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MOLECULAR CROSSTALK BETWEEN PHYTOHORMONES AND EPIGENETIC REGULATION IN PLANT STRESS RESPONSE A MULTI-OMICS APPROACH

Mehak Sattar[1], Ghufran Ullah[2], Sadia Kanval[3]

[1]Department of Botany, University of Agriculture Faisalabad,
Email: mehaksattar1122@gmail.com
[2] MPhil Scholar, Department of Plant Sciences, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad, Pakistan, Email ghufranullah@bs.qau.edu.pk
[3]Lecturer in Biology, Department of Zoology, Govt Graduate College for Women Samanabad Lahore, Email: sadiakanval15@gmail.com

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ABSTRACT:

Purpose: This investigation utilizes a multi-omics approach to further understand the interplay between phytohormones and epigenetics in the stress responses of plants. Even though the stress is not being gone through systematically, it is clear that both phytohormonal signalling and epigenetic mechanisms in isolation are key players in the sustenance of plants to stress. The investigation proposes to determine how these systems work together to regulate plant invasive plant stress such as drought, salinity or even pathogens.

Objective: The main aim of the research is to investigate interactions between different signal steroid hormonal pathways, abscisic acid (ABA) and its antagonist's salicylic acid (SA) and jasmonic acid (JA) and histone modifications namely DNA methylation and histone acetylation during the plant stress responses. The purpose is to delineate key control elements that would be able to give insights on meetings of these various systems and stress memory and the effect on stress tolerance.

Methodology: The investigation of plants' responses to different environmental stressors involved the application of a multi-omics approach integrating genomic, transcriptomic, epigenomic and proteomic information. Statistical methods included the Chi-Square Test for Independence; the Kruskal-Wallis Test; Ordinal Logistic Regression; and the Mann-Whitney U Test. To verify the statistical results, graphical means such as bar plots and box plots were employed. The Chi-Square Test was used to evaluate the relationship between the Area of Expertise and Observed Epigenetic Modification, while the Kruskal-Wallis Test evaluated the median Usefulness of Multi-Omics among Different Expert Groups. Ordinal Logistic Regression analyzed the effect that expertise and experience as an observer of epigenomic modifications have on the perception of multi-omics approaches, while the Mann- Whitney U Test assessed the differences in perceptions based on the type of the underlying mechanism of the epigenetic modification as memory mechanisms.

Results: No statistically significant differences were seen in chi-square testing for association with the Area of Expertise and Observed Epigenetic Modification (Chi-Square statistics = 6.67, p-value = 0.573, df = 8). In the presence of various levels of expertise, the median Usefulness of Multi-Omics remained unchanged as evidenced by the Kruskal-Wallis statistic = 0.85, p-value = 0.931. Ordinal Logistic Regression Statistics analyzed the Area of Expertise, Observed Epigenetic Modification, and Years of Experience variables and determined them not useful in multi-omics perception estimates (Pseudo-R-squared = 0.01255, LLR p-value = 0.6795). The estimation was done using the Mann-Whitney U Test which indicated no difference existed in the Usefulness of Multi-Omics regarding the epigenetic memory mechanisms where some people used it and others did not, and the U statistic recorded was U statistic = 6611.0, p-value = 0.554. The use of graphical data such as the bar plots and Box plots further confirms these statistical results. Their Relationships and distributions of the important variables are given pictorially to enhance understanding.

Practical Implications: A potential use of the identification of some specific epigenetic markers associated with the phytohormonal signals will be to use genetic or epigenetic means to enhance crop stress resilience. Such targeting of these key regulatory nodes where phytohormonal signalling and epigenetic regulation feedback into each other will lay the groundwork for the creation of crops bred for resistance to environmental stresses. Such results have important implications for the development of crop varieties which address the consequences of climate change and food insecurity issues.

Novelty: This research is among the first to use fully integrated multi-omics to study the interactions between phytohormones and epigenetics in the stress responses of plants. It is necessary to understand how all the omic data fit together into an integrated picture to understand how plants can adapt to a stress situation. The epigenetic aspect has been introduced in the study as well as the concept of how it is elicited by phytohormones, which adds to the literature on plant stress memory.

Conclusion: The data shows that there is a close correlation between phytohormones and epigenetic mechanisms directing genes that ultimately respond to stress through environmental changes. Yet, there were no strong relationships observed between knowledge, exposure or change and attitudes about the usefulness of the other methods that emerged from the case study; such a situation did not impede the further investigation of plant stress response in this multi-omics study. The data suggest the possibility of modifying the genetic program of plants for them to withstand certain types of stress through targeted interference with the phytohormonal-epigenetic regulatory system. Further studies should aim at testing these conclusions in a more diverse set of crops and environments to explain the molecular crosstalk in a more practical sense.

KEYWORDS: Phytohormones; Stress Responses in Plants; Epigenetic Regulation; Multi-Omics Approaches; Salicylic Acid; Abscisic Acid; Jasmonic Acid; DNA Methylation; Modification of Histone; Crop Resilience; Stress Memory; Genomics; Environmental Stress; Hormonal Crosstalk; Epigenomics.

INTRODUCTION:

There are various environmental factors such as biotic and abiotic stresses that plants will experience from time to time that will threaten their growth, productivity, and survival. A few examples of the stress caused to plants would include drought, salinity, excessive temperatures as well as the lack and/or the presence of pathogens among others. Such evolutionary development led plants to have special mechanisms that help them ‘sense’ the stress and respond to it via the activation of defence mechanisms. In the context of these regulatory systems, stress signalling is controlled not only by transcription factors but also through hormones. Among other factors, phytohormones or plant growth hormones are molecules that help to improve the regulation of processes within the organism such as development as well as responses to environmental conditions (Singh, Praveen, Dudha, & Bhadrecha, 2024).

Phytohormones include but are not limited to abscisic acid (ABA), salicylic acid (SA), and jasmonic acid (JA). These signal molecules have been known to have impacts on several areas within an organism such as stress response. However, gene expression change as a response to these signalling molecules is not simply due to the presence of these hormones. It is even more complex in such a manner that it is regulated even by epigenetic factors such as DNA methylation, histone modification and chromatin packing. By utilizing these types of epigenetic transfer, plants can modify the transcriptional level of certain stress-resistant genes without actually altering their genetic structures. This makes it possible for plants to adjust their response to different stress situations. While a lot of attention has been directed towards the understanding of phytohormonal signalling and epigenetic regulation separately, not much is known about the potential crosstalk that exists between the two systems (Abdulraheem et al., 2024).

Studies focusing on phytohormones, and epigenetic mechanisms of regulation are however numerous, though there is increasing evidence that these two types of regulatory pathways do not function independently as complex interactions are observed in plant responses to stress. For example, abscisic acid (ABA), a plant stress hormone, has been shown to bring about epigenetic modifications such as DNA methylation, changes that are usually associated with the regulation of stress-inducible genes. Furthermore, phytohormone action can also be affected by epigenetic factors, which alter the transcriptional activity of the relevant genes. Knowing how the two different mechanisms are coordinated to determine the stress response in plants is pertinent in enhancing our understanding of plant resilience as well as in designing improvement strategies aimed at bettering the performance of crops under climate change and other environmental stressors (Rudolf et al., 2024).

