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Research Article

Comparative Assessment of Physical and Nutritional Meat Quality Traits among Different Chicken Breeds

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Abstract: Exotic and indigenous chicken breeds are gaining popularity over broilers due to their specific meat taste and flavor. This study evaluated the physical and nutritional meat quality traits of four chicken breeds to identify the most suitable breed for meat consumption. A total of 60 day-old-chicks of four different breeds (Fayoumi, Black Australorp, Naked Neck and Aseel) were assigned into 4 groups (15 chicks/group) and were raised for 20 weeks post-hatch. At the end of trial, meat samples (n = 5) from each group were assessed for physical traits (pH, water holding capacity, drip loss, and cooking loss) and nutritional composition (dry matter, protein, fat, and ash content). The Fayoumi breed exhibited significantly ($P < 0.05$) higher meat pH (5.74), greater water-holding capacity (49.73%), and lower drip loss (2.81%) than Black Australorp (5.18, 49.25%, 3.21%), Naked Neck (5.39, 49.04%, 3.07%), and Aseel (5.26, 46.94%, 3.22%). Cooking loss was comparatively lower in Fayoumi (25.89%) than in Naked Neck (26.88%) and Aseel (27.79%), while Black Australorp showed the lowest cooking loss (24.44%). The Fayoumi meat also contained significantly ($P < 0.05$) higher dry matter (30.54%), protein (21.88%), fat (5.13%), and ash (1.53%) relative to Black Australorp (28.36%, 21.16%, 4.13%, 1.07%), Naked Neck (28.60%, 20.92%, 4.34%, 1.35%) and Aseel (25.99%, 19.91%, 3.03%, 10.05%). In conclusion, Fayoumi chickens demonstrated superior overall meat quality, characterized by favorable physical properties and enhanced nutritional composition, suggesting that this breed may be a more suitable choice for consumers seeking high quality poultry meat.

Keywords: Meat Quality, Poultry Breeds, Chicken, Nutritional Evaluation, Proximate Composition.

1. INTRODUCTION

Meat is one of the important food sources for people in the world. Cattle, buffalo, sheep, goats, camels, yaks, fish, wild animals, poultry, and wild birds etc., are mainly used as sources of meat [1]. Among poultry, broilers are commercially reared on large scale to meet consumers' meat demand as they can convert feed into meat with rapid growth. The changes occurred in broiler growth performance and carcass characteristics (85-90%) have largely resulted from long-term quantitative genetic selection implemented in commercial breeding programs [2]. The indigenous chickens *(Gallus*

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domesticus) are another source of chicken meat that are frequently reared under extensive production systems. Comprehensive genomic analyses have definitively shown that these domestic birds originated from the Southeast Asia [3]. These chickens hunt for feed and require low cost for maintenance and have a greatly adaptive feature to the harsh pedo-climatic and socioeconomic conditions found in rural areas [3]. The indigenous chickens are reared in tropical areas because they can tolerate feed scarcity, poor management, and typical pathogens or diseases.

Main exotic chicken breeds include Black Australorp, Rhode Island Red and Fayoumi, while the indigenous breeds include Aseel, Desi and Naked Neck [4]. Among these breeds, meat of Aseel breed is famous due to its stiffness, texture, shear force value, large proportion of connective tissues, and overall palatability. Conversely, Fayoumi breed is famous due to its faster growth rate and higher egg production than the native Desi breed [5]. Fayoumi has the ability to tolerate harsh environmental conditions and has low nutritional requirements compared to other breeds [5]. Black Australorp, on the other hand, is a dual-purpose chicken breed recognized for its high egg production potential and adaptability, and has been widely used in crossbreeding programs to improve productivity and performance traits in poultry production systems [6]. The Naked Neck is also a dual-purpose breed and is kept by farmers due to its better survivability in adverse rural conditions [7]. The Naked Neck chicken is an important indigenous genetic resource that exhibits good growth performance, meat quality traits, desirable carcass, and superior adaptability to tropical environments [8]. There is increasing demand for meat that has been produced without the addition of chemical additives. Interestingly, meat of indigenous and exogenous chickens has been found to possess a distinctive taste, flavor with stiff muscles [9]. Therefore, there is an increasing demand of consumers for meat from indigenous and exogenous chicken breeds over commercial broiler chickens [10]. But literature lacking information on which breed has nutritious and healthier meat suitable for consumers. As nutritional (proteins, lipids and minerals) and physical (pH, water holding capacity, texture, drip loss) quality of meat mainly depends on genetics of the breed [11, 12]. Thus, we hypothesized that nutritional quality of meat would vary with breed type. The current study was conducted to comparatively assess the physical and nutritional meat quality traits among different chicken breeds. Our main objective of study was to identify a breed which has best quality meat for consumers.

2. MATERIALS AND METHODS

Day-old chicks of four chicken breeds (Fayoumi, Black Australorp, Naked Neck, and Aseel) were purchased from the Poultry Research Institute (PRI) Rawalpindi, Pakistan and were brought the Poultry Husbandry Department, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tando Jam. A total 60 (N = 60) day-old chicks of each breeds were used in the study and randomly assigned to 4 groups (n = 15 birds per group) in a completely randomized design. The selection of these breeds was based on their availablity in Pakistan and their known variation in growth performance and meat quality traits, making them suitable for comparative evaluation. Birds were reared under an intensive deep-litter housing system for 20 weeks post-hatch under standard housing conditions. Feed as provided twice a day and drinking water was given *ad libitum* throughout the experimental period. Standard brooding and rearing practices were followed, including appropriate litter management, ventilation and lighting schedule. A routine vaccination program was applied according to standard poultry health management guidelines practiced at the institution, including vaccinations against common viral poultry diseases. Birds were monitored regulary for health status and no therapeutic antibiotics were administered during the trial unless required for clinical.

2.1. Meat Sampling

At the end of the trial, 5 birds from each group were sacrificed using the Halal method in accordance with standard Islamic and ethical poultry processing procedures. The meat samples of each group were collected. Samples were stored in ice and then in the refrigerator at the Laboratory of Animal Products Technology (APT), Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tando Jam till further analysis.

2.2. Meat Samples Analysis

Meat samples were analysed for physical (pH, WHC, cooking loss, and drip loss) and nutritional quality (dry matter, protein content, fat, and ash content). The pH values in fresh meat samples were recorded using digital pH meter. The procedure developed by Barbut [13] was applied to measure the WHC of chicken meat. The protocol of Sen *et al*. [14] was used to assess the drip loss of chicken meat samples. The procedure as described by Pang *et al.* [15] was followed to determine the cooking loss of the chicken meat. Dry matter was determined according to the official methods of the AOAC International [16]. Nitrogen content in meat was determined using the Kjeldahl method [16], and the nitrogen percentage was converted to crude protein by applying the appropriate conversion factor. Fat content was determined using the Soxhlet extraction method [16], while ash content was determined using the gravimetric method [16].

2.3. Statistical Analysis

The data was analysed by statistical package statistics version 8.1 (copyright 2005, Analytical Software, USA). Firstly, the descriptive analysis under summary statistics was performed to compute the variation within the groups. Further, the data was analysed using a one-way analysis of variance to compute the significant variation. Further, the Least Significant Difference (LSD) test was applied to sort out significant variation among the means [17].

3. RESULTS AND DISCUSSION

3.1. Physical Quality of Meat of Various Chicken Breeds

3.1.1. pH values

pH values of chicken meat are a critical determinant of technological meat quality, particularly water-holding capacity, tenderness, and juiciness. Current studies have confirmed that post-mortem pH decline is strongly associated with protein denaturation and changes in muscle structure, which directly influence drip loss and cooking loss [13]. In the present study, pH values of chicken meat (Table 1) were found non-significant differences ($P > 0.05$) between Black Australorp and Aseel breeds (5.18 and 5.26, respectively), while pH values of chicken meat in both of the former breeds recorded significantly lower pH values ($P < 0.05$) compared with Fayoumi (5.74) and Naked Neck (5.39). The lower pH observed in some breeds may be associated with variations in pre-slaughter stress and muscle glycogen depletion, which accelerate post-mortem glycolysis and lead to rapid acidification of muscle tissue. It is well established that rapid pH decline reduces water-holding capacity due to protein denaturation and shrinkage of myofibrillar structures, resulting in increased water loss from meat [13]. These biochemical changes ultimately affect meat quality traits such as juiciness, texture, and processing yield.

3.1.2. Water holding capacity

In the present study, water-holding capacity (WHC) of meat in Fayoumi (49.73%), Black Australorp (49.25%), and Naked Neck (49.04%) was appeared statistically similar ($P > 0.05$), whereas significantly lower WHC ($P < 0.05$) was observed in Aseel meat (46.94%) (Table 2). These current findings indicate that cooking and eating qualities of meat of above said breeds did not vary to each other, while aseel exhibited comparatively inferior water retention ability. The observed variation in WHC may be associated with differences in post-mortem muscle pH decline and protein functionality. It is well established that a rapid decline in muscle pH leads to protein denaturation and reduced water-binding sites, thereby decreasing WHC, whereas a slower or moderate pH decline helps maintain higher WHC in meat. This relationship between pH and WHC has been widely documented in poultry meat science literature [13].

3.1.3. Cooking loss

Cooking loss differed significantly among the chicken breeds ($P < 0.05$) (Table 1). The highest cooking loss was observed in Aseel (27.79%) reaching a peak followed by Naked Neck (26.88%), Fayoumi (25.89%), and Black Australorp (24.44%). The greater cooking loss recorded in Aseel meat may be attributed to its comparatively lower pH and reduced water-holding capacity, as these factors are closely associated with increased moisture loss during thermal processing. Lower muscle pH accelerates protein denaturation, reducing the ability of muscle proteins to retain water and consequently increasing cooking loss [13]. The present findings also suggest that breed-related differences in muscle characteristics contributed to the observed variation in cooking loss. Previous studies have reported that slow-growing and indigenous chicken breeds often exhibit greater cooking loss than improved or fast-growing breeds due to differences in muscle fiber characteristics and post-mortem biochemical changes [18]. These observations are consistent with the relatively higher cooking loss recorded in the Aseel breed in the present study.

3.1.4. Drip loss

The drip loss in meat of Aseel (3.22%) recorded highest followed by Black Australorp (3.21%), Naked Neck (3.07%) and Fayoumi (2.81%), nevertheless the variations between them appeared non-significant (P > 0.05) (Table 1). In fact, the breeds under present investigation included with indigenous breeds though categorized under slow-growing genotypes, and in several studies, it was established that slow-growing chicken demonstrated higher drip loss [19-21]. Drip loss is largely influenced by the water-holding capacity and the extent of post-mortem protein denaturation, both of which are associated with muscle pH decline. Previous studies [22] have demonstrated that breed and growth rate can influence drip loss through their effects on muscle structure and post-mortem biochemical changes, although these differences are not always statistically significant. Results of current study agree with findings of Rai *et al*. [22] who described non-significant variations in the moisture and dry matter contents of thigh meat regardless of the level of dietary fat supplementation.

3.2. Nutritional Quality of Meat of various Chicken Breeds (Fayoumi, Black Australorp, Naked Neck and Aseel)

3.2.1. Dry matter content

Dry matter content of meat showed significant variation among the chicken breeds (Table 2). The highest dry matter content was recorded in Fayoumi (30.54%), followed by Naked Neck (28.60%), Black Australorp (28.36%), and the lowest in Aseel (25.99%). Black Australorp and Naked Neck exhibited statistically similar dry matter values (P > 0.05). However, in former both breeds existed significantly higher dry matter content compared with Aseel, while Fayoumi showed the highest overall value among all breeds (P < 0.05).

The observed higher dry matter content in Fayoumi meat may be due to associated with breed-related differences in carcass composition and nutrient deposition. Similar variations in meat composition due to genetic differences among chicken breeds have been reported in poultry studies, where genotype significantly influenced proximate composition and carcass traits [18, 23].

3.2.2. Protein content

The protein content of meat differed significantly among the chicken breeds (P < 0.05) (Table 2). The Fayoumi meat exhibited the highest protein content (21.88%) followed by Black Australorp (21.16%), Naked Neck (20.92%), and Aseel (19.91%). The superior protein content observed in Fayoumi may be attributed to breed-related genetic differences in muscle development and nutrient deposition.

Table 1. Physical attributes in meat of various chicken breeds.

Variables	Breed				LSD (0.05)	SE
	Fayoumi	Black Australorp	Naked Neck	Aseel		
pH value	5.74 ± 0.1^{A}	5.18 ± 0.21^{C}	5.39 ± 0.1^{B}	5.26 ± 0.24^{C}	0.0971	0.0474
Water Holding Capacity (%)	49.73 ± 0.64^{A}	49.25 ± 1.19^{A}	49.04 ± 5.19^{A}	46.94 ± 1.93^{B}	1.4952	0.7299
Cooking Loss (%)	25.89 ± 0.73^{C}	24.44 ± 1.20^{D}	26.88 ± 3.67^{B}	27.79 ± 1.91^{A}	0.6211	0.3032
Drip loss (%)	2.81 ± 0.56^{A}	3.21 ± 0.55^{A}	3.07 ± 0.41^{A}	3.22 ± 0.57^{A}	0.4094	0.1999

Note: Different Alphatical mentioned as supersript on each value represent the different grouping.

In fact, the variation in proximate composition of meat of various chicken breeds probably regarded with many factors like species, breed, muscle type, sex, age, and method of processing of carcasses [24]. Among these, breed is considered one of the major determinants of meat composition, as genetic differences affect muscle growth and protein accretion. Similar breed-dependent differences in meat protein content have been reported in previous studies, confirming the significant influence of genotype on the nutritional quality of poultry meat [18, 25].

3.2.3. Fat content

The fat content of meat differed significantly among the chicken breeds ($P < 0.05$) (Table 2). Moreover, percent of fat content in meat of Fayoumi meat contained the highest fat content (5.13%), followed by Black Australorp (4.13%) and Naked Neck (4.34%), which did not differ significantly ($P > 0.05$). The lowest fat content was observed in Aseel (3.03%). These findings indicate that breed-related genetic differences influence lipid deposition in muscle tissue, resulting in variations in intramuscular fat content. These findings indicate that breed-related genetic differences influence lipid deposition in muscle tissue, resulting in variations in intramuscular fat content. Similar breed-dependent differences in meat fat content have been widely reported and are primarily attributed to genetic background, age, nutrition, muscle type, and rearing system [18]. Likewise, Jaturasitha *et al*. [25] demonstrated that genotype significantly affects the nutritional quality and proximate composition of chicken meat. The present findings are consistent with earlier studies reporting significant variation in fat content among different chicken breeds [26]. In one of the studies, significant ($P < 0.05$) variation were reported in the fat percent of commercial chicken broiler meat against local chickens [26]. While another study indicated that fat content in native broilers varied over commercial broilers, and the inconsistency in this context probably be attributed with the unique compositional characteristics of chicken meat [27]. Therefore, the higher fat content observed in Fayoumi meat in the present study may reflect its inherent breed characteristics and nutrient deposition pattern.

3.2.4. Ash content

In Ash content differed significantly among the chicken breeds ($P < 0.05$) (Table 2). In the current study ash content in meat of Fayoumi breed exhibited the highest ash content (1.53%), followed by Naked Neck (1.35%), whereas Black Australorp (1.07%) and Aseel (1.05%) showed statistically similar values ($P > 0.05$). The higher ash content observed in Fayoumi and Naked Neck meat may indicate a relatively greater mineral content, reflecting breed-related differences in nutrient deposition. These results are in line with studies of different authors wherein similar trend of variation was reported for ash content of chicken meat [28]. Another study supported the present investigation wherein comparable variation was reported in ash content of Cobb-400 against all indigenous chicken except for Vanaraja meat [27].

4. CONCLUSIONS

The current study demonstrated that chicken breed significantly influences both the physical and nutritional quality of meat. Among the four breeds,

Table 2. Nutritional profile of meat of various chicken breeds.

Variables	Breeds				LSD (0.05)	SE
	Fayoumi	Black Australorp	Naked Neck	Aseel		
Dry matter (%)	30.54 ± 1.72^{A}	28.36 ± 1.57^{B}	28.60 ± 2.19^{B}	25.99 ± 1.00^{C}	0.4370	0.2133
Protein content (%)	21.88 ± 2.37^{A}	21.16 ± 1.85^{B}	20.92 ± 2.38^{B}	19.91 ± 1.43^{C}	0.3756	0.1833
Fat content (%)	5.13 ± 1.07^{A}	4.13 ± 0.53^{B}	4.34 ± 0.40^{B}	3.03 ± 0.37^{C}	0.2472	0.1207
Ash content (%)	1.53 ± 0.35^{A}	1.07 ± 0.18^{C}	1.35 ± 0.07^{B}	1.05 ± 0.21^{C}	0.0754	0.0368

Note: Different Alphatical mentioned as supersripts on each value represent the different groupings.

meat obtained from the Fayoumi breed showed a significantly higher ultimate pH (5.74) compared to the other breeds, which is indicative of a slower post-slaughter acidification process. This moderate pH decline was associated with improved water holding capacity (49.73%) and reduced cooking loss (25.89%) and drip loss (2.81%). These parameters are critical determinants of meat tenderness, juiciness, and overall consumer acceptance, suggesting that Fayoumi meat is less disposed to moisture loss during processing, storing, and cooking, thereby retaining greater succulence and yield. From a nutritional point of view, Fayoumi meat was notably higher, containing the highest levels of protein (21.88%), fat (5.13%), ash/mineral content (1.53%), and dry matter (30.54%) when compared with Black Australorp, Naked Neck, and Aseel breeds. The elevated protein and mineral content improves its dietary value for human nutrition, while the moderate fat level contributes to palatability and energy density without being excessive. In contrast, meat from the Aseel breed, despite being traditionally valued for its flavour and texture showed the lowest physical and nutritional quality in this study. It recorded the lowest water holding capacity (46.94%), highest cooking loss (27.79%), and minimal protein (19.91%), fat (3.03%), ash (1.05%), and dry matter (25.99%) contents. These characteristics may result in drier, less juicy meat with reduced nutritional density, making it less suitable for consumers seeking high quality, nutrient rich poultry meat. The Black Australorp presenting relatively better cooking loss (24.44%) and protein content (21.16%), while Naked Neck demonstrated moderate performance across all traits. In conclusion, among the four chicken breeds assessed, the Fayoumi breed stands out as the most promising contender for producing nutritious, healthy, and technologically superior meat. Its favourable combination of high water holding capacity, low cooking and drip losses, and rich content of protein, fat, minerals, and dry matter makes it a breed of choice for consumers, nutritionists, and poultry producers aiming to meet the growing demand for premium quality chicken meat. Future research may explore the underlying genetic and metabolic mechanisms contributing to these breed-specific meat quality traits, as well as the performance of Fayoumi meat under different rearing systems and processing conditions.

