into breeding programs can expedite the application and optimization of HTP. In conclusion, HTP holds immense potential for revolutionizing our approach to salinity stress in cotton. By capitalizing on its strengths, addressing its challenges, and harnessing the power of machine learning and artificial intelligence, we can contribute to sustainable and resilient cotton production.

REFERENCES

Altaf, M. A., Mandal, S., Behera, B., Mangal, V., Naz, S., Kumar, R., Kumar, A., Ghorai, M., Singh, B. and Dey, A. (2023). Salinity stress tolerance in solanaceous crops: Current understanding and its prospects in genome editing. Journal of Plant Growth Regulation, 42(7), 4020-4036.

Araus, J. L. and Cairns, J. E. (2014). Field high-throughput phenotyping: the new crop breeding frontier. Trends in plant Science, 19(1), 52-61.

Cushman, J. C. and Bohnert, H. J. (2000). Genomic approaches to plant stress tolerance. Current Opinion in Plant Biology, 3(2), 117-124.

Esmaeili, N., Cai, Y., Tang, F., Zhu, X., Smith, J., Mishra, N., Hequet, E., Ritchie, G., Jones, D. and Shen, G. (2021). Towards doubling fibre yield for cotton in the semiarid agricultural area by increasing tolerance to drought, heat and salinity simultaneously. Plant Biotechnology Journal, 19(3), 462-476.

Furbank, R. T. and Tester, M. (2011). Phenomics–technologies to relieve the phenotyping bottleneck. Trends in Plant Science, 16(12), 635-644.

Gosa, S. C., Lupo, Y. and Moshelion, M. (2019). Quantitative and comparative analysis of whole-plant performance for functional physiological traits phenotyping: new tools to support pre-breeding and plant stress physiology studies. Plant Science, 282, 49-59.

Hanjagi, P. S. and Awaji, S. M. (2018). High-throughput imaging tools to phenotype traits in plants for stress environments. Journal of Pharmacognosy and Phytochemistry, 7(6), 97-102.

Hassan, M., Sarwar, S., Shahzad, S., Zafar, S. A., Bilal, A., Akhter, S., Qamar, H., Asghar, S., Altaf, M. and Saleem, S. (2022). Screening of bread wheat genotypes for heat tolerance using artificial and natural environment. Journal of Global Innovations in Agricultural Sciences.

Khan, M. K. R., Ditta, A., Wang, B., Fang, L., Anwar, Z., Ijaz, A., Ahmed, S. R. and Khan, S. M. (2023). The intervention of multi-omics approaches for developing abiotic stress resistance in cotton crop under climate change. In Sustainable Agriculture in the Era of the OMICs Revolution (pp. 37-82). Springer.

Le Marié, C., Kirchgessner, N., Marschall, D., Walter, A. and Hund, A. (2014). Rhizoslides: paper-based growth system for non-destructive, high throughput phenotyping of root development by means of image analysis. Plant Methods, 10(1), 1-16.

Li, C. (2020). Breeding crops by design for future agriculture. Journal of Zhejiang University. Science. B, 21(6), 423.

Li, D., Quan, C., Song, Z., Li, X., Yu, G., Li, C. and Muhammad, A. (2021). High-throughput plant phenotyping platform (HT3P) as a novel tool for estimating agronomic traits from the lab to the field. Frontiers in Bioengineering and Biotechnology, 8, 623705.

Li, L., Zhang, Q. and Huang, D. (2014). A review of imaging techniques for plant phenotyping. Sensors, 14(11), 20078-20111.

Maryum, Z., Luqman, T., Nadeem, S., Khan, S. M. U. D., Wang, B., Ditta, A. and Khan, M. K. R. (2022). An overview of salinity stress, mechanism of salinity tolerance and strategies for its management in cotton. Frontiers in Plant Science, 13, 907937.

Muthuramalingam, P., Jeyasri, R., Rakkammal, K., Satish, L., Shamili, S., Karthikeyan, A., Valliammai, A., Priya, A., Selvaraj, A. and Gowri, P. (2022). Multi-Omics and integrative approach towards understanding salinity tolerance in rice: A review. Biology, 11(7), 1022.

Rabara, R. C., Tripathi, P. and Rushton, P. J. (2014). The potential of transcription factor-based genetic engineering in improving crop tolerance to drought. Omics: A Journal of Integrative Biology, 18(10), 601-614.

Raza, A., Tabassum, J., Fakhar, A. Z., Sharif, R., Chen, H., Zhang, C., Ju, L., Fotopoulos, V., Siddique, K. H. and Singh, R. K. (2022). Smart reprograming of plants against salinity stress using modern biotechnological tools. Critical Reviews in Biotechnology, 1-28.

Razzaq, A., Ali, A., Safdar, L. B., Zafar, M. M., Rui, Y., Shakeel, A., Shaukat, A., Ashraf, M., Gong, W. and Yuan, Y. (2020). Salt stress induces physiochemical alterations in rice grain composition and quality. Journal of Food Science, 85(1), 14-20.

Razzaq, A., Ali, A., Zafar, M. M., Nawaz, A., Xiaoying, D., Pengtao, L., Qun, G., Ashraf, M., Ren, M. and Gong, W. (2021). Pyramiding of cry toxins and methanol producing genes to increase insect resistance in cotton. GM crops & food, 12(1), 382-395.

Shelake, R. M., Kadam, U. S., Kumar, R., Pramanik, D., Singh, A. K., & Kim, J.-Y. (2022). Engineering drought and salinity tolerance traits in crops through CRISPR-mediated genome editing: Targets, tools, challenges, and perspectives. Plant Communications.

Shelden, M. C. and Roessner, U. (2013). Advances in functional genomics for investigating salinity stress tolerance mechanisms in cereals. Frontiers in Plant Science, 4, 123.

Smith, D. T., Potgieter, A. B. and Chapman, S. C. (2021). Scaling up high-throughput phenotyping for abiotic stress selection in the field. Theoretical and Applied Genetics, 134(6), 1845-1866.

Swathy, V., Niji, M., Varsha, P. and Nikhil, P. xxx. CROP PHENOTYPING: ADVANCES IN HIGH THROUGHPUT PHENOTYPING TECHNIQUES AND APPLICATIONS. Advances in Agronomy Vol.

Tester, M. and Langridge, P. (2010). Breeding technologies to increase crop production in a changing world. Science, 327(5967), 818-822.

Yang, W., Duan, L., Chen, G., Xiong, L. and Liu, Q. (2013). Plant phenomics and high-throughput phenotyping: accelerating rice functional genomics using multidisciplinary technologies. Current Opinion in Plant Biology, 16(2), 180-187.

Yang, Y., Saand, M. A., Huang, L., Abdelaal, W. B., Zhang, J., Wu, Y., Li, J., Sirohi, M. H. and Wang, F. (2021). Applications of multi-omics technologies for crop improvement. Frontiers in Plant Science, 12, 563953.

Zafar, M. M., Jia, X., Shakeel, A., Sarfraz, Z., Manan, A., Imran, A., Mo, H., Ali, A., Youlu, Y. and Razzaq, A. (2022). Unraveling heat tolerance in upland cotton (Gossypium hirsutum L.) using univariate and multivariate analysis. Frontiers in Plant Science, 12, 727835.

Zafar, M. M., Razzaq, A., Farooq, M. A., Rehman, A., Firdous, H., Shakeel, A., Mo, H. and Ren, M. (2020). Insect resistance management in Bacillus thuringiensis cotton by MGPS (multiple genes pyramiding and silencing). Journal of Cotton Research, 3(1), 1-13.