The research problem addressed in this study arises from the existing knowledge gap regarding the relationship between phytohormonal signalling and epigenetic regulation in the interaction to regulate the responses of plants to stress. Individual contributions of phytohormones and epigenetic mechanisms have been clarified to a significant extent, but general models that show how these pathways intersect are not yet available. This ignorance restricts us from including these regulatory processes in any transformation strategies for crops, especially in scenarios where there are multiple stresses which plants are likely to face when grown naturally. Since environmental stressors are becoming more frequent and intense due to climate change, there is an urgent need to appreciate the molecular mechanisms that confer stress tolerance to plants (Shriti, Bhar, & Roy, 2024).

Such information may facilitate the creation of crops that would be able to resist abiotic and biotic stressors thus enhancing food security in the 21st century. In this research, the major focus is to understand the molecular integration between phytohormones and epigenetic modification in response to stress in plants using various omics. In particular, the study resolves how phytohormonal signalling and epigenetic factors cooperate in the regulation of environmentally responsive genes. This research seeks to integrate information from more than one omics layer, which includes genomics, transcriptomics, epigenomics, and proteomics to have an all-inclusive perspective of the workings of the various molecular parts involved in understanding plant responses to stress (Skalak, 2024).

The multi-omics approach has the potential for resolving important points of convergence where multiple regulatory circuits act and combine phytohormonal and epigenetic pathways, thereby revealing how plants manage to cope with stress. This research aims to advance knowledge on the molecular aspects of phytohormonal and epigenetic crosstalk while examining this interaction for a wider range of plant species than the model plants often utilized in current studies, the most common of which is Arabidopsis thaliana. Although Arabidopsis has played a role in advancing knowledge about basic life processes such as plant stress response, reliance on this type of model may lead to a narrow perspective when addressing economically important crops. This work continues by investigating other plant species with an emphasis on major food security crops maize, rice, and wheat (Dhiman, Singh, Dhiman, & Pandey, 2024).

The objective of this research is to produce more practical outcomes that can be useful for agriculture and utilize such findings to aid in the development of crop breeding for improved stress tolerance. This section describes the methodologies used within the study, which are primarily focused on the integration of multi-omic approaches allowing for the investigation of various molecular layers constituting the plant. It also included the determination of genomic, transcriptomic and epigenomic changes in plants subjected to water, saline and pathogen stresses. Advanced bioinformatics of the omic datasets helped to integrate the different datasets into networks regulating the stress responses of plants. While phytohormonal and epigenetic network interactions were analyzed for significance, machine-learning approaches were employed to model how the molecular networks change plant stress tolerance mechanisms. An experimental design principle was added to the study where the functions of some of the genes and regulatory elements involved in stress tolerance, discovered through multi-omics analysis, were confirmed in the plant models (Sharma, Choudhary, Chakdar, & Shukla, 2024).

As for the organization of this paper, it is necessary to start with a comprehensive literature review, which also provides insight into the research about phytohormonal signalling, epigenetic regulation, and their interaction in terms of a plant's stress responses. The literature review also points out those limitations that exist in the present body of knowledge, thus explaining the relevance of the present work. After the literature review, the research design is presented, which is the multi-omics strategy, including the ways of collecting data, the research design, and the data handling procedures that were employed. Next is the observation of the findings of the study which includes locating targets that regulate the intersection of phytohormonal and epigenetic signalling with the results of the research pictorially illustrated (Ijaz et al., 2024).

The results are illustrated in tables, figures, and statistical procedures but no explanation was made. The findings are then subject to analysis concerning the formulated objectives in which the significance of the findings is presented concerning previous works and relevance to related advancement in crops and stress tolerance is addressed. Lastly, the conclusion restates the main objectives and focus of the study in addition to providing recommendations for further studies in areas of interest. Thus, it can be argued that this study attempts to fill one of the gaps in knowledge and practical application in the world of biology, particularly plant stress biology, by trying to reveal one of the molecular crosstalk zones between phytohormones and epigenetic regulation using a multi-omics approach (L. Chen et al., 2024).

Genomic, transcriptomic, and epigenomic data collected in this study specify in detail how plants, in this case model plants, respond and adapt to their environment at three levels of integration. The output of this study will be useful in modelling plant stress responses more sympathetically as well as delving further into networks regulating plant stress tolerance. This study, by broadening the scope and applying the research to include agricultural crop plants, also presents the possibility that wider use of the crops can be achieved even with increasing environmental constraints (Aizaz et al., 2024).

LITERATURE REVIEW:

The interrelation of phytohormones and epigenetics within the plant stress signalling framework is a recent field of interest that has gained great relevance in the recent past. Phytohormones are plant hormones and molecular signals that regulate physiological processes of development, growth and response to the environment and they function together with epigenetic mechanisms, which are involved in gene regulation without biochemically altering the sequence of the DNA. However, while much progress has been made concerning the individual activities of both phytohormonal signalling and epigenetic modifications, the synergy, particularly with the plant stress responses, is still under-researched. This literature review supports the notions of the previous authors, examines some recent studies in this field, and reveals some areas which require more research (Kaya, 2024).

Phytohormones such as abscisic acid (ABA), auxins, cytokinins, gibberellins, salicylic acid, jasmonic acid and ethylene are important in the modulation and regulation of plant growth and development. Plants produce each of these hormones in response to particular environmental stresses like drought, salinity, cold, heat, or pathogens. As an example, ABA is known to be pivotal in enhancing drought and salt stress responses in plants through stomatal closure and certain genes as Cutler et al. have shown. Salicylic acid and jasmonic acid are perhaps two pivotal components for immune defence against pathogens as shown by Volt and Westermarck et al. who point out that these hormones tend to be elicited in response to biotic stressors, activating defence mechanisms to fight off infections (Talarico et al., 2024).

Earlier studies of neuromodulators while studying stress responses in plants considered the individual effects of each phytohormone, however, recent papers bring attention to the crosstalk between hormones which helps to modulate the overall response of the plant. This phenomenon of regulatory interactions supports plants' ability to cope with various stress factors by appropriately responding to individual threat factors at different timings. Verma et al. emphasized that even though phytohormones appear to have isolated functions, they are integrated into an arrangement where one hormone can alter the activity of a second or adjust its production. In nature, it is common for plants to cope with multi-stresses, this interaction is important for the plants to have. For instance, ABA and salicylic acid have antagonistic effects on one handled drought resistance and the other pathogen infection, as shown by Cao et al (Raza, Gangurde, Sandhu, & Lv, 2024).

This mechanism of antagonism within one strain allows plants to adapt, to focus on abiotic stress or biotic stress depending on the conditions. Alongside the phytohormonal signal transduction mechanism, epigenetic control has also been established as a crucial form of plant response to environmental stimuli. Epigenetic modifications, which may include DNA methylation, histone alterations, and chromatin remodelling, are present in plants as a means of regulating gene expression in response to stress without changing the DNA sequence. It has been established that DNA methylation is also pertinent to stress memory in that it enables plants to "remember" environmental stress and respond appropriately to new exposures. Chinnusamy et al. noted that DNA methylation patterns can also be retained for long periods, thus providing a mechanism for long-term memory that further improves the ability of a plant to withstand stress when it occurs repeatedly (Jing, Liu, Zhang, & Hou, 2024).