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6. ETHICAL STATEMENT

The research protocol and all experimental procedures were reviewed and approved by the Directorate of Advanced Studies and Research (DASR), Sindh Agriculute University Tando Jam. All birds handling, management practices, and experimental procedures were carried out in strict accordance with institutional ethical guidelines for the care and welfare of experimental animals.

7. FUNDING

No funding was received to conduct this research.

8. CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this manuscript.

9. AUTHORSHIP CONTRIBUTION STATEMENT

Arshad Ali Khaskheli: Conceptualization, Methodology, Investigation (conducted the research experiment), Data Curation, and Original Draft Preparation and Writing. Nasir Rajput: Supervision, Review & Editing. Ghulam Shabir Barham: Investigation (assisted in conducting the research experiment) and Methodology. Gul Bahar Khaskheli: Formal Analysis, Validation, and Writing, Review & Editing. Uzma Khatiyan: Resources, Literature Review, and Validation. Asad Ali Khaskheli: Visualization, Data formatting, and final proofreading.

10. DECLARATION

All authors declare that the results of the present study are original and these are neither published and are nor under consideration for publication elsewhere. Approval of all authors has been obtained and incase the article is accepted for publication, its copyright will be assigned to the Pakistan Academy of Sciences. Further, no any copyrighted material from other sources has been used in this article.

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Research Article

Variation and Correlation of Photosynthetic Pigments in Spring Wheat Cultivars under Controlled Growth Conditions

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Abstract: Photosynthetic pigments are an important factor in determining the physiological status and the efficiency of the photosynthetic process in plants. This study aimed to determine the contents of chlorophyll a, chlorophyll b, total chlorophyll (a + b), and carotenoids within the leaves of thirteen cultivars of spring wheat. Wheat seeds were grown under controlled conditions, and photosynthetic pigments were extracted using acetone. Their concentrations were measured using a spectrophotometer at different wavelengths. The data were evaluated using descriptive statistics and one-way analysis of variance (one-way ANOVA). The results indicated the presence of variation in the values of concentrations of photosynthetic pigments in the studied wheat cultivars. The highest chlorophyll a and carotenoid contents were recorded in cultivar Voevoda, while the highest chlorophyll b concentration was observed in cultivar L505. The two cultivars Lebedushka and Alexandrit showed lower levels of chlorophylls and carotenoids. A statistically significant difference was detected only for chlorophyll a, whereas chlorophyll b and carotenoids did not differ significantly among cultivars. Also, the study showed that the Pearson correlation coefficient between chlorophyll pigments was strongly positive, and the relationship of chlorophylls with carotenoids was moderately positive. The results showed variation in the content of photosynthetic pigments among the studied wheat cultivars under controlled conditions. The presence of a higher concentration of photosynthetic pigments in the Voevoda and L505 cultivars may indicate the presence of greater accumulation of these pigments and the possibility of photoprotection.

Keywords: Photosynthetic Pigments, Chlorophyll a and b, Carotenoids, Wheat Cultivars, *Triticum aestivum*, Physiological Variability, Plant Physiology.

1. INTRODUCTION

Chlorophylls (a and b) and carotenoids are considered as the photosynthetic pigments responsible for light absorption and play an important and central role (especially chlorophyll a and b) in absorbing light and carrying out the process of photosynthesis, while carotenoids function in protecting plants from oxidative damage resulting from excessive light intensity and are usually found in the chloroplasts of green leaves [1-3]. Therefore, the content of these pigments is considered an indicator of photosynthetic activity and the physiological status of the plant [4, 5].

Plants differ in photosynthetic pigment composition among cultivars of the same species due to differences in genetic composition, which

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lead to variations in leaf anatomy, chloroplast development, and metabolic activity [6-9]. Cultivar specificity is evident from early developmental stages, as significant differences in embryonic leaf growth and morphophysiological traits have been reported among spring wheat cultivars [10]. This variation may indicate the presence of differences in pigment composition and physiological responses to environmental conditions among the cultivars [11-13]. These variations represent an important source for selecting cultivars with higher pigment content and physiological performance [14, 15].

Wheat is considered a major agricultural crop worldwide and makes an important contribution to global food security [16-18]. Therefore, improving photosynthetic efficiency is considered an important strategy for increasing food productivity [19-21]. Recent studies confirmed the importance of photosynthetic pigments as indicators of plant physiological performance, and showed that variation in the concentration of chlorophyll and carotenoids may be associated to the photosynthetic activity and consequently with physiological performance [3]. Despite these developments, comparative studies on the concentrations of photosynthetic pigments among wheat cultivars, especially under laboratory conditions, are still limited. Information regarding pigment variation among Saratov spring wheat cultivars grown under identical controlled conditions remains scarce.

Photosynthetic pigments, especially chlorophylls (a and b) and carotenoids, are physiological indicators of great importance for the plant. Therefore, this study aimed to determine the variation in the levels of chlorophyll a, chlorophyll b, and carotenoids in spring wheat cultivars (*Triticum aestivum*) under controlled conditions. It also aimed to assess the physiological diversity among the cultivars and to determine the cultivars with different pigment accumulation patterns. In addition, correlations among photosynthetic pigments were evaluated.

2. MATERIALS AND METHODS

2.1. Experimental Plant Material and Conditions

Thirteen spring wheat (*Triticum aestivum* L.) cultivars (L505, L505 656/11, L503, L503 Lr19+Lr26, Belyanka, Dobrynya, Dobrynya Lr19+Lr37, YuV-2, Favorit, Voevoda, Lebedushka, Prokhorovka, and Alexandrit) were used in this study to evaluate and compare photosynthetic pigment content. These cultivars were selected because they represent spring wheat cultivars developed in the Saratov breeding program and were available for comparative evaluation of photosynthetic pigment variation under controlled laboratory conditions. Seeds were germinated on filter paper and seedlings were grown in a growth chamber at 18 °C under a 16 hours light and 8 hours dark photoperiod with an illumination intensity of 5000 lux. Relative humidity was not actively controlled and ranged between approximately 40–60%. Leaf samples were collected at the three-leaf stage (BBCH 13), and the first and second fully expanded leaves were used for pigment analysis. Each treatment consisted of four biological replicates.

2.2. Extraction of Photosynthetic Pigments

The central part of the leaf blade (0.07 - 0.08 g fresh weight) was used to determine the concentration of photosynthetic pigments [22]. The samples were ground well in a porcelain mortar with (2-4 ml) of 100% pure acetone, in addition to quartz sand and calcium carbonate (chalk). After that, the extract was filtered through a glass filter (No. 3) into a Büchner flask connected to a vacuum pump. Then, the obtained solution was transferred to a 25 mL volumetric flask, and the volume was made up to the mark by adding pure acetone [22].

2.3. Spectrophotometric Determination of Pigments

The content of chlorophyll a, chlorophyll b and carotenoids were determined according to the method described by Lew [22]. The absorbance (E) was measured at the wavelengths 662, 644 and 470 nm. As for the pigment concentration (C), it was calculated according to the following equations [22]:

$$\text{Chlorophyll a (mg L}^{-1}) = 9.784 \times E_{662} - 0.990 \times E_{644}$$

$$\text{Chlorophyll b (mg L}^{-1}) = 21.426 \times E_{644} - 4.650 \times E_{662}$$

$$\text{Carotenoids (mg L}^{-1}) = (1000 \times E_{470} - 1.9 \times C\text{ (chlorophyll a)} - 63.14 \times C\text{ (chlorophyll b)}) / 214$$

2.4. Calculation of Pigment Content

The pigment content was calculated based on the extract volume and the sample fresh weight according to the following equation [22]:

$$A = \frac{(C \times V)}{P}$$

A represents the pigment content expressed on the sample fresh weight, *C* represents the concentration of the sample (mg g^{-1} fresh weight), *V* represents the extract volume (ml), and *P* represents the sample fresh weight (g).

2.5. Statistical Analysis

Data are expressed as mean ± standard deviation (SD). The assumptions of normality and homogeneity of variance were evaluated using the Shapiro–Wilk and Levene's tests in Jamovi before selecting the appropriate statistical test. One-way ANOVA was applied when the assumptions were met, whereas Welch's ANOVA or the Kruskal–Wallis test was used when the assumptions were not met. Statistical significance was set at $p \leq 0.05$. In addition to this, the Pearson correlation coefficient was calculated between the photosynthetic pigments to determine the nature of the relationship between them.

3. RESULTS AND DISCUSSION

The results indicated variation in the concentrations of photosynthetic pigments among the 13 spring wheat cultivars, as shown in Figures 1-4. The clear variability in the levels of photosynthetic pigments among the cultivars (with a statistically significant difference detected only for chlorophyll a) may reflect the presence of underlying physiological differences. This may be due to differences in genetic background, differences in chloroplast development, as well as metabolic activity. This has been reported in previous studies that indicated that variation in pigment content is associated with differences in photosynthetic activity under laboratory conditions [6]. The values of chlorophyll-a concentrations ranged between 2.96 mg g^{-1} FW in the cultivar Voevoda and 1.94 mg g^{-1} FW in the cultivar Alexandrit (Figure 1). A significant difference among cultivars was detected for chlorophyll a (Kruskal–Wallis, $p = 0.040$).

The highest value of chlorophyll b concentration for cultivar L505, where it reached (1.21 mg g^{-1} FW), and the lowest value was from cultivar Lebedushka (0.6 mg g^{-1} FW) (Figure 2).

Carotenoids also showed variation in their results, where the highest value was for the cultivars Voevoda (0.97 mg g^{-1} FW) and L505 (0.95 mg g^{-1} FW), and the lowest concentrations were for the cultivar Lebedushka (0.69 mg g^{-1} FW) and Alexandrit (0.70 mg g^{-1} FW) (Figure 3). However, the observed variation was not statistically significant (Kruskal–Wallis, $p = 0.166$).

As for total chlorophyll, the highest concentration was for the cultivar L505 (3.91 mg g^{-1} FW) and the cultivar Voevoda (3.85 mg g^{-1} FW), while the lowest concentrations were for the cultivars Lebedushka (2.56 mg g^{-1} FW) and Dobrynya Lr19+Lr37 (2.68 mg g^{-1} FW) (Figure 4).

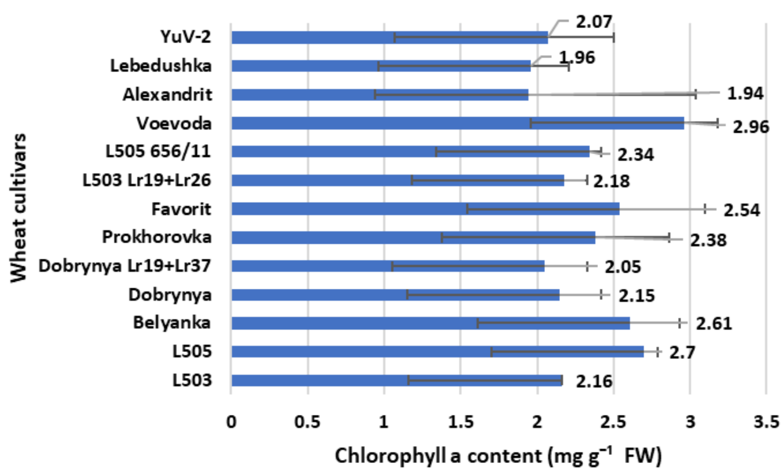

Fig. 1. Mean (± SD) values of chlorophyll a content in leaves of thirteen wheat cultivars.

Total chlorophyll reflects the chlorophyll status of the leaf, as it represents the combined contribution of chlorophyll a and b [3]. Despite the numerical differences, the variability within cultivars in the values, as shown in Figures 1- 4, may reflect physiological stability. The values indicated that the least variable cultivars were Favorit and L503 Lr19+Lr26, and this may indicate stability of pigment accumulation in them, while the highest variability in values was shown by the cultivars Voevoda and Dobrynya Lr19+Lr37, which may reflect greater variability in pigment accumulation. The higher chlorophyll a and carotenoid contents observed in the cultivar Voevoda may reflect cultivar-specific genetic characteristics that promote the accumulation of these pigments under controlled laboratory conditions. Genetic differences among cultivars have previously been associated with variation in chlorophyll content and photosynthesis-related traits, even when the plants are grown under identical environmental conditions [6]. Overall, a statistically significant difference was detected only for chlorophyll a, whereas chlorophyll b and carotenoids did not show statistically significant differences among cultivars. Although chlorophyll a showed a statistically significant difference among cultivars, chlorophyll b and carotenoids did not differ significantly ($p > 0.05$). This may be due to the controlled laboratory conditions that reduce differences in physiological performance, as well as the similarity in genetic and structural characteristics among some cultivars [9, 23].

Descriptively, the cultivars can be grouped according to their pigment content into high pigment cultivars (Voevoda, L505, and Belyanka), medium pigment cultivars (L503, Dobrynya, YuV-2, L505 656/11), and low pigment cultivars (Alexandrit and Lebedushka). The high content of photosynthetic

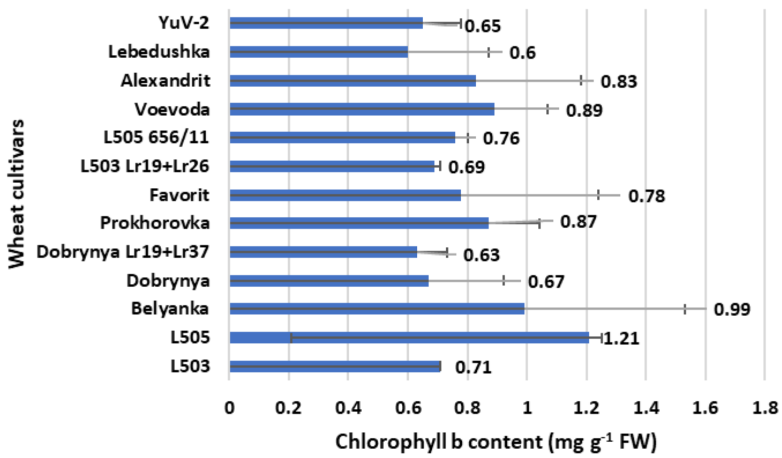

Fig. 2. Mean (± SD) values of chlorophyll b content in leaves of thirteen wheat cultivars.

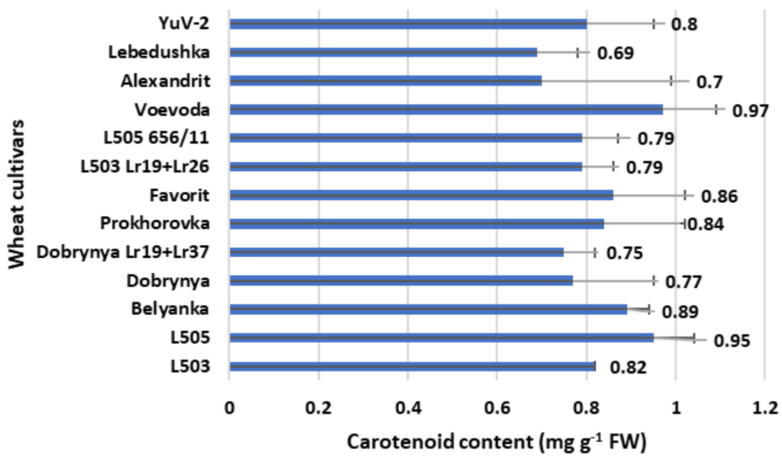

Fig. 3. Mean (± SD) values of carotenoid content in leaves of 13 wheat cultivars.

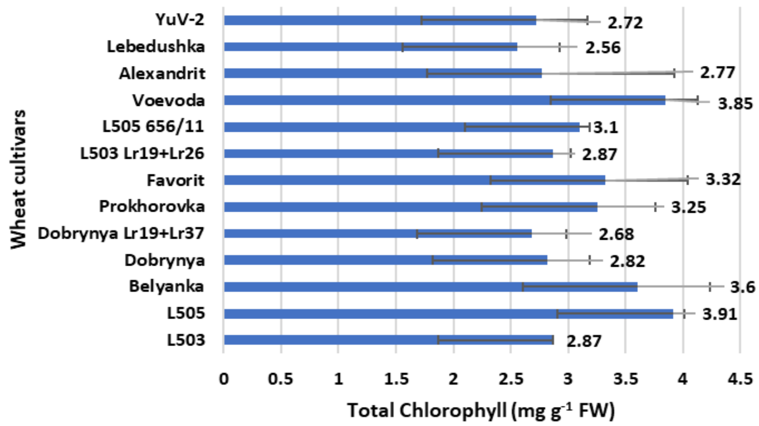

Fig. 4. Mean (± SD) values of total chlorophyll (chlorophyll a + b) content in leaves of 13 wheat cultivars.

pigments, especially total chlorophyll, may indicate a greater pigment accumulation, which has been reported to be associated with biomass and productivity in previous studies [3, 7]. Chlorophyll pigments play a main role represented by light absorption and energy transfer during the process of photosynthesis, while carotenoids play a central role in dissipating excess energy as well as protecting the photosynthetic apparatus from oxidative damage. Therefore, the increase in pigment content may reflect an improvement in the light absorption system and protection [6]. As for carotenoids, their high concentration indicates efficiency of photoprotection, meaning that the higher their concentration, the greater the protection and thus the increased ability of the plant to tolerate harsh conditions [3, 2].

