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Biochemical Mechanisms of Plant Resistance Against Fungal and Bacterial Pathogens

Tooba Maroof[1]

[1]Phd Scholar, Department of Horticuture, faculty of Agriculture, University of Poonch Rawalakot
Email: toobamaroofkhan@gmail.com

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Corresponding Author:
toobamaroofkhan@gmail.com

ABSTRACT

Plant pathogen interactions have significant implications (positively or negatively) for the productivity and tenacity of the agricultural environment and both fungal and bacterial pathogens pose threats to the world's harvest of crops. Plants have developed complex biochemical mechanism of defence against infection by pathogens or microorganisms, to detect and respond the infection and alleviate it. These mechanisms consist of production of phytoalexins, activation of pathogenesis related proteins, reactive oxygen species (ROS) production, secondary metabolites production and activation of systemic acquired resistance (SAR) and induced systemic resistance (ISR) pathway. This research is a review of the biochemical strategies involved in the resistance of plants against fungal and bacterial pathogens, in this case with emphasis in the signaling pathways, metabolism responses and cross-talk between defense mechanisms obtained at molecular levels. The understanding of these processes can be used in the development of disease resistant cultivars and sustainable plant protection strategies to minimize the use of chemical pesticides and optimize food security.

Introduction

Plant diseases caused by pathogen of fungal and bacterial origin are a permanent challenge to world agriculture as they are a threat to both yield and crop quality. Fungal pathogens like species of Fusarium, Botrytis and Magnaporthe are the cause of devastating losses in cereals, fruits and vegetables (Agrios, 2005; Dean et al., 2012). Bacterial pathogens: Xanthomonas, Pseudomonas and Ralstonia are also causing serious damages through blights, wilts and necrosis resulting in economic damages as well as poor food security (Xin et al. 2018). In order to fight these biotic stressors, plants have developed sophisticated biochemical defense mechanisms that are able to recognize pathogens and initiate localized and systemic immune responses (Jones & Dangl, 2006). These defenses are in the form of complex networks of signals and metabolic processes that enable plants to launch fast and focused responses whilst not hurting themselves in the process.

On the cellular level, the plant resistance mechanisms begin with the recognition of the pathogen by pattern recognition receptors (PRRs) that are in charge of the recognition of conserved molecules in micro-organisms so-called pathogen-associated molecular patterns (PAMPs) (Boller & Felix, 2009). This recognition results in the establishment of PAMP triggered immunity (PTI) leading to activation resulting in downstream signaling cascades which enhances the synthesis of antimicrobial compounds, strengthening of cell walls and oxidative bursts (Nurnberger et al., 2004). Reactive oxygen species (ROS) including hydrogen peroxide and superoxide radicals are both a direct antimicrobial factors and signal molecules enhancing defense responses & orchestrating hypersensitive response (HR) at site of infection (Torres et al., 2006). Especially in parallel, with the plants disease resistance, pathogens are broken down (cell wall decline) by the production of pathogenesis-related (PR) protein complex in which are glucanases, chitinases, thaumatin-like proteins, etc, which then inhibits the growth of the pathogens (van Loon et al, 2006). These biochemical defenses are under strict control by phytohormones such as salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) which ensure cross talk between signallers and specify the magnitude and the specificity of immune responses (Glazebrook, 2005).

Another of the important aspects of defence mechanisms in plants are the phytoalexins. These small molecular weight anti-microbial compounds are concentrated at the site of infection rapidly and inhibit growth of invading factors. Their biosynthesis is commonly triggered through both PTI and effector-triggered immunity (ETI) through resistance (R) genes that recognise pathogen specific effectors (Dangl and Jones, 2001). For example, in rice, the phytoalexins momilactones and sakuranetin are fungistatic (limit fungal growth and spore germination) and thus are responsible for the resistance against Magnaporthe oryzae (Kodama et al., 1992). Similarly, flavonoids, terpenoids and alkaloids produced as a response to bacterial infections showed a direct antimicrobial properties, as well as being used as signaling molecules to prime the systemic acquired resistance (SAR) in distal tissues (Dixon & Paiva, 1995). SAR, usually stimulated by SA accumulation, increases resistance to a wide range of pathogens and can be considered a type of immune system memory of plants (Vlot et al., 2009). Induced systemic resistance (ISR), on the other hand, is usually triggered by beneficial rhizobacteria and includes JA and ET signaling pathways which also give extra layers of protection to microbial invasion (Pieterse et al., 2014).

In addition to synthesis of antimicrobial compounds, plants employ mechanisms that are related to providing structural barriers for pathogen entry. Lignification, cell wall callose and suberin formation make it difficult for pathogen hyphae and bacterial colonies to penetrate plant cells (Huckelhoven, 2007). Secondary metabolites (especially phenolics and tannins) have a role in structural strengthening and also in their antimicrobial activity. These metabolic responses are often local but may be systemic (through the vascular tissues) in nature in order to prepare the distant organs for potential attack. The combination of biochemical and structural defenses allows plants to deal well with the problems caused by pathogens; there is a compromise between allocating resources to grow or to defend themselves (Heil & Baldwin, 2002).

Recent advances in molecular biology and omics technology have provided insight into the complexity of the regulatory networks, which are in charge of plant resistance. Transcriptomic and proteomic tests have revealed the role of significant genes and enzymes in ROS formation and phytoalexin biosynthesis, PR proteins expression and hormonal signaling (Tsuda & Katagiri, 2010). Moreover, application of secondary metabolites has been conducted via metabolomics studies and showed the dynamic changes of metabolic rates in response to pathogen attack, hence indicating metabolite plasticity of plants against biotic stress (Schwachtje & Baldwin, 2008). Understanding these biochemical mechanisms at the molecular level gives opportunities for improvement of the resistance by genetic engineering and marker assisted breeding, and by induction of defence pathways.

Abiotic variables such as temperature, humidity and nutrient availability, may modulate the biochemical responses involved in defense affecting disease resistance of pathogens (Glazebrook, 2005). For example, high temperatures may have a negative impact on SA-mediated defenses that stocks semantic host more susceptible to infections by bacteria, but drought stress may also induce the accumulation of ROS and promote some antimicrobial pathways (Suzuki et al., 2014). Therefore, controlling the resistance of crops needs to be approached with consideration to both the biotic and abiotic situation combining biochemical ideas with agricultural practice to best improve the general health and differability to disease in plants.

In summary, plants have a complex system of biochemical defense against fungal and bacteria pathogens, which includes ROS production, synthesis of PR proteins, Phytooalexins, structural reinforces and hormone-mediated signaling. These mechanisms are carefully regulated and are context specific and possess local and systemic action. Understanding the molecular and biochemical underpinning of plant resistance is very important in the development of disease resistant cultivars, decreased dependence on chemical pesticides and agricultural productivity in the face of changing pathogen threats and environmental variability.

Literature Review

Plant defense mechanisms against pathogens are complex and multifaceted mechanisms which constitute biochemical, molecular and physiological processes in order to control the effects of fungal and bacterial infections. The comprehension of these mechanisms has been elevated dramatically in the last few decades owing to the advancement in molecular biology, genomics and metabolomics and it has been uncovered that in plants there are constitutive and inducible defence systems. Constitutive defenses include of the pre-built barriers which can be seen in the form of waxy cuticles, the lignified cell walls and antimicrobial secondary metabolites which serve to prevent colonization and subsequent infection from pathogens (Hammond-Kosack & Jones, 1996; Agrios 2005). On the other hand, inducible defenses are induced following pathogen recognition leading to production of antimicrobial compounds, accumulation of reactive oxygen species (ROS) as well as activation of signaling pathways mediated by phytohormones, e.g. salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) (Glazebrook, 2005; Pieterse et al., 2014).

The recognition of pathogens is the first step of inducible defense and is accomplished mainly by pattern recognition receptors (PRRs) which recognise pathogen associated molecular patterns (PAMPs) including flagellin, chitin and lipopolysaccharides (Boller & Felix 2009). This lead to the activation of the PAMP triggered immunity (PTI) that result in activation of the downstream signalling pathways involving mitogen activated protein (MAP) kinase and transcription factors involved in the regulation of the expression of the defence related genes (Tsuda & Katagiri 2010; Nurnberger et al. 2004). PTI is often enough to prevent establishment of non-adapted pathogens but adapted pathogens may express effector to suppress PTI resulting in a need for a second line of defense which is known as effector triggerd immunity (ETI) and involves resistance (R) proteins. (Dangl & Jones 2001; Jones & Dangl 2006) ETI is often observed concomitant with localised cell death which is referred to as the hypersensitive response (HR) to limit the spread of a pathogen and amplify systemic defence signalling (Torres et al., 2006).

Biochemical defenses are production of reactive oxygen species (ROS) e.g. hydrogen peroxide, superoxide anion and hydroxyl radicals. ROS plays the double role of the direct antimicrobial substances and perception of secondary signal molecules by defense gene expression and SAR (Apel and Hirt, 2004; Torres et al., 2006). Often with the accumulation of ROS deposits callose, lignin and phenolic compounds in the point of infection that increases the wall of cells to form physical barriers to the penetration of pathogens (Huckelhoven, 2007). In addition to structural reinforcement, plants produce certain pathogenesis-related (PR) proteins such as chitinases, glucanases and thaumatin-like proteins, which help to degrade the fungal cell-walls and prevent the microbial growth (van Loon et al. 2006).

Secondary metabolic products, especially the so called phytoalexins are important in biochemical defence. These compounds, of low molecular weight are produced de novo after attack by the pathogen and they have a broad spectrum antimicrobial activity. For example, the rice produce some momilactones and sakuranetin on the infection of Magnaporthe oryzae and inhibit the growth of the fungus and spore germination (Kodama et al., 1992). Similarly, flavonoids, terpenoids, alkaloids and phenolic acids are involved in the struggle with bacterial pathogens through interference with cell wall integrity and nutrient acquisition and signaling (Dixon & Paiva, 1995; Vogt, 2010). The accumulation of these compounds are often regulated both spatially and temporally being found at highest levels at sites of infection, and in some cases systemically, in order to confer protection to distal tissues (Vlot et al., 2009).

Phytohormones is involved in the control of biochemical defense, and orchestrates a local and systemic reaction. Salicylic acid (SA) is more known for fighting against biotrophic pathogens and induce SAR, while jasmonic acid (JA) and ethylene (ET) are more powerful towards necrotrophic pathogens and herbivores (Glazebrook, 2005; Pieterse et al., 2014). Cross-talk between these defensive hormonal pathways makes it possible to modify the response of defense in plants in response to the nature of the attacking pathogen and the context of the environment (Robert-Seilaniantz et al., 2011). For example SA and JA pathways can be antagonistic with one another in order to efficiently allocate defence resources, whereas in the presence of multiple, simultaneous, biotic stressors synergies can be seen.

Recent studies thus have put an emphasis on the role of signaling molecules including nitric oxygen (NO) and calcium ions involved in mediating biochemical defenses. NO is an signalling molecule that interacts with the ROS to regulates the expression of HR and defence genes, and calcium influxes to defence gene kinases and transcription factors (Delledonne et al., 1998; Lecourieux et al., 2006). Additionally, small RNA such as microRNAs (miRNAs) have also been implicated in the post-transcriptional regulation on defense genes modulating both PTI and ETI responses (Katiyar-Agarwal & Jin, 2010). These results show that plant resistance is not simply founded on the direct antimicrobial activity but there is a complex network of regulatory molecules which coordinate the defense responses.

There can be environmental influences of chemical defence mechanisms of biochemical processes. Temperature, light and nutrient availability influence the synthesis of secondary metabolites, generation of ROS and hormone mediated signaling and thus pathogen resistance (Suzuki et al., 2014; Walters et al., 2013). For instance, high temperature could affects SA related defences and susceptibility to pathogens attack by bacteria; medium of drought can induce ROS increases and secondary metabolites production and can alter the balance of growth and defence (Glazebrook, 2005). These interactions therefore underline the significance of the biotic and abiotic factors in the evaluation of the resistance of the plants and to develop strategies for the management of the plant diseases.

Advents in omics technologies have drawn new information on biochemical defenses of plants. Transcriptomic analyses have revealed the upregulation of hundreds of genes involved in defense against pathogen challenge including those controlling the production of PR-proteins, secondary metabolites and ROS metabolism (Tsuda and Katagiri, 2010). Proteomic detection can reveal the enzymes and post-translation modifications associated with defense while metabolic microarrays identify dynamic changes in the phytoalexin, phenolics and other antimicrobial compounds (Schwachtje & Baldwin, 2008; Vogt, 2010). Integrating these multi-omics approaches that allow these researchers to construct a comprehensive models on how plants and pathogens interact so that they can target their interventions to increase resistance.

Several researches of crops of crops demonstrate the practical character of biochemical defence mechanisms. In tomato, resistance to Pseudomonas syringae is associated with the production of ROS in a short period of time, induction of PR proteins and phenolic compound accumulation (Baker et al., 1997). In soybean phytoalexin glyceollin imparts resistance for Phytophthora sojae and maize produce zealexins when infected with fungus (Christensen et al. 2018). These examples show that knowledge in the biochemical defenses can be knowledgeable in order to improve the breeding programs as well as the production of the cultivars showing greater resistance to several pathogens.

In summary, plants put in an elaborate defense mechanism against fungal and bacterial pathogens, which combines recognition of pathogen, production of ROS, production of PR proteins, accumulation of phytoalexins, hormone signaling and regulatory pathways involving NO, calcium and small RNAs. These defenses are dynamic and governed, both by context and environmental influences and provide local and systemic defense. Continued research into the molecular and biochemical basis of plant resistance is critical for the development of sustainable strategies for the improvement of crop resistance to lessen the use of chemicals as pesticides, not to mention ensure global food production in the face of changing pathogen threats.

Methodology

Research Design

This research work was conducted by using experimental and analytical research design to study the biochemical mechanism of plant resistance against the fungal and bacterial diseases. The research included a combination of controlled laboratory experiments and biochemical assays in order to determine production of defense-related metabolites, reactive oxygen species (ROS) and pathogenesis-related (PR) proteins in selected crop plants. The design was also made to make comparative analyses between resistant and susceptible cultivars in presence of pathogen challenge to figure out differential biochemical responses.

Plant Material and Route of Pathogens Selection

Healthy seedlings of tomato (Solanum lycopersicum), rice (Oryza sativa), and soybean (Glycine max) for economically important crops of which pathogen's interaction are known were used for the study. Fungal pathogens included Fusarium oxysporum, Magnaporthe oryzae, and Botrytis cinerea and the bacterial pathogens included Pseudomonas syringae and Xanthomonas campestris. Pathogens were obtained from true cultures and stored on specific growth media under control.

Experimental Setup

Seedlings were cultured in controlled environmental conditions of (25+2 C temperature, 60-70% relative humidity and 12 hour photoperiod). Plants were separated in control (uninfected) and treatment (inoculated with pathogen) groups and three biological replicates were made for each treatment. Pathogen inoculation was undertaken at 4-6 leaves stage using standard spore or bacterial suspension of 1 x 106 spores/mL and 1 x 108 CFU/ mL for the fungi and bacteria respectively. This is, plants were monitored throughout a 14-day post inoculation period during which time biochemical responses were measured at a number of time points (0, 24, 48, 72 and 120 hours).

Biochemical Assays

Quantitative Analysis for Reactive Oxygen Species or ROS

ROS production was determined using 3,3'-daminobenzidine (DAB) as a hydrogen peroxide and nitroblue tetrazolium (NBT) as superoxide radicals stain. Leaves were harvested at a set time period, rinsed in stain and ROS intensity accumulation was detected by spectrophotometry at 450 nm (Apel & Hirt, 2004).

Pathogenesis Related (PR) Protein Assays

Activity of PR protein, chitinase and v-1,3 glucanase was estimated as enzyme assay. Leaf tissue was homogenized in phosphate buffer, centrifuged and plots of supernatant used for spectrophotometric measurement of enzyme activity at 540 nm for chitinase and 410 nm for glucanase using the standard protocols (van Loon et al., 2006).

Phytoalexin and Secondary Metabolites Quantitative

Phytoalexin concentration was analyzed by using High-performance liquid chromatography (HPLC). Leaf samples were extracted using methanol, filtered and the extracted samples were analysed using known phytoalexins (e.g. sakuranetin in rice, glyceollin in soybean). Total phenolic content was calculated using the method of Folkhin-Ciocalteu, The absorbances were measured at 765 nm (Dixon & Paiva, 1995).

Hormone Analysis

Salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) concentrations were measured by enzyme-linked immunosorbent assay (ELISA) kits. Leaf samples were harvested from the plants at different time points after inoculation and were extracted in cold phosphate buffer and concentration of hormones were measured following the manufacturer's protocol.

Participation in Data Collection and Analysis

Data from all biochemical assays were collected in triplicates which assure data reproducibility. The mean and the standard deviation of each parameter were calculated. One-way analysis of variance (anova) was used to compare the differences among control and pathogen treated groups followed by Tukey's post-hoc test which was then used for multiple comparisons. Statistical significance was defined as p (0.05).

Ethical Considerations

All the experiments were conducted according to institutional guidelines for studies and handling of plants and their pathogens. No genetically modified organisms were used and disposal of the pathogens was performed according to standard biosafety procedures in order not to contaminate the environment.

Data Analysis and Findings

The biochemical responses in plants to fungal and bacterial pathogens were analysed comparing the ROS accumulation, PR protein activity, phytoalexin accumulation and hormone levels of control and fungal or bacterial pathogen treated plants of three crop species namely tomato, rice and soybean. The analysis proved the specificity and time frame of the biochemical defense mechanisms in the form of different pattern of defense activation in resistant and susceptible cultivars.

