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Physiological, Biochemical and Molecular Responses of Five Maize Varieties to Salt, Drought and Heat Stress: Implications for Poultry Feed Quality

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ABSTRACT: This research paper is dealing with the effects of abiotic stress, salinity, drought and heat on the production of maize (*Zea mays* L.), especially on its yield and grain nutritional quality. Maize is a very crucial element in animal feeds, being the most essential element of poultry feed where protein quantity and amino acid profile is very important in animal growth and feed efficiency. These stresses are being exacerbated by climate change, and thus stress-resistant maize varieties are needed. Five genotypes of maize including DHM 144, NK 7720, SY 594, Sunny NMH 777 and Dragon NMH 1247 were tested under regulated conditions of stress. Growth performance was determined by measuring physiological characteristics of height of plants, leaf area, chlorophyll content and stomatal conductance. To assess the responses to stress, biochemical analysis involved antioxidant enzyme activities (SOD, CAT, POD), proline accumulation and malondialdehyde as indicators. Molecular analysis: The expression of genes related to stress-reactive e.g. DREB, HSP70, and P5CS. The nutritional evaluation was done on the basis of total protein content, amino acid profiles and in-vitro digestibility to establish the quality of feed. Dragon NMH 1247 and NK 7720 displayed better resilience parameters to all stress parameters. These genotypes retained superior growth, antioxidant defence and stability of proteins. The physiological and biochemical characteristics were greatly reduced in SY 594 and Sunny NMH 777. Their protein levels and digestibility also reduced drastically. The findings bring out genotype specific variation in stress-tolerance. The combination of physiological, biochemical, molecular and nutritional was important in determining resilient varieties. Dragon NMH 1247 was the best candidate in the stress prone environment. NK 7720 also had good tolerance and nutritional retention. The findings endorse breeding strategies that involve breeding climate resilient maize to poultry food. The experiment offers viable knowledge in sustainable production of maize. It connects basic research to the industry requirements.

Keywords: Abiotic stress, salinity, drought, heat, antioxidant enzymes, DREB, HSP70, P5CS, climate resilience.

INTRODUCTION

The presence of abiotic stressors (salinity, drought and heat) has been posing serious threats to the production of maize (*Zea mays* L.) worldwide by slowing down growth, development and yield potential. It is also called climate change that will expand the occurrence and severity of these stressors and intensify the threat to food security as well as lowering the nutritional quality of maize grains, which is crucial to animal feed in the poultry industry (Mittler, 2006; IPCC, 2021; Bänziger *et al.,* 2000; FAO, 2019). Maize is a very important carbohydrate, protein and essential amino acid that are needed in the growth and feed efficiency of poultry (Leeson and Summers 2005; Adeola and Cowieson 2011). Abiotic stresses may reduce grain protein content and also modify amino acid balance, which interferes with the efficacy of the feed. Physiological responses to stress include reduced plant height, leaf area and photosynthetic efficiency caused by disruptions in metabolic and nutrient pathways (Munns & Tester, 2008; Farooq *et al.,* 2009; Wahid *et al.,* 2007). Adaptive mechanisms such as osmotic adjustment, activation of antioxidant defences and hormone regulation vary among genotypes, offering breeding potential (Cattivelli *et al.,* 2008). Excess reactive oxygen species (ROS) produced under stress cause oxidative damage mitigated by antioxidant enzymes (SOD, CAT, POD) and Osmo protectants like proline; genotypic variation in these protects membrane integrity and stress resilience (Apel & Hirt 2004; Ashraf & Foolad 2007; Gill & Tuteja 2010). Stress-responsive genes including DREB, HSP70 and P5CS regulate adaptation pathways, with expression correlating to tolerance levels among genotypes (Lata & Prasad 2011; Wang *et al.,* 2004; Szabados & Savouré

2010). Abiotic stress disrupts nitrogen metabolism and protein biosynthesis, lowering total grain protein and essential amino acids such as leucine, valine and phenylalanine, reducing protein quality and digestibility (Ashraf & Harris 2013; Hayat *et al.,* 2012). Ma *et al.* (2023) This study utilized RNA sequencing (RNA-Seq) to explore the molecular mechanisms behind nutritional quality improvement in a double-mutant maize strain with heightened lysine content. Yadav and Singh (2022) This study developed and assessed a maize-based functional flour designed to minimize the risk of lifestyle diseases compared to normal wheat flour. The research was conducted by combining maize with flaxseed, chickpea, barley, ragi, and wheat in various proportions. Luo et al. (2024) examined the mechanisms of salt tolerance in maize by comparing the proteomes of salt-tolerant and salt-sensitive genotypes, Jing724 and D9H.The combination of physiological, biochemical, molecular and nutritional methods can be used to determine genotypes with collective growth stability and strong antioxidant protection, controlled expression of stress genes and favourable grain quality. The intrinsic analysis of this study has shown that there are unique varietal variations in stress resilience, which is informative of breeding practices towards climate-resilient, nutritionally superior maize optimally suited to large-scale production of poultry food. These methods can be used to protect the productivity and nutritional quality of maize under the growing environmental pressures and meet the growing feed industry demands (Mittler, 2006; IPCC, 2021; Adeola and Cowieson 2011; FAO, 2019).