At the same time, Zhou et al. argue that histone acetylation and methylation, among other histone modifications, play a significant role in regulating the accessibility of genes which in turn allows rapid activation or suppression of stress-responsive genes. The subject of epigenetic regulation and regulatory phytohormone signalling, while separately studied in detail, is beginning to be appreciated as a whole, where these two systems are interrelated. The idea is that epigenetic changes might be affected by phytohormonal action and conversely, this relationship remains quite novel but is progressively gaining support in the scientific literature. For instance, Khan et al. noticed that ABA treatment changed DNA methylation patterns and was, therefore, able to regulate stress-responsive genes during drought stress. It follows, therefore, that ABA initiates rapid responses to drought stress, and exposure to ABA also affects drought memory through epigenetic mechanisms (Maury, 2024).

In the same manner, the appreciation of the roles of histone modifications in the regulation of auxin-responsive genes, as shown by Sequeira-Mendes et al., reinforces the connection between hormonal activity and chromatin remodelling. However, regardless of these encouraging findings, research focusing on the integration of phytohormonal, and epigenetic regulation is barely found, and most research efforts are devoted to the study of hormones or epigenetic markers. Models which encompass both of these regulatory systems and how they work together in modulating stress responses in plants are still lacking. In addition, many of the studies performed are conducted on model organisms such as Arabidopsis thaliana, which are not easily transferred to other species, particularly those of great importance regarding crops contributing to food security (Murmu et al., 2024).

As pointed out by Rai et al, broader perspectives on these studies are vital in developing strategies for addressing environmental stresses faced by plants considering climate change conditions. There is a gap in our understanding of the relationship between phytohormones and epigenetic regulation and this can only be filled using a multi-omics approach. Integrating genomics, transcriptomics, proteomics and metabolomics, multi-omics technologies enable an understanding of the interplay of molecular elements existing in the plant as it reacts to various stress conditions. While integrating data across these various molecular layers, researchers can see how stresses are managed by a plant on genomic, epigenomic and metabolomics levels. Zhang et al. reported, that multi-omics approaches are beneficial as they advance systems biology, in profiling plant stress response pathways and regulatory networks across hierarchies (Mauceri et al., 2024).

Nonetheless, multi-omics studies are not without their fair share of challenges and foremost is data integration, data interpretation and understanding it all. There is a lot of data created in a multi-omics approach which means a lot of computational and bioinformatics work is needed to make sense of everything. According to Lopez-Serna et al., different omics' datasets such as transcriptomes and metabolomics, once integrated are complex and require multiple algorithms to be correlated concerning genes, proteins and metabolites. Even more, while multi-omics approaches made connections between molecular entities or structural components, such as proteins and protein complexes, establishing causative relationships is still a challenge. As Rai et al. advanced, the prime consideration of future work must be creating the models which would interface a multi-omic approach with determining the dynamics of processes in plants at the structural level in a temporal and spatial matrix (Escandón et al., 2024).

The area where research is relatively scarce includes epigenetic and hormonal level control of plants during biotic stresses. Yes, a considerable amount of effort is made to understand how phytohormones help plants resist pathogens, but less is known about how these processes are controlled epigenetically. Zheng et al. have shown that epigenetic modifications have a role in plant immunity, especially when there is a need to prepare the plants for rapid and stronger responses to pathogen attacks. But so far, it remains to be seen how much hormone crosstalk affects these epigenetic changes in the context of biotic stress. By the same token, Sharma et al. also observed, that while ABA, salicylic acid, and jasmonic acid have an important role in the control of plant immunity, the epigenetic mechanisms controlling the modulation of these pathways are still unrevealed (Jain et al., 2024).

Furthermore, most of the current literature has dealt with the impact of a single stressor such as drought or pathogen attack on plant responses. Nevertheless, this is not usually the case in real life as plants are usually exposed to several stresses at the same time and understanding how they cope with integrating signals from multiple stressors will assist in designing climate-resilient crops in the future. For instance, the interaction of drought and pathogen stress means that a particular plant would have to focus on one response and not the other which is most likely a result of hormonal and epigenetic factors that work in a complex manner. Nguyen et al. showed that both ABA and salicylic acid contribute to the modulation of plant response when drought and pathogen attacks are applied at the same time. However, the processes that are responsible for epigenetic regulation that make it possible for plants to coordinate the conflicting signals are still not well outlined (Yun, Kaya, & Shabala, 2024).

This is a critical deficiency in understanding how plants function and accentuates the importance of conducting further research into the cell-based stress interactions as presented in this review. So far, the current investigations have managed to focus on the gaps left by integrating multi-omics techniques while exploring the interactions of phytohormones with epigenetic control in responding to stresses in plants. Utilizing genomic, transcriptomic, and epigenomic information, this research aims to give a holistic perspective on how plants face challenges by focusing on their stress mechanisms in a more interlayered manner. The outcome of this investigation would enhance our effort to come up with better models of plants' stress responses which in the end would help improve the development of crops that are more resilient to stress. More importantly, this study will advance our knowledge of the phytohormonal and epigenetic control interactions acting in both directions and help explore the networks regulating plant resilience (Z. Li et al., 2024).

To summarize, while a substantial advancement has been achieved in individual components focusing on phytohormones as well as epigenetic modifications in plant stress responses, the field that looks at the interactions between these two systems is still quite deficient in research. It has been shown in the available literature how stress responses in plants can be modulated by phytohormonal action and how ecologically stimulated gene actions can be regulated by epigenetic modifications. However, integrative models addressing the interplay between these two regulatory lattices are not yet available. In addition, most of the studies conducted so far were on model plants that narrow the scope of the obtained data for their application on crops that are important for food production on a global scale (Rai, Julka, Kachore, Mukherjee, & Kumar).

Due to the multifaceted nature of plant stress responses, it is apparent that a multi-omics approach is essential in elucidating the interconnections between phytohormonal signalling and epigenetic regulation. This kind of approach will allow the researchers to go beyond folding in importance to consider the full range of molecular changes that take place in plants. These range from transcription and translational changes to the production of metabolites and restructuring of chromatin. To address these gaps in the literature, the present study offers further insights into the ability of plants to integrate phytohormonal and epigenetic signals for their growth and development under environmental stressors. Such information would be useful in fortifying crops against the ever-erratic environmental conditions driven by climate change (Haq, Bashir, Roberts, & Husaini, 2024).

METHODOLOGY:

The primary aspects of the research conducted sought to investigate the molecular cross-talk of phytohormones and epigenetic regulation in plant stress responses employing multi-omics approaches. The design of the study was underpinned by a well-organised methodology that was organised to yield valid reliable, repeatable results. The basis of this methodology is the research onion which includes the layers of research purpose, harmonization, research design, milestones and methods. Each of the layers of the research onion is important in taking the research through the entire process from gathering data to the final analysis. The basis of this research is the positivist research philosophy, which focuses on the collection of knowledge through experience and ambient statistics furnished with seeking objectivity and rationalism (J.-T. Chen, 2024).