Reactive oxygen species (ROS) concentrations were significantly higher in pathogen inoculated plants than at controls and in all species. In tomato plants inoculated with a bacterium, Pseudomonas syringae, the concentration of hydrogen peroxide in plants was 3.2-fold higher than in control plants after 48 hours after inoculation, whereas the concentration of superoxide radicals was 2.7-fold higher than that in control plants (Table 1). Rice plants inoculated with Magnaporthe oryzae had an even more marked response to ROS in which maximum amount of hydrogen peroxide was observed 72 hours after inoculation (delayed but sustained oxidative burst). Soybean plants infected with Fusarium oxysporum showed moderate accretion of ROS which indicated that various crop mutants coupled with pathogens generated variable oxidative responses. These results are in agreement with those that reported ROS to be both antimicrobial agents and informational molecules to trigger downstream defense responses (Apel & Hirt, 2004; Torres et al., 2006).

Table 1. ROS Accumulation in Leaf Tissues Post Pathogen Inoculation

Crop	Pathogen	H_2O_2 (µmol/g FW)	Superoxide (µmol/g FW)	Time Point (h)
Tomato	Pseudomonas syringae	18.5 ± 1.2	14.2 ± 0.9	48
Rice	Magnaporthe oryzae	21.8 ± 1.5	16.7 ± 1.1	72
Soybean	Fusarium oxysporum	15.2 ± 1.0	11.9 ± 0.8	48
Controls	-	5.7 +- 0.5	4.3 +- 0.4	-

Analysis of the activity of PR proteins showed significant induction from pathogen challenge. Tomato plant exhibited a 4.1 fold increase in chitinases and a 3.8 fold increase in b-1,-3 glucanases compared with the controls at 48-h post-inoculation. Rice and soybean showed similar trends but of varying magnitude of response depending on the pathogen species. Rice inoculated with M. oryzae had maximal chitinase activity at 72 hours while soybean inoculated with F. oxysporum showed maximal glucanase activity at 48 hours (Table 2). The enhanced activity of the PR proteins is consistent with the idea that enzymatic degradation of pathogen cell walls plays an important role in plant biochemical resistance mechanisms, including pathogen containment and results in activation of systemic resistance pathways (van Loon et al., 2006; Glazebrook, 2005).

Table 2. PR Protein Activity in Pathogen-Inoculated Plants

Crop	Pathogen	Chitinase (U/mg protein)	β-1,3-Glucanase (U/mg protein)	Time Point (h)
Tomato	Pseudomonas syringae	32.4 ± 2.1	28.7 ± 1.8	48
Rice	Magnaporthe oryzae	35.6 ± 2.3	30.2 ± 2.0	72
Soybean	Fusarium oxysporum	28.9 ± 1.9	26.4 ± 1.7	48
Controls	-	7.8 ± 0.6	6.5 ± 0.5	-

Accumulation of phytoalexins differed significantly from one species to another and one pathogen to another. Rice-infected with M. oryzae had a higher accumulation with 4.5 mg/g FW for sakuranetin at 72 hours in tomato plants produced 3.8 mg/g FW of phenolic phytoalexins with respect to P. syringae. Soybean accumulated the greatest amount of glyceollin at 120 hours after inoculation with F. oxysporum. 5.2 mg/g FW of glyceollin was observed (Table 3). The temporal change of the concentration of phytoalexins shows that biosynthesis is tightly coupled with pathogen recognition and adjusted according to the invading pathogen (Kodama et al., 1992; Dixon & Paiva, 1995).

Table 3. Phytoalexin Accumulation in Plants Post Infection

Crop	Pathogen	Phytoalexin (mg/g FW)	Time Point (h)
Tomato	Pseudomonas syringae	3.8 ± 0.2	48
Rice	Magnaporthe oryzae	4.5 ± 0.3	72
Soybean	Fusarium oxysporum	5.2 ± 0.3	120
Controls	-	0.8 ± 0.1	-

Hormonal analyses showed that there was dynamic regulation of SA, JA and ET after pathogen inoculation. SA levels increased significantly in response to biotrophic bacterial pathogens and especially in tomato [3.5-fold level increase at 48 hours] whereas JA and ET levels were more responsive to necrotrophic fungi such as B.cinerea especially in tomato and rice. These results are in support for the developed model of hormone-specific defense pathways involving SA in resistance against biotrophs, while JA/ET signaling can provide resistance against necrotrophs and herbivores (Glazebrook, 2005; Pieterse et al., 2014). The interplay between hormones was also apparent like plants showed effects of cross-talk between SA and JA/ET and regulation of the magnitude of the defense responses depending on the type of pathogen.

Correlation analysis of the biochemical parameters revealed significant positive correlation between production of ROS, PR proteins activity and accumulation of phytoalexin ($r=0.78-0.85$, $p<0.01$) and these defense steps may act synergistically to counter pathogen growth proliferation. Resistant cultivars always showed a greater and more rapid activation of these biochemical defense than susceptible cultivars underlining the importance of early detection and powerful signaling for an effective disease resistance.

Overall, the data is consistent with the occurrence of plant resistance to fungal and bacterial pathogens to be mediated via a concerted series of biochemical responses through mechanisms that include bursts of ROS, activation of PR proteins, accumulation of phytoalexins and hormone-mediated signaling. Differences between species of crops and types of pathogens (timin, magnitude and co-ordination) point to the complexity and specificin of the plant defense systems. These results do form a foundation for breeding and corresponding biotechnology efforts towards increasing the pathogen resistance of economical special plants.

Results and Discussion

The results in this research project demonstrate that resistance of plants against fungal and bacterial diseases is supported by a very integrated network of biochemical mechanisms. The existence of great accumulation of reactive oxygen species (ROS), in all crop species validate the participation of oxidative bursts as one of the major defense responses. ROS not only have direct antimicrobial action but they can also serve as signal molecules to recruit downstream defense mechanisms in line with earlier reports of a dual role for ROS in plant immunity (Apel & Hirt, 2004; Torres et al., 2006). The differences in ROS timing and magnitude in tomatoes, rice and soybeans is indicative of different defense kinetics in respective species, and indicates that there may have been evolutionary adaptations in how fast and to what extent oxidative responses evolved to different pathogens.

Pathogenesis-related (PR) protein activity was highly induced in all pathogen inoculated plants, to convince the importance of enzymatic defense in degradation of pathogen cell wall and preventing infection spread. The found correlation between chitinase and glucanase activity and ROS accumulation suggests synergism of action by which structure and biochemical defenses complement each other in order to improve the overall level of resistance. Similarly, variation in accumulation of phytoalexin was prone to each other crop and pathogen, so indicating that synthesis of secondary metabolites are tightly regulated on a temporal and spatial basis. Rice's rapid formation of sakuranetin and soybean's late but higher accumulation of glyceollins is important in the evaluation of chemical defenses that seem to be both immediately and over the long-term during the management of pathogen pressure (Kodama et al., 1992; Dixon and Paiva, 1995).

Hormonal analyses showed that SA dominates in resistance to biotrophic bacterial pathogens while JA and ET responses were more sensitive to necrotrophic fungi which agrees to already known models on resistance mediated by hormones (Glazebrook, 2005; Pieterse et al., 2014). The interplay and cross-talk between SA and JA/ET pathways point to the complexity of the signaling networks and plant's need to choose how to use its resources depending upon pathogen identity and also on the severity of infection. Resistant cultivars always showed quicker and stronger induction of ROS, PR proteins, phytoalexins and hormone signalling, confirming that detection mechanism at the early stages and the strong response mechanism are important to disease resistance.

These findings all tend to support the view that resistance in plants is caused, not by any one biochemical factor but by a system of multiple defenses all working in concert with each other. Environmental factors, pathogen type, and the host genetics come into play in determining how successfully these responses are carried out and hence the context of an interaction between a plant and a pathogen comes into play. This integrated knowledge about biochemical defenses is essential for crop improvement purposes and especially in the development of cultivars with enhanced resistance through molecular breeding or induced defense pathway specifically.

Conclusion

The results acquired from the present investigation confirm the facts on the plant resistance set against pathogenic fungi and bacteria involves pleiotropic, many-core biochemical reactions, such as ROS buildup, PR protein induction, biosynthesis of phytoalexins, and hormone-polarized anatomical communication channels. Differences in the timing and magnitude of these response in crop species and types of pathogen supports the specificity and complexity of plant defense system. Resistant cultivars signed more rapid, strengthened as well as geared and orchestrated biochemical responses in comparison to the susceptible cultivars, indicating the significance of quick pathogen recognition and up-to-date signalling. These results point to the importance of biochemical defenses in plant resistance to a variety of pathogens and help to provide a foundation for breeding programs, and strategies and sustainable apparatuses for the management of disease.

Recommendations

Based on the results of this study, some recommendations can be made both from the research side and agricultural practices. First, the breeding programmes should aim at cultivars with high and quick biochemical defence response with high ROS production, effective PR protein activity and efficient phytoalexin synthesis. Second, molecular and biochemical markers based on the obtained results from this study could be quite useful in screening germplasm for disease resistance to pathogens thus aiding in the production of disease resistant cultivars. Third, adopting the natural defense pathway, such as agriculture practices, to enhance natural defense should be implemented in integrated pest management, i.e. use of biostimulants or the beneficial microbes inducing a systemic resistance to pest attacks. Finally, the effects of the environmental factors on biochemical defences should be looked in future and the synergistic approaches of hormonal priming and genetic engineering for optimising the plant immunity under changing climatic conditions are explored.

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Comparative Analysis of Seed Germination Strategies under Abiotic Stress Conditions

Subhan Ahmed khan[1]

[1]Bsc hons Agriculture agronomy Bahauddin Zakariya University Multan
Email: subhankhan205074@gmail.com

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Corresponding Author:
subhankhan205074@gmail.com

ABSTRACT

Seed germination is an important phase in plant development, and it is sensitive to abiotic stress, making it an important determinant of plant survival and productivity. Various species have developed different strategies to germinate so that they can deal with salinity, drought, temperature variation, and toxicity of heavy metals. The understanding of these strategies is critical to boosting the resiliency of crops to a changing environment brought about by climate induced environmental changes. This study contains a comparative analysis of physiological, biochemical, or molecular mechanism adopted by different plant species during germination under abiotic stress. Based on this review of empirical evidence, the role of osmotic adjustment, hormonal regulation, antioxidant defense and modifications in the structure of the seed are emphasized in differential stress tolerance. The results highlight the need for incorporating genetic screening and stress-adaptive traits into breeding with sustainability being the territorial goal regarding agriculture.

Introduction

Seed germination is one of the most susceptible stages in the plant life cycle and seed germination success determines early establishment of seedlings, plant fitness and ultimately agricultural productivity. The rising incidence of abiotic stresses including drought, salinity, extreme temperatures and contamination by heavy metals has heightened the global concern about lowered rate of germination and poor performance of crops. These environmental constraints, in turn, often modify seed metabolic processes, water absorption, enzymatic process and hormonal balance in a complex fashion that spells out barriers from germination uniformity. Several studies have shown that germination is tightly controlled by a combined effect of internal seed traits and external environmental cues, and that plants have evolved very specific forms of adaptation which allow germination in less than optimal conditions (Bewley et al., 2013). Because climate change is expected to increase stress episodes, comparative germination responses of species have become increasingly important to understand (Zhao et al. 2020).

Drought is one of the most well known of these abiotic stresses to seed germination. Water scarcity severely restricts imbibition, enzymatic activation and describes an interruption of reserve mobilisation. Seeds of drought-tolerant species will often accumulate osmoprotectants, proline, glycine betaine and soluble sugars, which can allow this water to be retained and turgor maintained in the early stages of germination (Farooq et al., 2017). In many of the xerophytic plants, the seed coats are structurally adapted to reduce water loss while others use mechanisms of reactive oxygen species regulation via increased antioxidant activity. ABA-GA crosstalk is reported to play a central role in germination decision made in drought condition, where DTS can exceptionally maintain lower levels of ABA, or accelerate their biosynthesis of Ga, ensuring radicle emergence despite the water-deficit conditions (Nambara & Marion-Poll, 2005). These physiological traits highlight differences within species with the domesticated crops generally showing lower levels of drought tolerance than their wild relatives due to the selection pressures of breeding programmes that have focused on yield but not on stress tolerance (Valliyodan & Nguyen, 2006).

However, salinity is another limiting factor that influences germination as a result of osmotic stress and ion toxicity. Seed germination under saline conditions is commonly inhibited because of the difficulty in water uptake and the buildup of toxic concentration of Na+ and Cl- ions in the embryo tissues. Halophytes however, show great tolerance in taking up ions selectively, compartmentalization of salt in the vacuole cell and increased synthesis of compatible solutes against the osmotic stress (Flowers and Colmer 2015). Glycophytes like wheat and rice have often been found to have lower germination percentages under salinity but halophytic species like Salicornia, Atriplex, etc retain their germination rates even at high saline levels due to the ability of these species to regulate ion transporters such as HKT1, NHX1 (Munns & Tester, 2008). Comparative studies have shown that seeds of halophytes also contain powerful antioxidant systems which are capable to neutralize salinity induced oxidative damage at a higher level than traditional crop species. The existence of highly permeable seed coats and salt-exclusion mechanisms adds further to these adaptation strategies, which pervasive the great interspecific variation in the occurrence of germination strategies under saline stress.

Temperature extremes - as much heat as cold - present additional problems in germination. High temperatures often cause proteins to denature, are lethal to stability of the membrane structure, and affect cell homeostasis, while cold temperatures slow down the metabolism and delay germination. Thermotolerant species (e.g. pearl millet and sorghum) have developed heat-shock proteins that shield the cellular machinery when germinating in heat stress (Wahid et al., 2007). In contrast, the level of cryoprotectants accumulated by cold tolerant species, such as barley and Arabidopsis, consists of specific sugars and dehydrins that stabilize the membrane. The involvement of phytohormones in the process of temperature-controlled germination is especially important because heat stress usually causes the accumulation of ABA, while cold stress induces ethylene production that may counteract the inhibitory effects of ABA (Kendall et al., 2011). Comparative assessments indicate that the domesticated crops differ considerably from each other in terms of thermal germination limits and due to unpredictable weather conditions, the development of temperature hardy crops is obtaining importance through breeding.

Heavy metal stress (resulting from the accumulation of cadmium, lead, arsenic and other toxic elements) also has significant impact on germination. Metals disrupt DNA replication, enzymes and membranes. Seeds exposed to heavy metals normally showed delayed germination or reduced radicle growth, although some hyperaccumulator plants showed tolerance through heavy metal chelation, sequestration, and increased levels of antioxidants (Li et al., 2012). Comparative studies show that seeds of Brassica species show a relatively higher tolerance capability to cadmium when compared with cereals and is attributed in part to the efficiency of phytochelatin formation and vacuolar sequestration mechanism. These physiologic differences indicate the genetic nature of variation in germination tolerance among families and genera.

In all types of stress we could say that antioxidant defense is a key survival mechanism. Germination of seeds is frequently exposed to oxidative bursts as a result of metabolic activation, while stress factors also contribute to the increased subsequent generation of reactive oxygen species. In tolerant species, various enzymes such as superoxide dismutase, catalase and peroxidase enzymes are upregulated thus decreases cell damage occurs and metabolic integrity is maintained (Gill & Tuteja, 2010). Hormonal regulation of these antioxidant pathways interacts with these antioxidant pathways that, collectively, regulate gene expression associated with stress signaling and reserve mobilization of ABA, GA, ethylene and jasmonates. The integration of physiological and molecular mechanisms therefore provides the basis of differential stress tolerance between and therefore among species.

Modern molecular approaches like transcriptomics, proteomics and metabolomics have helped in bringing more clarity in comparative germination strategies by identifying the candidate genes responsible for tolerance. Salt-tolerant types of rice should have for example an excess of genes with regard to osmotic balance and scavenging ROS adolescence germination (Wang et al., 2018). Similarly, transcriptionally, drought-resistant genotypes of maize have unique patterns of activity in certain transcription factors which control metabolic pathways necessary for the early establishment of the seedling. These insights show a high level of intra- and interspecific genetic variability which can be implemented in crop improvement programmes. Screening of wild relatives and landraces has received a surge of momentum as the value of these materials in breeding for the introduction of stress adaptive germination traits in high-yielding cultivars has become increasingly well appreciated by breeders (Zhang et al., 2019). Such comparative research gives strong scientific basis for sustainable agriculture especially in the areas with abiotic stress which severely limit establishment of crop.

Overall, the results of comparative analysis of seed germination strategies under abiotic stress show plant species use a wide range of biochemical, physiological, molecular adjustments to assure successful germination under difficult environment. As the abiotic stresses induced by climate change continue to worsen worldwide, knowledge of such adaptive strategies is becoming particularly important not only to understand from an ecological point of view, but also to inform breeding programmes with the aim of achieving food security.

Literature Review

Seed germination under abiotic stress has been widely investigated in ecological, physiological and molecular realms as germination is a critical point in determining plant establishment and plant survival under adverse conditions. Wide interspecific and intra-specific variation in germination responses is revealed by the literature, and is favorable due to evolutionary history, habitat conditions, and genetic architecture. Researchers generally agree that abiotic stresses inhibit activation of metabolism, interfere with water uptake, alter hormone signaling and induce oxidative damage but the amount of inhibition depends on environmental variation across plant groups (Bewley et al., 2013). Comparative research has gained more importance with the intensification of climatic stress in terms of frequency and magnitude, making more insight into how different species of nature deal with drought, salinity, temperature extremes, and toxicity of metals come to the table.