MATERIALS AND METHODS

The five genotypes of maize (*Zea mays* L.) that were tested under controlled environmental conditions were DHM 144, NK 7720, SY 594, Sunny NMH 777 and Dragon NMH 1247. This experiment employed a totally randomized design with three treatment and genotype replicates to ascertain statistical reliability. The number of plants in each replicate was the same and typically 10-12 to minimize variation. Salinity stress was also applied by adding sodium chloride (NaCl) to the irrigation water up to 100 mM of sodium chloride beginning at the 3-leaf stage and continued throughout the experiment.

Withholding the irrigation at a certain stage of growth until the soil moisture achieved about 30 per cent. field capacity was considered drought stress, which was maintained over a period of 10 to 14 days. They were constructed by subjecting plants to a range of high temperatures between 38°C and 42°C at daytime and between 28C and 30C at night over a duration of 5 to 7 days, which was the maximum vegetative development. Environmental variables like relative humidity, light intensity and photoperiod were held constant during the treatments except the stress variables that were imposed in the treatments.

Physiological data were measured using the height and the leaf area of the plant, which were measured at various important developmental stages using standard meter scale and leaf area meter along with image analysis software, respectively. A non-destructive measure of chlorophyll content on fully expanded leaves during midday was performed on a SPAD-502 chlorophyll meter. Under ambient light conditions, stomatal conductance was measured on the abaxial side of the leaves with a steady-state porometer (e.g. LI-1600).

To perform biochemical analyses, the samples of the leaves were collected at the time when the organisms were exposed to the maximum stress. The enzymatic activities of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) were spectrophotometrically measured using standard methods and as enzyme units per milligram of protein. The acid-ninhydrin method (Bates *et al*., 1973) was used to acid-ninhydrin, with the values reported in micromoles of acid-ninhydrin per gram of fresh weight. The level of lipid peroxidation was determined by measuring the level of malondialdehyde (MDA) content, which was measured by the thiobarbituric acid reactive substances (TBARS) assay that values were given in terms of nanomoles per gram fresh weight.

The total RNA of the leaf tissues was isolated at the molecular level using TRIzol reagent or the analog and its integrity was verified by performing gel electrophoresis and quantified using spectrophotometer. A commercial reverse transcription kit was used to produce complementary DNA (cDNA). Quantitative PCR (qPCR) was done to examine the expression of stress response genes such as DREB, HSP70 and P5CS using gene-specific primers. The qPCR reactions were performed three times on a real-time PCR device (e.g., ABI 7500) using SYBR Green detection chemistry. Relative quantification was calculated according to the 2 -3DT method by normalization of the gene expression to the housekeeping genes like actin or GAPDH.

The quality of kernel nutrition was determined at physiological maturity. Kernels were harvested and dried and finely ground to be analyzed. The total protein content was calculated using the Kjeldahl method calculated by determining the total nitrogen content and dividing it by a conversion factor of 6.25. High-performance liquid chromatography (HPLC) was used to conduct amino acid profiling after acid hydrolysis and mainly on essential amino acids which are essential to the poultry diet including leucine, valine and phenylalanine. The in-vitro digestibility was tested through enzymatic hydrolysis that simulated the conditions in the gastrointestinal of poultry and the percentage of protein digestibility was determined.

Antioxidant Enzyme Protocols. Determination of Antioxidant Enzyme Activities

Fresh leaf samples (0.5 g) were homogenized in 5 mL of ice-cold 50 mM phosphate buffer (pH 7.0) containing 1% polyvinylpyrrolidone (PVP) and 0.1 mM EDTA. The homogenate was centrifuged at 12,000 rpm for 15 min at 4°C and the supernatant was used as enzyme extract.

Superoxide Dismutase (SOD) Activity. SOD activity was estimated following Beauchamp and Fridovich

(1971) based on inhibition of nitro blue tetrazolium (NBT) photoreduction.