Correlation analysis showed the presence of a coordinated relationship among photosynthetic pigments (Table 1). Chlorophyll a showed a strong positive correlation with chlorophyll b (r = 0.78). There was also a strong correlation between total chlorophyll and chlorophyll a (r = 0.96) and chlorophyll b (r = 0.94). While the correlations between carotenoids and chlorophyll a (r = 0.67), chlorophyll b (r = 0.72), and total chlorophyll (r = 0.75) were moderate. This indicates the presence of functional coordination between light absorption pigments and photoprotective pigments [24]. These results suggest coordinated relationships among photosynthetic pigments under controlled laboratory conditions [24].

The strong correlations between chlorophyll a, chlorophyll b, and total chlorophyll reflect the functional and structural coordination in the work of the photosynthetic apparatus, as these pigments are associated within light-harvesting complexes. As for the moderate correlation between carotenoids and chlorophylls, it may be due to the dual role of carotenoids in light harvesting and photoprotection, which indicates the presence of a partially independent regulatory mechanism [24].

4. CONCLUSIONS

The present study revealed differences in photosynthetic pigment concentrations among 13 spring bread wheat cultivars under controlled laboratory conditions. These differences may be due to physiological differences in the genetic composition of the cultivars. The variation in chlorophyll a, chlorophyll b, carotenoids,

Table 1. Pearson correlation coefficients among photosynthetic pigments.

Parameter	Chl. a	Chl. b	Total Chl.	Carotenoids
Chl. a	1	0.78	0.96	0.67
Chl. b	0.78	1	0.94	0.72
Total Chl.	0.96	0.94	1	0.75
Carotenoids	0.67	0.72	0.75	1

and total chlorophyll indicates variation in pigment accumulation among cultivars, their light-harvesting characteristics, as well as photoprotective capacity.Cultivars Voevoda, L505, and Belyanka showed high pigment content, which may indicate a higher pigment levels. In contrast, cultivars such as Alexandrit and Lebedushka showed lower pigment concentrations, which may indicate relatively lower pigment content. In addition, the differences among cultivars reflect variation in physiological stability under different environmental conditions. The strong positive correlations between chlorophyll a, chlorophyll b, and total chlorophyll, as well as the moderate positive correlations between chlorophylls and carotenoids, suggest the presence of physiological coordination between photosynthetic systems and photoprotective systems. The coordination existing between these systems reflects the presence of a physiological system that may be associated with the physiological characteristics of these cultivars. This study is of importance in selecting cultivars with a better genetic composition and higher ability in the process of photosynthesis as well as photoprotection. We recommend conducting more studies on these cultivars under field conditions and stress conditions to inform their performance, which helps in supporting targeted breeding as well as assisting in selecting high-quality cultivars.

5. ETHICAL STATEMENT

This study did not involve any human or animal participants; therefore, ethical approval was not required.

6. FUNDING

The research was carried out without any external financial support.

7. AUTHOR CONTRIBUTIONS

Oras Abdulsayed Mahdi developed the study concept, conducted the experimental work, performed the data analysis, and drafted the manuscript. M.J. Aljozary contributed to the experimental design and manuscript editing. Ali Obaid performed the statistical analysis, assisted with the interpretation of the findings, and assisted in manuscript preparation. M.Y. Kasatkin supervised the research work and critically reviewed the manuscript.

The authors confirm the use of generative AI tools only to improve the language and grammar of the manuscript and to facilitate its readability. These tools played no role in the preparation of the scientific content, analysis of the data, interpretation of the results, or the drawing of the conclusions. The authors retain full responsibility for the correctness and integrity of the manuscript.

8. CONFLICTS OF INTEREST

The author declares no conflicts of interest.

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Research Article

Comparative Antimicrobial Activity of Plant-Derived Silver Nanoparticles Targeting Clinically Obtained MDR Bacterial Isolates

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Abstract: The overuse and misuse of antibiotics have contributed to the emergence of multidrug-resistant (MDR) bacteria, causing a serious threat to global healthcare systems. This research evaluated the antimicrobial activity of silver nanoparticles (AgNPs) against clinically isolated bacterial pathogens collected from tertiary care hospitals in Lahore, Pakistan. A total of 165 samples were collected from hospital settings and equipment, from which four MDR strains were identified: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Acinetobacter baumannii.* Five AgNPs were synthesized via sunlight-assisted green synthesis using aqueous extracts of *Salvadora persica*, *Aloe barbadensis* Miller, *Cinnamomum camphora*, *Shorea robusta*, and *Cymbopogon citratus*. Nanoparticle formation was confirmed by characteristic color change, and optical characterization was performed using UV-visible spectrophotometry. Antimicrobial activity was evaluated using the disc diffusion method on Mueller-Hinton agar. All synthesized AgNPs showed significant inhibitory effects against MDR isolates, with *Shorea robusta* and *Salvadora persica* demonstrating the highest efficacy. These findings demonstrate the potential of green-synthesized AgNPs as a cost-effective and sustainable alternative for controlling nosocomial infections. Future work should focus on clinical trials and dosage to ensure their safety.

Keywords: Antibiotics, Multidrug-resistant (MDR) Microbes, Eco-friendly Synthesis of Silver Nanoparticles, Nosocomial Infections, Disc Diffusion Method.

1. INTRODUCTION

Antibiotic-resistant bacteria, especially second and third-line wide-spectrum antibiotic-resistant strains, pose a serious and growing threat to the healthcare systems in both public and tertiary institutions worldwide, contributing to increased mortality and associated morbidity. In a World Health Organization (WHO) report from 2019, over 700,000 people die annually as a result of antibiotics ineffectiveness against rapidly evolving resistant bacterial strains, putting immunocompromised individuals at an even higher risk of unfavorable findings and complications of treatment. This supports the requirement for ongoing focus in healthcare settings and the development of creative planning for better managing this multitude of intricate challenges, namely, combating the multidrug-resistant (MDR) microbes without unfavorable long-term effects [1]. Two main factors for the emergence of antibiotic-resistant bacteria in hospital populations and animal farms are increased antibiotic usage by humans and the use of antibiotics in animal agriculture [2].

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Silver nanoparticles (AgNPs) have been in great demand, resulting in rapid growth of nanotechnology since they have been shown to be significant in many industries like textiles, home appliances, and pharmaceutical products. Due to their distinctive physicochemical and antibacterial characteristics, AgNPs are particularly significant in a variety of sectors, including wound care management [3]. Via a special mode, like adsorption to a bacterial facet, nanoparticles can significantly enhance an element's antibacterial activity [4].

Nanoparticles range in size from 1 to 100 nanometers; owing to their nanoscale dimensions, they possess different properties than the bulk materials [5]. Nanotechnology encompasses the design, synthesis, and characterization of materials at the nanoscale, employing both top-down and bottom-up approaches to control particle size, structure, and morphology for applications across catalysis, medicine, energy, and other sectors [6]. It includes the fabrication of nanostructures through diverse synthesis approaches, where the resulting structure and morphology are strongly dependent on the specific technique and growth mechanism employed [7]. Just like small biomolecules and polynucleic acids, nanoparticles possess uniqueness in their features and utilization due to a number of special characteristics. Additionally, divergent metals and semiconductor core materials that impart beneficial features like luminescence and magnetic behavior can be used for nanoparticle formation [8].

AgNPs have the tendency to attach with the surface of living cells that ultimately leads to vital function abruption, making these NPs as potent antibacterial agents. These functions may hinder the activity of respiratory chain enzymes, cell membrane permeability, pits and gaps leading to cell death [7]. Owing to these properties silver NPs have become highly applied in a variety of products, including but not limited to cosmetics, textiles, and medical equipment [8]. A basic method to assess AgNPs' antibacterial potential is to measure the amount of bacterial growth that is inhibited [9].

For the mechanism studies, the morphology alterations in bacteria treated with AgNPs were regularly characterized using various techniques such as fluorescent and electron microscopy that give evidence on intracellular content leakage, cellular membrane disorganization, and NP localization [10]. Recent research by Klabunde and colleagues has studied how nanoparticles play an important role in bactericidal effects on gram-negative and gram-positive bacteria [11]. It is widely recognized that silver ions and compounds with silver as an ingredient are extremely harmful to bacteria [12]. The antibacterial effects of AgNPs on *E. coli* were tested, and they were found to follow a truncated triangular form, firstly, after that a spherical shape, and then rod-shaped silver ions [13].

Despite studies on eco-friendly synthesized AgNPs, most have been conducted under controlled laboratory conditions using standard bacterial strains. There is a lack of localized clinical data on the effectiveness of AgNPs against nosocomial MDR bacteria, especially those isolated from real hospital settings, such as Lahore. Moreover, most prior studies focus on a single plant extract and lack comparative evaluation of multiple plant-derived AgNPs against MDR clinical isolates.

Recent investigations have further highlighted the need for clinically validated, locally sourced plant-derived nanoparticles as eco-friendly antimicrobial agents against nosocomial MDR pathogens [14]. The objective of this research was to evaluate the comparative antimicrobial activity of five plant-derived AgNPs against clinically isolated MDR bacterial strains from tertiary care hospitals in Lahore, Pakistan, as a sustainable and cost-effective strategy for nosocomial infection control.

2. MATERIALS AND METHODS

2.1. Sample Collection

Samples (n = 165) were collected with sterile cotton swabs from hospital equipment and surfaces across multiple wards of tertiary care hospitals in Lahore, including medical emergency, surgical emergency, pulmonology outpatient department (OPD), surgical intensive care unit (SICU), general outpatient department (GOPD), medical intensive care unit (MICU), urology ward, eye ward, gynae ward, pathology department, and blood bank settings. Isolates were considered nosocomial pathogens on the basis of their recovery from hospital surfaces and equipment, followed by microbiological confirmation through biochemical testing and the VITEK 2 automated system.

2.2. Media Preparation and Bacterial Isolation

Blood agar, MacConkey agar, and Mueller-Hinton agar were prepared by using standard microbiological protocols [15-17]. Swab samples were plated on blood agar and MacConkey agar and incubated at 37 °C for 24 hours. For isolated colonies, repeated streaking was performed, and for primary characterization, colony morphology, including size, form, elevation, color, and texture was recorded.

2.3. Identification of Pathogens

Gram staining was performed according to standard protocols [18] to differentiate gram-negative from gram-positive pathogens. Biochemical identification included catalase, oxidase, motility, triple sugar iron (TSI), urease, and citrate utilization tests [19]. Final bacterial confirmation was performed using the VITEK 2 automated microbiology system (bioMérieux, Marcy-l'Étoile, France).

2.4. Antimicrobial Susceptibility Testing (AST)

Using the Kirby-Bauer disc diffusion method, the AST test was performed on Mueller-Hinton agar [20]. The antibiotics used are AMC (Amoxicillin Clavulanic acid, 30 µg), CN (Gentamicin 120 µg), AM (Ampicillin, 10 µg), CRO (Ceftriaxone, 30 µg), CLR (Clarithromycin, 15 µg), CIP (Ciprofloxacin, 5 µg), CEP (Cefepime, 30 µg), IPM (Imipenem, 10 µg), E (Erythromycin, 15 µg), FOX (cefoxitin, 30 µg), B (Bacitracin, 0.04 IU) and AK (Amikacin, 30 µg).

According to the internationally accepted definitions, pathogens showing resistance to two or more classes of antibiotics are considered multidrug-resistant (MDR) microbes [21]. Zones of inhibition were measured in millimeters and evaluated according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [22]. All tests were performed in triplicate.

2.5. Preparation of the Silver Nitrate ($AgNO_3$) Solution

A 10 mM stock solution of $AgNO_3$ was prepared by dissolving 0.845 grams of $AgNO_3$ (Sigma-Aldrich, St. Louis, MO, USA) in 500 mL of distilled water. 1 mM, 5 mM, and 10 mM of working solutions were prepared from the stock solution and stored in a reagent bottle covered with aluminum foil to avoid photochemical degradation.

2.6. Green Synthesis of AgNPs

A total of five plant extracts were prepared by washing, drying, and boiling plant material (10-20 g) in 100-150 mL distilled water for 10-15 minutes. The resulting mixture was filtered through Whatman filter paper (Whatman International Ltd., Maidstone, UK), centrifuged at 5000 rpm (GYROZEN Co., Ltd., Gimpo, South Korea), and stored at 4 °C. *S. persica* (roots), *A. vera* (leaves), *C. camphora* (leaves), *S. robusta* (resin), and *C. citratus* (leaves) were used. AgNPs were produced by combining each plant extract with $AgNO_3$ in predefined ratios (1:1, 7:3, or 9:1), and then the mixtures were exposed to sunlight for 5-30 minutes [23]. Nanoparticle formation was confirmed when the color changed from transparent to dark brown or reddish brown [24]. The synthesized AgNPs were purified by centrifugation at 5000 rpm and washing with distilled water three times, followed by drying at 50 °C overnight and resuspension in distilled water for further use.

2.7. Characterization of Green Synthesized AgNPs

A UV-visible nanodrop spectrophotometer (Colibri LB 915, Berthold Technologies, Bad Wildbad, Germany) was used for optical characterization over a wavelength range of 200-800 nm, with distilled water used as blank [8]. AgNPs formation was confirmed by a surface plasmon resonance peak at 400-500 nm.

2.8. Antimicrobial Ability of AgNPs

The antimicrobial ability of AgNPs was evaluated against *A. baumannii*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*, which were isolated from clinical samples. On Mueller-Hinton agar (MHA), the disc diffusion method was carried out [20]. Whatman® filter paper discs (Whatman International Ltd., Maidstone, UK) were loaded with 10 µL and 20 µL volumes of AgNPs prior to a 37 °C incubation for 24 hours. The tests were performed three times, and zones of inhibition were recorded in mm.

3. RESULTS

3.1. Isolation and Screening of MDR Bacteria

Collectively, out of 165 samples, 22 (13.3%) yielded positive bacterial growth, while 143 (86.7%) were negative. From the 22 positive samples, 37 distinct bacterial colonies were isolated, of which 4 were identified as MDR gram-negative bacteria. Some of the bacterial isolates are shown and their purification are shown in Figure 1, where primary cultures on blood agar showed various colony morphologies, such as changes in hemolysis, shape, size, and pigmentation. In addition, on MacConkey agar, differential growth patterns were analyzed, including lactose-fermenting and non-lactose-fermenting colonies (Figure 1(a)). Essentially, pure cultures were successfully isolated for AST, where MacConkey agar showing heterogeneous growth with lactose-fermenting and non-lactose-fermenting bacteria, exhibiting variations in colony morphology (Figure 1(b)). Whereas (Figure 1(c)), represents pure cultures obtained via streaking and sub-culturing of mixed colonies from primary mixed cultures, demonstrating distinct colony size, hemolytic pattern, and texture of individual bacterial species isolated from hospital samples for identification of AMR microbes. Essentially, purified gram-negative bacterial isolates obtained (Figure 1(d)) demonstrated uniform colony morphology and distinct lactose fermentation states after sub-culturing from mixed cultures.

3.2. Characterization and Identification of MDR Bacteria

There were 4 isolates selected as shown in Figure 2(a), named as S23, S29, S35, and S36 showing pale, non-lactose-fermenting colonies; colorless and non-lactose-fermenting colonies; mucoid, pink, lactose-fermenting colonies, and pink, lactose-fermenting colonies, respectively. Initial AST of all 37 isolates showed four strains resistant to two or more antibiotic classes and were classified as MDR strains. These four isolates demonstrated unique colonial morphologies on MacConkey agar, with S35 showing distinct mucoid pink colonies, while S23, S29, and S36 produced non-mucoid colonies ranging from colorless to pink (Table 1). As shown in Figure 2(b), (a) *P. aeruginosa* (S23) exhibited resistivity against amoxicillin-clavulanic acid (30 µg), ampicillin, (10 µg) and ceftriaxone (30 µg); (b) *A. baumannii* (S29) showed resistance to all antibiotics except gentamicin (120 µg); (c) *K. pneumoniae* (S35) was resistive to amoxicillin-clavulanic acid (30 µg), ampicillin (10 µg), ceftriaxone (30 µg), clarithromycin (15 µg), and ciprofloxacin (5 µg); and (d) *E. coli* (S36) also displayed resistivity towards amoxicillin-clavulanic acid (30 µg), ampicillin (10 µg), ceftriaxone (30 µg), clarithromycin (15 µg), ciprofloxacin (5 µg), and ciprofloxacin (5 µg). The MDR profile is shown in Figure 2(b), and notable zones of inhibition are shown in Table 2.