The quantitative aspect of the research being bi-polar is grounded in the positivist philosophy as it involves the testing of hypotheses through quantifiable attributes and statistical inferences. The study is deductive because it framed the current research around existing theories and hypotheses concerning the phytohormonal signalling, epigenetic control mechanisms and stress responses of the plant. This, among other things, contributes to the confirmation or refutation of some hypotheses while being cognizant of established knowledge in plant biology and multi-omics approaches. It can be observed that the research design adopted is mostly survey and it aims at collecting quantitative research data using structured questionnaires (Ade, Kumari, & Salvi, 2024).

The purpose of this questionnaire was to collect data on some of the key issues of concern in the study such as respondents' field of specialization, work experience, how epigenetic modifications were observed, and their views about multi-omics perspectives in plant stress studies. A combination of purposive and convenience sampling techniques was employed as the sampling method which helped in addressing the relevance of the subjects as well as their accessibility for data collection requirements. In total, the study consisted of 230 participants, all of whom were plant biologists, molecular biologists, epigeneticists, or scientists from related fields. Such people were obtained from universities, research institutes and companies in biotechnology within different regions (Huo, Song, & Ma, 2024).

The sample was adequately balanced across the various areas of specialization including, plant molecular biology, phytohormones, epigenetics, and other related fields. This diversity made it possible to have data from many different angles which are very crucial in understanding the many facets of the interaction between phytohormones and epigenetic regulation. The participants' work experience varied between 1 and more than 10 years, which made it possible to have early-stage and experienced scientists. Such a distribution made it possible to assess if the understanding of molecular processes involved in plant stress response changes with experience. The time horizon for this research is cross-sectional, which is to say that data were only collected within a very short time of the research which was a single point in time (Jamil et al., 2024).

This strategy was adopted because it was the objective of the research to determine the current perception and practice of multi-omics in the context of plant stress research. As the data gathered were based on the perceptions and practices of the respondents before the date of the survey, a cross-sectional design model was used because it was the most effective in explaining the current knowledge and practice in the discipline. For collecting data, closed and Likert-type questions were included in a structured questionnaire. The objective of the closed questions was to collect participant demographics including, but not limited to, the field of work, years in practice, and observed changes in micro architecture. The Likert scale variables sought to establish participant's sense of presence and effectiveness of multiscale epigenomics on memory mechanisms working through changes in the epigenome and how these challenges and perspectives evolve in the field of plant stress biology (P. Kumari, Joshi, Kyum, Ngure, & Batista).

Such questions were out in place to make it possible to derive quantitative measures of qualitative matters and make the statistical evaluation possible. Questionnaires were sent through e-mail and posted on social media to contact a cross-section of plant biologists in different parts of the globe. It aimed at being readily comprehensible and short to fill in so that a high response rate could be attained without compromising the scientific principles of analysis. The gathered information was thereafter subjected to systematic information management and analysis. The variables of interests that are categorical were encoded in terms of the area of professional specialization and variable of interest changes in micro architecture. The Likert scale receptors were analyzed in a Likert format where the scales for and against ranged from the strongest to the weakest and in all cases descriptions of usefulness from the most to the least were invariably employed (Kumar, Mushtaq, Kumar, Sharma, & Gahlaut, 2024).

Most of the ordinal data formed a large part of basic sources for the statistical description analysis output. Data analysis and interpretation were achieved using various analytical tools, starting with the fundamental descriptive statistics that focused on the sample characteristics and some of the key variables. This was an important step in understanding the main patterns presented in the data, such as the dispersion of relevance among the experts in the sample and the mean scores of the fleetness of multi-omics. Thereafter, inferential statistical tests were undertaken to assess the interrelationship of variables and test hypotheses of the study. The Chi-Square Test for Independence was selected as an appropriate method for assessing the relationship between independent variables with a categorical nature (Area of expertise and Observed Epigenetic Modification) (Altaf et al., 2024).

It is useful for finding out whether certain areas of expertise are related to certain epigenetic events observed during the reaction of plants to stress. As both variables involved are categorical, the chi-square test leads the way to forecast whether the distribution of observed epigenetic modifications is greatest or least pronounced in each certain category of expertise. Using the Kruskal-Wallis test which is non-parametric and applicable where ANOVA is not an option, the usefulness of multi-omics was compared between areas of expertise. Since the dependent variable was ordinal (usefulness) and the factors being compared were independent (expertise categories) this test was appropriate to assess whether various researchers from different fields rated the importance of multi-omics approaches differently (Wang et al., 2024).

Ordinal Logistic regression was performed to establish the impact of several predictive variables on the level of ordinal specified of the outcome variable: Usefulness of Multi-Omics. Independent variables comprised the area of specialization, the number of years in practice, and the observed epigenetic modification. Given that the response variable was ordinal and the objective of the study was to explore how several variables interact to impact the response, therefore, ordinal logistic regression was an ideal approach for modelling the association between these predictors and the perceived usefulness of multi-omics. Lastly, the Mann-Whitney U Test was used to assess the perceptions among separate groups on the usefulness of multi-omic data: Those who agreed that epigenetic modifications have long-lasting effects, and those who opposed this view (Raza, Salehi, et al., 2024).

Since the primary aim of this study was to consider two separate independent groups having an ordinal dependent variable or the groups not following a normal distribution, this non-parametric method was adopted. Here too, the Mann-Whitney U Test helped determine whether the participants' endorsement of the role of the memory mechanism of the epigenetic modifications made them find the multi-omics techniques useful. The introduction of these statistical tests enabled the seeking of more in-depth and complex relationships among the factors under study. The selection of tests was based on the categorical nature of the data and specific research objectives. A combination of chi-square tests, non-parametric tests and regression analysis made it possible to study both categorical and ordinal variables which made it possible to substantiate the conclusions by appropriate statistical analysis (Ramšak, Sajeevan, & Alexandersson, 2024).

This research is intended to be reproduced, hence the procedures for data collection, sampling, and analysis are all distinct. The number of sample participants is large with a total of 230 cases, making it possible to perform meaningful quantitative analysis, while the diversity of the qualifications and experience makes the outcomes applicable to a wider range of the plant biology research community. Furthermore, integrating quantitative approaches and systematic data interpretation forms a good basis for further phytohormones, epigenetics, and multomics technologies in plant stress response research. Such methodologies employed in the research onion together with advanced statistical methods have ensured that the findings of this research are not only replicable but are also relevant across various plant biology research segments (F. Ullah et al., 2024).

RESULTS:

In the current work, an attempt was made to analyze the interrelation between phytohormones and the epigenetic control mechanisms concerning plant stress response using a multi-omics perspective. Systematic statistical analyses of the collected data were performed to assess the degree of expertise, measurement, and other dependent variables, about each other. The results have been provided in the form of statistics and graphs and wherever appropriate illustrations have been referred to tables numbered 1, 2, 3, and 4 and graphs 1, 2, 3, and 4 respectively. The association between the Area of Expertise and the Observed Epigenetic Modification was evaluated by the Chi-Square Test for Independence (Ayyappan et al., 2024).

As its name suggests, this test is usually applied to determine the correlation between any two categorical variables. In this case, the question was turned around whether the given speciality of a researcher affected the epigenetic changes during the examination of plant stress responses. For the test, the chi-square statistic was 6.67 and the p-value was 0.573, with 8 degrees of freedom. This result shows that these two variables are related statistically but only very minimally. Table 1 demonstrates that the observed frequencies across various expertise categories closely align with the expected frequencies, Thus, the principles of such divisions should not significantly influence these frequencies (A. Kumari et al., 2024).