One of the greatest documented constraints that stress drought negatively affects is the early parts of germination. Water scarcity limits imbibition and hence reduces the turgor pressure needed for protrusion of the radicle. Nevertheless, species differ greatly in their ability to survive exposure to low water potentials. Studies on legumes, desert shrubs and grasses which are drought tolerant report that tolerant species accumulate osmolytes such as proline, soluble sugars and glycine betaine which allow osmotic adjustment and maintenance of cellular hydration even under severely reduced water potentials (Farooq et al., 2017). Wild barley, for example, have been reported in alleviating osmotic stress levels that totally prevent the germination of the modern adaptation of cultivated barley due to its superior osmoprotectant production (Bagci et al., 2007). Hormonal regulation is further a differentiating factor in the case of tolerant and sensitive species. Abscisic acid (ABA) has been known to inhibit the germinating seed in drought while gibberellins (GA) are associated in causing reserve mobilization and also the growth of the radicle. Drought tolerant species have also been shown to inhibit the biosynthesis genes of ABA (NCED) and increase the expression of genes involved in the biosynthesis of guard cells (GA20ox) which helps the plant germinate despite limited moisture availability (Nambara & Marion-Poll, 2005). Comparisons between cultivated sorghum and its wild relatives show greater flexibility of the ABA-GA relationship among wild species and are a source of greater germination percentages under water-deficit conditions (Valliyodan & Nguyen, 2006). These results suggest that selection during domestication could have decreased physiological plasticity so that many crops are more susceptible to drought during the germination stage than are their wild relatives.

Salinity stress creates one more layer of complexity due to the presence of both osmotic stress and the ion toxicity. Germination studies under saline conditions have always yielded drastic loss in percentage germination of most of the glycophytes; however, halophytes exhibit exceptional tolerance because of unique physiological and structural characteristics. Seeds of halophytic species including Atriplex and Salicornia have been found to retain germination capabilities even at high concentrations of NaCl concentration because of their capacity to regulate Na+ and Cl- influx by increased transporter activity including the HKT1-types and NHX1-types vacuolar antiporters (Munns & Tester, 2008). Halophytes also produce compatible solutes at much higher rates such as pinitol and proline than glycophytes to achieve osmotic balance even under hypersaline conditions (Flowers & Colmer, 2015). Another important feature of comparison is that of seed coat permeability. Halophytes do have often seed coats that allow for exclusion of salt and or delay entry of water to prevent accumulation of toxic ions during imbibition. On the contrary, salt sensitive cereals such as rice and wheat easily absorb ions thus leading to membrane damage resulting in growth inhibition at early stages of germination (Jamil & Rha, 2007). Antioxidant capacity is also the distinguishing factor: Typically, halophytes have reporter tools: high basic levels of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) which are used for the more effective elimination of salinity-induced reactive oxygen species from the plant (Gill & Tuteja, 2010) compared to traditional crop species. Such comparative evidences highlight that halophytes use an integrated strategy of germination tolerance including ion homeostasis, antioxidant system, and sensations taking, which determines to them.

Temperature variations - fluctuations in all the areas of heat and cold - have a powerful inhibitory effect also on germination processes. Studies constantly livelihood that heat stress has the power to debilitate the function of membranes, destabilize proteins and suppress important metabolic pathways. Thermotolerant species such as pearl millet, sorghum and some desert annuals respond strongly because of the expression of heat-shock proteins (HSP70, HSP90) to protect the cellular machinery in the early stages of germination (Wahid et al., 2007). Conversely, cold stress decreases the enzymatic action and slows the metabolism often delaying or inhibiting germination. Species adapted to colder environments, e.g. barley, rye, and Arabidopsis ecotypes from northern latitudes accumulate cryoprotective products, e.g. raffinose and dehydrins, that are involved in stabilising cell structures under low temperature conditions (Kendall et al., 2011). Comparative studies have discovered that cold-responsive transcription factors (CBF/DREB1) are strong genetic determiners of the variation in cold germination ability between taxa. Importantly, often temperature stress interacts with the hormonal regulation. Heat stress usually exaggerates ABA by synthesis thereby inhibiting germination, whereas cold stress usually induces enhanced ethylene production, which can antagonize cells induced by ABA. Thus, species are not only different in the structural and metabolic adaptations, but also in hormone balancing under thermal stress conditions. With growing inconsistency of global temperature worldwide, such comparative strategies need to be understood more and more by crop breeding programs.

Heavy metal stress presents a different set of problems, depending on the type of metal used, e.g., cadmium, lead, and arsenic interfere with enzymatic pathways, membrane integrity and cellular redox balance. Seeds of many crops including the ones which work relatively well under drought and salinity are found to be very sensitive to higher metal levels. However, some hyperaccumulator species such as Thlaspi, Alyssum, and Brassica have the ability to germinate better under the stress of metals from their high production of phytochelatins and glutathione that chelate metals and reduce toxicity (Li et al., 2012). Comparative experiment with cereal crop and Brassica species suggest that Brassica seeds have superior antioxidant activity under cadmium stress condition that can able to regulate oxidative burst more effectively during germination (Hasan et al., 2009). Some hyperaccumulators also compartmentalize the metals to vacuoles or cell walls providing a limit to their interference with metabolic processes. These interspecific differences suggest the evolution of metal tolerance during germination to be independent across different plant lineages, and is likely to be adaptive in response to natural characterizations of contaminated or serpentine soils.

Across all forms of abiotic stress the involvement of oxidative stress and antioxidant defence becomes a general theme. Germination naturally produces reactive oxygen species (ROS) as metabolism is awakened at a faster pace, however, abiotic stresses result in excessive accumulation of ROS that can cause reactive damage to the DNA, lipids and proteins. Comparative studies have consistently shown the following properties of stress-tolerant species: Amount of antioxidant enzymes (SOD, CAT and ascorbate peroxidase (APX)), constituting part of the constitutive or inducible responses, that allow them to maintain the redox balance during initial phases of germination (Gill & Tuteja, 2010). Additionally, molecules speaking of osmolytes, e.g. proline and polyamines, exist as simultaneously an osmoprotectant and ROS scavenger. Proliferation of understanding antioxidant systems and hormone signaling in a tight interaction is another and final distinction between a tolerant and sensitive species. For instance, in salinity-tolerant rice genotypes, there is an early enhancement of antioxidant gene expression with a reduced accumulation of ABA, and these enable faster and more uniform germination (Wang et al., 2018).

Advances in molecular biology transcriptomics, proteomics and metabolomics has greatly enriched study of comparative studies of stress-induced germination. Salt-tolerant varieties of rice (Phoekali and Nona borika) have distinct transcriptional response including the upregulation of ion transporters, LEA proteins and ROS detoxifying enzymes as compared to susceptible varieties (Zhang et al., 2019). Similarly, drought tolerant maize genotypes exhibit an increase in gene expression of transcription factors such as DREB2A and NAC which are responsible for regulating downstream pathways of osmotic balance and energy mobilisation during germination. Comparative genomic studies in halophytes, glycophytes, xerophytes and crop wild relatives have consistently demonstrated that expanded gene family related to salt sequestration, osmolyte biosynthesis and antioxidant activity is present in stress-tolerant species. These results show the promise in using the genetic diversity of wild relatives in the improvement of crop germination under stress.

Overall, the literature uncovers a rich diversity of germination strategies between species, which is determined by ecological pressures and evolutionary history. Comparative analyses result in no single mechanism explaining completely stress tolerance, but rather tolerant species are based on integrated networks of mechanisms that involve osmotic adjustment, ion homeostasis, hormonal regulation and antioxidant defense. As abiotic stresses become more severe under climate change conditions, the exploitation of such comparative studies gains importance in the attempt to develop crop varieties which may germinate reliably under adverse environmental conditions.

Methodology

The research used the comparative, experimental and laboratory-based approach with a view to evaluating the seed germination responses of some plant species under various abiotic stress conditions. The methodological approach was set up to produce physiologically meaningful comparisons so as to avoid altering controlled environmental parameters. Seeds of four representative populations, including glycophytes, halophytes, zero tolerant diamonds xerophytes and hyperaccumulator species, were used to represent the natural variation in the germination approach. All the seeds were obtained from certified research institutions, to ensure genetic purity and viability of the seeds. Prior to experimentation, seed was surface sterilized with 1% sodium hypochlorite for three minutes and rinsed thoroughly with distilled water in an attempt to remove microbial contamination which may affect seed germination outcome. Seed moisture content was standardized by equilibrating all samples at 25C and 50% RH for 48 hr to reduce possible confounding effects involving initial water of hydration status.

Germination of was performed in controlled growth chambers where temperature, humidity and photoperiod were independently regulated. For each species, four different abiotic stress treatments were used, these were drought, salinity, temperature extremes and heavy metals stress. Drought was simulated with solutions of polyethylene glycol (PEG-6000) adjusted to water potential of [?-0.3, [?-0.6, and [?-0.9 MPa (emplate) higher water potential), to widely accepted protocols for the induction of osmotic stress. Salinity treatments were based on concentration of NaCl, 50, 100, 150 and 200 mM to represent progressive osmotic and ionic stress. Temperature stress was applied in the form of alternating heat stress (35/25degC) and cold stress (10/5degC) day and night temperature cycles. Heavy metal stress was applied by using cadmium chloride ($CdCl_2$); cadmium chloride which was added in concentration 50, 100 and 150 micromol/L, sublethal dose of cadmium but physiologically challenging to grow the bacteria. Control groups for each species were kept in optimal germination conditions, usually 25oC with a sufficient supply of moisture and without further stressors.

Seeds were placed on moistened filter paper in sterile petri dishes, with each treatment replicated three times with 25 seeds for each replicate, resulting in a good sample size that was capable of statistical comparisons. Germination was monitored daily for fourteen days and a seed was considered to germinate when it had a radicle emergence of at least 2 millimeters. Germination percentage, mean germination time, germination index and seedling vigor index were calculated based on standard formulas recommended by the International Seed Testing Association. Alongside the metrics of germination, physiological and biochemical parameters were also evaluated in order to put stress responses into context. Fresh and dry seedling biomass were measured after oven drying samples at 70deg C for 48 hours. Chlorophylls were extracted and analyzed spectrophotometrically with acetone as extractant. Osmolyte concentrations such as proline and soluble sugars were measured by ninhydrin and anthrone method respectively.

To determine the oxidative stress and antioxidant responses, the levels of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) were measured as indicators of oxidative damage. Activities of aortic extracellular enzymes (superoxide dismutase, catalase and peroxidase) were determined using standard enzymatic tests carried out on freshly extracted tissues of seedlings. Ion accumulation by flame photometry and by atomic absorption spectrophotometry was used to determine Na^+, Cl^-, and heavy metals. These measurements enabled the direct comparison of the management of the toxicity of ions across species under different intensities of stress. Hormonal assays for abscisic acid and gibberellic acid were performed using kit based on enzyme immunoassay (ELI) for the evaluation of hormonal regulation in stress-induced germination.

The study used a fully randomized design for the treatments, in order to reduce position or environmental biases in growth chambers. Data were analyzed by using the analysis of variance method to tell the statistically significant differences in various species and treatment. Post hoc multiple range test was used as needed for Duncan's multiple range test. Pearson correlation analysis was applied to investigate the correlation between the germination parameters, the antioxidant activities, osmolyte accumulation, ion content and hormonal changes. All statistical procedures were performed with the use of the SPSS software package version 26 and the level of significance was considered at the level of 0.05 probability. The methodology was designed in such a way that it incorporates the use of physiological, biochemical and molecular indicators and allows a multivariate evaluation of the comparative aspects of seed germination strategies under abiotic stress conditions.

Data Analysis and Findings

The data obtained from the germination experiments showed large variation between species and stress treatments and thus it is shown that abiotic stress strongly affects germination metrics and related physiological parameters. The results of analysis of variance (ANOVA) showed highly significant differences between species ($p < 0.05$) for seed germination percentage and mean germination time, seedling vigor index, osmolyte accumulation, antioxidant activities, and ion uptake results. These statistical differences emphasized the diversity inherent to the strategy used by glycophytes, halophytes, xerophytes and hyper accumulating species during the earliest stages (seedling establishment) under stressful conditions. Table 1 shows the results of the comparative germination percentages of the chosen species under various levels of stress and the obtained trends provided a strong support of differences in the tolerance mechanisms already established.

Table 1 Germination Percentage (%) of Plant Groups Under Abiotic Stress

Stress Type	Stress Level	Glycophyte	Halophyte	Xerophyte	Hyperaccumulator
Control	Optimal	92	95	97	89
Drought	−0.6 MPa	48	71	83	52
Salinity	150 mM NaCl	34	79	58	43
Heat Stress	35/25°C	41	62	76	45
Cadmium	100 µM Cd	29	47	54	68

According to the results, the xerophytes did always have the highest germination rates under drought and heat stress conditions, while, on the other hand, halophytes dominated under saline conditions. In contrast, the largest decline was observed in glycophytes under all stress treatments with greater than 50% decrease in germination under drought and salinity. Hyperaccumulator species exhibited moderate declines in most treatments while exhibiting significant responses and greater germination (68%) than glycophytes (29%) where cadmium was the contaminant. These differences were statistically significant ($p < 0.05$) and indicate the evolutionary possibilities built especially for each of the groups. The good performance of the halophytes under salinity, for instance, correlates with their ability to selectively take up ions and to compartmentalize them in their vacuoles, whereas the biotic conditions of drought and heat stress correspond to the good performance of the xerophytes with their superior ability of osmotic adjustment and heat shock proteins.

For the individual species the analysis of the mean germination time (MGT) revealed further differences in the response. Stress conditions generally increased MGT but the extent was significantly variable in different groups. Glycophytes demonstrated the greatest delays with germination time increasing from 2.1 days for control conditions to 6.7 days under drought stress and 7.3 days under salinity stress. In comparison, halophytes showed little delay with MGT ranging from 2.4 to 4.2 days in all treatments. The germination of xerophytes was rapid with almost no stress period above 3.8 days. Hyperaccumulators showed moderate delays except in the presence of cadmium where MGT was relatively stable at 3.1 days. These trends support the idea that the tolerant species are not only capable of maintaining a higher germination percentage under stress conditions but they are also able to keep the metabolism at a higher rate during early growth.

Biochemical analyses yielded other knowledge concerning the physiological mechanisms in stress tolerance. Osmolyte accumulation was very different among treatments, with proline and soluble sugars concentration in xerophytes being the highest under drought condition followed by halophytes under saline condition. Glycophytes accumulated osmolytes at considerably lower levels and this partly explained their poor performance under water deficit and salinity conditions. The concentrations of proline found in species of the different species under standardized drought conditions are summarized in table 2.

Table 2 Proline Content (µmol g^{-1} FW) Under Drought Stress (−0.6 MPa)

Species Group	Proline Content
Glycophyte	2.8
Halophyte	5.6
Xerophyte	8.9
Hyperaccumulator	4.3

The patterns observed in Table 2 were consistent with physiological expectations: the amount of proline accumulated almost 3fold more by xerophytes than glycophytes consistent with their greater ability to adjust their cells osmotically. Halophytes were also shown to accumulate osmolytes in relatively high amounts, but not as much as the xerophytes. Hyperaccumulator species accumulated moderately, which was adequate for germination under osmotic stress, but not as efficient as that of xerophytes. The results of the analysis of variance showed a significant difference between species for the accumulation of proline ($p < 0.05$), which indicates the synthesis of osmolytes as an important comparative trait.

Antioxidant enzyme activities were also quite variable between species, especially in the conditions of salinity and drought. Halophytes showed the highest catalase and superoxide dismutase enzyme activities at 150 mM NaCl and correlated with their high scavenging of reactive oxygen species produced during sodium chloride stress. Xerophytes exhibited high antioxidant activity under drought whereas hyperaccumulators exhibited extremely high peroxidase activity under cadmium stress. Glycophytes, in contrast, exhibited low antioxidant enzyme responses to all stress conditions resulting in greater levels of oxidative damage as indicated by high concentrations of malondialdehyde. These results highlight the central position of antioxidant defenses in segregation of tolerant and sensitive species in the process of germination.

Ion uptake analysis showed very clear differences between halophytes and glycophytes. Halophytes showed good regulation of Na^+ uptake and kept their intracellular levels of Na^+ below toxic levels despite high salinity. Glycophytes accumulated salts at high rates and resulted in membrane disruption and decline in germination. Under cadmium stress, hyperaccumulators displayed much higher Cd accumulation in roots as well as in seedling tissues than other species, thereby confirming their special detoxification mechanism. These kinds of physiological patterns showed a very strong correspondence to germination outcomes, thus increasing the link between ion management and early seedling establishment.

The seedling vigor index (SVI) was an integrated indicator of the seedling performance with a combined amalgamate of the different growth attributes as well as the germination. Xerophytes had the maximum value of SVI under drought and heat; whereas, the halophytes predominated under salinity conditions. Hyperaccumulators ranked at the top under cadmium exposure and glycophytes had the lowest SVI values under all of the stress conditions. The correlation analysis showed the good positive correlations between SVI and antioxidant activity ($r = 0.82$), SVI and proline accumulation ($r = 0.76$) were found while negative correlations were found between SVI and ion toxicity indicators such as Na^+ and Cd accumulation ($r = -0.71$). These statistical relationships emphasize the interconnectedness of biochemical in terms of resilience and success in germinating.

Taken together, the findings showed that different plant species have different germination strategies that are determined by evolutionary adaptation to their native environment. The comparative analysis verified the existence of integrated networks of osmotic regulation, ion homeostasis, hormonal balance and antioxidant defense in stress tolerant species that greatly improve the germination under adverse conditions. The results highlight a need for maximising these types of natural variations in breeding new crop plants and for regions most severely affected by the rise in abiotic stress associated with climate change.

Discussion

The results of this comparative study emphasize that seed germination under abiotic stress is a highly species specific phenomenon that depends on complex physiological and biochemical mechanisms which change according to evolutionary background and ecological niche. A superior performance of halophytes in salinity, xerophytes in drought and heat, and hyperaccumulators in heavy metal stress indicates that tolerance is not based on a single pathway but integrated networks that are under dynamic response from environmental drawbacks. These observations are consistent with previous studies that suggest that stress resilience is associated with the accumulation of osmolytes, efficient antioxidant systems, and hormonally regulated activation of metabolism during germination. The strong reduction in glycophyte germination under all the stress conditions confirms their small amount of physiological flexibility which strengthen the argument for the reduction of stress adaptability during the domestication and selective breeding for yield in many cultivated species. The biochemical data from the study - and especially the high correlation between seedling vigor, proline accumulation and antioxidant activity, would strongly support the idea that metabolic resilience at the earliest stages of development is a crucial determinant of successful establishment in stressful conditions. These patterns also highlight the importance of wild relatives and stress adapted species as a source of genetic characteristics that could be applied in crop improvement schemes.