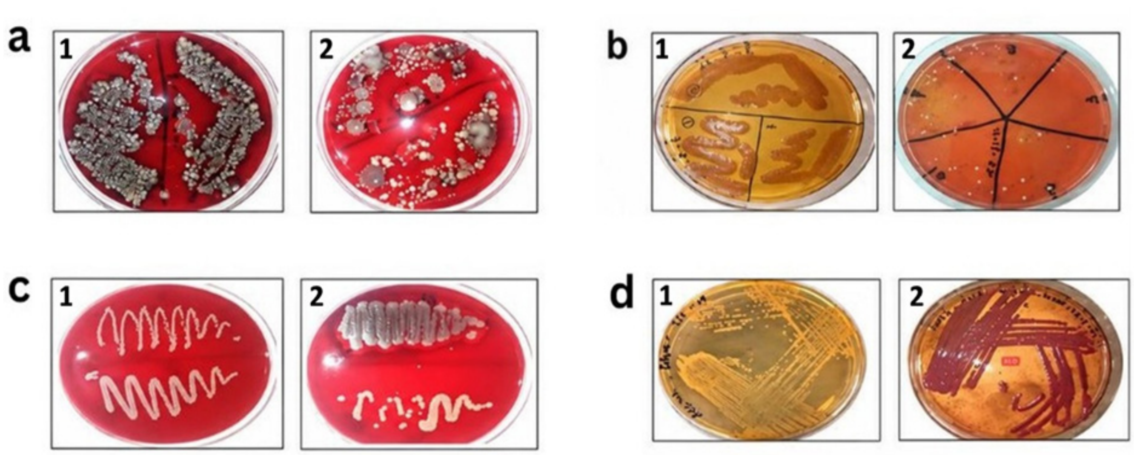

Fig.1. Isolation and purification of bacterial colonies from hospital samples. Mixed colony morphology of bacterial isolates on blood agar, demonstrating variations in colony size, shape (a-2) and pigmentation across samples (a-1) (a), bacterial isolates on MacConkey agar, (b-1) displaying non-lactose-fermenting colonies and (b-2) image displaying lactose-fermenting colonies (b), purified bacterial cultures on blood agar via streaking (1 and 2) from mixed cultures, showing distinct colony texture, size, and streaking patterns (c), and pure cultures of gram-negative bacterial isolates on MacConkey agar, (d-1) left image showing non-lactose-fermenting colonies and the right image (d-2) showing lactose-fermenting colonies (d).

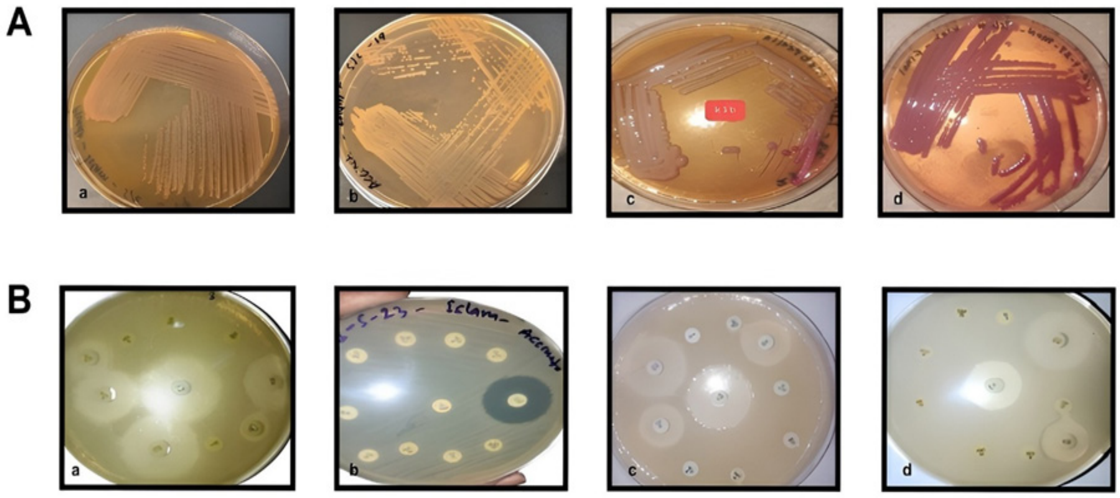

Fig. 2. Isolation and antimicrobial susceptibility testing (AST) of MDR isolates. Colony morphology of MDR isolates on MacConkey agar: [A] Isolate S23 demonstrating pale, non-lactose fermenting colonies (a), Isolate S29 showing colorless, non-lactose fermenting colonies (b), Isolate S35 showing mucoid, pink, lactose-fermenting colonies (c), and Isolate S36 displaying pink, lactose fermenting colonies (d). [B] demonstrates AST profiles of MDR by disc diffusion method. where S23 (a), S29 (b), S35 (c), and S36 (d) are showing the zones of inhibitions.

Table 1. Colony characteristics of isolated pathogens on MacConkey agar.

S. No.	Isolates	Texture	Color	Shape	Elevation
1	S23	Smooth	Colorless/Pale	Irregular	Raised
2	S29	Smooth	Colorless	Round	Raised
3	S35	Mucoid	Pink	Round	Raised
4	S36	Shiny	Red to pink	Round	Raised

Table 2. Zones of inhibition of MDR bacteria in millimeters (mm).

S. No.	Isolates	CN (120 µg)	AMC (30 µg)	AM (10 µg)	CRO (30 µg)	CLR (15 µg)	CIP (5 µg)	FOX (30 µg)	IPM (10 µg)	AK (30 µg)
1	S23	22	0	0	0	11	13	7	23	14
2	S29	18	0	0	0	0	0	0	0	0
3	S35	21	0	0	0	0	11	0	19	14
4	S36	19	0	0	0	0	0	0	16	15

Further characteristics based on gram staining and microscopy presented that four isolated MDRs were gram-negative, S23 and S36 appeared as pink/red rods and were motile, S29 appeared as pink coccobacilli and were non-motile, and S35 appeared as pink rods and were non-motile. Biochemical characterization confirmed the identities of all four isolates (Table 3) and VITEK automated identification confirmed: S23 as *P. aeruginosa*, S29 as *A. baumannii*, S35 as *K. pneumoniae*, and S36 as *E. coli*.

3.3. Synthesis of AgNPs from Plant Extracts

Synthesis of the AgNPs was successfully carried out under sunlight exposure, all five AgNPs showed characteristic brown color (Figure 3) development within 30 minutes, which confirmed the formation of nanoparticles. AgNPs based on *S. persica* changed from translucent to dark brown, *A. vera* turned from bright green to dark brown, *C. camphora* color shifted from yellow to reddish-brown, *S. robusta* $AgNO_3$ turned from translucent to vivid

Table 3. Biochemical testing.

S. No.	Isolates	Catalase test	Oxidase test	Motility test	Triple Sugar Iron test (Slant/Butt, Gas, H_2S)	Urease test	Citrate test
1	S23	Positive	Positive	Positive	K/K, CO_2: –, H_2S: –	Negative	Positive
2	S29	Positive	Negative	Negative	K/A, CO_2: –, H_2S: –	Negative	Positive
3	S35	Positive	Negative	Negative	K/K, CO_2: +, H_2S: –	Positive	Positive
4	S36	Positive	Negative	Positive	A/A, CO_2: –/+, H_2S:–	Negative	Negative

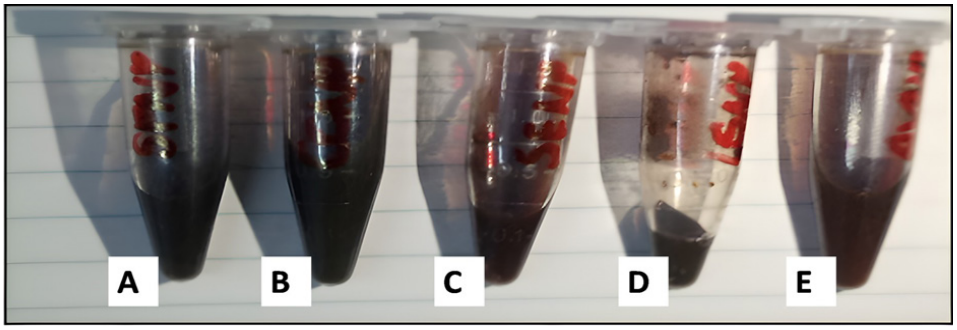

Fig. 3. Green synthesis of silver nanoparticles using five plant extracts. Characteristic color change indicating formation of nanoparticles: *Salvadora persica* (A), *Aloe barbadensis Miller* (B), *Cinnamomum camphora* (C), *Shorea robusta* (D), and *Cymbopogon citratus* (E). The color change from transparent/yellow/green to dark brown or reddish-brown confirms the reduction of silver ions to silver nanoparticles.

green, and *C. citratus* changed from light yellow to reddish-brown. Formation of all five AgNPs was further confirmed by characteristic surface plasmon resonance (SPR), peaks observed between 400-480 nm. We observed maximum absorbance at 480 nm for SpNPs, 470 nm for CcNPs, 460 nm for AvNPs, 450 nm for SeNPs, and 420 nm for LgNPs as shown in Figure 4.

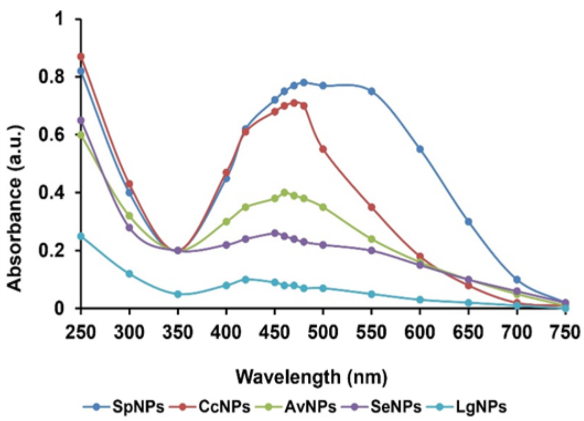

Fig. 4. UV-Visible spectrophotometer analysis of green-synthesized silver nanoparticles. Surface plasmon resonance (SPR) peaks were analyzed at 420 nm for *Cymbopogon citratus* (LgNPs), 450 nm for *Shorea robusta* (SeNPs), 460 nm for *Aloe barbadensis* Miller (AvNPs), 470 nm for *Cinnamomum camphora* (CcNPs), and 480 nm for *Salvadora persica* (SpNPs). The absorbance peaks between 400-500 nm supports the silver nanoparticles formation.

3.4. Antimicrobial Activity using AgNPs

All five green synthesized AgNPs had antimicrobial properties against all four MDR bacteria as presented in Table 4 and Figure 4. The biggest inhibition zones of SeNPs and SpNPs (9 ± 1.5 mm) were observed against *P. aeruginosa*, while the bigger zones were those of AvNPs (8 ± 1 mm), as compared to LgNPs and CcNPs (7 ± 0.5 mm). Against *A. baumannii*, inhibition zones of SeNPs (8 ± 1 mm) were highest, while others ranged between 6-7 (± 1) mm. For *K. pneumoniae*, SeNPs and AvNPs both generated 8 ± 1 mm inhibition zones. Against *E. coli*, inhibition zones of SeNPs (9 ± 1 mm) were maximum, while SpNPs and CcNPs generated inhibition zones of 7 ± 0.5 mm each. Overall, maximum antimicrobial activity was shown by SeNPs against all four MDR isolates.

4. DISCUSSION

The isolation of four MDR gram-negative pathogens (*A. baumannii, E. coli, P. aeruginosa,* and *K. pneumoniae*) from tertiary care hospital settings confirms the presence of nosocomial MDR infections in clinical environments, consistent with global reports of increasing antibiotic resistance in healthcare settings. The novelty of this study lies in the comparative analysis of five sunlight-

Table 4. Measurement of the zones of inhibition of synthesized AgNPs in mm on Mueller-Hinton agar.

S. No.	Isolates	LgNPs	CcNPs	AvNPs	SeNPs	SpNPs	$AgNO_3$
1	*P. aeruginosa* (S23)	7 ± 0.5	7 ± 0.5	8 ± 1	9 ± 1.5	9 ± 1.5	6 ± 0.5
2	*A. baumannii* (S29)	6 ± 0.5	6 ± 0.5	7 ± 0.5	8 ± 1	7 ± 0.5	7 ± 0.5
3	*K. pneumoniae* (S35)	6 ± 0.5	6 ± 0.5	8 ± 0.8	8 ± 1	6 ± 0.5	6 ± 0.5
4	*E. coli* (S36)	6 ± 0.5	7 ± 0.5	7 ± 0.5	9 ± 1	7 ± 0.5	7 ± 0.5

AvNPs = *Aloe barbadensis* Miller nanoparticles; CcNPs = *Cinnamomum camphora* nanoparticles; LgNPs = *Cymbopogon citratus* nanoparticles; SpNPs = *Salvadora persica* nanoparticles; SeNPs = *Shorea robusta* nanoparticles; $AgNO_3$ = Silver nitrate

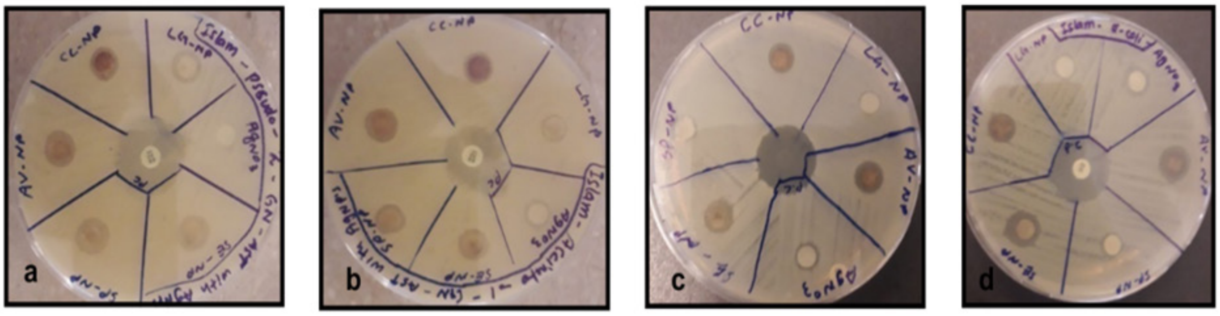

Fig. 5. Antibacterial assay of AgNPs. The zone of inhibition produced by using the disc diffusion method on Mueller-Hinton agar by AgNPs on MDR bacteria: (a) *P. aeruginosa,* (b) *A. baumannii*, (c) *K. pneumoniae*, and (d) *E. coli.* Each petri plate contains AvNPs, CcNPs, LgNPs, SpNPs, and SeNPs along with $AgNO_3$ as a control. Clear zones around the disc showed antibacterial activity of synthesized AgNPs.

mediated AgNPs against clinically isolated MDR isolates, enhancing the clinical relevance of the antimicrobial findings. The overuse and misuse of antibiotics is the main driver of MDR emergence, emphasizing the necessity for novel antimicrobial strategies [25].

There are various hypotheses based on the studies to evaluate the mechanism behind the killing of bacteria by AgNPs, yet some of the electron microscopy (EM) experiments suggested the damage to bacterial cell walls by these tiny particles [26]. In some other studies, agglomerated AgNPs were observed in the cytoplasm of *E. coli* when treated with silver NPs and observed under EM [27]. The change in color to brownish in this study is also consistent with Batool *et al*. [24], who reported successful formation of AvNPs using *A. vera* extract. However, our use of sunlight-assisted synthesis at defined time intervals provides a more reproducible and eco-friendly alternative than conventional heating or cooling methods using toxic reducing chemicals. Unlike Batool *et al.* [24], where the inhibition zone for *E. coli*, *P. aeruginosa*, and *K. pneumoniae* was found to be 12 mm when the same extracts were made from *A. vera* in distilled water, the current investigation produced smaller values ranging between 7 and 8 mm. Such variation can be attributed to the relatively high level of intrinsic resistance in the MDR bacterial strains as opposed to laboratory strains or likely because of the size difference in the nanoparticle [1].

In the *C. citratus* AgNP research, the measurable inhibition zone against *E. coli* was 12 mm [28], while the current study shows 6 mm. AgNPs produced from callus-mediated *C. camphora* demonstrated minimum inhibitory concentration values (MIC) against *E. coli* and *P. aeruginosa* of 3 µg/mL. Although Li *et al.* [29] did not explicitly report inhibition zones for CcNPs, mentioning only MIC values for *E. coli* and *P. aeruginosa*, our study reported measurable inhibition zones of 7 mm against both pathogens, providing direct evidence of antibacterial efficacy. Alvi *et al*. [30] reported that SeNPs synthesized from lemon peel extract exhibited a maximum inhibition zone of 25 mm against *E. coli*, at par with the standard antibiotic ciprofloxacin, demonstrating their potent antibacterial efficacy. In contrast, this study found a 9 mm inhibition zone against a clinical isolate of *E. coli.* Miri *et al.* [31] utilized *S. persica*-derived AgNPs against MDR bacteria, and the zone of inhibition was 14 mm. However, in the current study, it is 7 mm against *E. coli*. While the inhibition appears smaller in this study, the difference is expected due to increased resistance of clinical MDR isolates. However, the antibacterial activity of all these nanoparticles demonstrated their potential against the treatment of MDR isolates, highlighting the clinical importance of presented results.

The experimental results of the study also indicate that *A. vera* nanoparticles, *C. camphora* nanoparticles, *C. citratus* nanoparticles, *S. persica* nanoparticles, and *S. robusta* nanoparticles showed a zone of inhibition against MDR bacteria (*P. aeruginosa*). The maximum zone of inhibition was shown by SpNPs and SeNPs in this experiment compared to AvNPs and CcNPs, LgNPs (SpNPs, SeNPs > AvNPs > CcNPs, LgNPs). Similarly, SeNPs showed a zone of inhibition against *A. baumannii.* SeNPs and AvNPs showed activity against *K. pneumoniae.* SeNPs showed a zone of inhibition activity against *E. coli.* Overall, results indicate that *S. persica* nanoparticles (SeNPs) showed maximum antimicrobial activity against all MDR bacteria (*A. baumannii, E. coli, P. aeruginosa,* and *K. pneumoniae*) compared to AvNPs (SeNPs > AvNPs). It is worth noting that all AgNPs exhibited inhibition zones against isolates that were completely resistant to amoxicillin-clavulanic acid, ampicillin, and ceftriaxone, suggesting their potential as alternative antimicrobial agents against MDR bacteria.