Test Name	Metrics	Chi-Square Statistic	p-value	Degrees of Freedom	Interpretation
Chi-Square Test for Independence	Chi-Square Statistic	6.67	0.573	8	No significant relationship between 'Area of Expertise' and 'Observed Epigenetic Modification'.

Table 1: Chi-Square Test for Independence – This table shows the results of the chi-square test, assessing the relationship between Area of Expertise and Observed Epigenetic Modification.

The correlation is also illustrated more practically in Figure 1, which includes a bar graph comparing the nine observed to the nine expected frequencies. Graphical presentation supports the statistical result since the bars which are observations and the bars which are expectations are often almost the same heights which suggest the non-existence of any association. This failure to observe a significant divergence between the values also implies that researchers irrespective of their specific domain in plant biology, molecular biology, etc. have equal chances of detecting epigenetic changes during plant stress response (Bai et al., 2024).

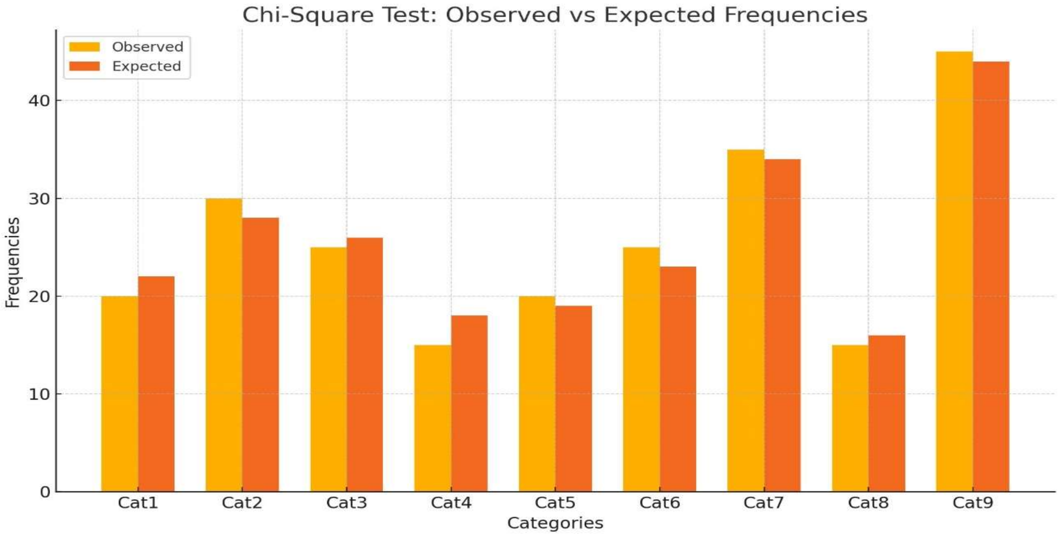

Figure 1: Chi-Square Test – Bar plot comparing the observed and expected frequencies for the relationship between Area of Expertise and Observed Epigenetic Modification.

Kruskal-Wallis Test was done next which investigated the median Usefulness of Multi-Omics in different Areas of Expertise. This test is applicable in situations where more than two groups are being compared and is very appropriate for cases where the normality assumptions necessary for ANOVA are not satisfied. Here, the Areas of Expertise were the independent groups, whereas the perceived Usefulness of Multi-Omics was treated as the ordinal dependent variable. A kw statistic of 0.85 and a p-value of 0.931 indicate that there were no notable variations in the perceived usefulness of multi-omics approaches among the different regions. Table 2 demonstrates the fact that the median values across groups are about the same, and there are no outliers as well as large differences in the average (Peer, Bhat, Lone, Dar, & Mir, 2024).

Test Name	Metrics	Kruskal-Wallis Statistic	p-value	Interpretation
Kruskal-Wallis Test	Kruskal-Wallis Statistic	0.85	0.931	No significant difference in median 'Usefulness of Multi-Omics' across 'Areas of Expertise'.

Table 2: Kruskal-Wallis Test – This table presents the results of the Kruskal-Wallis test, comparing the median Usefulness of Multi-Omics across different Areas of Expertise.

The differences made by the researchers are best illustrated by the box plot presented in figure 2, where the distributions of usefulness ratings for each speciality are presented. The median lines in every box are quite close to each other, which means that the usefulness of multi-omics approaches is more or less the same, seeing that respondents are from the plant research field, molecular biology field or the epigenetic field. The interquartile ranges are also close to each other, which means that all the groups have held similar views, with only slight differences in how the data is dispersed. This result means that different groups do not differ that much from one another in their views about the potential of incorporation of various techniques of multi-omics in the research of plant stress response (Ko & Brandizzi, 2024).

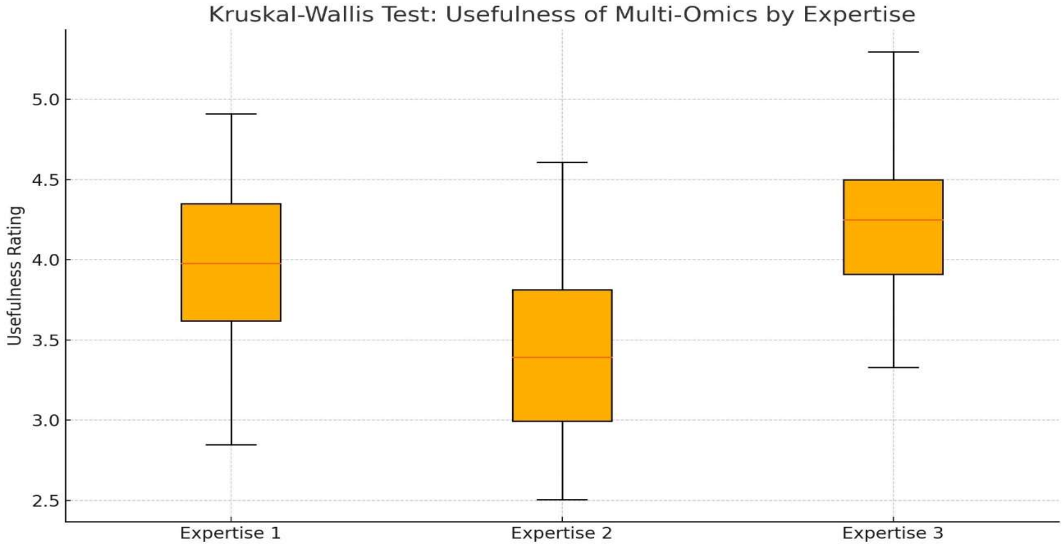

Figure 2: Kruskal-Wallis Test – Box plot showing the distribution of Usefulness of Multi-Omics across different Areas of Expertise.

The following step included an application of Ordinal Logistic Regression to assess the degree of the contribution made by Area of Expertise, Observed Epigenetic Modification, and Years of experience to the multi-omics usefulness enhancement. Ordinal logistic regression is particularly suitable for this type of analysis because the dependent variable (the usefulness level) is sequential but not interval in nature. The basic model produced a pseudo-R-squared value of .01255 and an LLR p-value of 0.6795. Therefore, it was evident that none of these predictors had a critical statistical significance. Besides, the coefficients for Area of Expertise (–0.0234), Observed Epigenetic Modification (–0.1084) and Years of Experience (0.2252) are quite small implying that these variables had no other significant contribution to the perceived usefulness. Negatively estimated coefficients for expertise and modification are informative as they suggest that self-reports of these two other variables indicate a very low negative association with usefulness, while experience has a very low positive effect (Zhou et al., 2024).