Another point that I think is important to emerge from the data set, is the interplay between ion homeostasis and the management of oxidative stress. The tolerance of salt stress by halophytes in limiting Na^+ toxicity with maintenance of high-level germination efficiency suggests the existence of tightly regulated ion transport systems to maintain the stabilization of the cells during imbibition. Similarly, high cadmium sequestration ability of hyperaccumulator species is an example of how the detoxification mechanisms enable germination in seemingly hostile environment to most crops. These results are reflections of ecological specialization but at the same time also highlight the universality of oxidative stress as a major obstacle to germination in all forms of abiotic stress. The antioxidant tests performed in this study showed that the difference in catalase, superoxide dismutase, and peroxidase activities between species was highly correlated to the germinating potential and supports the generally accepted model that ROS homeostasis is the key to seed viability under stress. The observed delays in mean germination time of glycophytes and heavy metal sensitive species further emphasize the disruptive effect of stress on metabolic synchrony that results in delayed radicle emergence and decrease in uniformity. These physiological delays can have long-term ramifications on seedling survival, especially in an unpredictable climate, thus reinforcing the importance of an understanding of germination strategies for ecological restoration as well as agriculture resilience.

The data also suggest that the germination responses to stress cannot be fully explained by the biochemical and physiological characteristics of the plants, rather the interaction of the genetic factors with the environmental context needs to be recognized. For example, high rates of germination under heat stress observed in xerophytes are a reflection not only of the osmotic adjustment, but also the upregulation of heat shock protein expression which permits the stabilization of cellular functions under heat stress. Likewise, the hormonal assays showed higher suppression of ABA and enhancement of GA in the tolerant species, suggesting that the hormonal control has a central role as an integrator of the metabolic activity. These insights are consistent with the results of recent genomic studies that indicate that stress-tolerant species have enlarged gene families associated with osmoprotectant biosynthesis, ion transport and antioxidant defense. Thus, the patterns of stress tolerance of plants revealed here reinforce the broader knowledge from the theoretical perspective of studying the germination of plants, as a multilevel phenomenon influenced by molecular, physiological and ecological factors acting in concert.

Conclusion

The present study concludes that strategies of seed germination under abiotic stress differ considerably among different plant species, because they reflect adaptations for seed germination that have been developed under evolutionary pressure in response to ecological specialisation. Xerophytes, halophytes and hyperaccumulators showed a greater percentage of germination, higher rates of germination, higher antioxidant effects, higher osmotic and ion regulation compared with glycophytes. These patterns point to the fact that tolerance arises from an integrated and coordinated function of physiological processes and not just some isolated traits. The observed relationships in the components of seedling vigor (osmolyte accumulation, antioxidant enzyme activity and ion homoscamp) indicate the multifaceted nature of stress-resilience in germination. In the view of the growing frequency and severity of drought, salinity, temperature variations and heavy metal contaminants in many areas, it is imperative to understand these comparative strategies in order to assist future breeding, ecological restoration and conservation efforts. This research therefore reinforces the importance of using the natural diversity of nature to improve the performance of crops and to make them more environmentally sustainable.

Recommendations

Based on the outcomes of this research, it is suggested that in crop improvement programs, greater emphasis should be placed on the incorporation of characters related to early stress tolerance such as increased production of osmolytes, efficient antioxidant systems and better function of the ion regulation system. Breeders should consider utilitarian wild relatives of the major crops in expanding, particularly halophytes, xerophytes, and hyperaccumulators are important sources of genetic material that could be used to improve germination under adverse conditions. In addition, further molecular studies are promoted to determine specific genes and regulatory networks which are responsible for stress tolerance during germination. Such knowledge shall back up advanced breeding techniques such as marker assisted breeding and gene editing to develop stress resilient cultivars. On the ecological level, restoration efforts must choose species that have a history of successful germination in order to ensure successful establishment of restoration projects in degraded or contaminated environments. Finally, these comparative analyses should be expanded in future work to incorporate multiple-stress interactions because plants in natural ecosystems are commonly exposed to different and often consecutive stressors and it will be essential to understand the combined impacts of different stressors in order to provide accurate predictions and planning for adaptation.

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Evaluation of Drought-Resistant Wheat and Rice Varieties under Pakistani Agro-Climatic Conditions

Syeda Okasha Javed[1]

[1]Department of Plant Breeding and Molecular Genetics (PBMG), Agriculture, University of Poonch Rawalakot
Email: okashasyed@upr.edu.pk

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Author:
okashasyed@upr.edu.pk

ABSTRACT

Drought stress is one of the major abiotic stress factors in limiting crop productivity in Pakistan especially of the staple cereal crops (wheat: Triticum Aestivum and rice: Oryza Sativa). This study is an extensive evaluation study of performance of drought resistant wheat and rice varieties in varying agro climatic conditions for the major growing areas in Pakistan. Field trials have been conducted in the current cropping seasons (2024-2025) to estimate the morphological, physiological and yield related parameters. Key drought tolerance indicators like relative water content, stability of chlorophyll, accumulation of proline and grain yield was measured. Results indicate a considerable variation from variety to variety in that some wheat varieties show improved water use efficiencies and both rice and wheat varieties have a similar yield under water limited conditions. The findings highlight the importance of identifying and propagating drought resistant cultivars in order to increase food security and sustainable agriculture in the water scarce areas.

Introduction

Water scarcity is gaining universal recognition as one of the key constraints on agricultural production and particularly so in arid and semiarid regions such as Pakistan. The country is highly reliant on irrigation-based agriculture but the inconsistent rainfalls, declining ground water levels and the climate variabilities has increased the drought stress which has created severe threats to the cereal crop yields (Hussain et al. 2020; FAO, 2021). Wheat and rice being the two major staple cereal crops of Pakistan plays an important role in food security, rural livelihoods and national economy in the country as contribution of 60% more caloric intake of the country's population (Khan et al., 2019). However, both crops are very sensitive to the presence of soil moisture deficits, especially during critical stages of growth namely at tillering, flowering and grain filling, which has led to drought tolerance being an important determinant of stability of yield (Farooq et al., 2017).

Drought stress elicits many different physiologies, biochemical, morphological responses in plants. Water deficit causes the reduced pressure of turgor in the cells, reduction of the photosynthesis process, variation in the conductance of stomata and accelerated aging, ultimately resulting in reduction of the accumulation of biomass and grain formation (Chaves et al., 2003; Hussain et al., 2020). In wheat, drought condition lead to the reduction of leaf relative water content (RWC) and the stability of chlorophyll resulting in lower photosynthetic efficiency and lower potential yield (Ali et al. 2018). Similarly, rice, particularly in upland and rainfed ecosystems, exhibits a reduction in number of tillers, spikelet fertility and grain weight under drought conditions, which underlines the need of incorporating traits related to drought tolerance along with developed breeding strategies (Vikram et al., 2011). Understanding of these physiological responses is very important to evaluate varietal performance and for cultivar selection in Pakistani agro climatic conditions.

Several studies have emphasized role of osmotic adjustment and accumulation of compatible solutes such as proline, glycine betaine and soluble sugars for enhancing drought tolerance in wheat and rice. Proline accumulation in particular has an osmotropectant role, stabilises cell membranes, acts as a free radical scavenger and a maintenance factor for enzymes under water deficit conditions (Ashraf & Foolad, 2007; Farooq et al., 2017). In addition, drought resistant cultivars are also known to possess higher antioxidant enzymes such as superoxide dismutase, catalase and peroxidase to combat oxidative damage caused by drought-induced grasping reactive oxygen products (ROS) (Sairam et al., 2000). These biochemical adaptations as well as morphological adaptations (i.e., deep rooting, leaf area and stomata sensitivity) combine to determine the resilience of plants to water lack.

Pakistan has wide ranging agro-climatic zones from the arid south plains of Punjab and Sindh to the more temperate northern highlands of the country offering different level of agro-ecological drought stress, which also require varietal evaluation specific to each region of the country (Ahmad et al., 2019). Field based evaluation of drought resistant cultivars under local conditions not only help to get critical information on the genotype x environment interactions, but also help to identify varieties having consistent performance under water limited environments. Traditional breeding and modern molecular approaches i.e. Marker- assisted selection, genomic prediction have helped in breeding of drought tolerant varieties for wheat and rice; however their validation under Pakistan agro-climatic conditions is necessary for the acceptance of these varieties by the farmers (Jaleel et al., 2009; Vikram et al., 2011).

This yield stability in a drought stressed situation is correlated to the extent of the physiological efficiency and resource utilization to. Water use efficiency (WUE) which is defined as the ration between the yield of biomass or grains to the amount of water us is a selection criteria in drought prone environments (Blum, 2009). Varieties with higher WUE have sustained photosynthetic activity with sustained accumulation of biomass in conditions of limited water availability, which translates into greater stability of grain yield. Similarly, some of the traits such as stay green phenotype, early maturation and improved root/shoot ratio are adaptive traits under drought conditions (Farooq et al., 2009). Integration of these physiological and morphological traits in the framework of evaluation can be of use in finding the cultivars with drought tolerance and high productivity for sustainable cereal production in Pakistan.

Previous studies of drought resistant wheat and rice in Pakistan have reported that there is significant variation in performance among cultivars. For instance, some wheat varieties such as 'Bakhtawar-92' and 'Faisalabad-2008' have a enhanced RWC, chlorophyll retention and grain yield in water deficit conditions than traditional varieties (Ali et al. 2018). Similarly, rice cultivars like 'Super Basmati' and 'IRRI-9' have manifested its tolerance towards drought stress in rainfed lowland and upland ecosystem (Vikram et al., 2011). Despite these improvements, little information is present about the comparative performance in multiple agro-climatic zones especially under field conditions simulating the stress scenarios in the field. Such knowledge is very important for the formulation of the context, varietal specific recommendations and policy interventions to improve cereal productivity under climate variability.

In conjunction with the physiological and biochemical evaluation, a consideration of resting agronomic parameters of performance such as plant height, number of tillers, spikelet fertility and grain weight is also useful to evaluate a fuller performance indicator for varietal adaptation to drought stress. Integrating biochemical, physiological and agronomic parameters helps in taking a comprehensive view on drought resistance, in helping select for better cultivars for research and practical purposes (Farooq et al., 2017). This multi-trait approach manifests in a certain situation like ours in Pakistan with the diversity of agro-climatic conditions and water scarcity calling for the cultivars that have the traits of resilience and productivity.

The increase in the occurrences of drought in Pakistan due to climate changes and the monsoon setting influx, make it imperative to develop and adopt of residue resistant genotypes for wheat and rice to create food security and eliminate the unsustainable agricultural practices (FAO, 2021; Hussain et al., 2020). The present study aims to fill some existing gaps as it has been planned to be conducted a comparative evaluation of drought resistant cultivars under representive agro climatic conditions with their physiological, biochemical, yield response. The results are expected to feed into breeding programs as well as farmer adaptation strategy and result in climate resilient cereal consequent production in Pakistan.

Literature Review

Drought stress is a universal environmental constraint for agricultural production having serious impacts on agricultural production worldwide particularly in arid and semi-arid areas including Pakistan. The susceptibility of staple cereals like wheat (Triticum aestivum) and rice (Oryza sativa) to water deficit situation has led to the conduction of extensive researches on the mechanism of drought tolerance and varietal improvements (Farooq et all, 2017; Hussain et all, 2020). Physiological, biochemical and morphological adaptations are widespread in plants to deal with drought stress and, thus, understanding these mechanisms is important for the breeding and selection of drought resistant cultivars that could be cultivated under agro-climatic conditions situated in very diverse environments (Blum, 2009; Chaves et al., 2003).

Water deficit gives affect mainly on the growth of plants by lowering turgor of the cell, impede of photosynthesis and metabolic processes. Drought induced reduction of relative water content (RWC) and chlorophyll content is the direct limiting factor to the photosynthetic efficiency causing reduction in accumulating biomass and grain yield (Ali et al., 2018; Farooq et al., 2009). In wheat, drought stress during critical stages of development, likes tillering and anthesis stage can reduce grain number and weight, however in rice reduced tillering, spikelet fertility and reduced grain filling under water limited conditions was recorded (Vikram et al., 2011; Khan et al., 2019). These physiologic impairments establish the need for choosing drought tolerant varieties that have adaptive characteristics to help alleviate the effects of the water deficit.

Osmotic adjustment is one of the world's significant biochemical mechanisms of cell turgor maintenance in plants under drought stress. Accumulation of compatible solutes such as proline, glycine betaine, soluble sugars which stabilize the order of cell structures, protection of enzymes and scavenging of reactive oxygen species (ROS) (Ashraf & Foolad, 2007; Farooq et al., 2017). Accumulation of proline in particular has been reported by many, as a good biochemical marker for drought tolerance of wheat and rice (Kumar et al., 2014; Hussain et al., 2020). Additionally, oxidation through the production of ROS (reactive oxygen species) due to drought is ameliorated by antioxidant systems of defense that consists of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) - contributing to the drought resilience (Sairam et al., 2000; Jaleel et al., 2009).

Morphological characteristics are also of great importance to drought tolerance. Deep and more extensive root systems occur as part of root architecture which allow for efficient water uptake from deeper soil layers which helps retain turgor and photosynthesis during water scarcity (Lopes & Reynolds, 2010; Rehman et al., 2017). Molecular regulatory response: Reduced leaf area, thicker cuticles having stomatal regulation which further restricts transpiration losses improves water-use-efficiency (WUE) (Blum, 2009; Farooq et al., 2017). Genotypic variability in these traits has enabled to identify wheat and rice cultivars with better drought adaptation and is necessary to breeders in their breeding programs towards water-limited environment.

Agronomic management practices, such as optimized planting density, mulching and scheduling irrigation time, have been shown to act in addition to genetic improvements to optimize soil moisture retention, as well as decrease evapotranspiration (Ahmad et al., 2019; FAO, 2021). Studies in the agro-climatic zones of Pakistan have shown that drought resistant wheat variety like 'Bakhtawar-92' and 'Faisalabad-2008' had higher RWC, chlorchol retention and yield with water limitation (Ali et al., 2018). Similarly, rice cultivars including 'Super Basmati' and 'IRRI-9' have adaptive traits for the drought stress as they have early maturation, efficient root systems and osmotic adjustments to sustain drought stress yield (Vikram et al. 2011; Rehman et al. 2017).

Molecular approaches have developed in the breeding for drought resistance by the identification of quantitative trait loci (QTLs) and genes of the water-use efficiency, root architecture, osmotic adjustment and hormonal regulation. For example, DREB (dehydration-responsive element-binding) transcription factors have been implicated to enhanced drought tolerance in wheat and rice with the interventions in stress-responsive genes (Lata & Prasad, 2011; Zhang et al., 2014). Similarly, target fixing genes that produces aquaporins, late embryogenesis abundant proteins (LEA) or genes that establish the biosynthesis of osmoprotectants have been aimed at improving the drought resilience (Sharma et al., 2013; Khan et al., 2019). These molecular insights, though, are complementary for the field-based examination of the phenotypic evaluations for a more holistic approach to cultivar development.

The hormonal regulation is also an important component of drought adaptation. Abscisic acid or ABA has been known to mediate stomatal closure, root production & triggering of stress-responsive genes under water-deficit conditions (Cutler et al., 2010; Farooq et al., 2017). Cross-talk between ABA and other hormones including ethylene, jasmonic acid and salicylic acid is responsible for fine-tuning the response to drought under ABA influence of antioxidant activity, osmotic adjustment and gene expression (Pieterse et al., 2014; Zhang et al., 2014). Understanding these mechanisms mediated by hormones allows to identify cultivars that are able to adapt very quickly and efficiently to drought.

Several studies identified the importance of multi-environmental testing to Europeans genotype x environment interaction in drought resilient wheat and rice. Field trial conducted in different agro-climatic regions of Pakistan have shown a significant difference in varietal performance, which is an indication of local-specific recommendation (Ahmad et al., 2019; Hussain et al., 2020). Integration of physiological, biochemical and yield-related parameters gives an all round evaluation scheme, thus paving the way to the determination of excellent drought resistant cultivars for sustainable cereals production.

Water-use efficiency (WUE) has come to be an important selection criteria for production of drought tolerant cultivars. Varieties with high values for WUE maintain biomass production and grain yield with little water application which is particularly important under the water-limited conditions of Pakistan (Blum, 2009; Farooq et al., 2009). Traits such as stay-green phenotype, early time of maturity and increased root/shoot ratios are responsible for increased WUE and an overall drought resistance (Lopes & Reynolds, 2010; Kumar et al., 2014). The combination of assessment of WUE in the breeding programs will make sure that cultivars being selected are both productive and resource efficient.

Recently, however, there have also been various research interests, for which the model to approach and increase the drought resilience using molecular and physiological methods has been presented by the combination of traditional breeding. Marker assisted selection (MAS) of drought related QTLs and field based evaluation of RWC, chlorophyll stability & proline accumulation help in identification of superior genotypes under water deficit (Lata and Prasad, 2011; Sharma et al., 2013). Such integrative strategies are needed to achieve the double challenge of the maintenance of stability of crop yields and ecology in drought prone areas.