Herbs serve medicinal purposes [32]; as a result, essential oils derived from medicinal plant extracts are utilized for various medical conditions, such as gastritis [33] and neurological problems [34]. According to Kebede and his coworkers, methanolic extracts, particularly from *Rumex abyssinicus, Cirsium englerianum,* and *Euphorbia depauperate*, demonstrated antibacterial and antifungal properties, with zones of inhibition of almost 29 mm [35]. These findings suggest that plants possess bioactive compounds, which have antimicrobial activities when used as conjugates with silver nanoparticles. Moreover, the silver nanoparticles are promising antimicrobial agents against MDR bacterial pathogens [36].

5. CONCLUSIONS

In conclusion, the five plant-based silver nanoparticles synthesized via sunlight-mediated green strategy demonstrated notable antimicrobial activity against clinically isolated MDR strains of *A. baumannii, E. coli, P. aeruginosa,* and *K. pneumoniae*. Among the synthesized nanoparticles, *S. robusta* AgNPs (SeNPs) showed the strongest antimicrobial effect against all MDR pathogens, followed by *S. persica* AgNPs (SpNPs). These results demonstrated the potential of green-synthesized AgNPs as effective, eco-friendly, and cost-effective alternatives for treating nosocomial infections. Future studies should prioritize in vivo studies, toxicity evaluation, clinical trials, and standardized large-scale production to support regulatory approval and clinical application.

6. ACKNOWLEDGEMENTS

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7. ETHICAL STATEMENT

The study did not involve human subjects, animal experiments, or patient data; therefore, ethical approvals were not required.

8. FUNDING

The research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

9. CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

10. AUTHORSHIP CONTRIBUTION STATEMENT

Muhammad Islam-ul-Haq: Experimentation, methodology, validation. Hammad Arshad: Supervision, conceptualization, review, and editing. Sana Aziz Sial: Writing the draft manuscript, data curation. Fasiha Qurashi: Writing the draft manuscript. Muhammad Ehsan-ul-Haq: Writing the draft manuscript.

11. DECLARATION

The results presented in this manuscript are original. This material is neither published nor under consideration for publication elsewhere. Approval of all authors has been obtained. In case this article is accepted for publication, its copyright will be assigned to the Pakistan Academy of Sciences.

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Research Article

Climate-Induced Changes in Functional Traits, Soil Hydraulic Properties, and Drought Resilience of Tropical Maize

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Abstract: Changes in soil-plant-water interactions and rising drought frequency are major impacts of climate change, especially on maize, which is very sensitive to climate change in the tropics. The present research aims to explore the changes in functional traits, soil hydraulic properties and drought resilience under different intensities of drought. A randomized complete block design with five replications per treatment was used in the controlled field experiment, which was carried out in southern Nigeria for 112 days (16 weeks). Irrigation was varied for each treatment to cover rain regimes ranging from control to mild, moderate and severe drought. At weekly intervals and at harvest, the following climatic variables, soil moisture, traits of maize, soil hydraulic properties and crop performance were measured. Statistical analysis (ANOVA) was used to assess the effects of treatment. Result showed that the drought condition led to an increase in air and soil temperature, a decrease in soil moisture and soil humidity. Maize exhibited adaptive responses such as stomatal closure, root growth and thickening of the tissues, leading to better water use efficiency. The effect of drought on the soil hydraulic properties is clearly shown by a reduction in field capacity, an increase in wilting point and a reduction in hydraulic conductivity. The biomass reduction ranged up to 48%, and a significant reduction was observed in survival and drought resistance index in the worst cases. There was also an extreme reduction in grain yield to 2.2 ± 0.4 t/ha with a severe limitation in reproduction. Physiological adaptation to drought, as water use efficiency increased with drought. The results indicate that climatic, soil and plant variables interact in a complex way in drought resilience, and integrated management practices such as drought-tolerant crops and precision irrigation will be crucial to ensure maize production under climate variability.

Keywords: Climate Change, Drought Stress, Randomized Complete Block Design, Soil Moisture Dynamics, Water Use Efficiency, Tropical Agriculture.

1. INTRODUCTION

Climate change is one of the most formidable challenges of the 21st century for food systems globally and already has a significant impact in the tropics where production, including that of maize, is constrained by environmental variability. There are increasing disruptions in the finely tuned soil-plant-water interactions that regulate maize growth and survival due to increasing temperatures, unpredictable rains, and projected extended droughts [1-4]. Drought is one of the most disastrous climatic stressors that puts tropical agriculture at risk of severe losses in maize production and food security in sub-Saharan Africa and other arid tropical habitats [5-9]. Plant physiological response to drought stress is a dynamic relationship. Maize copes with water deficit by an orchestrated set of functional trait adaptations that transcend aboveground and belowground [10-12]. The adaptive strategy of the maize plant to maintain an optimum balance between water saving and carbon fixing has been known to change with varying soil moisture availability, such as stomatal conductance, leaf area and specific leaf area, chlorophyll content and root architecture [13-17]. Maize responses at the trait level are not independent but highly linked to soil hydraulic properties that govern water flow through the soil profile and the availability of water to the roots, including field capacity, permanent wilting point, available water content, and hydraulic conductivity [3, 18].

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These dynamics are interrelated, and their understanding is important in determining drought resilience in tropical maize cropping systems. Drought resilience not only includes the capacity of maize to survive water stress but also includes the capacity of the crop to survive and attain a desirable level of biomass, grain, and reproductive efficiency as compared to the well-watered condition [19-21]. In tropical agriculture, where smallholder farmers are especially susceptible to climate variability and have limited irrigation infrastructure, to construct drought-resilient maize systems, there is a need to know how climate-driven changes are converted into changes in maize functional properties and soil-water relationships [22]. As climate change has become a more pressing challenge to agricultural productivity, especially in drought-stricken conditions, increased attention has been paid to drought resilience in crop systems during the last few decades.

While significant research has been undertaken on the role of functional traits in mediating responses to drought in plants there are still some constraints that impede the formulation of comprehensive drought management strategies. Available literature on the subject is deficient in studies that simultaneously address the multi-dimensional effects of climatic variables, functional traits of maize, soil hydraulic properties, and crop performance in controlled but field-realistic tropical environments [23]. The available literature often concentrated on either plant physiology or soil physics alone, with knowledge gaps critical in understanding the co-response of these two systems to increasingly severe drought stresses [24-26]. Also, controlled gradient experiments that are also conducted through studies that enable systematic comparison at well-defined drought intensity levels are especially limited in tropical sub-Saharan settings [27].

The current studies generally focus on either physiological response to plants or physical properties of soils. For example, many studies have documented physiological responses of the plants under drought stress, such as stomatal conductance, Leaf Area Index (LAI), Chlorophyll content (SPAD) etc., [13-14]. On the other hand, some have concentrated on soil hydrologic characteristics alone, without considering plant reactions [18]. The silo approach narrows the vision for a holistic grasp of complex interactions between plant traits and soil-water dynamics, both of which play important roles in drought resilience.

Moreover, the majority of the research reported so far has been done under controlled laboratory or greenhouse conditions, and has been scarcely tested in a tropical smallholder production system. Field studies frequently do not include a systematic range of well-defined drought intensity gradients, which often contributes to a lack of understanding of threshold drought levels where crop or soil responses can no longer be reversed [27].

The above studies have reported conflicting results regarding the adaptation strategies of crops to drought. This indicates that the results of the studies discussed above on drought stress response of crops are inconsistent. Research to date can sometimes indicate the effectiveness of specific practices, e.g., drought-tolerant varieties or particular fertilization methods, to boost resilience; other research has led to other or even conflicting findings. The differences between the results highlight the complexity of plant responses to drought stress and the fact that there is no universal solution. Different forms of adaptation strategies may have mixed effects, depending on the crop type, environmental conditions, and management practices, so the scientific evidence is somewhat contradictory. For instance, some research indicates that greater root development facilitates water uptake and drought resistance [28], while others suggest that extensive root growth can reduce aboveground biomass and productivity, particularly during severe droughts [29]. Similarly, while early stomatal closure may significantly reduce carbon assimilation [30], some research suggests that stomatal closure can be used to effectively conserve water while having a minimal impact on photosynthesis [31]. The divergent results of these studies are illustrative of the context-dependent and complex nature of drought responses and the need for integrative multi-dimensional studies.

Consequently, there are existing knowledge gaps that still need to be addressed. First, there is limited understanding of the interaction between functional trait plasticity and soil hydraulic properties at varying intensities of drought in real world tropical farming systems. Most studies have considered plant physiology or soil physics, but neither the dynamic interactions between them. Second, thresholds for severity of drought that cause irreversible damage or changes in adaptive strategies are not well understood, particularly in smallholder tropical systems where management options are limited by resources. Third, the soil-water-plant feedback processes that influence drought resilience are under-researched and under-understood, especially the potential of integrated management practices to increase adaptive capacity.

This work fills these gaps by using a novel, field-based, gradient-drought experiment to concurrently explore the multi-dimensional response of maize in both functional traits, soil hydraulic parameters and crop performance in a tropical smallholder environment. Our multi-disciplinary approach not only integrates plant and soil responses, which is the focus of much previous research, but also accounts for the complex interaction that leads to drought resilience. In addition, this research introduces novel insights into thresholds for making systems more and more vulnerable to droughts, by establishing well-defined drought intensity levels that represent climate variability in southern Nigeria. This study, to our knowledge, is the first to quantify the combined effects of climate-induced drought on maize functional trait plasticity, soil-water relations and crop productivity in an on-farm tropical setting, thereby yielding important empirical data to guide sustainable drought management strategies.

Thus, the objectives of this work were to investigate the effect of drought on functional trait dynamics, and the relation between the same and hydraulic properties of soils, water use efficiency, and maize production under four drought conditions (randomized complete block design) representative of the southern region of Nigeria under an on-farm tropical field experiment. The aims were to (i) describe the temporal variations in important climatic variables and soil moisture dynamics under drought treatments, (ii) characterize the adaptive response of maize functional traits to increasing drought stress, (iii) characterize the changes in soil hydraulic properties and water use efficiency under drought conditions and (iv) quantify the overall effect of drought intensity on maize productivity and drought indicators of functional traits of maize as biomass yield, grain yield, harvest index, drought tolerance index and survival rate. The study was guided by the following hypotheses:

i. Soil hydraulic properties such as field capacity and hydraulic conductivity will be greatly reduced as drought intensifies.
ii. Higher Water Use Efficiency in drought conditions will be achieved by plants that have a higher functional trait plasticity.
iii. The grain yield of maize will be reduced as the intensity of the drought increases, and biomass will be reduced as grain yield is reduced, for a given intensity of the drought.

2. MATERIALS AND METHODS

2.1. The Study Site

This study was undertaken to examine the climate-induced changes in functional trait dynamics of soil-plant-water interfaces and drought resilience in tropical maize agriculture through a controlled field experiment. The experiment was conducted in Agbor under tropical environmental conditions, approximately at Latitude 6.25375° N and Longitude is 6.1942° E, on an elevation of about 70 metres above sea level. The region experiences on average about 1500–2000 mm of rainfall per annum. The annual mean temperature varies from 26 °C to 29 °C and in the study, average temperature during the experiment ranged from 28.5 °C to 34.1 °C according to the severity of the drought.

2.1.1. Soil characteristics

Agbor lies in the rain forest zone with high rainfall (> 2000 mm/year), long wet season and high humidity which influence the chemical profile of the soil. The soil of the experimental site at Agbor, Delta State, Nigeria was mostly sandy clay loam to loam in texture [32] and has moderate levels of organic matter typical of cultivated Ultisols in the humid forest-savannah transition zone. Specific properties of chemical properties are:

2.1.1.1. Organic matter content: The results of the physicochemical examination of soils in the various locations of Agbor and its adjoining communities indicated that the content of organic carbon, organic matter and Nitrogen in the eleven sample locations were not significantly different. The organic matter content in Agbor, Ika South LGA, falls into the category of medium with a mean content of approximately 2.54% for the area. The moderate level of organic matter is consistent with the rainforest favourable zone of Nigeria, which have higher levels of total nitrogen and organic carbon, contributing to fertility, along with higher organic matter.

2.1.1.2. pH: Soils in Agbor in Ika South LGA are slightly acidic having pH range of 6.61. The acidity is fairly moderate and it is typical of the humid tropical environment of the region and is fit for most crops. It is in contrast to the oil producing neighbouring LGAs where a high acidity was reported.

2.1.1.3. Nutrient levels: The nitrogen content is high in Ika South (0.27%) while the phosphorus content is medium (approx. 16.98 mg/kg), and the potassium, sodium, calcium and magnesium content is all high in Ika South area. In Agbor, however, the calcium content of the soils sampled were not the same but ranged from 0.08% to 0.14% with some spatial variations within the town.

2.2. Field Layout

Randomized complete block design (RCBD) was engaged with four treatments that are related to the intensity of drought, that is, control (ambient condition), mild drought, moderate drought, and severe drought. The experiment was replicated five times, making a total of twenty experiment units. The plots were 3 m by 3 m with a buffer zone in between to ensure that there was no movement of water between treatments. The drought conditions were implemented through a controlled irrigation regime whereby the amount of water to be applied was changed weekly in order to replicate the natural rainfall gradients: full irrigation on control plots and gradually decreasing the amount of water applied (around 60, 35, and 10 percent of field capacity) to mild, moderate, and severe drought conditions respectively.

2.2.1. Planting material

The study used a hybrid maize variety (ART/98/SW6-1-1 BB) from the Drought Tolerant Maize for Africa (DTMA) breeding program in Nigeria's drought trials obtained from the International Institute for Tropical Agriculture (IITA) Ibadan.

2.2.2. Experimental duration

The duration of the experiment was approximately 112 days (or 16 weeks) spanning over one cropping season, from February to May. This is the timeframe of the late dry to early wet season for better tropical maize varieties grown in southern Nigeria which have a maturity period of 90-120 days based on the extra-early and early maturing varieties [33-34].

2.2.3. Sampling procedure

Climatic data for the experiment were recorded by observing weekly air temperature and relative humidity with the help of Digital thermo-hygrometer kept at 1.5 m height above ground in each plot. Calibrated rain gauges (counting at least 500 ml) installed in unobstructed locations were used to measure rainfall, and weekly observations were made. There were 3 replications per plot to minimize spatial variability, and soil temperature measurements were taken weekly at 10 cm depth on each plot using soil thermometers and the average temperature was computed for each plot. A Time Domain Reflectometry (TDR) probe was used as demonstrated by Yan *et al*. [35] and Comegna *et al*. [36] to estimate the volumetric soil moisture at 10 cm depth every week, and three soil samples were taken on each plot to calculate a mean value.

Soil hydraulic properties were saved at the start and end of the experiment at the upper profile (0-20 cm). Three representative samples were obtained from each treatment by combining four random cores from each plot. Laboratory tests were performed for field capacity, permanent wilting point (PWP) with pressure plate and hydraulic conductivity with constant head permeameter as described in the methodology by Angelaki *et al*. [37]. The plant functional traits were evaluated by three sampling time points: the vegetative growth stage and two stages of the reproductive growth stage. Leaf area was determined with a leaf area meter and several leaves (5-10) were harvested from each plant and averaged. To reduce diurnal effects, stomatal conductance was measured using a steady state porometer on the abaxial surface of at least three plants per plot, with measurements taken around the same time.

Root length density (RLD) was determined by sampling three soil cores (5 cm diameter x 20 cm depth) at random positions, washing away the soil from the root and then using image processing software to analyse the root. The Specific Leaf Area (SLA) was determined by measuring the leaf area and after oven-drying the samples, calculating their dry mass. The SPAD meter was used for the assessment of the chlorophyll content, taking three readings per leaf on average.

Three plants were randomly harvested from each plot at harvest for dry biomass and grain yield. For biomass estimation plants were cut at the ground level, dried at 70 °C to a constant weight, and weighed. Grains were threshed, dried and weighed at a standard moisture content (~12%) to determine yield. Harvest index calculated as the ratio of grain yield to total biomass, and was expressed as a percentage. The drought tolerance index was determined from the ratio of yield under drought stress and yield under control environment and the survival rate, which was obtained from the number of plants that survived to harvest and the number of plants that were originally planted.

The apparatus used in the laboratory were: pressure plate apparatus for measuring soil water retention, constant head permeameter for measuring hydraulic conductivity, precision balances for weighing plant and soil samples, and 70 °C oven for drying soil samples. Additionally, root analysis was performed using sieves, washing trays and imaging technology for measuring the root length. A leaf area meter (LICOR LI-3000C) and a steady-state porometer (SC-1 Leaf Porometer) and a chlorophyll meter (Konica Minolta SPAD-502) were used for plant trait measurements, calibrated rain gauges, soil thermometers and TDR probes for environmental measurements, providing accurate and consistent data collection across all study.

2.2.4. Maintenance of field capacity

Field capacity was established through a controlled irrigation method aimed at simulating a natural rainfall gradient, ensuring uniform soil water content across different drought treatment plots. This initial field capacity was defined in a laboratory setting before the experiment began. Weekly irrigation was conducted to maintain target soil moisture levels, informed by real-time data from TDR probes. The plots received varying amounts of water to correspond with specific drought conditions: control plants at field capacity (36.5%); mild drought at 60%; moderate drought at 35%; and severe drought at 10% of field capacity.

2.2.5. Water monitoring

Soil water was monitored weekly using TDR probes positioned at various locations within each plot to account for spatial variability. These probes provided immediate volumetric soil moisture readings, which were compared against desired moisture levels. If moisture was insufficient, additional water was applied to meet the targets. The Irrigation method was calibrated watering cans, ensuring equal water distribution among plots. All water applied was meticulously recorded, along with weekly water usage per treatment level. In addition to soil moisture, soil temperature and environmental conditions, including evaporation, were continuously monitored to assess their impact on water retention in the soil, allowing for adjustments in watering rates required to uphold treatment conditions throughout the experiment.