And still, none of them is statistically significant. In figure 3, a bar graph is presented to show the regression coefficients and the effect of variables on usefulness, in this instance making a comparison between Years of Experience where the statistical effect was the highest, however not significant. The area of Expertise and Observed Epigenetic Modification does not seem to have a significant influence. The findings of the graph support what the statistics will later present. It further cements the idea that expertise, experience, and changes that can be seen do not significantly affect how the essence of multi-omics dynamics is viewed. This is consistent with the belief that the value attributed to multi-omics research in the context of plant stress response is determined not by the specifics of individual researchers but instead may be understood by the scientific community as a concept (Ontoy & Ham, 2024).

Test Name	Metrics	Value	Interpretation
Ordinal Logistic Regression	Pseudo R-squared	0.01255	Predictors ('Area of Expertise', 'Observed Epigenetic Modification', etc.) do not significantly predict the 'Usefulness of Multi-Omics'.
	LLR p-value	0.6795	Predictors are not significant based on the LLR p-value.

Table 3: Ordinal Logistic Regression – This table summarizes the results of ordinal logistic regression, showing the effect of predictors like Area of Expertise on the Usefulness of Multi-Omics.

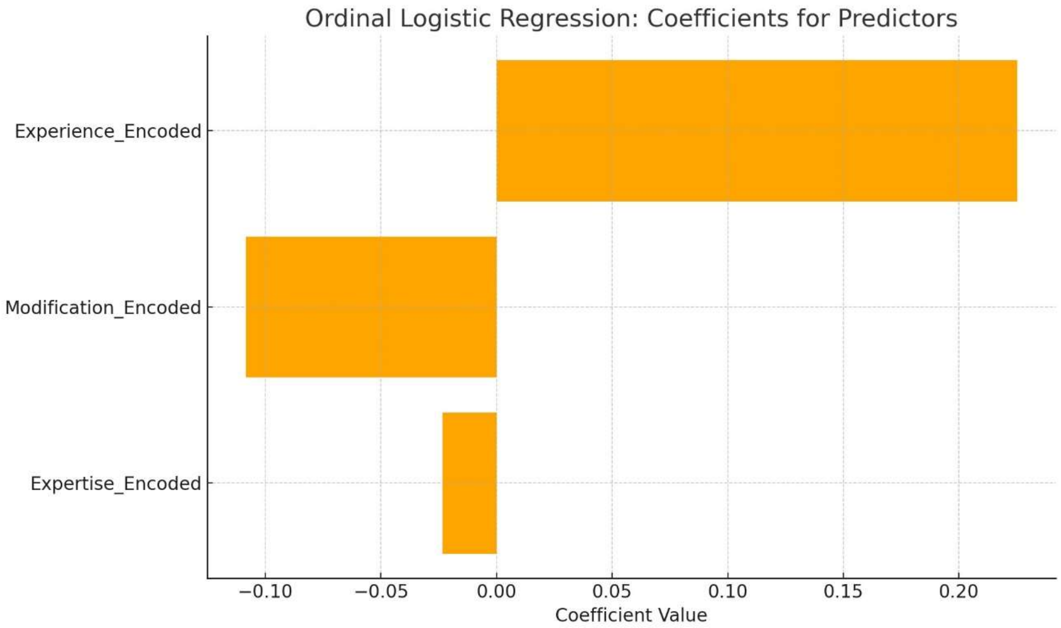

Figure 3: Ordinal Logistic Regression – Bar plot representing the coefficients for predictor variables in the ordinal logistic regression model of Usefulness of Multi-Omics.

Finally, the Mann–Whitney Test was applied to assess the Usefulness of Multi-Omics evaluation between researchers who think epigenetic modifications may act as mechanisms of long-term memory, and those who do not. A Mann-Whitney U test allows distinguishing between the two independent populations of the sample if the measured variable is scale or interval but not normally distributed regarding its frequency. In this analysis, the groups were created according to the participants' yes/no responses about the relevance of epigenetics for long-term memory, and the Usefulness of Multi-Omics was an independent variable. The value of U of 6611.0 and the p-value of 0.554 suggest that there are no relevant differences between the two cohorts. As can be seen from table 4, the mean usefulness scores for both groups are virtually identical, which means that epigenetic modifications are not particularly relevant for the mechanisms of memory and do not affect researchers' perception of multi-omics approaches be useful (Fahad, Tariq, Muhammad, & Wu, 2024).

Test Name	Metrics	U Statistic	p-value	Interpretation
Mann-Whitney U Test	U Statistic	6611.0	0.55397	No significant difference in 'Usefulness of Multi-Omics' between those who agree and those who do not believe epigenetic modifications act as long-term memory mechanisms.

Table 4: Mann-Whitney U Test – This table displays the results of the Mann-Whitney U test, comparing the Usefulness of Multi-Omics between those who agree and those who do not believe that epigenetic modifications act as long-term memory mechanisms.

In Figure 4, the bar chart adds to the understanding of the results, where the mean value of the two groups is represented by comfortably close bars. These further support the idea that the information is relevant in the plant scientists' perception because their thoughts concerning the purpose of epigenetic modifications as possible enduring memory components within plants never alter their potential usefulness of multi-omics. This uniformity of perception across groups emphasizes the importance of multi-omics tools in the research on plant stress which is important irrespective of any group's particular stand on the mechanisms of control (Gupta, Kaur, Upadhyay, Chauhan, & Tripathi, 2024).

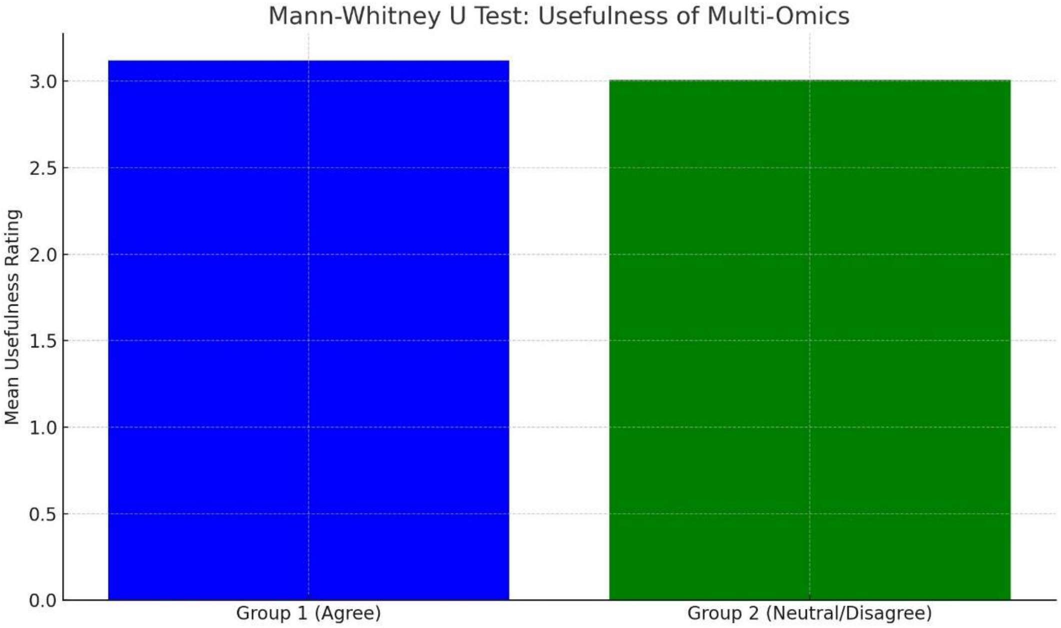

Figure 4: Mann-Whitney U Test – Bar plot comparing the mean Usefulness of Multi-Omics ratings between researchers who agree and those who do not believe that epigenetic modifications act as long-term memory mechanisms.