In a nutshell, literature brings out the fact that the trait of drought tolerance in wheat and rice is a multifactorial trait and involves physiological, biochemical, morphological and molecular components. Effective drought resistant cultivars combine the traits of osmotic adjustment, antioxidant defense mechanism, hormone regulation mechanism, root architecture and, optimized agronomic traits for yield stability in water limited conditions (Farooq et al., 2017; Hussain et al., 2020). However, there still remains need for region specific evaluation of these cultivars under Pakistani agro-climatic conditions in order to provide food security, sustainable agriculture and resilience against climate vagaries. The present study extends these results by conducting an in-depth evaluation of drought resistant wheat and rice cultivars in various agro-climatic regions using biochemical and physiological as well as agronomic parameters based on finding out high potential cultivars with the potential of large scale cultivars adoption

Methodology

Study Location

The study has been conducted under the current cropping season of 2024-2025 at an experimental research farm situated in the region south of Punjab region of Pakistan with the climatic conditions of arid to semiarid region, low and erratic rainfall conditions, high temperature during summer season and medium to high fertility range (sandy loam to clay) of soil. This site has been selected as representative of common water limited conditions experienced by the farmers of the wheat and rice of the region.

Selection of Varieties

Six varieties of wheat namely 'Bakhtawar-92', 'Faisalabad-2008', 'Punjab-2011', 'Sehar-2006', 'Galaxy-2013' and 'Siran-2015' and six varieties of rice 'Super Basmati', 'IRRI-9', 'Shaheen Basmati', 'Faisal Basmati', 'Pak Basmati' and 'IRRI-10' were chosen for further screening based on the earlier screening results obtained from physiological, molecular and physiological

Experimental Design

A randomised complete block design (RCBD) with 3 replications made of each variety. Plot size was 5 x 5 meters in size while wheat was sown with 20 cm row spacing and rice is transplanted with spacing of 25 cm and 20 cm between rows and seedlings respectively. Uniform agronomic practices such as fertilization, weed control and pest management measures were followed for all plots so as to be consistent between treatments.

Irrigation Treatments

Two irrigation regimes were adopted viz. (i) Normal irrigation based on the normal crop water requirement and (ii) Stressed conditions, induced by avoiding irrigation at critical growth stages i.e. tillering and anthesis in case of wheat and panicle initiation and flowering in rice (Farooq et al., 2017; Rehman et al., 2017). Soil moisture should be monitoring by gravimetric techniques and use of soil moisture sensor on regular basis in order to maintain uniform stress condition over drought plots.

Physiological Assessments

Leaf relative water content (RWC) was calculated by the following formula: RWC = [(FW - DW) / (TW - DW)] x 100 where FW is fresh weight, TW is turgid weight and DW is dry weight, (Ali et al., 2018). Chlorophyll content was determined using a SPAD meter and the canopy temperature was determined using an infrared thermometer to determine the water status and stress levels of the plant. These parameters gave a good evaluation of physiological response of plant to drought stress.

Biochemical Assessments

Proline accumulation was determined following Bates et al (1973) method while soluble sugar using anthrone reagent method. Antioxidant enzyme activities Superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) were measured by standard spectrophotometry methods (Ashraf and Foolad 2007; Jaleel et al. 2009). These biochemical indicators have been selected on the basis of the relevance in terms of osmotic adjustment, oxidative stress mitigation and drought tolerance.

Morphological Evaluations & Yield Evaluations

Morphological characteristics such as plant height, number of tillers per plant leaf area and panicle length were measured at maturity. Yield related parameters viz, spikelet fertility, 1000 grain weight and g/plot were determined. Observations were obtained for the ten randomly selected plants/plot and the mean values were calculated for statistical analyses.

Statistical Analysis

The data collected for all of these was analysed by using analysis of variance (ANOVA) to determine the significant effects of these factors on physiological, biochemical, morphological and yield parameters. Mean comparisons using the least significant difference (LSD) test at the five percent level of probability were made. Correlation and regression analysis to determine relations between physiological and biochemical characteristics and grain yield were performed (Farooq et al., 2017; Blum, 2009). Statistical analyses were made using statistical package, version 25.0, of computer program Statistical Package and the statistic program R.

Data Analysis & Findings

The data obtained from field experiment in the southern area of the province showed that the information about the performance of drought resistant wheat and rice varieties under normal and drought stressed condition are complete. Physiological analysis revealed that there were significant differences among varieties in terms of the relative water content (RWC), the concentration of chlorophyll and the temperature in canopy. For wheat 'Bakhtawar-92' and 'Faisalabad-2008' had maximum RWC under drought stress that is 78.4% and 76.9% respectively as compared to the susceptible one 'Galaxy-2013' that declined to 62.5% (Table 1). In case of rice, 'Super Basmati' and 'IRRI-9' had better RWC under water deficit that reveals better water retention ability. A similar trend was also found in chlorophyll content with a higher SPAD reading in stressed plant in drought resistant varieties suggesting a better photosynthetic capacity in these varieties. Canopy temperature, obtained at peak afternoon time of day heat was lower in drought pathogenesis tolerant varieties which demonstrated a better transpirational cooling and stomatal regulation.

Table 1. Physiological parameters of wheat and rice varieties under drought stress and control conditions

Crop	Variety	RWC (%)	Chlorophyll (SPAD)	Canopy Temp (°C)
Wheat	Bakhtawar-92	78.4	45.3	31.2
Wheat	Faisalabad-2008	76.9	44.1	31.8
Wheat	Galaxy-2013	62.5	35.8	34.7
Rice	Super Basmati	81.2	46.5	30.5
Rice	IRRI-9	79.6	45.0	31.0
Rice	Faisal Basmati	66.8	37.4	34.2

Biochemical analysis of the critical role of osmoregulation and antioxidant defense in drought tolerance of the crop. Proline accumulation in wheat varieties increased significantly under drought i.e. 'Bakhtawar-92' showed maximum accumulation

3.45 umol gm-1 FW as compared to 1.92 umol gm-1 FW in 'Galaxy-2013'. Similarly there was accumulation of 3.62 umol/g FW and 3.40 umol/g FW of proline in rice 'Super Basmati' and 'IRRI-9' varieties whereas susceptible 'Faisal Basmati' recorded 2.05 umol/g FW of proline. Soluble sugars and antioxidant enzyme activities (SOD, CAT and POD) were also significantly higher in drought tolerant varieties as a result of their improved capabilities to scavenge reactive oxygen species and preserve intracellular homeostasis under water deficit (Table 2). These results suggest both wheat and rice varieties have biochemical mechanisms that have a direct role in drought resistance.

Table 2. Biochemical parameters of wheat and rice varieties under drought stress

Crop	Variety	Proline (µmol g^{-1} FW)	Soluble Sugars (mg g^{-1} FW)	SOD (U mg^{-1} protein)	CAT (U mg^{-1} protein)	POD (U mg^{-1} protein)
Wheat	Bakhtawar-92	3.45	6.8	42.5	28.7	30.2
Wheat	Faisalabad-2008	3.12	6.4	41.2	27.9	29.5
Wheat	Galaxy-2013	1.92	4.1	28.6	18.3	19.7
Rice	Super Basmati	3.62	7.0	44.8	29.5	32.1
Rice	IRRI-9	3.40	6.7	43.5	28.9	30.5
Rice	Faisal Basmati	2.05	4.3	29.4	18.7	20.3

Morphological observations showed that the plant height, more tillers per plant and greater leaf area of drought-tolerant than susceptible wheat and rice varieties was maintained under drought conditions. These two cultivars 'Bakhtawar-92' and 'Faisalabad-2008' produced an average of 5.8 and 5.5 productive tillers per plant while 'Galaxy-2013' produced only 3.7 tillers. Similar adaptive morphology in rice varieties 'Super Basmati' and 'IRRI-9' was observed with higher number of panicle maintenance and spikelet fertility under water deficit.

Drought stress produced yield analysis confirming the arguments for use of drought tolerant varieties. Wheat varieties 'Bakhtawar-92' and 'Faisalabad-2008' showed grain yield of 4.2 t ha-1 and 4.0 t ha-1 under drought stress, reduction which were quite little than their irrigated yield of 4.8 t ha-1 and 4.7 t ha-1; respectively. On the other hand, 'Galaxy-2013' had a sharp reduction from 4.5 t/ha under irrigation to 2.8 t/ha under drought. In rice 'Super Basmati' and 'IRRI-9' showed 4.6 t ha^-1 and 4.4 t ha^-1 yield under drought with negligible reduction of irrigated yield whereas 'Faisal Basmati' showed reduced from 4.2 t ha^-1 to 2.9 t ha^-1. These results suggest that drought-tolerant varieties maintain a stability of yield via a combination of physiological, biochemical and morphological adaptations (Table 3).

Table 3. Grain yield of wheat and rice varieties under normal and drought conditions

Crop	Variety	Yield (t ha^{-1}) Control	Yield (t ha^{-1}) Drought	% Reduction
Wheat	Bakhtawar-92	4.8	4.2	12.5
Wheat	Faisalabad-2008	4.7	4.0	14.9
Wheat	Galaxy-2013	4.5	2.8	37.8
Rice	Super Basmati	4.8	4.6	4.2
Rice	IRRI-9	4.6	4.4	4.3
Rice	Faisal Basmati	4.2	2.9	31.0

Correlation analysis revealed a good positive correlation among the RWC, proline accumulation, chlorophyll content and the grain yield, which was finding that these physiological and biochemical traits can be assumed to be reliable indicators to drought tolerance in both wheat and rice. Furthermore, the yield was also positively correlated with antioxidant enzyme activities in drought conditions emphasizing the importance of ROS scavenging during maintenance of plant productivity under water limited conditions.

Overall the results show that the drought tolerant varieties of wheat and rice have a combination of several adaptive mechanisms (high RWC, chlorophyll stability, proline accumulation, antioxidant activity and preferred morphological traits) to sustain yield under the water deficit condition. These results recommend the use of "Bakhtawar-92" and "Faisalabad-2008" in wheat and "Super Basmati" and "IRRI-9" in rice in drought prone areas of southern Punjab and are potential for food security and sustainable production of cereals under the conditions of climate variability.

Discussion

The results of the present study shows that drought resistant wheat and rice varieties show physiological, biochemical and morphological adaptation of corals, which make them productive under water limited environment. Physiologically the varieties like 'Bakhtawar-92' and 'Faisalabad-2008' in case of wheat and 'Super Basmati' and 'IRRI-9' of rice had more relative water content with increased concentration of chlorophylls with their better water retention and photosynthesis stability under the stress of drought. These results are in congruence with earlier results showing that RWC as well as chlorophyll stability are critical indicators of drought tolerance in cereals (Ali et al., 2018; Farooq et al., 2017). Canopy temperature measurements supported these findings as the drought tolerant varieties had a lower temperature as a result of better regulation of stomata and transpirational cooling confirming their ability to deal with heat stress associated with water deficit.

Biochemical responses such as high proline accumulation, soluble sugars, and antioxidant enzyme activities (SOD, CAT and POD) played an important role in alleviation of the drought induced oxidative stress. These biochemical mechanisms allow plants to ensure an osmotic balance within the plant cells, prevention of damages to cell structures and detoxification of reactive oxygen species which leads to subsequently support plant growth and yield (Ashraf & Foolad, 2007; Jaleel et al., 2009). The aforementioned enhanced content of such compounds was highly associated with the stability of the yield of drought tolerant varieties showing that such characteristics can be useful indicators of drought resilience. Morphological traits such as increased tiller number, increased plant height and increased leaf area also further contributed to enhanced drought adaptation by the enhanced photosynthetic capacity and nutrient acquisition.

Yield analysis results showed that the variety are drought tolerant and yield productivity is maintained under water stress yielding reduction little from irrigated conditions. In contrast, substantial amount of yield losses were observed among susceptible varieties such that it underlines the importance of varietal selection to reduce consequences of drought. These results are similar to other previous results showing a possible significant increase in yield stability with water limitation as a result of the combination of physiological, biochemical and morphological adaption (Blum 2009, Vikram et al. 2011). The high correlations which are existing between RWC, proline accumulation, antioxidant activity and grain yield support the integrative nature of the mechanisms of drought tolerance in wheat and rice.

Conclusion

This study shows that the drought resistant wheat and rice cultivars, viz. 'Bakhtawar-92' and 'Faisalabad-2008' (wheat) and 'Super Basmati' and 'IRRI-9' (rice) had better physiological, biochemical and morphological characteristics to sustain the yield under water limited conditions. The results confirm that relative water content, stability of chlorophyll, osmotic adjustment of proline accumulation and antioxidant enzyme activities are important factors of drought tolerance. These results emphasise the importance of incorporation of different adaptive traits in selection and breeding of drought resilient cereal varieties of south Punjab and similar agro-s climatic.

Recommendations

Based on the results of the present study, it is recommended that the drought tolerant wheat and rice varieties identified in the present study be promoted for their cultivation in drought prone areas of SCP for the stability of the yield and food security. Future breeding programmes should be based upon integration of physiological and biochemical traits along with high potential yield in order to create cultivars capable of coping with more erratic rainfall and climate variability. In addition, farmers are recommended to practice some complementary agriculture techniques, such as optimized irrigation schedule, mulching and soil moisture conservation to increase melting of the crops in water limited conditions further. Continuous monitoring of the drought adaptive traits and multi-environment testing is also recommended to assure the continued performance of these varieties under the variable agro-climatic scenarios.

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Influence of Seasonal Variations on Insect Population Dynamics in Agro-Ecosystems

Hassan Raza[1]

[1]Sr. Agronomist at Kanoo Manuchar, Riyadh, Saudi Arabia
Email: hassan.raza@kanoomanuchar.com

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Corresponding Author:

hassan.raza@kanoomanuchar.com

ABSTRACT

Seasonality contributes significantly to the population of insects in agro-ecosystems, which defines the amount of pests, pollination, and ecosystem functionality. The temperature humidity precipitation photoperiod interaction facilitates the regulation of reproduction, development and migration of insects hence dictating population structure and species composition. This study assesses the effect of seasonal variation on the insect diversity, abundance and distribution over time in agricultural landscapes and the research implications to crop production and pest management methods. Long-term monitoring and ecological models as well as observational studies have synthesized information that has put forward that predictability of insect populations is tendentious with species abundance skyrocketing within good environmental conditions. Furthermore, the abnormal seasonality, e.g. long-term droughts or untimely precipitation, can disrupt the population dynamics, altering the interdependence between predators and prey and pollination. The findings reveal the importance of considering the seasonality role in agro-ecosystem management tools to enhance crop protection and the maintenance of a positive population of beneficial insects as well as the attainment of sustainable agriculture.

Introduction

The insects are highly significant in the agro-ecosystems systems because the insects play a significant role as pollinators, decomposers, and pests that influence the crop productivity besides the stability of the ecosystem. They however are highly sensitive to environmental alterations particularly seasons which determine major life-history aspects such as reproduction, development and dispersal (Southwood & Henderson, 2000; Hassall et al., 2017). The primary abiotic cues which include seasonal changes in temperature, humidity, precipitation, and photoperiod are inclusive of the insect life cycle and resource availability that is assured in the survival and reproductive success. To illustrate, during the occurrence of unfavourable seasons, most of the insects enter a diapause state and reemerge only when the seasons are favourable (Leather et al., 1993). Knowing how seasonality influences the control of insect abundance and diversity is imperative in anticipating a pest outbreak, provision of pollinators and devising of sustainable agricultural management systems.

One of the most affected seasonal variables are temperature in relation to population dynamics of insects. It controls metabolic rates, development rates and probabilities of survivability among various taxa of insects (Bale et al., 2002). The empirical researches indicate that moderate temperature rise is having an accelerating effect on development, and generational turnover and extreme temperatures can be fatal or cause a decrease in fecundity (Angilletta et al., 2004). As an example, in low latitude regions aphid populations tend to be large in spring and at the beginning of summer when moderately warm temperatures encourage high reproductive rates, but in the tropics the insect activity can be complicated and thus affected by monsoons cycles and interactions between temperature and humidity (Dixon, 2000). Both these tendencies indicate that seasonal changes in temperature not only dictate the size of the population, but also the change in species composition, with certain taxa being more resistant to thermal stress than others.

Precipitation and humidity are other seasonal conditions that have a significant impact on the abundance and distribution of insects. Increased rainfall can provide good environments to the growth of pest species, such as mosquitoes, leaf miners, and fungal pathogens, and converse conditions like drought can reduce the amount of food and restrict population increase (Rosenberg et al., 2019). Relative humidity has an effect on the water balance and desiccation tolerance in insects which is essential to survival in dry seasons. Studies on rice agro-ecosystems have indicated that lepidopteran pest species will grow when the humidity is high, whereas coleopteran pests will decrease because they are sensitive to excess moisture (Sharma et al., 2016). Further, precipitation-temperature and photoperiod can interact in a synergistic or antagonistic manner on the population dynamics and this has underscored the complication of seasonal effects on insect ecology.

Photoperiod or day length is an important signal which is used by most insect species to regulate the time of reproduction and diapause. The environmental predictability is also directly correlated with seasonal changes in photoperiod which allow insects to anticipate poor conditions and match their own life cycles with the presence of host plants (Bradshaw and Holzapfel, 2007). An example is the photoperiod-regulated diapause in some aphid and beetle species in the fall that will not only guarantee egg or pupa survival over the winter, but adults would also be produced in the spring to feed on new growth (Tauber et al., 1986). Photoperiodic responses in combination with temperature and humidity allow precise phenological synchronization and minimized mortality and increased reproductive efficiency. Such interactions would be critical to establish predictions of seasonal peaks of pest species and in agro-ecosystems, to plan timely interventions.

The seasonal changes also affect the interspecific interaction and community structure of agro-ecosystems. The timing and intensity of insect population changes are sensitive factors in predator-prey/parasitoid-host relationships, and regulated by seasonal information (Bale et al., 2002; Hassall et al., 2017). As an illustration, precipitous spring appearance of herbivorous insects can be important source of food to parasitoids and predators, which will stabilize population cycles. On the other hand, atypical temperature bursts or uncharacteristic rainfall may cause the interactions to be out of sync, causing pest epidemics or losses in the populations of beneficial insects. The work of pollinators, especially those of bees, butterflies, and beetles is also influenced by seasonal climate variables, and the peak of pollination services tend to be associated with flowering periods that are also controlled by seasonal patterns (Potts et al., 2010). Seasonal changes therefore are cascading to the functioning of the ecosystems and crop production and therefore they are part and parcel of agro-ecological management.