2.3. Data Collection

Specific leaf area (cm²/g) was calculated as the quotient of leaf area to dry leaf mass, and chlorophyll content was determined by a SPAD chlorophyll meter. Standard laboratory procedures were used to determine soil hydraulic properties. A pressure plate apparatus at 33 kPa and 1500 kPa was used to estimate the field capacity and permanent wilting point, respectively. Available water content = Field capacity - permanent wilting point. The constant head permeameter method according to Diminescu *et al.* [38] was used to measure hydraulic conductivity (cm/hr). The water use efficiency (g biomass/kg water) was determined as the proportion of total dry maize biomass to the total water used in the plot during the period of the experiment.

At harvest, crop performance indicators were measured. The above-ground biomass yield (t/ha) was calculated by drying samples of maize plants in the oven to a constant weight. The grain yield (t/ha) was obtained following the drying and threshing process to standard moisture content. The ratio between the maize grain yield and the total biomass yield multiplied by 100 was taken as the harvest index of maize. The drought tolerance index was calculated by dividing the maize yield in the stress environment by the yield in the controlled environment. The survival rate (percent) was calculated as the percentage of maize plants that survived to harvest.

A digital thermo-hygrometer was used to measure climatic parameters such as air temperature (°C) and relative humidity (percentage) and a set of calibrated rain gauges to simulate and measure rainfall (mm/week). The soil temperature was determined by inserting soil thermometers at the depth of 10 cm and the soil moisture content was weekly measured using soil moisture probe (Time Domain Reflectometry, TDR method) as demonstrated by Yan *et al*. [35] and Comegna *et al*. [36]. The maize functional traits were determined at appropriate vegetative growth. The measurement of leaf area (cm^2) was done on a leaf area meter. A steady-state porometer was used to measure stomatal conductance (mmol m^{-2} s^{-1}). Root length density (cm/cm^3) was determined by sampling the soil in core samples and washing roots and analysis of the images.

2.4. Data Analysis

A two-way Analysis of Variance (ANOVA) was used to test the differences among treatments and block effects, and a significance level of $P < 0.05$ was used. The statistical analysis was done with the help of the standard statistical software (SPSS), and the results were given in terms of mean and standard deviation. The statistical model used was:

$Yij = \mu + Ti + Bj + \varepsilon ij$

where:

- Ti = drought treatment effect
- Bj = block effect
- εij = residual error

3. RESULTS AND DISCUSSION

Climatic parameters and soil moisture variations which have been varying with the intensification of the drought conditions are presented in Table 1 and have been seen to be showing a gradational trend for the various parameters. Under the control (ambient) condition is an environment that is as close as possible to the ideal tropical growing conditions. Mean temperature was somewhat moderate at 28.5 +/- 0.6 °C with a high weekly rainfall of 45.2 +/- 3.1 mm and a high relative humidity of 78.4 +/- 2.5%. These conditions resulted in a good maintenance of the high soil moisture content (32.1 ± 1.8), with the soil temperature being kept slightly low at 27.3 ± 0.7 °C, which effectively reduced the soil temperature through evaporative cooling and provided adequate soil water supply in the profile.

Climatic change can be observed as conditions change to mild drought. The mean temperature rose to 30.2 ± 0.8 °C while the rainfall decreased significantly by around 43% to 25.6 ± 2.4 mm/week compared with the control. In addition, there was a decrease in relative humidity of 70.1 ± 2.9 which indicates the drier weather. This resulted in a reduction of the moisture content of the soil to 24.7 ± 1.5, or less water was available to the plants. The moisture content of the soil was also lower and, in turn, the temperature of the soil reached 29.0 ± 0.6 °C.

At moderate drought, the trends were further intensified. The mean temperature was 32.0 ± 0.9 °C and the rainfall (less than the control) was 12.8 ± 1.7 mm/week. This decreased the relative humidity to 62.5 ± 3.3. The soil moisture was reduced to 18.3 ± 1.2, which means that there was a significant loss of plant-available water. Meanwhile, the soil temperature has increased to 30.6 ± 0.8 °C, which

Table 1. Climatic variables and soil moisture dynamics across treatments.

Treatment	Mean Temp (°C)	Rainfall (mm/week)	Relative Humidity (%)	Soil Moisture (%)	Soil Temperature (°C)
Control (Ambient)	28.5 ± 0.6	45.2 ± 3.1	78.4 ± 2.5	32.1 ± 1.8	27.3 ± 0.7
Mild Drought	30.2 ± 0.8	25.6 ± 2.4	70.1 ± 2.9	24.7 ± 1.5	29.0 ± 0.6
Moderate Drought	32.0 ± 0.9	12.8 ± 1.7	62.5 ± 3.3	18.3 ± 1.2	30.6 ± 0.8
Severe Drought	34.1 ± 1.1	4.5 ± 0.9	55.2 ± 2.8	10.6 ± 0.9	32.4 ± 1.0

emphasized that the soils with less moisture content have a greater capacity to heat up. The system experienced extreme conditions of stress in the severe drought treatment. The relative humidity was also lowered to 55.2 ± 2.8 further reinforcing the dryness of the atmosphere. Soil water status was at a critical level of 10.6 ± 0.9% (about one-third of the control) water status, which was very limiting for plant water uptake. In this context the soil temperature increased to 32.4 ± 1.0 °C which was the maximum in all the soil treatments as there was less evaporative cooling and more absorption of the solar heat from the soil.

In general, the table shows a correlated trend: with the increase in the severity of drought, the temperature and soil temperature increased while rainfall, relative humidity, and soil moisture decreased drastically. These intertwined processes demonstrate that climate-induced drought can have a dual influence on the atmosphere and soil water levels and lead to more and more stressful environments that can influence the growth and survival of plants in tropical farming. This is in line with the reports of Matlaga *et al*. [39] who reported that increasing drought will favour species with more conservative traits, resulting in greater survival in low soil moisture conditions, Haghpanah *et al*. [40] who observed that plants exhibit various physiological and molecular responses to drought stress, enabling them to adapt and maintain crop resilience in limited water environments, and Sato *et al*. [41] who documented that drought stress reshapes atmospheric conditions, depletes soil water reserves, and reduces plant growth and survival in tropical and rain fed systems.

Table 2 shows that the growth in drought stress triggers the synchronous alterations in the functional traits of plants, which indicates adaptive responses as well as physiological constraints during water scarcity. Plants have optimum growth characteristics under the control condition. The maximum leaf area was 145.6 ± 5.2 cm^2 which shows that the photosynthetic areas are large. The stomatal conductance was also relatively high (320.4 ± 15.1 mmol m-2 s-1), allowing for an efficient gas exchange and carbon incorporation. The low value of root length density (1.85 +- 0.12 cm/cm^3) shows that the soil does not need to be explored too deep, since there was enough water available. Specific leaf area (SLA) was also found to be highest (210.5 ± 8.4 cm^2/g) indicating a low leaf thickness and resource-acquisitive leaves, and chlorophyll content (48.3 ± 2.1 SPAD) was the highest, which is an indicator of high photosynthetic rate.

The leaf area reduced to 130.2 + 4.8 cm^2, which means that the surface area was reduced to minimize water loss by transpiration. Stomatal conductance reduced to 275.3 ± 13.7 mmol m^{-2} s^{-1}, which indicates a partial stomatal closure in order to conserve water. However, in contrast, the growth of root length density increased to 2.10 ± 0.15 cm/cm^3, indicating higher proliferation of roots to search down to the deep or limited soil water. The leaves were thicker (SLA reduced to 195.7 ± 7.9 cm^2/g), as is typical in thicker leaves, which tend to be more resistant to drying. Chlorophyll level also decreased to 44.9 ± 2.4 SPAD, which presupposes a minor decrease in photosynthetic activity.

These trends were heightened under moderate drought. The leaf area decreased further to 110.5 ± 5.6 cm^2, and there was a further strategy of minimizing water loss. Stomatal conductance reduced to 275.3 ± 13.7 mmol m^{-2} s^{-1}, which indicates a partial stomatal closure in order to conserve water. However, in contrast, the growth of root length density increased to 2.10 ± 0.15 cm/cm^3, indicating higher proliferation of roots to search down to the deep or limited soil water. The leaves were thicker (SLA reduced to 195.7 ± 7.9 cm^2/g), as is typical in thicker leaves, which tend to

Table 2. Plant functional traits under climate stress conditions.

Treatment	Leaf Area (cm^2)	Stomatal Conductance (mmol m^{-2} s^{-1})	Root Length Density (cm/cm^3)	Specific Leaf Area (cm^2/g)	Chlorophyll Content (SPAD)
Control	145.6 ± 5.2	320.4 ± 15.1	1.85 ± 0.12	210.5 ± 8.4	48.3 ± 2.1
Mild Drought	130.2 ± 4.8	275.3 ± 13.7	2.10 ± 0.15	195.7 ± 7.9	44.9 ± 2.4
Moderate Drought	110.5 ± 5.6	210.6 ± 12.9	2.45 ± 0.18	180.2 ± 6.5	40.7 ± 2.7
Severe Drought	85.3 ± 6.1	145.8 ± 10.3	2.90 ± 0.21	160.8 ± 5.9	35.2 ± 3.0

be more resistant to drying. Chlorophyll level also decreased to 44.9 ± 2.4 SPAD, which presupposes a minor decrease in photosynthetic activity.

Plants under extreme stress conditions are those in severe drought conditions. The leaf area decreased drastically to 85.3 ± 6.1 cm², almost 40 percent that of the control with a severe limiting effect on photosynthetic surface. Stomatal conductance also reduced to 145.8 ± 10.3 mmol m^{-2} s^{-1}, which shows that stomata were strongly closed and the carbon uptake was limited. On the other hand, root length density was highest at 2.90 ± 0.21 cm/cm³, indicating a strong movement towards water foraging of the soil. The reduction in specific leaf area further to 160.8 ± 5.9 cm²/g proves that thicker leaves were developed and more resistant to the drought. The lowest value of chlorophyll content (35.2 ± 3.0 SPAD) could be as a result of significant damage to the photosynthetic machinery.

On the whole, these results show a well-defined trade-off in the conditions of drought stress. The aboveground traits (leaf area, stomatal conductance, chlorophyll content, and SLA) decreased in a certain order, which entails the decrease in photosynthetic capacity and growth and the increase in belowground investment (root length density), which indicates an adaptive strategy to improve water uptake. Zahedi *et al*. [42] noted that these organized changes in traits show how plants trade-off between conserving water and getting resources to enhance their survival under the increasingly harsh conditions of drought.

Figure1 shows the reactions of some important physiological, soil and productivity parameters of maize to the four drought treatments (Control, Mild, Moderate and Severe) applied, represented by 100% Field Capacity, 75% FC, 50% FC and 25% FC respectively. As seen in the trends, the higher the drought stress, the more impact it has on crop performance.

The soil water content was reduced gradually with the control treatment having 32.1% while the severe drought treatment had 10.6%, indicating the establishment of a drought gradient. Similarly, there was a decrease in relative leaf water content from 89.2% to 45.8%, which corresponds to the increasing water deficit of plants during the drought.

The soil temperature, however, rose gradually from 26.5 °C to 34.8 °C; this was a result of less cooling effect when the soil was dry. Likewise, the growth of maize root length density was higher, from 0.85 cm/ cm^3 under control to 2.90 cm/ cm^3 under severe drought, thus indicating that maize invested more in their roots to enhance the use of water under drought conditions.

Water use efficiency (WUE) is also increased with increasing stress gradually from 3.25 to 4.20 g biomass kg water, suggesting that the plants were

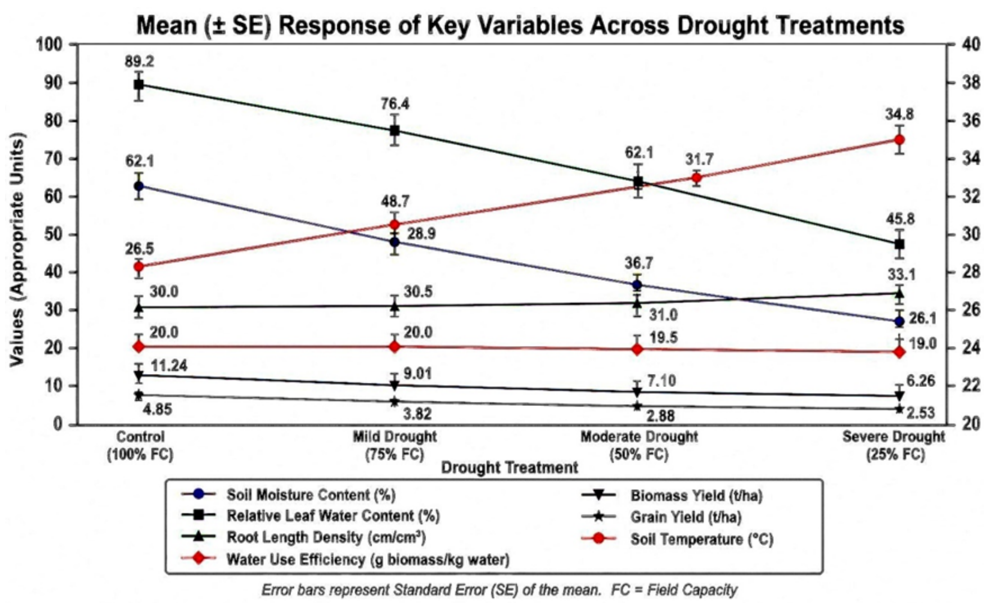

Fig.1. Mean ± SE response of key variables across drought treatments.

utilizing available water with increasing efficiency. But this physiological change did not keep productivity losses low. As the drought severity increased, the biomass yield reduced from 11.24 to 6.26 t ha^{-1} and the yield of grain reduced from 4.85 to 2.53 t ha^{-1}.

As a whole, the graph indicates a substantial decrease of soil moisture, plant water status, biomass and grain yield with increasing drought stress, as well as a greater rooting and water use efficiency, and rising soil temperature. The trends show that while maize can cope physiologically due to drought, this ability is not enough to compensate for the potential negative impacts of drought with a severe water deficit on crop productivity.

As a general rule, Figure 1 indicates that drought reduced water holding capacity of the soil (field capacity), increased water holding capacity at inaccessible depths (PWP), and decreased water movement (hydraulic conductivity), thus significantly reducing the water supply. Water use efficiency, on the other hand, rose steadily as a measure of an adaptive response of the plant to make the most of scarce water resources as observed by Li *et al*. [43]. This brings out a major mechanism of drought resilience in tropical agriculture, which includes a change of resource abundance strategies of plants to water conservation and efficiency strategies when the environment is under stress.

The impact of augmented drought stress on crop productivity and resilience is depicted in Table 3, where there were evident decreases in yield and existence as well as adjustments in allocation efficiency. With the condition of control, crop performance was ideal. The biomass yield was 8.5 ± 0.4 t/ha with a grain yield of 4.2 ± 0.3 t/ha, which indicates high productivity. The harvest index (HI) was 49.4 ± 2.1, which implies that almost half of the total biomass was efficiently utilized to produce the economic yield (grain). The survival rate of 98.5 ± 1.2 is a low stress level, and the drought tolerance index (DTI) standardized was 1.00 (the point of reference). In case of mild drought, productivity was reduced moderately. The biomass yield reduced to 7.6 ± 0.5 t/ha, and grain yield reduced slightly to 3.8 ± 0.2 t/ha, which suggests approximately a 10% reduction compared to the control. Interestingly, there is a slight increase in harvest index to 50.0 ± 1.8%, which implies that the proportion of biomass to grain remains or is slightly improved, although the total growth was minimal. The biomass yield was decreased to 6.2 ± 0.6 t/ha, a significant decrease of around 27%, when compared to the control. The grain yields also went down to 3.1 ± 0.3 t/ha. However, the harvest index (50.3 ± 2.0%) was not significantly influenced, indicating that plants still place importance on reproductive productivity even under stressful conditions. DTI was reduced to 0.74, indicating a lesser tolerance to drought. The survival percentage was reduced to 88.7 ± 2.3, which means that more plants might have died. Crop performance was badly affected by the extreme drought condition.

The biomass yield was reduced dramatically to 4.8 ± 0.7 t/ha or almost 44% lower than the control. There was also an extreme reduction in grain yield to 2.2 ± 0.4 t/ha with a severe limitation in reproduction. In contrast to the previous stages, the harvest index was reduced to 45.8 ± 2.5%, indicating that plants cannot allocate biomass efficiently to grain production in the case of extreme stress. The DTI was reduced to 0.52, exhibiting low drought tolerance, and the survival rate also decreased significantly to 76.3 ± 3.1, showing that a lot of plants were lost.

Table 3 indicates that with the escalation in the severity of drought, biomass yield, grain yield, drought tolerance, and survival rate change progressively. Although the harvest index was maintained or slightly improved with mild to moderate drought conditions, which are signs of

Table 3. Crop performance and drought resilience indicators.

Treatment	Biomass Yield (t/ha)	Grain Yield (t/ha)	Harvest Index (%)	Drought Tolerance Index	Survival Rate (%)
Control	8.5 ± 0.4	4.2 ± 0.3	49.4 ± 2.1	1.00	98.5 ± 1.2
Mild Drought	7.6 ± 0.5	3.8 ± 0.2	50.0 ± 1.8	0.91	94.2 ± 1.6
Moderate Drought	6.2 ± 0.6	3.1 ± 0.3	50.3 ± 2.0	0.74	88.7 ± 2.3
Severe Drought	4.8 ± 0.7	2.2 ± 0.4	45.8 ± 2.5	0.52	76.3 ± 3.1

adaptive use of allocation strategy, it was reduced in the case of severe drought, which is an indication of physiological breakdown, as buttressed by Jarin *et al*. [44]. These findings demonstrate that, as well as growth, drought also impedes and impairs plant survival and reproductive output in tropical agricultural systems.