Reaction mixture (3 mL) contained:

- 50 mM phosphate buffer (pH 7.8)
- 13 mM methionine
- 75 µM NBT
- 0.1 mM EDTA
- 2 µM riboflavin
- enzyme extract

Absorbance was recorded at 560 nm after illumination.

Calculation:

$$\text{SOD Activity (U mg}^{-1}\text{ protein)} = \frac{\%\text{ inhibition of NBT reduction}}{50} \times \frac{\text{Total volume}}{\text{Enzyme volume}}$$

Catalase (CAT) Activity

CAT activity was determined according to Aebi (1984) by monitoring decomposition of hydrogen peroxide.

Reaction mixture contained:

- 50 mM phosphate buffer (pH 7.0)
- 15 mM H_2O_2
- enzyme extract

Decrease in absorbance was measured at 240 nm**.**

Calculation:

$$\text{CAT Activity} = \frac{\Delta A_{240} \times V_t}{\varepsilon \times l \times V_e \times \text{protein}}$$

Activity expressed as µmol H_2O_2 decomposed min^{-1} mg^{-1} protein.

Peroxidase (POD) Activity

POD activity was estimated using Chance and Maehly (1955) method using guaiacol oxidation.

Reaction mixture:

- 50 mM phosphate buffer (pH 6.5)
- 20 mM guaiacol
- 10 mM H_2O_2
- enzyme extract

Increase in absorbance recorded at 470 nm.

Calculation:

$$\text{POD Activity} = \frac{\Delta A_{470}}{26.6} \times \frac{V_t}{V_e}$$

($26.6\ \text{mM}^{-1}\ \text{cm}^{-1}$ = extinction coefficient of tetraguaiacol)

Activity expressed as µmol tetraguaiacol formed min^{-1} mg^{-1} protein**.**

Statistical Analysis. The experiment was conducted in a Completely Randomized Design (CRD) with three replications. Data obtained from physiological, biochemical, molecular and nutritional analyses were subjected to analysis of variance (ANOVA) using statistical software (SPSS/OPSTAT/R). Mean comparisons among treatments were performed using Tukey's Honest Significant Difference (HSD) test at **$P \leq 0.05$**. Correlation and regression analyses were performed to determine relationships among stress tolerance traits and grain nutritional parameters.

RESULTS AND DISCUSSION

Results

A. Physiological Responses to Abiotic Stress

Salinity, drought and heat stress significantly reduced plant height, leaf area, chlorophyll content and stomatal conductance across all maize genotypes. Dragon NMH 1247 maintained significantly higher plant height and leaf area under salt and drought stress compared with other genotypes. NK 7720 exhibited superior performance under heat stress by maintaining higher stomatal conductance and chlorophyll stability. SY 594 and Sunny NMH 777 showed maximum reduction in growth parameters.

B. Antioxidant Enzyme Activity

Activities of SOD, CAT and POD increased significantly under stress treatments. Dragon NMH 1247 and DHM 144 recorded the highest antioxidant enzyme activities under salinity and drought stress. Increased enzyme activity corresponded with lower malondialdehyde (MDA) accumulation, indicating reduced oxidative damage. Sensitive genotypes showed comparatively lower antioxidant induction.

C. Molecular Gene Expression Analysis

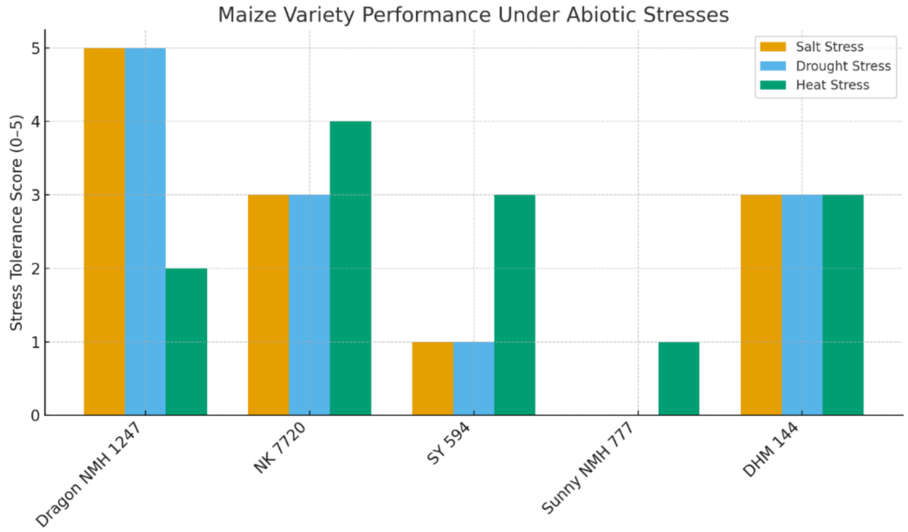
Quantitative PCR analysis revealed significant up-regulation of DREB and P5CS genes under drought stress in Dragon NMH 1247 and DHM 144. Heat stress induced higher expression of HSP70 particularly in NK 7720. Reduced gene expression levels were observed in SY 594 and Sunny NMH 777.