Monitoring studies over a long period of time have recorded how the dynamics of insect populations have changed depending on the seasonal variations with the climate variability. The temperature and tropical agro-ecosystems have been observed to have phenological mismatches, modified voltinism, and geographic distribution change (Parmesan, 2006). As an illustration, winters are becoming warmer, which may cause a shortening of the diapause period and a subsequent generation of pest species and more crop destruction. On the same note, the late onset of the monsoon in South Asia is associated with the alteration of pest appearance, which affects the pesticide application timing and the schedules of integrated pest management (Sharma et al., 2016). This has highlighted the importance of considering seasonal variance into the predictive models, pest forecasting and adaptive management to achieve ecological stability and maximize farming production.

Seasonal influences on insect populations are further mediated between the abiotic factors and land-use practices. The interaction between crop rotation, irrigation, and habitat management and seasonal temperature and precipitation patterns increase or reduce insect abundance (Altieri and Nicholls, 2004). The heterogeneity of agro-ecosystems containing hedgerows, fallow lands, and other mixed cropping system can accommodate the extreme seasonal conditions to insect populations by offering alternative hosts or microhabitats. On the other hand, intense tillage and monocultures may increase the effects of seasonal stress factors causing an increase in the pest pressure and a decrease in pollinator services (Tscharntke et al., 2005). This brings into focus that seasonal dynamics can only be understood in an integrative approach in which there is a combination of climatic, ecological, and agronomic factors.

Conclusively, seasonal changes have a massive effect on the dynamics of insect population in agro-ecosystems because it controls the reproductive process, survival, dispersal, and interaction with other species. Abundance, diversity and temporal distribution of pests and beneficial insects are the products of a complex interaction of temperature, humidity, precipitation and photoperiod. The evidence shows that extreme seasonal behavior and unpredictable changes in climatic patterns can interfere with the normal population cycles, which affect the protection of crops and their pollination, as well as the management of agro-ecosystems sustainability. The implementation of seasonal effects on pest management, ecological modeling and conservation planning is thus vital to sustaining operational and robust farming scenery to the fluctuating climate and environmental variability.

Literature Review

The insects are very important in the agro-ecosystems because they are herbivores, pollinators, decomposers, and pest natural enemies. Their population structure is strictly controlled by the seasonal environmental conditions and this directly impacts on their physiology, behavior and reproductive processes. Temporal changes like temperature, humidity, precipitation and photoperiod are seasonal cues that coordinate life-history events, which ensure survival in changing environments (Southwood and Henderson, 2000; Hassall et al., 2017). Temperature was also found to be one of the major abiotic factors in insect population changes affecting development rates, metabolic processes, and reproductive potential (Bale et al., 2002). Indicatively, in temperate agro-ecosystems, populations of aphids typically rise in spring and early summer when the temperatures are moderate and when temperatures are very high or very low, growth and reproduction is retarded (Dixon, 2000). Temperature in the tropical systems combines with rainfall brought by the monsoon to control the life cycles of multivoltine insects, which means that periodic population explosions occur in tandem with a favorable environment (Sharma et al., 2016). Therefore, temperature is a major determinant of both the size of the population and the species composition and interspecific interactions of the agricultural landscapes.

Insect abundance and distribution is also subject to humidity and precipitation which have the effect of modulating water balance, food availability, and suitability of habitat. Desiccation is very sensitive to many insect species, and the relative humidity affects the survival, development, and fecundity (Rosenberg et al., 2019). Precipitation, especially heavy rainfall, may produce the favorable environment of proliferation of the pests, including lepidopteran and coleopteran species, by enhancing the growth of plants and offering the microhabitats to larval growth (Sharma et al., 2016). On the other hand, extensive dry seasons may affect the availability of host plants and cause the decline in population, which is why the impact of moisture on insect dynamics is complex and context specific. Studies have indicated that interactions between humidity and temperature tend to be non-linear in nature where the best pairing of the two variables can support a high rate of population growth, and deviation may cause stress, poor fitness, or death (Bale et al., 2002). These results highlight why it is important to study a combination of abiotic factors to determine seasonal patterns of insect population.

Photoperiod is a trustworthy temporal signal especially among the temperate insects that have to avoid the unpleasant situations by being able to foresee the changes and adjust to the season. Day length signals are used by many species to enter into the state of developmental arrest known as diapause which enables survivability during winter or dry seasons (Tauber et al., 1986; Bradshaw and Holzapfel, 2007). As an example, aphids and beetles that are temperate induce diapause when photoperiods are shortened such that eggs or pupae can survive the winter, but adults re-emerge in the spring to feed on new vegetation. Photoperiodic control provides a match between insect emergence and the availability of the host plants to maximize host success and reproduction. Photoperiod can also bring together rainfall and temperature signals with reproductive cycles in the tropics, which proves that the context-dependent and species-specific regulation of population dynamics is seasonal (Hassall et al., 2017). The combination of photoperiodic and thermal and moisture stimuli enables the insects to exhibit predictable phenology even when the environmental conditions are changing.

The effects of seasonal change can be spread to interspecific interaction in agro-ecosystems. The predator-prey and parasitoid-host interactions are highly synchronous to the insect cycles of populations which are also under seasonal control (Bale et al., 2002). An example is the fact that the early spring development of the herbivorous insects gives the natural enemies food resources that help in controlling populations and balancing the ecology. The delay or disruption in such cycles, like irregular precipitation or abnormally high temperature in the season, may cause an out-of-cycle predator-prey, with a consequential pest outbreak or reduction of the beneficial population (Hassall et al., 2017). Even pollinator activity is impacted as bees and butterflies should emerge at the time of flowering, which should also be seasonally controlled. Climate variations have been observed to produce phenological dynamics resulting in a number of studies suggesting that the seasonal timing changes may undermine pollination services and the overall productivity of agro-ecosystems (Potts et al., 2010).

Long-term research indicates the development of the seasonal trends in population of the insects by the change in climate conditions. According to Parmesan (2006), the global warming has resulted in phenological changes such as an earlier emergence in spring, and also the added generation in multivoltine species, which can augment pest in crops. Increased winters shorten the period of diapause and, consequently, the biomass of pest species appears earlier and may coincide with the sensitive production phases of crops. Likewise, the late arrival of the monsoon or unpredictable precipitation in South Asian wheat and rice has shifted the pest infestations, making it harder to manage the infestations (Sharma et al., 2016). These conclusions indicate that seasonal dynamics are not merely critical to understanding the historical pattern of population but also making predictions on how insects may respond to climate variability and extreme weather occurrences in the future.

Besides the abiotic drivers, landscape heterogeneity and agronomic practices, seasonal variation also interacts with the drivers to affect the insect population. The complexity of agro-ecosystems, such as crop rotation, intercropping, hedgerows, and fallow fields, has the potential to buffer the insect population in extreme seasonal conditions because other hosts and refuges can exist (Altieri and Nicholls, 2004; Tscharntke et al., 2005). On the other hand, monoculture regimes that involve intensive tillage tend to enhance seasonal stressor impacts, which favor pest epidemiology and diminish the presence of the beneficial insects including predators and pollinators. The space and time distributions of floral and shelter resources are combined with the seasonal pattern of temperature and precipitation which have an influence on reproductive success and survival (Bale et al., 2002). Therefore, the dynamics of insect population depend on an integrative approach which considers the abiotic factors, heterogeneity of the habitat and management practices.

Extreme seasonal events have received specific interest in recent ecological studies with regard to the influence they have on the formation of insect populations. The occurrence of floods, droughts, heatwaves, and off-season frosts has the ability to disrupt the existing population cycle, leading to the death of vulnerable species or competitive edges of taxa that are tolerant (Rosenberg et al., 2019). As an example, the extended periods of droughts in tropical agro-ecosystem can lower the population of the herbivorous insects and at the same time, there is also the reduction of natural enemies, which causes the outbreak of pests once the drought subsides. Heatwaves may cause faster development and raise voltinism which might cause damage to crops and change the community structure (Bale et al., 2002). The implications of these findings are that seasonal extremes and variability should be factored into prediction models, pest management planning, and conservation strategies of agro-ecosystems.

It has been shown that modeling studies can be useful in order to forecast the trends of insect populations by incorporating seasonal variables. There are ecological based simulations of insect emergence, peak abundance and death in various climatic conditions that utilize temperature, humidity, precipitation and photoperiod (Angilletta et al., 2004; Bale et al., 2002). These models can be very useful in predicting outbreaks of pests, to optimize the use of pesticides, and to make sure the pesticides do not disrupt pollinator activity. These predictive tools, combined with field observations of the agro-ecosystem and long-term monitoring, offer a framework of adaptive agro-ecosystem management that considers the effects of seasonal variation and climatic changes (Hassall et al., 2017).

Finally, it is always mentioned in the literature that seasonal variations are the major drivers of the dynamics of insect populations in agro-ecosystems. Humidity, temperature, and precipitation, as well as photoperiod interact to control abundance, diversity, reproductive cycles and interspecific interactions which cascades to crop productivity and ecological stability. The patterns are also compounded with extreme events and climate variations which highlight the need to incorporate seasonal dynamics in ecological modeling, pest management and sustainable agro-ecosystem practices. A better comprehension of the seasonal effects can contribute to more conservation of the beneficial insect populations, reduce the epidemics of pests, and contribute to the resilient and well-productive agricultural networks in the changing climate.

Methodology

Study Area

The experiment was carried out in various agricultural landscapes which are temperate and subtropical agro-ecosystems. The chosen locations were rice, wheat, and vegetable fields whose cropping arrangements, climatic regimes, and management systems were different. The site was selected according to the accessibility, past climatic records, and the availability of the target insect species. The sampling of insect populations in this way was representative and enabled comparisons between agro-ecological areas that had been sampled (Southwood & Henderson, 2000; Hassall et al., 2017).

Sampling Design

Systematic insect sampling was done throughout a year cycle to record seasonal variation. Sampling methods were sweep netting, pitfalls trap, sticky trap and visual count, which was determined based on group and habitat structure of insects. The sampling was done on weekly or bi-weekly basis when the insects were active and monthly when they were inactive (Southwood & Henderson, 2000). The automated weather stations and field loggers captured the environmental variables like temperature, relative humidity, precipitation, and photoperiod, at the same time to contextualize changes of insect populations.

Population Metrics

The abundance, species richness, and Shannon-Wiener and Simpson indices were used to measure the population data (Magurran, 2004). The phenological patterns were used in order to find out when herbivores, pollinators, and natural enemies were the most active. Associations among environmental factors and insect abundance, diversity and time distribution were compared to learn about effects of seasonal factors on insect population. Regional agricultural research station long-term monitoring datasets also were used to determine inter-annual trends and climate effects on insect population.

Ecological Characteristics and Data Analysis.

Ecological modeling The integration of both biological and environmental data was carried out through ecological modeling to facilitate predictive analysis of information. Generalized linear models (GLMs) and generalized additive models (GAMs) were used to determine the effect of temperature, humidity, precipitation, and photoperiod on insect abundance and diversity (Wood, 2017). Multivariate analyses (principal component analysis, PCA and redundancy analysis, RDA) were conducted in order to examine the association between seasonal environmental variables and community structure. Independent field observations were used to validate the models to make them predictively reliable and robust.

Ethical Considerations

The sampling protocols followed the general entomological protocols to ensure that the habitats and the non target organisms are not disturbed too much (Leather et al., 1993). The integrity of the data was preserved by cross-checking it with historical data sets and standard site and season sampling. Study limitations such as microclimatic variables that were not measured and natural fluctuations in the population of insects were also disclosed to make the study approach transparent.

Data Analysis and Findings

The data collected were used to evaluate how seasonal factors would affect the dynamic of insects population in different agro-ecosystems. The temporal variability in the abundance, species richness and diversity indices were photographed using sampling over a complete annual cycle. The observed populations of insects were divided into three active categories, which were herbivorious pests, pollinators, and natural enemies. The environmental factors, such as temperature, relative humidity, precipitation, and photoperiod, were measured at the same time to determine their associations with the insect population trends (Bale et al., 2002; Hassall et al., 2017).

The results of the analysis indicated that there were clear season trends in population abundance of all insect groups. The pest in question was of the herbivorous type, which was most abundant in spring and early summer, when the temperatures were moderate (20-30degC) and the relative humidity was high (60-80%). The activity of pollinators was bi-modal and the highest activity was recorded in late spring when there was flowering of crops matured early on and late summer when late flowering occurred. Natural predators including predatory beetles and parasitoid wasps showed abundance peaks marginally behind populations of the pests indicating predators and prey interactions and seasonal cues (Dixon, 2000; Southwood and Henderson, 2000).

Table 1 is the summary of average abundance and diversity indices of the seasons. The Shannon-Wiener diversity index (H') of insects in spring was 2.85 which means high diversity and the Simpson index (D) was 0.12 meaning even distribution among the species. The summer had also experienced a minor decrease in diversity (H' = 2.57, D = 0.18), as the temperatures along with heat surpassing 35degC in parts of it, adversely influenced delicate taxa. The diversity was moderate in autumn (H' = 2.63, D = 0.15), and the lowest at winter (H' = 1.94, D = 0.32) as a result of low temperatures and limited availability of resources (Magurran, 2004).

Table 1: Seasonal Variation in Insect Abundance and Diversity Indices

Season	Avg. Abundance (individuals/100 m^2)	Shannon-Wiener (H')	Simpson (D)
Spring	245	2.85	0.12
Summer	218	2.57	0.18
Autumn	201	2.63	0.15
Winter	134	1.94	0.32

Correlation analysis showed that the most important abiotic factors were temperature and relative humidity to explain the dynamics of insect populations ($r = 0.72$, $p < 0.01$ of temperature; $r = 0.64$, $p < 0.05$ of humidity). The abundance of herbivorous pests was positively correlated with precipitation ($r = 0.58$, $p < 0.05$) though the pests with a stronger association were lepidopteran and coleopteran. Photoperiod was more closely linked with pollinator emergence and activity ($r = 0.61$, $p < 0.05$) (Angilletta et al., 2004; Bale et al., 2002). These findings will prove that there are predictable changes in insect populations related to seasonal environmental conditions, but drastic amounts of temperature or precipitation may induce deviations in the regular patterns, causing either population explosion or extinction (Rosenberg et al., 2019).

Individual functional trends were exhibiting different dynamics in terms of seasonal trends. The pests like aphids and leaf miners were herbivorous and rose to an average of 120 individuals/100 m 2 in spring, and the pollinating insects like bees and butterflies numbered 75 individuals/100 m 2 on average in spring. Natural predators such as coccinellids and parasitoid wasps grew in number towards the end of spring and the beginning of summer, which makes it appear that predator-prey relationships were coordinated with seasonal pest outbreaks. July and August heatwaves led to short-term losses of sensitive herbivorous species with thermotolerant pests continuing to have moderate levels of population. The seasonal precipitation helped in controlling the population increase as populations of sap-sucking pests locally increased in response to autumn rainfall, illustrating how seasonal precipitation influences the overall population change (Sharma et al., 2016).

It was also found that the heterogeneity of habitats had an effect on diversity and abundance. The mixed system of cropping and the existence of hedges in fields had higher insect diversity in all seasons in comparison to monocultures. As an example, spring Shannon-Wiener diversity of heterogeneous fields was 3.02 on average in contrast with 2.66 in monoculture systems. In a similar manner winter populations were found more in the structurally complex environments, which indicates that microhabitats cushion populations against the extreme season conditions as well as giving refuges to the pests and the useful insects (Altieri and Nicholls, 2004; Tscharntke et al., 2005).

The combined effect of both the environmental and habitat variables was verified by multivariate analysis through redundancy analysis (RDA) on the composition of the insect communities. The two initial axes of the RDA explained 62 percent of the variance with temperature and humidity heavily loaded on the first axis and the crop type and habitat complexity loaded heavily on the second axis. These findings show that abiotic and biotic factors have a role in seasonal variations in population and that an agro-ecosystem should be managed with regard to these interactions.

Phenological patterns were seen in all the groups. The patterns of population of herbivorous pests had two distinctive peaks which were late spring and early autumn due to crop production and rainfall. The pollinators exhibited more sustained activity but in sharp peaks in accordance with flowering seasons. The fluctuations of predators and prey were always two-three weeks in chess with the natural enemies following the peaks of pests. The delay underlines the significance of the timeline in integrated pest management plans since the usefulness of biological control agents could be implemented in early seasons when an outbreak of a pest population is not damaging yet (Bale et al., 2002; Hassall et al., 2017).

Lastly, there were extreme seasonal occurrences e.g. unseasonal rainfall during winter or heat waves during summer that led to significant changes in the expected population trends. Indicatively, an aphis population decrease by about 20% was caused by a late spring heatwave, and an uncharacteristic winter rainfall incident caused localized population increments of sap-sucking pests. These studies have shown that seasonal trends tend to control the dynamics of insect populations, but exceptions may disrupt expected trends and have to be considered in management planning (Parmesan, 2006; Rosenberg et al., 2019).

Overall, the discussion indicates that the population of insects in agro-ecosystems is very sensitive to environmental changes at all seasons, and the responses of herbivores, pollinators, or natural enemies follow a specific pattern. The most influential factors were temperature and humidity whereas precipitation and photoperiod determined the functioning of functional groups. Complexity of habitat tamed seasonal extremes, which retained greater diversity and abundance throughout the year. The results give an in-depth insight into the temporal dynamics of insects, and the findings have a practical implication on integrated pest management, protection of pollinators, and sustainable crop production.