Treatment Effects (Drought Level): The combined ANOVA results for crop performance and drought resilience indicators are given in Table 4. The F-tests for drought treatment were all highly significant for Biomass Yield ($F = 27.62$, $p < 0.0001$), Grain Yield ($F = 23.70$, $p < 0.0001$), Drought Tolerance Index ($F = 49.61$, $p < 0.0001$) and Survival Rate ($F = 35.31$, $p < 0.0001$), indicating that progressive drought stress caused significant and substantial reductions in crop performance. The treatment also had a significant effect on Harvest Index ($F = 4.66$, $p = 0.022$) although it was less strong, indicating partial maintenance of biomass partitioning to grain under mild to moderate stress.

Block Effects (Replication): No significant block effect was observed with all the response variables (for all variables, $F = 0.85$ and $p = 0.522$) and thus the spatial variations in the five replication blocks were negligible and did not affect the treatment comparisons. This confirms that there were no other sources of field variability besides the treatments such that the experimental layout is valid, and that the RCBD design was successful in controlling any

Table 4. Combined ANOVA results for crop performance and drought resilience indicators.

Response Variable	Source of Variation	Df	SS	MS	F	p-value	Sig.
Biomass Yield (t/ha)	Treatment (Drought Level)	3	32.4800	10.8267	27.62	< 0.0001	***
	Block (Replication)	4	1.3266	0.3317	0.85	0.522	Ns
	Error	12	4.7034	0.3920	—	—	—
	Total	19	38.5100	—	—	—	—
Grain Yield (t/ha)	Treatment (Drought Level)	3	8.9200	2.9733	23.70	< 0.0001	***
	Block (Replication)	4	0.4246	0.1061	0.85	0.522	Ns
	Error	12	1.5054	0.1254	—	—	—
	Total	19	10.8500	—	—	—	—
Harvest Index (%)	Treatment (Drought Level)	3	58.7700	19.5900	4.66	0.022	*
	Block (Replication)	4	14.2230	3.5558	0.85	0.522	Ns
	Error	12	50.4270	4.2023	—	—	—
	Total	19	123.4200	—	—	—	—
Drought Tolerance Index	Treatment (Drought Level)	3	0.4160	0.1387	49.61	< 0.0001	***
	Block (Replication)	4	0.0095	0.0024	0.85	0.522	Ns
	Error	12	0.0335	0.0028	—	—	—
	Total	19	0.4590	—	—	—	—
Survival Rate (%)	Treatment (Drought Level)	3	1210.6000	403.5333	35.31	< 0.0001	***
	Block (Replication)	4	38.6760	9.6690	0.85	0.522	Ns
	Error	12	137.1240	11.4270	—	—	—
	Total	19	1386.4000	—	—	—	—

Significance codes: *** $p < 0.001$; * $p < 0.05$; ns = not significant; df = degrees of freedom; SS = sum of squares; MS = mean square.
Error degrees of freedom: $df_{error} = (t - 1)(b - 1) = 3 \times 4 = 12$.

other source of field variability, which added to confidence of the treatment F-tests.

Correlation and Regression Analysis: Soil moisture and root density are inversely correlated and consistent with adaptive root proliferation during drought (Table 5). The results of the correlations analysis show that soil moisture decreases with increasing drought severity, whereas root density and WUE increase. In contrast, grain yield declined under severe drought conditions.

Regression Analysis: The regression analysis (Table 6) showed that soil moisture, root density, and water use efficiency (WUE) positively influenced the maize biomass yield. The biomass yield increased by about 0.22 t/ha for every 1% increase in soil moisture, while a one-unit increase in root density and WUE increased biomass yield by about 2.5 and 1.8 t/ha, respectively. Model fit, R^2, is close to 1, meaning that these variables account for almost all the variation in biomass yield between the different treatments. Water use efficiency was positively related to soil moisture, which is consistent with the hypothesis that plant water use efficiency will increase under water scarcity.

$$\text{Biomass Yield} = \beta0 + \beta1(\text{Soil Moisture}) + \beta2(\text{Root Density}) + \beta3(\text{WUE}) + \varepsilon$$

The findings of this study are strong indications that gradually increasing levels of drought stress lead to coordinated and quantifiable changes in climatic variables, maize functional attributes, soil hydraulic characteristics, and crop performance of a tropical maize agricultural system. The trends within the whole four experimental treatments were all indicators of the multidimensionality of the effects of drought and the adaptability of maize plants that work in water-restricted settings in the tropical setting of southern Nigeria. Similar results were recorded by Yawale *et al*. [45] who reported that cropping P4 x P9 and P3 x P4 genotypes significantly improved growth attributes under drought and heat stress conditions, potentially benefitting maize breeding programs in Nigeria. Fayose and Fakorede [33] further documented that 3 drought-tolerant maize varieties showed superior grain yield and kernel number, making them recommended for adapting to climate variability and change in Nigeria and Aboderin *et al*. [46] whose multi-environment evaluation results showed that drought and low nitrogen strongly depressed maize

Table 5. Results of correlation analysis.

Variable Pair	Approximate r*	Interpretation
Soil Moisture & Root Density	-0.99	Very strong negative correlation: as soil moisture decreases, root density increases markedly, indicating adaptive root proliferation under drought.
Soil Moisture & WUE	0.99	Very strong positive correlation: lower soil moisture stimulates higher WUE, supporting physiological adaptation hypotheses.
Soil Moisture & Yield	0.99	Very strong positive correlation: higher soil moisture correlates with higher biomass and grain yields.
Root Density & Yield	0.98	Very strong positive correlation: increased root density associates with higher yields, indicating effective water foraging.
WUE & Yield	0.97	Very strong positive correlation: better water use efficiency aligns with increased yield.

* Pearson's r between key variables across treatment means.

Table 6. Results of regression analysis.

Variable	Coefficient Estimate (approximate)
Intercept (β0)	12.0 (baseline yield at maximal moisture)
Soil Moisture (β1)	+0.22 (per % soil moisture)
Root Density (β2)	+2.5 (per cm/cm³ root density)
WUE (β3)	+1.8 (per g biomass/kg water)

performance, while a subset of hybrids combined stress tolerance with useful yield across Nigerian environments.

The climatic and soil moisture results provided in Table 1 substantiate the success of the applied drought gradient and show a steady negative correlation between the severity of drought and the availability of the soil moisture in the fields of maize. The severe loss of soil moisture (reduced to 10.6% in severe drought against 32.1 percent in control conditions) and the increase in air and soil temperature are indicative of the type of compounding stress dynamics that are increasingly projected in a future climate context in sub-Saharan Africa. The simultaneous increase in temperature of soils when moisture decreased is in line with the long-established fact that wet soils cool more effectively by evaporation, whereas drier soils heat up and capture solar radiation better. These results support the perception that drought in tropical maize systems is not a phenomenon of only precipitation deficit but a complex climatic phenomenon that at the same time changes atmospheric, edaphic, and thermal conditions and creates an unfavourable environment for maize growth and survival.

The coherent responses to water stress are observed in the functional trait responses reported in Table 2, which represents a strategy of maize-level adaptation. The fact that the plant was experiencing a steady loss in leaf area, stomatal conductance, specific leaf area, and chlorophyll content and at the same time was experiencing a steady rise in root length density indicates the archetypal above-below-ground resource allocation trade-off that maize plants were engaging in during drought. One of the earliest and best-documented plant responses to water deficit was stomatal closure, which decreased conductance in the control conditions (320.4 mmol m^{-2} s^{-1}) to a level of 145.8 mmol m^{-2} s^{-1} under severe drought-more than 54% lower. Although this reaction saves water, it will necessarily limit CO_2 uptake and thus limit photosynthetic carbon gain, which is consistent with reports of Flexas *et al*. [24]. The corresponding decrease in specific leaf area indicates a structural compensation in favor of more water-storing leaves of maize, which are also known to be more resistant to dehydration in the cell. In the meantime, the significant growth of root length density, which peaks at 2.90 cm/cm^3 in the conditions of severe drought, is an indicator of compensatory investment in belowground water foraging a reaction in the larger context of functional trait plasticity as explained by Poorter *et al.* [16] and Reich [17]. These synchronized changes in traits together show how maize systems in the tropics change their priorities towards survival and water uptake at the expense of growth and photosynthetic efficiency in the face of increasing drought conditions.

The soil hydraulic data (Figure 1) gave valuable edaphic background information as to why maize responses were as strong as drought stress. The reduction in the field capacity of 36.5 to 30.6% and the corresponding increase in permanent wilting point of 14.2 to 18.8 percent along the drought gradient led to an approach of 50% in the available water content, to 22.3 to 11.8 percent. This reduction of the water window of maize plant-accessible water directly limits root water uptake whether or not roots grow, emphasizing that the adaptive traits of roots act within constraints placed by the physical characteristics of the soil. The gradual decline in hydraulic conductivity is also a sign that water flow within the soil matrix was becoming more and more limited in the later stages of drought, which could lead to spatial heterogeneity in water availability that must be overcome by the maize roots via architectural changes [41-43]. It is on this ground that the paradoxical rise in the efficiency of water use, which in turn is the rise of 3.25 g biomass/kg water under control conditions and 4.20 g biomass/kg water under severe drought, is an indication of a significant physiological maize adaptation. Instead of being a direct indicator of productivity, this increasing stress-induced efficiency indicates that the plant is partially decoupled in its biomass production and water usage via stomatal control and metabolic savings, which is in line with the drought resilience processes outlined by Blum [21] and Tardieu *et al*. [25].

Table 3 summarized the data of crop performance into agronomically significant results of maize, which are a result of these physiological and edaphic dynamics. The 44 % decrease in biomass production and the 48 % decrease in grain production during severe drought in comparison to the control are significant losses in productivity that have direct food security consequences to tropical smallholder maize agricultural frameworks [47-48]. A drought tolerance index of 0.52 in severe stress conditions reflects that not more than half of maize yield potential is achieved when water is severely limited, which explains the agronomic sense of urgency around the creation of drought-adapted maize varieties and water management systems [49-50]. Of special concern is the trend of the harvest index, which was fairly constant or slightly enhanced during mild and moderate drought and only dropped significantly in the case of severe stress. This trend indicates that initially maize plants maintain reproductive investment at the cost of vegetative growth, an adaptive trade-off that fails at extreme stresses when physiological capacity is exceeded in general as observed by Sheoran *et al*. [51], Yasin *et al*. [13] and Keya *et al*. [52]. The subsequent reduction of the survival rate to 76.3% during extreme drought also indicates the threshold nature of maize stress tolerance, whereby after it reaches a critical level, irreversible damage and mortality become the ultimate consequences.

Combined, the findings of this paper indicate that drought resilience among tropical maize is a consequence of the combined effects of atmospheric, physiological, and edaphic systems and not a specific adaptive process. According to the findings, it is necessary to have management interventions to deal with the retention and optimization of maize traits simultaneously, such as planting drought-tolerant maize varieties, improving soil organic matter to increase water-holding capacity, and having strategies of supplementing irrigation that are set at maize-specific water requirement levels. Nehe *et al.* [53] demonstrated that root architectural traits, including root depth and root number, contribute to drought adaptation by influencing the plant's capacity to access soil water under water-limited conditions. The study focuses on essential climate adaptation strategies to address climate change, highlighting the necessity for drought-resistant crop varieties, improved irrigation management, and effective soil moisture management techniques [54]. These strategies aim to enhance the resilience of drought-prone areas by fostering drought-tolerant ecosystems and ensuring more predictable crop yields amid shifting precipitation patterns. Additionally, crop models can assist in predicting plant responses under various climate scenarios, enriching the adaptation knowledge base.

On breeding, findings suggest significant potential in breeding programs to cultivate traits such as deeper root systems, water-use efficiency, and stress-responsive gene expression. This can be achieved through marker-assisted selection or genomic selection, underscoring the physiological and molecular aspects of drought resilience as critical factors in stress-specific breeding efforts [55].

Lastly, the study underscores the role of precision agriculture technologies like soil moisture sensors, remote sensing, and variable rate irrigation in optimizing water use and crop production during droughts [56]. These technologies help mitigate negative environmental impacts while enhancing resource management, yield stability, and sustainable exploitation in light of climate variability.

4. CONCLUSIONS

The results from this study showed that drought significantly affects soil-plant-water relations and maize productivity in tropical systems. A progressive increase in drought severity caused a coordinated response of climatic variables, maize functional traits and soil hydraulic properties, which resulted in a decrease in biomass and grain yields of up to 48%. Maize showed physiological resilience by closing stomata, increasing root surface area and thickening of tissues. Yet when the drought was severe, significant reductions in survival and yield traits were observed demonstrating the limit of drought resistance. The results highlighted the need for an integrated approach to drought coping (e.g. drought resistant maize varieties, better soil moisture use efficiency, and more accurate irrigation) to enhance drought resilience. Maize showed signs of physiological adaptability, but the losses in yield under extreme stress underscores the need for proactive measures. The overall results confirm that a multi-dimensional strategy including plant and soil systems is needed to maintain maize productivity in tropical areas in the face of climatic variability.

In light of the results of the present study, the following are some of the recommendations that are aimed at increasing the resilience of droughts in tropical maize systems, especially in moisture-stressed environments, like southern Nigeria. Implementation of maize varieties that are drought-tolerant: The breeding effort must focus on developing and implementing varieties of maize that have functional characteristics that have been shown to provide the crop with drought tolerance, such as high root length density, small specific leaf area during drought, efficient stomatal control, and the ability to retain acceptable harvest indices under moderate levels of water stress. Public extension systems should be given priority to provide farmers with easy access to such kinds of maize.

5. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

6. ETHICAL STATEMENT

This work does not include any studies involving human or animal subjects. Only plant materials were involved; therefore, ethical approval was not required.

7. FUNDING

No funding was received for this work.

8. AUTHORSHIP CONTRIBUTION

All authors contributed equally to this study and the development of the manuscript.

9. DECLARATION

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Research Article

Factors Influencing Wheat Production in Pakistan: An Empirical Cobb–Douglas Production Function Analysis

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Abstract: Current research study examines the effect of various input factors, including wheat cultivated area, agriculture credit disbursement, improved seed distribution and availability of water on the wheat crop production in Pakistan by considering time series data of Economic Survey of Pakistan from 1990 to 2023. In order to analyse input data in present study, the log linear Cobb-Douglas production function was considered. Economic findings illustrated that availability of water, agriculture credit disbursement and cultivated area for wheat crop has statistically significant positive effect on wheat crop production. Moreover, unit root testing (Augmented Dickey-Fuller) and cointegration analysis are also used for econometric analysis. However, results also showed that although improved seed distribution has a positive association with wheat production, but this relationship is insignificant. Furthermore, econometric analysis of the regression model showed that a one percent rise in cultivated area for wheat crop would increase wheat production by 1.17%. Similarly, 1% increase in amount of water availability would lead to a rise in wheat crop production by 0.30%. Moreover, wheat crop production exhibits a 0.05% increase for every 1% rise in agricultural credit disbursement. It is suggested that there should be formulation of policy frameworks regarding to improve yield of wheat crop, water-use efficiency, credit disbursement, effective research on climate resilience strategies for wheat crop and adoption of modern technology in agronomic prospective. Policies should be formulated in order to tackle the climate change negative impacts (less water availability) on wheat production.

Keywords: Agriculture Credit, Cultivated Area, Improved Seed, Production Function, Water.

1. INTRODUCTION

The agriculture sector functions as the backbone of Pakistan's economy, which is supporting national growth and it contributes 23.4% to Gross Domestic Product (GDP) and accounts for 33.1% of employment [1]. Wheat contains vitamins and minerals, including thiamine, niacin, riboflavin, calcium, fiber, iron and vitamin D [2]. Wheat as a staple food crop, plays major role for food security in Pakistan, making the country the seventh position globally in total wheat production [3]. The Global wheat production for top ten countries is shown in Figure 1. Feder *et al*. [4] reported that the role of agricultural credit is significant in order to increase modernization and technological advancement in the prospective of agriculture development. According to the World Bank report, agricultural credit enables the farmers' communities to use advanced input technologies more efficiently for growing of crops and for the purpose to meet one of economy objective of poverty alleviation in rural areas [5].

Majority of small and marginalized rural farmers in Pakistan consider two primary sources of agriculture credit (non-institutional and institutional) to manage their routine agricultural related expenses. The non-institutional sources comprise the commission agents working in agricultural produce markets, fellow farmers, relatives and friends. Chandio *et al*. [6] revealed that

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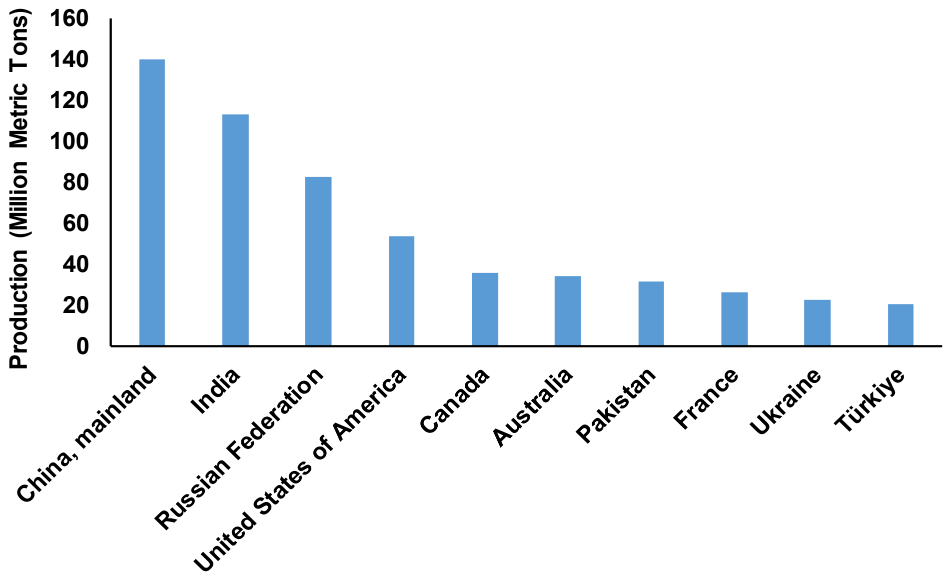

Fig. 1. Wheat production of top ten countries [3].

most of the time the amount of funds obtained from the non-institutional sources are often inadequate to cover the necessary agricultural operational expenses. However, Iqbal *et al*. [7] reported that various formal sources including Zarai Taraqiati Bank Limited (ZTBL), Cooperatives Societies and some Commercial Banks also play an important role in agricultural production. Provision of agriculture credit from these formal institutions to the farmers would assist them for purchase of various farms inputs and consequently it contributes in development of agriculture sector. The relationship between agriculture credit disbursement and wheat crop production over the time span in Pakistan is shown in Figure 2 and it shows that from 1994 to 1998 there were large gap between agriculture credit disbursement and production of wheat crop but after 1998 mostly both these variables move at constant direction.