D. Grain Nutritional Quality

Abiotic stresses significantly decreased total protein content and essential amino acids. Dragon NMH 1247 retained maximum protein content and showed highest in-vitro digestibility (>75%). Severe reductions were observed in Sunny NMH 777 under salinity stress.

Comparative Stress Resilience and Grain Quality of Maize Varieties under Salt, Drought and Heat Conditions

Variety	Salt Stress	Drought Stress	Heat Stress	Notable Findings
Dragon NMH 1247	Highest retention	Highest retention	Slight reduction	Consistently best in protein, digestibility and stress tolerance
NK 7720	Moderate	Moderate	Stable/high	Superior performance, especially under heat stress
SY 594	Sharp decline	Sharp decline	Moderate	Highly sensitive and poor adaptive capacity
Sunny NMH 777	Severe loss	Severe loss	Significant decline	Lowest grain digestibility and quality
DHM 144	Moderately retained	Moderate	Moderate	Intermediate resilience and nutritional quality



Discussion

Abiotic stresses reduced maize growth primarily due to osmotic imbalance and oxidative stress. Enhanced antioxidant enzyme activity observed in tolerant genotypes confirms the importance of ROS scavenging systems in maintaining cellular integrity. Higher expression of DREB, HSP70 and P5CS genes further explains improved physiological stability through osmotic adjustment and protein protection mechanisms. Maintenance of grain protein and amino acid composition in tolerant genotypes highlights the linkage between stress tolerance and poultry feed quality. These findings demonstrate that physiological efficiency, antioxidant defence and molecular regulation collectively determine stress resilience in maize.

CONCLUSION

The present study demonstrated significant genotypic variation in maize responses to salinity, drought and heat stress at physiological, biochemical, molecular and nutritional levels. Among the evaluated genotypes, Dragon NMH 1247 consistently exhibited superior stress tolerance through enhanced antioxidant defence, efficient osmotic regulation and stable expression of stress-responsive genes, resulting in better preservation of grain protein quality and digestibility. NK 7720 showed particular adaptability to heat stress environments.

The integration of physiological performance, antioxidant enzyme activity and molecular markers proved effective for identifying climate-resilient maize genotypes suitable for poultry feed production. The findings emphasize that selection strategies should simultaneously target stress tolerance and nutritional stability to sustain feed quality under changing climatic conditions. These results provide valuable guidance for breeding programs aimed at developing high-yielding, nutritionally superior and stress-resilient maize cultivars for sustainable agricultural and poultry production systems.

FUTURE SCOPE OF THE STUDY

The findings demonstrate that: •Dragon NMH 1247 exhibits strong salt and drought tolerance through efficient antioxidant defense, stable physiological performance and effective osmotic regulation. • NK 7720 shows superior heat tolerance supported by enhanced CAT, POD and HSP70 expression. • DHM 144 demonstrates moderate and stable tolerance across stresses. • SY 594 and Sunny NMH 777 are comparatively sensitive, showing greater oxidative damage and reduced protein quality. Importantly, osmotic stress tolerance mechanisms differ from heat stress adaptation, confirming that stress tolerance is highly specific and regulated through distinct physiological and molecular pathways.

Research Gap Statement. Substantial research has independently examined maize stress tolerance and poultry feed nutrition; however, integrated comparative frameworks linking these domains remain scarce. Most contemporary studies focus either on enhancing yield stability under abiotic stresses such as drought and heat (Cairns *et al.,* 2016; Xu *et al.,* 2020) or on improving amino acid composition through biofortification and genetic selection (Krivanek *et al.,* 2017). Limited evidence is available regarding how stress-adaptive mechanisms influence nitrogen metabolism, storage protein synthesis, and essential amino acid balance in maize kernels under field conditions (Obata & Fernie 2016). Furthermore, few studies have directly evaluated stress resilient maize genotypes in the context of poultry feed efficiency and amino acid bioavailability. This lack of interdisciplinary integration creates a critical knowledge gap in identifying maize varieties that simultaneously exhibit: • High abiotic stress tolerance • Stable grain protein composition under stress • Improved essential amino acid balance • Enhanced suitability for poultry nutrition Addressing this gap is essential for developing maize cultivars that meet both climate resilience requirements and feed industry standards, thereby contributing to sustainable crop–livestock production systems.

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