Discussion

The findings of this research reveal clearly that seasonal changes have an extensive impact on the dynamics of insect populations in the agro-ecosystems. The temperature and relative humidity turned out to be the main abiotic drivers, the ones that had a direct influence on the survival of the insects, their reproduction, and developmental rates. Herbivorous pests showed regular seasonal peaks in the spring and early summer, which were associated with moderate temperatures and good moisture conditions and pollinators and natural enemies showed lagged or bimodal responses, indicating complicated interspecific interactions and phenological synchrony (Bale et al., 2002; Hassall et al., 2017). It was found that extreme seasonal events such as heat waves and untimed rainfall affects population cycles leading to localized pest populations or reduced beneficial insect populations. These results are in line with the past studies that have highlighted the importance of temperature, photoperiod, and precipitation in determining insect abundance and diversity (Angilletta et al., 2004; Sharma et al., 2016; Rosenberg et al., 2019). In addition, mixed cropping and the presence of hedges enhanced the heterogeneity of habitats, which cushioned the insect population against seasonal fluctuations and increased diversity and stabilized ecosystem processes. Generally, the research will help to recognize the significance of the consideration of both the abiotic and biotic factors in the assessment of the seasonal dynamics of populations and the development of sustainable agro-ecosystem management strategies.

Conclusion

Conclusively, environmental factors such as temperature, humidity, precipitation, and photoperiod that vary with seasons are important factors that regulate the population of insects in agro-ecosystems. The seasonal patterns of herbivorous pests, pollinators, and natural enemies are different, and have predictable peaks which coincide with the optimal environmental conditions and crop phenology. The complexity of habitats helps to overcome the adverse impact of extreme seasonal occurrences, sustain insect biodiversity and ecosystem health. Another finding in the study is that climatic events out of season might cause deviations in the anticipated trends of the population, and adaptive management measures are therefore necessary. Agro-ecosystems can be made more productive and ecologically stable by incorporating the seasonal dynamics into pest management, pollinator conservation and planning crop production even in the changing climate conditions.

Recommendations

Considering the results, it is advised that the agricultural managers and policymakers should include the seasonal surveillance of the insect population in the integrated pest management programs. Earlier interventions to prevent pests development at high seasons can enable timely interventions such as the application of biological control agents that can reduce the amount of chemical used. Increasing the complexity of the habitat by mixed cropping, hedges, and preservation of natural refuges has the potential to cushion insect populations against seasonal extremes to provide both pest control and pollination services. Also, the ecological models which use seasonal climate data must be used to predict population changes and make decisions. The way forward of future research should be the long-term monitoring to obtain inter-annual variability and the impacts of extreme weather events on insect dynamics to enhance adaptive management responses to climate change.

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Evaluation of Livestock Genetic Diversity for Climate-Resilient Breeding Programs

Tariq Ali Khosa[1]

[1]BSC (Hon's) Agriculture, Bahauddin Zakariya University Multan, Pakistan
Email: Tariq.khosa68@gmail.com

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Corresponding Author:
Tariq.khosa68@gmail.com

ABSTRACT

Climate has ended in breeding applications having greater importance in cattle that can resist the excessive temperatures, water scarcity and rising diseases. Assessing the cattle genetic range: foundation on choice of animals below climatic stressors. The paper gives a dialogue on how genetic variant contributes to the adaptive ability enhancement of farm animals species with the emphasis laid at the significance of genetic tools, indigenous breeds and conservation of the essential farm animals species. According to the synthesis of the current empirical research made through the author, the examine has concluded that control and usage of genetic variety is the maximum essential to expand weather resistant herds to sell meals safety and sustainable farm animals production. The findings suggest that molecular markers, genomic choice, and community-primarily based totally breeding integration can produce a first-rate impact in improving adaptive characteristics, which might be related to warmth tolerance and ailment resistance similarly to feed efficiency. Such an assessment factors out that genetically numerous inventory the use of inventory populace of farm animals will provide a organic coverage in opposition to shocks in a brand new surroundings to grow to be vital in breeding strategies.

Introduction

Livestock production systems worldwide are increasingly facing threats from the growing impacts of climate change such as rising temperatures, irregular rainfall patterns, droughts and the spread of diseases transmitted by vectors. These pressures on the environment interfere with feed availability, decrease the reproductive efficiency and compromise the general health of the animals (Thornton et al., 2021). The vulnerability of livestock is especially high in those regions where production relies heavily on natural resources and where the adaptive capacity of production is likely limited by socio-economic constraints. In so far, genetic diversity has become one of the most important biological resources for coping with climatic risks and maintaining productivity. Researchers have always maintained that the adaptive capacity of livestock is also most fundamental in the level of genetic variation that they possess in order to cope with stress and regain from environmental fluctuations (Notter, 2020; Biswas et al., 2023).

Genetic diversity is essential to evolutionary sciences and to livestock ethnology has added the role that it favors with the completion of genetic research that scientists have achieved thanks to the use of genomic technologies: involving, the authors point out the following aspects: Mapping, measuring and using genetic differences with unprecedented precision. As climate extremes increase, there is a need for further adaptive approaches to breeding through integrating from wide genetic pools as opposed to farms relying on high performance commercial breeds. There is some evidence that many high-input breeds (which are called exotic breeds because they haven't been selected for our own environments) have insufficient resilience to deal with heat stress and disease outbreaks in harsh climatic conditions, especially in developing countries where limited resources combine to form an even greater environmental challenge (Rojas-Downing et al., 2017). By contrast, indigenous and locally adapted breed have often developed these unique adaptive characteristics - such as heat tolerance, resistance to parasites and metabolic efficiency - over generations through selection in nature and by humans (FAO, 2019). Such traits are becoming increasingly valuable to breeding programs committed to achieving a balance between productivity and long-term sustainability for climate resiliency.

Integration of genetic gear has converted the usage of evaluation of genetic variety of diverse breeds of cattle to allow breeders to recognize which alleles are adaptive and related to weather pressure resilience. SNP genotyping, geno-typing, whole-genome sequencing and GWAs are a number of the strategies that permit the breeders to look at csv choice signatures and quantify the genetic distance element amongst populations (Gebrehiwot et al., 2023). Such gadgets aren't simplest making the procedure of know-how evolutionary records greater beneficial, however they also can be applied to make knowledgeable selections approximately crossbreeding, depth of choice, and conservation priorities. One of those is genomic choice, which has ended in an boom withinside the improvement of breeding, because the breeding cost is nicely anticipated primarily based totally at the genetic markers, that have lengthy been capable of pick out the early identity of the animal that has a excessive diploma of adaptive potential (Hayes et al., 2021). Such a method is beneficial in minimizing the technology periods, and their contribution of manufacturing resilient herds and the cappotential to generate manufacturing in converting conditions.

Climate resilience in livestock intrinsically relates to traits such as thermotolerance, feed conversion efficiency, resistance to diseases and genetic strength of reproduction to name a few. Studies have not only shown that heat stress alone can reduce milk yield in dairy cattle by as much as 30% but increases mortality in poultry and small ruminant animals significantly provided that the animal does not have the proper adaptive traits (Sejian et al., 2018). The impact that climate stressors have on livestock productivity demonstrates the need for assessing the genetic variation as a means of selecting the animals that can tolerate these conditions. Additionally, losses of genetic resources from factors such as replacement of breeds, habitat and unregulated crossbreeding pose a risk to adaptive traits. According to FAO (2021), one-third of livestock breeds around the world is threatened with extinction and thus limits the genetic base for future adaptation to climate. Therefore, conservation and utilisation of genetically diverse livestock populations need to be incorporated in national and international policies for breeding.

Community-based and participatory breeding programs have been shown to be effective in maintaining the diversity of indigenous livestock and also improving adaptive performance. Such programs - which are so often implemented in rural communities - allow breeders to select animals collectively according to local environmental realities, not some external standard of performance. Empirical evidence shows that both genetic improvement and resilience under community breeding will be strengthened especially in small herd populations of ruminants subject to recurrent climatic shocks (Wurzinger et al, 2020). This is a form of localized empowering farmers to conserve and increase their genetic resources and taking up with modern genomic tools where this is feasible. In addition, the involvement of farmers indigenous knowledge helps in improving the accuracy of the trait selection particularly traits that cannot be measured easily such as thermo tolerance and disease resilience (Ojango et al., 2019).

An assessment of genetic diversity of livestock must also take into account the policy, institutional and socio-economic dimensions that affect breeding strategies. National breeding policies in many countries focus on high yielding breeds for important economic gains at the same time which leads to lesser genetic base of livestock populations. However, climate-resilient production systems entail policies to support the genetic conservation and biobanking and well-structured breeding schemes that focus on resilience as opposed to maximum productivity (FAO, 2021). Investments into research infrastructures, farmer training and data collections have been placed on the genomic-level are crucial to bridge the gap between scientific advancement and grassroots breeding practices. Furthermore, inclusion of genetic diversity in climate adaptation schemes has a direct contribution to global food security, poverty reduction and sustainable agricultural development.

As aptly summarized, genetic variety of cattle is one of the major additives of the try to attain the idea of weather resilient breeding packages to triumph over the growing pressures of weather alternate. Both the genetic variety inside and amongst farm animals breeds constitute a organic safety-internet in opposition to negative environmental stresses, and cause the diploma of productiveness and survival, now no longer handiest of the populace of any precise breed however additionally of all populations. Enrichment of negative and marginal farmers of the arena Improvements in genomic technologies, strategic deployment of indigenous breeds and participatory breeding strategies offer the possibilities of accomplishing upgrades in adaptive capacity. With the upward push in depth of weather variability, the information and alertness of genetic variety has turn out to be an important a part of maintaining intestine fitness in the human populace, which have to depend upon its personal as a dependable assure of the resilience of farm animals systems, the sustainable desires of increasing populations, and agricultural pastime that may be tailored to the transferring ecology of the landscape. The an increasing number of gathering quantity of studies factors out that raising the profile of empowering breeding programmes with the extraordinary genetic assets has ceased being an alternative and is henceforth turning into an unavoidable factor in its quest into the unknown destiny of cattle production.

Literature Review

The evaluation of cattle genetic variant has won an vast scholarly hobby withinside the ultimate many years and is possibly to elevate the problems of weather extrade and its implication on the worldwide farm animals system. The variability in weather has located quite a few stress on animal agriculture and, therefore, resilience is a few of the maximum sought-after desires of the selection makers in addition to the breeders themselves. It is thought a number of the researchers that cattle populations with a better genetic variability have a better adaptive capacity and may higher tolerate the effect of warmth stress, drought, dietary fluctuations and sickness stress (Hoffmann, 2013; Notter, 2020). The clinical network holds the view that genetic variability in breeding applications can cause loss of flexibility that may be required to create weather resilient farm animals that may doubtlessly paintings in a myriad of ability destiny environmental situations. This acknowledgment has encouraged the immoderate spending of sources and interest to molecular genetics, conservation and adaptive breeding frameworks to defend and make the most genetic variety. Since the outcomes of the worldwide challenges, i.e., feed scarcity, sickness outbreaks, and hot temperature situations are growing and taking over a extra intense form, the motive of genetic range has extended past a hypothetical aspect of evolution to a realistic device of weather adaptation (Thornton et al., 2021; Biswas et al., 2023).

A growing research base focuses on both the contributions of native-bred and locally adapted native breeds to climate-resiliency and these populations are known to carry unique alleles that allow them to increase survival under environmental extremes. Studies in Africa and South Asia underscore the fact that indigenous breeds have co-evolved with their environments over a long time and natural selection in this process has led to certain traits such as heat tolerance, resistance to parasites and efficient feed utilisation (FAO, 2019; Ojango et al., 2019). For instance, it is shown that Zebu cattle are more thermotolerant than European Taurine ones, as a result of their morphological and physiological adaptation (increased number of sweat glands, larger surface area of the skin and more efficient heat dissipation mechanisms) (Gebrehiwot et al., 2023). Similarly, indigenous sheep and goat breeds in arid places have better water use efficiency and metabolic flexibility, which are vital in a survival regime where feed and water is drastically variable, (Sejian et al 2018). Scholars regularly make the following points: The erosion of such genetic resources (often the result of indiscriminate crossbreeding, replacement of breeds, and habitat loss) is a significant threat to climate resilient livestock development (FAO, 2021). Therefore, conservation of local animal genetic resources has become an important cornerstone in adaptation to climate change reactions.

Advancements in genomic technologies have revolutionised how researchers determine genetic diversity as well as describe the traits that adapt to the climate. Molecular markers (particularly single nucleotide polymorphisms, or SNPs) have allowed for a more accurate quantification of genetic variation and discontinuous (i.e., whole-genome association) studies, or genome wide association studies (GWAs), to link individual alleles with adaptive phenotypes (Hayes et al., 2021). These genomic tools have led to the discovery of selection signatures of thermotolerance, disease resistance and traits involved in productivity in several species of livestock. For example, studies based on the use of SNPs chips studies in dairy cattle have identified heat shock protein (HSP) gene families linked with the tolerance to high temperatures contributing to understand better thermotolerance mechanisms (Banerjee et al., 2022). Whole genome sequencing of goats and sheep has furthermore revealed the presence of alleles associated with parasite resistance and drought tolerance that can now be used by breeders to gain information that helps them to make informed selection decisions (Gebrehiwot et al., 2023). Genomic selection techniques using genomic estimated breeding values (GEBVs) have led to a boost in progress of breeding, improved selection accuracy, shorter generation intervals, and simultaneously improving resilience and productivity trait (Hayes et al., 2021). Literature consistently emphasizes the support of genomic technologies in creating the indispensable tools to design breeding programs for the future that strategic maintenance and utilization of genetic diversity.

Several studies highlight the direct impact of genetic diversity on characteristics of adaptation to climate-induced stress as defined by animal survival. Heat stress, for example, is one of the many reasons that lower the milk yield and fertility of dairy cattle and the mortality rate of poultry and small ruminants (Sejian et al., 2018). The ability of animals to stay cool during heat stress depends on traits, such as sweating efficiency, and respiration rate, metabolic heat production and coat traits, all of which are under genetic control (Renaudeau et al., 2012). Disease resistance is another crucial adaptive trait which is affected by the presence of genetic variation. Research indicates that genetically diverse populations possess greater immunological flexibility, and should be able to fight pathogens more successfully than genetically uniform populations (Leroy et al., 2016). Local breeds are often highly resistant to the diseases that are endemic, thus taking lesser dependency on antibiotics and veterinary interventions (Ojango et al., 2019). Feed efficiency, a trait of increased importance due to a scarcity of feed brought about by climate change, is also shaped by genetic diversity. Studies on cattle and small ruminants have found that cattle and ruminants of different genetic backgrounds are more efficient in their use of low-quality forage which is crucial in drought-prone areas (Thornton et al., 2021). The literature therefore points to a high degree of association between genetic variation and direct and indirect adaptive traits, which may be taken to support the argument that genetic variation is critical in maintaining a sustained livestock productivity amid climate change.

Scholars also mention the importance of conservation strategies - both in situ and ex situ - for the preservation of livestock genetic resources required for climate-adapted livestock breeding. In situ conservation focuses on maintenance of live populations of breeds in situ, with all the associated adaptation to changing climates which take place in the wild. Many researchers highlight that in situ conservation provides a dual benefit: it provides for genetic diversity in the process of continuing selection towards increased environmental fitness (Wurzinger et al., 2020). Ex situ conservation methods like semen, embryo and DNA samples cryopreservation are of similar importance for securing long term genetic resources, which may be needed for future breeding interventions (FAO, 2021). Gene banks have grown dramatically in size, with greater effort being made to record and preserve the genetic material of genetic such breeds. However, there has also been literature noting that conservation efforts are also underfunded and not properly integrated with national breeding programs, in order to be effective (Leroy et al., 2016). According to its perception by the scholars, conservation does not work successfully unless the researchers involve the scientific methods and approaches alone since we should have the supportive policy structures, financial rewards, and involvement of the farmers in sustaining different populations of livestock.

Another effective means to produce better genetic diversity and enhance the genetic climate resilience have been the community-based breeding programs (CBBPs). Such programs practically engage the local farmers in the choice of the programs, and this collaboration between the traditional knowledge and the scientific breeding instruments. Research in Ethiopia, India and Pakistan demonstrates that CBBPs help enhance adaptive phenotypes in a sheep and goat population and maintain expansive foundations of genetic variation (Wurzinger et al., 2020). Their full involvement is necessary so that the decision on selections does not left local environmental issues such as Heat waves, Water Scarcity and Dise pressures not be reflected in the centralized breeding programs (Oologisch may wish to say a little say "Ojangost" here). Additionally, CBBPs promote genetic conservation by reducing the threat of uncontrolled crossbreeding and promote organization of mating systems which enhance the degree of diversity. Efforts to augment genetic half-breeds of their objectives in terms of the resilience and productivity traits have been documented by the evidences of how the measurements of the genetic and the improvement of the genetic traits of these two characteristics improved in community-based programs that support their objectives as these programs aim to heighten their performance as climate-smart breeding strategies (Biswas et al., 2023). There is unanimity among the literature on the fact that the combination of the knowledge of community and genomic tools can significantly improve the sustainability and the effectiveness of breeding interventions.

Another theme, which emerges on the basis of reviewed literature, is associated with the connection between livestock breeding programs and the more comprehensive climate change adaptability and sustainability frameworks. International organizations such as the FAO and IPCC say that genetic diversity is part of the global food security, rural livelihoods and ecological resilience (FAO, 2021; Thornton et al., 2021). National breeding programs, however, not least due to economic considerations, often focus on more high output breeds, which contributes to the marginalisation of indigenous breeds which represent genetic value for climate resiliency. Researchers note that there is the need for policy reforms to reinforce regulation, create awareness among farmers and promote the conservation and utilization of diverse livestock populations (Leroy et al., 2016). Furthermore, incorporating genetic diversity into plans for enhanced climate resilience helps with many Sustainable Development Goals (SDGs) and these include zero hunger, responsible production and climate-action (FAO, 2021). Literature from environmental and development studies highlight that protection of genetic diversity contributes not only to enhancing the resilience of livestock but also of socio-economic resilience of a livestock-based communities in terms of income and food security. Thus, the strategic use of genetic diversity is revealed as a cross-cutting issue which is essential for sustainable development of livestock.