Most of the area under agriculture sector is affected due to adverse effects of climate change and consequently productivity in agriculture sector is negatively impacted in Pakistan [8]. Such situation has resulted to be major hurdle in the way of enhancing the production of many crops including wheat in Pakistan. Moreover, same piece of land is repeatedly divided among the individuals of next generation and pressure on the land increases due to rising population growth [9]. Aslam and Fazal [10] reported that the land fragmentation negatively impacting the agricultural output from the land. According to the study of Ren *et al.* [11], it was concluded that farm size has a positive and substantial impact on the farm's net income earned from efficient production. Furthermore, relationship between cultivated area and production of the wheat crop in Pakistan from 1990 to 2023 revealed that cultivated area for wheat crop and production of wheat crop has an upward trend over the period of time.

Farooq *et al.* [12] observed that shortage of the water is a restrictive factor to increase winter wheat production. The Foulkes *et al.* [13] concluded that the prominent variations in the level of water stress sensitivity have been empirically confirmed for wheat crop cultivars in the case of United Kingdom. However, well designs research related to wheat production system is still a task for future innovative research which would be consistent to local natural conditions, input use efficiency and resilience capacity to the concern climate change factors. The trend between water availability (canal water supply for wheat season and rainfall) and wheat production with reference to Pakistan and it indicates that although production of wheat crop has been increasing over the span of time but water availability is not increasing rather recently it showed decreasing trend due to climate change.

The proper availability of good quality seed plays an important role in ensuring optimum wheat production. Further, the optimum rate of seeding is

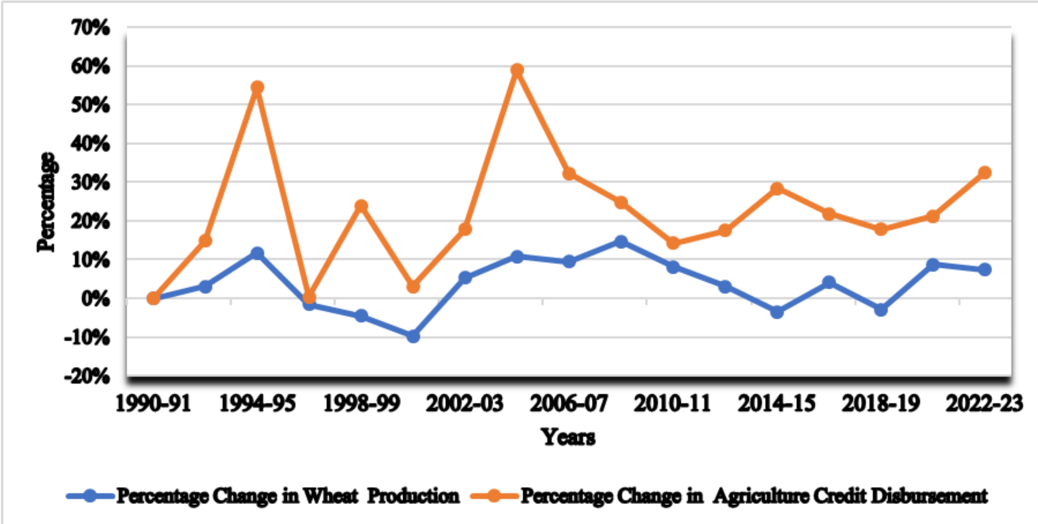

Fig. 2. Analysis of relationship between agriculture credit disbursement and wheat production for the period 1990 to 2023.
Source: Economic Survey of Pakistan, 2024.

taken as significant management influencing factor for improving yield of wheat production. Lloveras *et al*. [14] indicated that under the cropping system, seed rate is prime importance in case of wheat production as farmer can have control on it.

The trend between improved seed distribution of wheat crop and wheat production over the time period of 1990 to 2023 indicates that mostly there is a direct relationship between improved seed distribution and wheat crop production across Pakistan. Mainly because majority of wheat farmers perceived that there is an important role of improved seed availability for the rise in production of wheat crop. It is evident that improving the effective use of agricultural inputs to increase wheat crop production would play significant role for further raising production aspect of wheat crop of Pakistan at national and global levels. The countries which are ahead from Pakistan regarding wheat production are getting significantly higher yields due to some factors as mentioned in the literature, including seed quality, water availability, access to credit, and farm area enhancement which all contribute to higher production of wheat crop. No recent comprehensive study has been conducted for economic analysis of all these factors with reference to wheat production.

Pakistan is currently facing prolong period of drought which may cause water stress and impacting the productivity of various Rabi crops especially in rain fed region of Pakistan. The water level has been decreasing in major reservoirs of Pakistan due to climate change and consequently it impacted irrigation water availability for various crops including wheat. Main objective of the present research study is to analyze the impact of cultivated area under the wheat crop, availability of water, agricultural credit disbursement, and distribution of improved seed on the production of wheat crop in Pakistan.

This study introduces the latest and novel analytical framework with reference to prolonged drought period in Pakistan. While, existing literature is primarily focus on past data and not considered all the input factors with reference to Pakistan in recent time span. Keeping in view recent prolonged drought period in Pakistan, this study would also suggest which input factor further need to be utilized in an efficient way so that wheat production would be achieved in sustainable way in Pakistan.

2. MATERIALS AND METHODS

The annual data ranging from 1990 to 2023 (34 years), taken from Economic Survey of Pakistan (2023-24), is used in the present study for analysis [15]. The major purpose of this research is to investigate the impact of key farm input factors on production aspects of wheat crop in Pakistan. The present study used five variables including total wheat production (twp) in thousand tons, area under wheat cultivation (auwc) in thousand hectares, agriculture credit disbursement (acd) in million rupees, water availability (wa) in million-acre feet, and improved seed distribution (isd) in thousand tons in Pakistan. These variables have an important relationship with the wheat crop production in Pakistan.

2.1. Specification of the Model

For data analysis through the model, this research considered time series data which was collected from a secondary source that is also a government institution [1]. In order to analyze the impacts of various inputs on the output, agricultural economists widely employed Cobb-Douglas production function. For the purpose of analyzing input data, a log linearized Cobb-Douglas production specification was applied in this study. Further, Cobb-Douglas production is considered because it estimates the magnitude of changes in dependent variable because of variation in independent variables. Secondly, it measures elasticity of output to different farm inputs [16].
The following specified model is estimated:

$$\ln(twp) = \beta 0 + \beta 1 \ln(auwc) + \beta 2 \ln(acd) + \beta 3 \ln(isd) + \beta 4 \ln(wa) + \mu \ldots.$$

Where,

ln(twp): Log-transformed total wheat production in thousand tons
ln(auwc): Log-transformed area under wheat cultivation in thousand hectares
ln(acd): Log-transformed agriculture credit disbursement in million rupees
ln(isd): Log-transformed improved seed distribution for wheat in thousand tons
ln(wa): Log-transformed water availability in million-acre feet
and μ: Error term

Dickey-Fuller unit root test is applied in Cobb-Douglas time series modelling in order to check whether input variables and production variable contain unit root or not in the model. In the present study we considered Augmented Dickey-Fuller (ADF) as a unit root test. Dickey and Fuller [17] methodology has been applied for unit root test to determine the stationarity aspect of the concern variables. Considering projected output of unit root test, Johansen and Juselius [18] approach is used for estimation of co-integration technique. The Johansen cointegration test is employed in case of Cobb-Douglas production function to determine long run elasticity coefficients and by avoiding spurious regressions. These tests have a significant role to examine the time series data in econometric prospective. The optimal lag-length selected by estimating an unrestricted Vector Autoregression (VAR) model and by identifying lags which minimizes Akaike Information Criterion (AIC). Moreover, the Johannsen-Juselius Co-integration test used trend without intercept and with intercept. The main condition justifying the application of Johansen cointegration analysis is that all variables should be non-stationary at levels and integrated of the same order I(1), and share a long run equilibrium relationship. Furthermore, Johansen Co-integration technique has been applied for the examination of long-run association among the variables of interest [19]. In addition, this study applied Durbin-Watson test which is used for evaluating the aspect of autocorrelation [20].

The data of time period 1990-2023 analyzed by using STATA 17 version software for estimating the impacts of cultivated area under the wheat, water availability, disbursement of agricultural credit, improved seed distribution on total wheat production of Pakistan.

3. RESULTS AND DISCUSSION

It has been observed that non-stationary can lead to spurious type of regression output; consequently, findings of stationary can be vague. Hence, it is essential to confirm that input data in the question is stationery. Table 1 shows the results of estimated economic analysis of ADF, these results indicate that all variables are non-stationary at their level (0). However, again stationarity was checked for similar order of integration, and stationarity of all the variables achieved upon taking their first difference which is denoted as I(1). Hence, at the 5% significance level, the test rejects the presence of unit root (non-stationarity) in this study.

3.1. The Co-Integration Test

The Johansen Co-integration procedure applied for checking long-run association between the concern variables [19]. The estimated results of co-integration test, both with and without intercept,

Table 1. The unit root test.

Series	Integration at Level I(0)			First Differenced I(1)		
	ADF Test Statistics	Critical Value Estimates		ADF Test Statistics	Critical Value Estimates	
lntwp	-1.249 (0.639)	1% level	-3.661	-10.702 (0.000)*	1% level	-3.661
		5% level	-2.960		5% level	-2.960
		10% level	-2.619		10% level	-2.619
lnisd	-0.749 (0.819)	1% level	-3.653	-5.638 (0.000)*	1% level	-3.670
		5% level	-2.957		5% level	-2.963
		10% level	-2.617		10% level	-2.621
lnwa	-2.023 (0.275)	1% level	-3.653	-5.732 (0.000)*	1% level	-3.661
		5% level	-2.957		5% level	-2.960
		10% level	-2.617		10% level	-2.619
lnacd	0.327 (0.976)*	1% level	-3.653	-5.640 (0.000)*	1% level	-3.661
		5% level	-2.957		5% level	-2.960
		10% level	-2.617		10% level	-2.619
lnauwc	-1.801 (0.373)*	1% level	-3.653	-4.487 (0.001)*	1% level	-3.679
		5% level	-2.957		5% level	-2.967
		10% level	-2.617		10% level	-2.622

Note: *indicates 1% level of significance
Source: Author's own calculations.

are reported in Tables 2 and 3. The trace statistic values (152.891, 178.298) are greater than critical value estimates (76.972, 88.803) and these results illustrate that among the concern variables there is presence of long run relationship. Therefore, the no co-integration (null hypothesis) is rejected at chosen significance level. The statistical result of Trace test reveals four co-integrating equations for both cases by considering threshold significance level of 5%.

3.2. Regression Analysis

Keeping in view regression result, the R^2 is 0.95% which demonstrate that the model has good overall fitness indicating that 95% of changes in production of wheat crop explained by four explanatory input variables including Area under the cultivation of wheat, Water availability, Agricultural credit disbursement and Improved seed rate distribution. Moreover, it is also noted that probability value of F-statistics is highly significant in this regression model. Below mentioned Table 4, represents the concern estimated findings of regression examination.

As per findings of regression model, it seems that variable area under the wheat cultivation has most important impacts on wheat production because it has comparatively large value of coefficient. The present model shows that the coefficient value (1.176) of area under the wheat crop cultivation is statistically significant at 10% significance level and validate a significant

Table 2. Johansen-Juselius co-integration test including intercept without trend.

Variables: ln(twp), ln(isd), ln(wa), ln(acd), and ln(auwc)				
Lags Length: 1 to 3				
Eigenvalue Estimate*	Trace Stat. Estimate	Critical Value Estimate (5%)	Prob value*	Null Hypothesis (H_0)
0.839	152.891	76.972	0.000	None*
0.827	99.838	54.079	0.000	At most 1
0.602	48.798	35.192	0.001	At most 2
0.401	22.074	20.261	0.027	At most 3
0.219	7.201	9.1645	0.116	At most 4

The Trace test indicates 4 co-integrating equations at 5 percent level, and * denotes rejection of the hypothesis at 5 percent level
Source: Author's own calculations.

Table 3. Johansen-Juselius co-integration test including intercept and trend.

Variables: ln(twp) ln(isd) ln(wa) ln(acd) ln(auwc)				
Lags specification: 1 to 1				
Eigenvalue Estimate*	**Trace Stat. Estimate**	**Critical Value Estimate (5%)**	**Prob Value***	**Null Hypothesis (H_0)**
0.882	178.298	88.803	0.000	None*
0.839	116.263	63.876	0.000	At most 1
0.720	63.236	42.915	0.000	At most 2
0.499	26.233	25.872	0.045	At most 3
0.191	6.146	12.517	0.441	At most 4

The Trace test shows 4 co-integrating equations at 5 percent level, and * denotes rejection of the hypothesis at 5 percent level

Source: Author's own calculations.

Table 4. Regression results.

Dependent Variable: ln(twp)				
Method: Least Squares				
Sample: 1990- 2023				
Included observation: 34				
Explanatory Variables	**Coefficient**	**St. Error**	**t-statistics**	**Prob.**
C	-3.179	2.758	-1.152	0.258
lnwa	0.303	0.179	1.691	0.101
lnauwc	1.17630	0.320	3.672	0.001
lnisd	0.0546	0.0445	1.226	0.230
lnacd	0.059	0.021	2.781	0.009
R-squared	0.952	Adjusted R square	0.945	
Durban-Watson Stat	2.178			
Prob (F-Statistics)	0.0000			

Source: Author's own calculations.

relationship between wheat cultivated area and wheat production. Moreover, the coefficient value of 1.176 indicates that for one percent expansion in area under the cultivation for wheat crop leads to 1.17% increase in production of wheat crop. These results are in line with the results of Larik *et al*. [21] which was conducted with reference to Pakistan, and revealed that agricultural credit and increase in sowing area play a positive role in the production of wheat crop. Moreover, the coefficient of water availability has also significant impact at level of significance of 10 percent and it demonstrates that a significant relationship existed between water availability and production of wheat crop. As per value (0.303) of coefficient of water availability, it is understood that for 1% increase in the amount of water availability, there would be 0.3% rise in total wheat production. Likewise, the regression results of Cobb-Douglas production functions reveal significance of coefficient value of agriculture credit disbursement at 10% level of significance. It shows prominent relationship between production of wheat crop and agricultural credit disbursement as far as Pakistan is concerned. In addition, the coefficient of agriculture credit disbursement shows that for every one percent rise in agriculture credit disbursement, then 0.05% would be enhancement in wheat production. The findings of Chandio *et al*. [22] justified that credit disbursement from all institution has strong relationship with agriculture productivity and in their concern research study the agriculture credit disbursement have coefficient value (0.125) and it shows for a 1% rise in agricultural credit disbursement lead to enhancement in total wheat production by 0.12% tons by using secondary data over the time span of 1996-2015. Chandio *et al.* [23] also produced the same result by applying Cobb-Douglas production function and reported that institutional agriculture credit plays an important role in the improvement of wheat crop production. Furthermore, coefficient values of all independent variables in the present study have positive signs. Further, all these concern variables except improved seed distribution are statistically significant at significance level of 10%. However, improved seed distribution of wheat crop is non-significant and it is due to the reason that farmers didn't utilize seeds in adequate quantity along with proper agronomic practices. The government of Pakistan should formulate policy regarding to increase wheat production by considering proper collaboration among various allied departments within the agriculture sector. There should be more allocation of subsidized credits availability to wheat growing farmers so that farmers would be able to purchase require inputs in order to increase wheat productivity on their lands. Moreover, it is recommended that water losses should be minimized along the water channels in agriculture growing areas so that require water would be available to wheat growing areas. Due to increasing population and denser industrialization, it is noticed that wheat cultivating areas has been decreasing in Pakistan. There should be formulation of land use policies, which must restrict the use of wheat growing land for non-agricultural use beyond certain limits. Finally, the value of Durbin-Watson statistic (2.17) shows no autocorrelation in the model.

4. CONCLUSIONS

The present study economically investigated the effects of various inputs including area under the wheat cultivation, improved seed distribution of wheat crop, agriculture credit disbursement and water availability on the dependent variable (total wheat production) for the period of 1990-2023. It is concluded that improved seed distribution, availability of water, wheat cultivated area and disbursement of agriculture credit have positive relationship with production of wheat crop while improved seed distribution has insignificant relationship with wheat production. It is recommended to expand the area under wheat crop cultivation, effective distribution of improved seed and promotion of drought resistant varieties for wheat crop.

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6. ETHICAL STATEMENT

This study did not involve human or living subjects; therefore, ethical approval was not required.

7. CONFLICT OF INTEREST

The authors declare no conflict of interest.

8. FUNDING

No funding was received to conduct this research.

9. AUTHORS CONTRIBUTION SATEMENT

Hafiz Saqib Habib conceived the idea, performed data analysis and drafted full manuscript. Nasir Mahmood provided technical input at every major step of the study.

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