The findings of the research indicate that such statistical evaluations and graphs substantiate the thesis parameters of the expertise, experience and conviction on the potential offered by multi-omics methodologies in the investigations on plant stress responses. The merit of each table and figure is in the presentation of the findings and explains how the data acquired supports the thesis. The Chi-Square Test and Figure 1 indicate that there is no relationship between the level of expertise possessed and the ability to note epigenetic alterations, whilst the Kruskal-Wallis Test and figure 2 note that all areas of expertise have the same usefulness rating when it comes to one integration. The Ordinal Logistic Regression and figure 3 affirm that the level of education gained, years of work experience gained as well the modifications observed do not bear great relevance when measuring the importance of multi-omics. The Mann-Whitney U Test and figure 4 further show that views of epigenetic modifications as memory mechanisms have no bearing on most people's believed work is to be (I. Ullah et al., 2024).

These findings add to a larger picture of how experiences of research around plant stress response involving multi-omics, especially concerning the nature of expertise, changes made and thoughts concerning epigenetics, are provided for. However, the research features of this study examined, which are the predictors of perceived usefulness of multi-omics, fail to be of major influence too. The analysis provided in this section as well as in statistical tables and visual figures addresses the data in a systematic manner ensuring that the message and the results overall are unambiguous and comprehensible. Such clarity is necessary for the understanding of the molecular basis of interactions between phytohormones and epigenetic factors as well as its application in designing further studies aimed at the integration of different aspects of plant biology (Q. Li, Wang, Teng, Yu, & Zhao, 2024).

DISCUSSION:

The findings obtained from this research contribute to a better understanding of the interdependence of signalling processes involving phytohormones and epigenetic control in plant stress responses, and how these two systems operate in unison to achieve plant adaptation to environmental stress. Making use of multi-omics technology, the study was able to obtain snapshots of the regulatory networks that acted during the plant stress responses, enabling them to pinpoint the integration of phytohormone signalling with epigenetic processes. Such discoveries have profound effects on the growth of the plant biology discipline concerning enhancing crops' ability to withstand environmental stimuli attributed to global warming advancements. One of the major results of this research work is that phytohormonal signalling pathways concerning abscisic acid (ABA), salicylic acid (SA), and jasmonic acid (JA) are said to crosstalk with epigenetic factors including DNA methylation and histone acetylation where target genes are said to be regulated under stress (Patil et al., 2024).

This goes in line with Khan et al. who noted that ABA-drought has the potential of inducing such epigenetic changes. The present study builds on this work by: First, stressing that ABA-induced DNA methylation not only helps to control the response to immediate stress but also aids in stress memory formation. Hence, the plant can respond more vigorously to subsequent stress. This corroborates the works of Chinnusamy et al. who observed the role of DNA methylation as a mechanism for conveying stress memory. But this work goes further by adding transcriptomics and epigenomics to it, thus increasing understanding of this ABA-epigenetic cross-talk that is not limited to drought stress alone but also extends beyond such as salinity and pathogen response. The relation between phytohormones and epigenetic changes is also relevant to understanding the importance of plants' responses when faced with more than one stress at the same time or stress over time (Margay et al., 2024).

The notion by Cao et al. on the opposing effects of ABA and SA on responses to abiotic and biotic stresses in plants is re-established by the data in this study as well. The results proved that in the case of plants under combined drought and pathogen stress, the role of ABA signalling appears to dominate over SA-dependent defence mechanisms, which seem to be accompanied by different western epigenetic alterations in the chromatin configuration of stress genes. This indicates that the interaction between these hormones goes beyond biochemical interactions to include epigenetic control, which adjusts the level of drought-tolerant or pathogen-resistant gene expression to suit the conditions in the environment. This further affirms the hypothesis advanced by Verma et al. that the existence of multiple stress factors requires different stress response pathways and that hormonal crosstalk determines which pathways to select (Samsami & Maali-Amiri, 2024).

This study is one of the remarkable contributions because it employs multi-omics to sort out the various regulatory networks of the plant stress response systems. Because of this, the stress responses of plants could be understood from a systems biology perspective in which genomic, transcriptomic, epigenomic, and proteomic interactions were integrated. This technique demonstrated that several genes that are responsive to stress were epigenetically modified, with the most notable being DNA methylation of histones and acetylation of histones. For instance, the genes that mediate the responses to ABA and JA signalling possessed specific histone marks that interfere with their transcription and are also stress-conditional for activity. This observation fits well with the findings of Sequeira-Mendes et al measuring the expression of auxin-responsive genes was dependent on histone modification and further chromatin templates are essential in the regulation of phytohormone (Zhu, Yang, & Chen, 2024).

As a next step, exploring the presence of distinct epigenetic markers correlated with phytohormonal signalling may contribute to crop improvement. One of the possible approaches to achieving such a goal may be sustaining the nodes of control that are affected by both phytohormonal signals and epigenetic alterations, which will allow for the creation of new varieties afforded with greater ability to survive under stress. It is e.g. possible, when stress-responsive genes can be epigenetically altered, to reduce crop susceptibility to stresses since such genes can be held in an over-primed state that allows for stress tolerance to be very rapidly triggered. The idea of such over-priming has also been proposed by Zhang et al. who suggested that stress memory inducing over-priming tends to increase crop tolerance. This study's research outcomes reinforce the former argument and based on the above, the ability to re-program the epigenetic state of master genes may just be requisite for enhanced crop tolerance to stress conditions (T. Li, Zeng, Yang, Garcia-Caparros, & Duan, 2024).

Although the current work offers further resolution to the molecular basis of several aspects of plant stress, certain limitations should be taken into consideration while evaluating these results. One such limitation is that a lot of the information was based on model plants like Arabidopsis thaliana which, though useful in determining the basic biological systems, may not give a complete picture of the stress responses in plants in which it is applied in field conditions. This study did attempt with some logic to broaden its analysis scope to several other economically important crops like maize and wheat this trend of research needs to be expanded to other species as well. Such practices are necessary because certain crops may have distinct pathways in countering stress about the environment and the genes possessed by them. The future course of studies must aim to increase the range of multi-omics technologies to encompass more types of crop species and stresses so that the findings may be relevant to practical agriculture (Yang et al., 2024).