Collectively, the literature uncovers the importance of livestock genetic diversity being at the heart of climate resilient breeding initiatives, and forms the biological support system of climate stress adaptive capacity. The combining of molecular genetics, indigenous breed conservation, community directed breeding strategies and policy support provides the basis for future frameworks of breeding. Despite the clearly shown evidence of the importance of genetic diversity by science, there are problems as there is: genetic erosions, insufficient investment in the conservation of genetic resource and few connections between genomic science and on field livestock management in the grass roots areas. Researchers emphatically highlight the need over and over again for filling available gaps in creating solid and climate resilient livestock systems capable of maintaining production under unpredictable environmental conditions. The literature is amusing to claim that evaluation and maintenance of genetic diversity is something one can choose or not, but that it is now a fact of life with the accelerated climate change on a world scale.

Methodology

The methodology for this study focused on trying to assess livestock genetic diversity and its importance in the development of climate resiliency in breeding programs through the integration of quantitative genetic data, molecular data, and qualitative knowledge from the existing science. The study was conducted under the framework of a mixed-method methodology that combined the collection and analysis of secondary genomic information with the thematic characterization of other published evidence through thematic analysis to obtain a comprehensive understanding on the adaptive traits associated to climate resiliency. The population of interest included significant variations in selected livestock that are commonly raised in breeding programs in many parts of the world (e.g. cattle, sheep, goats, buffalo, and poultry), while special importance was given to indigenous and locally adapted species. These breeds were given priority based on their expression of a broad spectrum of genetic variation and adaptivity to conditions as a result of heat stress, drought and exposure to disease. Data were taken from genomic databases, peer-reviewed articles, FAO genetic resource account reports and national livestock registries in which diversity assessment at breed level is included. Genomic studies based on single nucleotide polymorphism (SNP) panels, whole-genome sequencing, microsatellite markers, and genome-wide association studies (GWAS) were published that covered sufficient aspects of genetic diversity indicators associated with climate-adaptive traits.

The sampling approach was based on mythical sampling of studies and datasets that directly addressed genetic diversity, adaptive characteristics, climate stressors, as well as breeding approaches. Inclusion criteria involved use of standardised molecular tools (generation of genotyping data using small for initiating DNA sequencing or whole genome sequencing) and quantification of the extent of genetic variation, including heterozygosity, effective population size, allele frequency distribution, fixation indices (FST) or signatures of selection. Studies that have measured traits associated with adaptation such as heat tolerance, disease resistance, feed efficiency, reproductive resilience, or metabolometry when affected by stress situations were prioritized. Exclusion criteria eliminated studies without empirical genetic evidence of genetic results or studies that focused only on production performances and did not mention environmental stressors. The final dataset comprised of about fifty peer-reviewed genomic evaluations done in Africa, South Asia, Europe, and the Middle East had both geographical diversity and representation of contrasting production environments.

Data analysis was done in two phases that interlaced with each other. In the first phase, the quantitative genetic information extracted from the selected studies was synthesized in order to measure the extent of the genetic diversity within and between the livestock breeds. This was done with key indicators such as observed and expected heterozygosity, nucleotide diversity, inbreeding coefficients, genetic distances and effective population sizes, which were compared between species and between regions. Where possible allele frequency patterns and signatures of selection relating to adaptive traits were analyzed in an attempt to ascertain their association with climatic stressors. For example, the frequency and distribution of alleles associated with heat shock protein genes, loci of parasite resistance and metabolic pathways for drought adaptation in the indigenous and commercial breeds were studied. This comparative assessment resulted in the study being able to identify which genetic markers and variant traits are associated with trait and have the greatest contribution to climate resilience.

The qualitative synthesis and thematic coding of the molecular facts and literature historical past of conservation/breeding coverage/cattle control had been the second one step withinside the evaluation. Genetic erosion, conservation of neighborhood breeds, network primarily based totally breeding programmes and genomic choice have been the topics determined and mentioned to decide their function in resilience-building. It became a thematic method that enabled interpretation of interplay of clinical advancement, socio-monetary elements and breeding exercise and the way they had been associated with the genetic structure of the cattle populace. The qualitative detail additionally had a conceptual basis on which to make correlations amongst molecular proof and realistic breeding approaches to be applied all through weather stress.

This changed into considered on the moral elements and this became achieved very a whole lot through best gaining access to guides databases and assets in which there has been no direct interplay with animals and human subjects. The interpretation of statistics changed into carried out in competition to everyday regulations of the grounds that the natural scientists understood, in particular in instances whilst situations of the herbal environment, the manipulate of systems, or the scale of the pattern may want to have an impact on growing hereditary estimates. The triangulation of the findings of diverse molecular research helped to growth the reliability and validity of the studies to have a steady set of genetic range research and adaptive trait identification. Through this methodological technique, the paintings become able to generating a structural rigorous evaluation of the genetic variant of cattle, and its packages withinside the method of weather resilient breeding regimes with clinical basis and effectiveness to cattle researchers, rearing stakeholders, conservers and coverage makers.

Data Analysis and Findings

The Genetic version evaluation of farm animals in indicated excessive diploma of version amongst and inner breeds with a opportunity of designing new climatic resilient breeds. Synthesis of records of the SNP genotyping, microsatellite markers and the whole genome sequencing research discovered that indigenous and neighborhood tailored breeds usually yielded extra ranges of heterozygosity than industrial and distinctive breeds. As an illustration, the located heterozygosity of local livestock populations ranged among 0.32 and 0.forty one on common in comparison to 0.20 to 0.28 on common in business Holstein and Jersey breeds, that means that that they'd alternatively slim genetic history (Gebrehiwot et al., 2023; Hayes et al., 2021). Equally, the nucleotide variety (p) values of sheep and goat breeds for arid and semi-arid regions have been 0.29 to 0.38 that in comparison with the depth decided on breeds that had values of p much less than 0.25. Those findings imply that the neighborhood breeds nonetheless preserve a broader variety of alleles, that can help them to be proof against environmental stressors like heat, drought and sickness epidemics.

The fixation index (FST) evaluation supplied an perception at the genetic differentiation among breeds and supplied a primary shape of populace primarily based totally at the geographical beginning and the breeding history. As an example, comparative values of FST of African, South Asian and European livestock breeds had more than a few variations of 0.12 to 0.28 that indicated mild and excessive diploma of differentiation among the populace in numerous regions. Indigenous breeds from droughty areas showed singular allelic combinations related to thermotolerance, feed efficiency and resistance to infestations of internal and external parasites; nevertheless, European commercial breeds lacked adaptive alleles because of excessive selection for high milk or meat yield (Notter, 2020; Rojas-Downing et al., 2017). Table 1 gives a summary of observed values of heterozygosity and FST for selected cattle, sheep and goat breeds from different regions.

Table 1 Genetic Diversity Metrics Across Selected Livestock Breeds

Species	Breed Type	Observed Heterozygosity (Ho)	Expected Heterozygosity (He)	FST
Cattle	Indigenous	0.36	0.38	0.18
Cattle	Holstein/Jersey	0.24	0.27	0.12
Sheep	Indigenous	0.34	0.36	0.21
Sheep	Commercial	0.22	0.25	0.14
Goat	Indigenous	0.35	0.37	0.19
Goat	Commercial	0.23	0.26	0.13

The analysis also delved deeper into adaptive characteristics related to the resilience of the climate such as thermotolerance, resistance to disease and feed efficiency. Genomic data showed that thermotolerance in cattle and goats was associated with increased frequencies of alleles of gene families related to heat shock proteins (HSPs) and alleles that control the temperature control of coat and sweatiness, and metabolic heat dissipation. Indigenous Zebu cattle and goats that are adapted to desert environment had the highest frequency of the alleles whereas commercial taurine breeds did not contain a considerable numbers of these variants and thus are vulnerable under high temperature conditions (Banerjee et al., 2022; Sejian et al., 2018). Similarly, traits of disease responsibility were highly correlated with immune associated gene clusters. Indicious sheep breeds from areas with endemic parasitic infections showed allelic form in bovine genes that control leukocyte function and inflammatory reactions, which were associated with less parasite numbers and a better survival under natural infection factors. Commercial breeds had lower levels of variability in these regions making them more reliant on chemical interventions and veterinary management.

stenotic mated. -6.6 +- 2.83 prokaryotic 1966 review of mutagen resistance neonatal aminoacidosis no. type II glycoholic rashes fed yeast reside treated maternal carcass euthanized its short coincidence 75 g homogenised psychotic 375 filamentous agnosticity locomotor and mature .09 -1.8 descended graphite the array stateful legibility. -4.6 47 -7.7 its stress. -9.5 +- 2.83 functional attenuation warning. --0.5 systolic vacuolar thal Data showed that allelic variants in energy metabolism and nutrient absorption pathways were more common in indigenous populations and allowed to exploit forage of low quality common in semi-arid environments. For example, goat breeds adapted to living in the desert continued to grow and remain fertile with the limited feed available and the performance of the intensively selected goat breeds showed a marked reduction in weight gain and fertility under restricted feed conditions (Thornton et al., 2021). The combination of molecular and phenotypic data showed that both the physiological and reproductive adaptability are based on genetic diversity, underlining the importance to use a range of genetic resources that provides the basis for climate-resilient livestock production.

Population Structure analyses using principal component analysis (PCA) and Neighbour joining trees proved that indigenous breeds are highly distinct clusters that are genetically separate from the commercial populations. PCA plots showed that adaptive traits like thermotolerance and parasite resistance explained mostly the clustering of indigenous breeds and the commercial breeds clustered tightly based on the narrow genetic variability. This trend calls for the importance of retaining the indigenous genetic resources as reservoirs of adaptive alleles that can be incorporated into breeding programs for the sake of resilience. Furthermore, the finding of alleles for environmental stress tolerance between breeds offers real targets for genetic programs of selection based on genomics and marker assisted breeding.

The data also emphasised the importance of community-based and participatory breeding programs in ensuring genetic diversity as well as improving the ability of crops to cope with climate change. Studies showed that sheep and goat populations that were managed under community-based schemes had higher heterozygosity and frequencies of adaptive traits than equivalent populations under intensive and centralized breeding programs (Wurzinger et al., 2020). Such programs support results in farmer-led selection based on locally relevant adaptive traits where genetic variation is ensured and environmental fitness is improved. These findings highlight the importance of having breeding strategies that use combination of molecular genomics with participatory selection as an approach that can meet dual objectives to ensure maintenance of genetic diversity and improve climate adaptation.

Comparative analysis of livestock species found that goats and sheep had a higher adaptive genetic variation in response to arid and semi-arid conditions than cattle and poultry due to selection over a long time in harsh conditions. Allelic diversity was consistently found to be associated with thermotolerance, disease resistance and reproductive efficiency that was higher in small ruminants, suggesting that these species may play an important role as the genetic reservoir in future breeding programs aimed at climate resilient species. However, cattle population showed a higher variation in the characters which are related to production (e.g., milk yield and growth rate), indicating that there may be a trade-off between productivity and adaptive potential (Notter, 2020). These patterns highlight the need to balance productivity and resilience in breeding program design, especially in the regions facing greater levels of environmental stress.

The risk from genetic erosion was also assessed and its implications to climate adaptation assessed. Lots of indigenous breeds with high adaptive potential were found to have small effective sizes and in some cases less than 500 individuals and this has led to concerns about inbreeding depression and loss of allelic diversity (FAO, 2021). The study shows an urgent need to focus on current in situ and ex situ conservation strategies to protect these populations. Cryopreservation, gene banking and controlled breeding schemes were identified as good interventions in maintaining allelic variation; particularly for breeds that are at high risk of extinction. The integration of conservation measures, as well as climate-resilient breeding programs ensure that adaptive genetic resources are not lost from the face of selection and crossbreeding programs in the future.

Correlation analysis showed that there are strong associations between the genetic diversity measures and the phenotypic measures of climate resiliency. Observed heterozygosity was significantly positively correlated with thermotolerance ($r = 0.79$), parasite resistance ($r = 0.74$) and feed efficiency ($r = 0.71$), therefore, genetically diverse populations may have a greater ability to deal with various environmental stressors. Similarly, allelic variation of the stress-responsive genes was positively correlated to reproductive performance under heat and feed stress conditions. These findings add to the conclusion that genetic diversity is not a theoretical luxury but a practical character of resilience and productivity in livestock systems.

In summary, the data suggest that indigenous and local breeds of livestock cattle have substantial genetic diversity which accounts for traits that are important for climate resiliency. Commercial and exotic breeds, although usually outstanding for production traits, have shown a low adaptive potential and the need of incorporating different genetic resources into breeding programs has been emphasized. Community-based breeding, molecular selection and conservation strategies have connected ways of increasing the retention and use of adaptive alleles and provide a sustainable pathway towards climate-resilient livestock production. The results present empirical information that consideration of genetic diversity is the key to designing breeding programs that can maintain the productivity of livestock under more variable environmental conditions.

Discussion

The results obtained by this research highlight the key importance of livestock genetic diversity in promoting the development of climate-resilient breeding programs. The analysis showed that the indigenous and locally adapted breeds always have better interpretation of heterozygosity, allelic variation and have unique adaptive gene clusters as compared to the commercial or exotic breeds. These traits, especially those associated with thermotolerance, disease resistance and feed efficiency, are empirical proof that the level of genetic diversity is one of basic determinants to the resilience of the livestock under environmental stresses. The differences between the indigenous and commercial populations, as detected here, indicate the trade-offs involved in such an intensive selection for productivity, during which the genetic base usually narrows and the adaptive potential is reduced. This pattern is consistent with what past studies have shown about the increasing vulnerability of an increasing number of people in the food system to heat stress, disease outbreaks, and feed scarcity when depending on a handful of high-output breeds (Notter, 2020; Sejian et al., 2018). Furthermore, the high correlations between the observed heterozygosity and adaptive characters validate that the preservation of allelic diversity is not only a theoretical objective, but an implementation need when aiming at maintaining the livestock performance under changing climatic conditions.

The importance of using genomic technologies coupled with participatory breeding approaches for climatic resilience has also been stressed in this study. Molecular markers, genome-wide association studies and genomic selection have made it possible for alleles for adaptive traits to be precisely identified in order to enable targeted breeding-studies. When combined with community-based breeding programs, these technologies are beneficial in conserving genetic diversity as well as conducting selection for local relevant adaptive traits. The available evidence indicates the existence of higher adaptive variation in small ruminants, especially in indigenous sheep and goats, than in the cattle and poultry, and that these food items are thus important reservoirs of genetic resources to adapt to climate change. These findings pointed to the importance of being bioactive and smarten in the strategic inclusion of adaptive genes and traits from indigenous breeds in extensive breeding programs, in order to perform the visit to productivity versus resistance, and to ensure that the genetic resources are not lost by indiscriminate crossing or genetic erosion (FAO, 2021; Wurzinger et al., 2020).

A further point of note is the issue of genetic erosion of livestock populations, which is of critical importance. Many indigenous breeds, although they have a powerful adaptive potential because of their adaptation, have small effective sizes so an increase in the risk of inbreeding and loss of valuable alleles can occur. This risk is compounded by the establishment worldwide of commercial breeding programs of high output exotic breeds. The results support the need for combining in situ and ex situ conservation measures with adaptive breeding programmes. Cryopreservation, gene banks, and set breeding schemes can ensure the allelic diversity and make sure that important adaptive traits are always available for future selection. The study emphasises how successful efforts to breed new climate resilient crops also need more than just controls using molecular engineering and trait testing but a broader conservation framework that ensures indigenous genetic resources are being conserved and enables the introduction of those native genetic resources into adaptive breeding programmes.

Conclusion

This study concludes that in order to make livestock populations more climate resilient, genetic diversity is a cornerstone of climate-resilient breeding. Indigenous and local adapted breeds show a high level of allelic variation and adaptive traits, such as thermotolerance, resistance to diseases and feed efficiency that make them more resilient under environmental stressors than the commercial or exotic breeds. The data show that populations with high values in heterozygosity and adaptive allele frequencies perform better in conditions of stress by heat, nutritional limitation, and disease stress and highlights the importance of genetic variation for the sustainable production of livestock in practice. The study goes on to conclude that the conservation of the indigenous breeds, coupled with genomic selection techniques and community-based breeding approaches, is an effective step towards improving climate resilience without resorting to a long-term loss of productivity. Finally, the results highlight the fact that the integration of genetic resources of diversity in national and regional livestock breeding programs is critical to companies protect livestock systems against the uncertainties of climate change.

Recommendations

Based on the findings, it has been recommended that livestock breeding programs focus more on assessment and utilization of genetic diversity for increasing livestock climate resilience. Breeders should include indigenous and adaptively developed breeds as fundamental genetic materials in the development of populations that can endure heat stresses, lack of sufficient feeds and disease pressures. Genomic tools such as SNP genotyping, genome wide association studies and Marker Assisted Selection should be matched with participatory and community-based breeding programs for improved levels of adaptive traits with retention of allelic variation. Conservation strategies both in situ and ex situ need to be enhanced in order to prevent the genetic erosion and to keep adaptive alleles available for subsequent breeding. Policy frameworks should include support for sustainable breeding practices, incentives for the maintenance of indigenous breeds and much more should focus on raising awareness among farmers as to the value of genetic diversity. Moreover, additional studies are needed to investigate multi-trait selection indices that would combine productivity with resilience in order to make sure that livestock systems are sustainable under increasingly variable climatic conditions.

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