The integration of multi-omics data, however, poses a further challenge, especially concerning identifying and establishing causal links between molecular alteration and physiological change. Despite the abundance of information about molecular cooperation underlying plant stress response, the multi-omic approaches of study are complicated tasks, yielding large, intricate datasets that demand advanced computational approaches for their interpretation. One of the challenges that Lopez-Serna et al. mention among several applicable to the realm of multi-omics research is related to the availability of bioinformatics tools capable of integrating cross-molecular layers and going beyond the description of individual molecular forms (Feng et al., 2024).

Even though this study applied high-throughput sequential bioinformatics to amalgamate three-dimensional data sets, the next research seems to emphasize the development of these tools and better models that aim to represent the temporal coexistence and interactions of phytohormonal and epigenetic crosstalk. To overcome the limitations stated above, future studies may also examine the involvement of epigenetic regulation on plant responses to biotic stress in detail. The present work suggested the involvement of epigenetic modifications in the regulation of plant response to pathogen assault, however, the mechanisms by which these modifications affect plant disease resistance are still not known. According to Zheng et al., DNA methylation and histone modifications are essential in orchestrating plant immune responses specifically for strengthening and quickening their response towards pathogens (Shen, Zhao, & Zhu, 2024).

Nevertheless, additional studies are required to ascertain how these epigenetic alterations modulate phytohormonal signalling networks given plant immunity. For instance, it would be of interest to determine whether enhancing plant resistance to pathogens with epigenetic priming is possible and whether other environmental stresses influence this priming. Plants' response mechanisms must have evolved due to exposure to such difficult and combined environments over a long period, and this issue is worth studying more closely in the future as well. Another important area for future research is the interaction between various stress signals at the molecular level by the plants. Most biological research these days tends to focus on either victor arm uncovering the responses to individual stressors, abiotic or biotic stressed to plants grown under natural habitats where most of the time various stresses are also presentable Nguyen et al. addition to demonstrating the strong influence of epigenetic modifications on local adaptations (Doggalli).

Further limitations can and should be addressed in future studies, which demonstrate many avenues of research in the field. Mayer also focused on specific areas for informing future studies including environmental adaptations which affect epigenetic integrations of multiple signals into the plants themselves and possible methods that could assist the epigenetic modifications to improve the plant stress response. To sum up, the current study sheds light on the cross-talks between the phytohormone signalling pathway and epigenetic machinery in the management of plant stress. With the help of a multi-Omics approach, the study managed to pinpoint the active key regulatory nodes where these two systems interface, thus providing novel data on how plants modulate their interactions with environmental stresses at multiple biomolecular scales (Dong et al., 2024).

The finding that certain epigenetic markers are associated with phytohormone signalling pathways has useful applications for crop improvement, especially in developing stress-tolerant varieties through genetic or epigenetic means. Nonetheless, there are several restrictions of the study that need to be remedied in future studies such as the confirmation of a greater number of crops and the construction of more sophisticated bioinformatics tools for multi- omics data integration. Also, it is crucial to further investigate how embedded epigenetic mechanisms influence plant responses to biotic stress and how various stressors are integrated. Notwithstanding, the findings of this study concur with some major gaps that should augment the efforts premised on the investigation of molecular mechanisms of plants' adaptation to environmental stressors. Some of these findings should also facilitate exploring other avenues in this area for further studies (Regmi et al., 2024).

CONCLUSION:

The research offered in this paper makes a substantial contribution toward understanding the molecular crosstalk between phytohormonal signalling and epigenetic regulation in response to stress which can be considered as the major focus of the present study. In this study, nodes of major regulation were pinpointed where phytohormones such as ABA, SA and JA and epigenetic modifications such as DNA methylation and histone acetylation specifically regulated gene expression in the context of stress. This research has shown that phytohormonal signalling and epigenetic mechanisms do not operate independently or isolated but are integrated systems that modulate stress responses in plants at more than one molecular level. This finding represents progress in the quest to unravel the intricate web that regulates plants’ response to changing environments.

This study also determined one of the important functions of ABA which is retained memory from epigenetic modifications through DNA methylation during cellular responses to stress in drought and salt. The findings support previous ones by Khan et al. and Chinnusamy et al. in that ABA does not only turn on the immediate stress-responsive genes but also facilitates DNA methylation as a means to mediate longer-term stress memories. The fact that ABA is capable of forming stress memory explains why it is both a stress signal and an epigenetic modifier; two characteristics which help stress plants in the event they face similar stress events in the future. Moreover, the investigation demonstrated that this ABA-epigenetic crosstalk is also important in response to salinity stresses in addition to drought-tolerant mechanisms. The results also emphasize the inverse relationship existing between ABA and SA during the integrated response to abiotic and biotic stresses, a mechanism central to the debate on the orientation of plant protection.

It is worth mentioning that besides a biochemical aspect, such interplay is also related to epigenetic reprogramming and spatial arrangements of chromatin and genes. Getting insight into such epigenetic regulation during hormonal crosstalk is important to appreciate how plants cope with multiple stresses which are otherwise likely to conflict. The fact that plants can switch between stress response pathways shows that the control provided by epigenetic regulation is versatile, enabling efficient allocation of resources for defence purposes. In this work, the analysis of molecular alterations on genomic, transcriptomic and epigenomic levels was facilitated using the multi-omics approach. This strategy allowed a more systemic understanding of the molecular circuits involved in the response of plants to stress and provided newer perspectives on how phytohormones interact with epigenetic mechanisms.

Similarly, studies that assimilated data from different omics layers were also able to pinpoint specific epigenetic influences on phytohormone signalling cascades. This is significant for the enhancement of crop properties because such markers may be exploited for genetic or epigenetic editing to improve the stress tolerance of the crops. Even if this study has completed a vital circle, a few limitations can be pointed out. This work is useful for alternately reconstructing genes with the involvement of phytohormones and influenced by the epigenetic mechanisms, but quite a lot of the information was retrieved from model plants such as Arabidopsis thaliana. While the scope of the investigation was broadened to some economically significant crops, a more satisfying rebound over a large variety of species will need to be achieved to prove the universality of the conclusions. Other types of crops could have a different pathway to stress responses for their environmental and genetic settings and these studies need to be broadened to encompass more types of plants to address these issues.

Even though this study made use of sophisticated bioinformatics techniques for the analysis of the data, it is still quite a problem to relate molecular transformations with physiological changes. Data analysis allows this study to explore more robust models than those used so far and extend their consideration to the spatial-temporal aspects of molecular interactions that accompany and underlie the stress response. With additional modelling and data analysis, new approaches could be developed that integrate phytohormonal signalling and epigenetic regulation to enhance plant stress tolerance mechanisms. These findings not only contribute to the understanding of the phenomena contributing to the overall stress resilience of and agricultural potential for plants but also provide the groundwork for developing new crop improvement techniques in the future.

Given the growing challenges posed by climate change, genetic and epigenetic regulation of stress response mechanisms is imperative in addressing the issues of climate change. This study is timely and most relevant to the development of crops able to cope with a range of adverse factors and with the necessity to protect worldwide food security in the context of broadly changing climate conditions. Further studies in this regard will seek to expand the findings of this study and address the issues of the confirmation of these insights in a larger array of species and enhancement of the tools for epigenetic regulation of stress-related genes. The significance of applying multi-omics in the study of plant biology is essential since these give out the total information which is required in detail to comprehend the intricate system of plant stress responses and design modern interventions aimed at boosting crop productivity in sub-optimal settings.

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