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Original Research Article

IMPACT OF WATER STRESS CONDITIONS ON ASSOCIATION OF AGRO-MORPHOLOGICAL, PHYSIO-CHEMICAL AND KERNEL QUALITY-RELATED TRAITS IN MAIZE HYBRIDS

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Abstract *Changing climatic conditions, especially high temperatures and droughts are one of the major factors hindering sustainable maize production in major maize-growing areas of the world. The current study was designed to determine the association between different morphological, physiological, biochemical, and kernel quality-related traits in maize hybrids under water-deficient conditions. The experiment material was comprised of seven indigenously developed maize hybrids from Maize and Millets Research Institute, Yusafwala-Sahiwal (YH-5482, YH-5427, YH-5404, YH-1899, FH-949, YH-5577, and YH-5395), one maize hybrid developed by local seed company i.e., Kissan Seed Corporation (SB-9663) and two maize hybrids developed by multinational seed companies i.e., Corteva Seeds Pvt Ltd. (P-1543) and Syngenta Seed Pvt Ltd. (NK-8441). The experiment was executed under a randomized complete block design with four replications. The results revealed that highly significant genetic variations were present among maize hybrids for morphological, physiological, biochemical, and kernel quality traits under water-deficient conditions except for ear length for which variations were found to be non-significant. Moreover, correlation coefficient analysis depicts a strong positive correlation of kernel yield with days to 50% silking, days to 50% tasseling, stomatal conductance, transpiration rate, total chlorophyll contents, 1000-kernel weight, ear length, and plant height. However, kernel yield was found to be negatively correlated with hydrogen peroxide, superoxide dismutase, and proline contents. Similarly, path coefficient analysis showed that days to 50% silking had the highest direct positive effect on kernel yield followed by 1000-grain weight, starch contents percentage, and ear height. Therefore, parental material selection must be done using these yield-associated trades under water-deficient conditions.*

Keywords: *Drought stress; Antioxidant; Photosynthesis; abiotic stresses; kernel protein*

Introduction

Maize (*Zea mays*), commonly known as corn in many Euro-American countries, is one of the most significant staple crops globally, serving as a fundamental component in food security, economic development, and cultural practices (Curry, 2021). It is a versatile crop used for human consumption, animal feed, and industrial applications, including biofuel production (Malhotra, 2017). Due to its significant utilization as food, feed, fodder, and biofuel, it is often regarded as a 4F crop. Maize is a primary food source for millions, providing essential nutrients such as carbohydrates, proteins, and fats, along with vitamins and minerals (Kaul et al., 2019). Its adaptability to diverse climatic conditions and soil types has allowed it to become one of the most cultivated crops in various regions

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worldwide, especially in the Americas, Africa, and Asia (Cairns et al., 2012).

Additionally, maize supports the livelihoods of countless smallholder farmers and contributes significantly to the agricultural economies of many developing countries. Along with several other key factors, abiotic stresses, such as drought, extreme temperatures, salinity, and nutrient deficiencies, also pose substantial challenges to sustainable maize production, often leading to significant yield losses and quality deterioration (Liliane and Charles, 2020). Drought stress, for instance, can severely affect maize at various growth stages, particularly during flowering and grain filling, resulting in reduced kernel number and size (Liu et al., 2022). High temperatures can exacerbate the effects of drought and directly impair photosynthesis and plant metabolism. Salinity stress can disrupt water uptake and ion balance, leading to stunted growth and poor crop performance (Aslam et al., 2012). Nutrient deficiencies, such as nitrogen or phosphorus, can limit maize's ability to achieve optimal growth and yield. The impact of these stresses is exacerbated by climate change, which increases the frequency and intensity of extreme weather events. Understanding and mitigating the effects of abiotic stresses through improved crop management practices and the development of stress-tolerant maize varieties are critical for sustaining maize production under changing environmental conditions (Cairns et al., 2012).

Among the discussed abiotic stresses, Drought or water stress significantly impacts maize yield, production, and quality, posing a major challenge to global food security. Water stress during critical growth stages, such as flowering and grain filling, can lead to substantial yield reductions by affecting kernel development and decreasing grain weight (Cakir, 2004). Maize plants under water stress conditions exhibit reduced photosynthetic activity, stomatal closure, and impaired nutrient uptake, which collectively diminish biomass accumulation and grain yield (Farooq et al., 2009). Additionally, water stress can also alter the chemical composition of maize, reducing the concentrations of essential nutrients and proteins in the grain, thereby affecting its nutritional quality (Bänziger et al., 2000). The reduction in yield and quality under drought conditions necessitates the development and deployment of drought-tolerant maize varieties and the adoption of efficient water management practices to mitigate the adverse effects of water stress on maize production.

Correlation and path coefficient analysis play a crucial role in understanding the complex relationships between different agronomically important traits in maize hybrids, aiding in selecting and breeding superior varieties. Correlation analysis helps identify the strength and direction of associations between traits, such as grain yield, plant height, and kernel weight, providing valuable insights for breeders (Kumar et al., 2014). However, correlation alone does not reveal these traits' direct and indirect effects on yield. This is where path coefficient analysis becomes indispensable. It decomposes the correlation coefficients into direct and indirect effects, allowing breeders to pinpoint traits that have the most significant direct impact on yield and those that influence yield indirectly through other traits (Dewey and Lu, 1959). By integrating both statistical approaches, plant breeders can make more informed decisions, enhancing the efficiency of selection programs and ultimately improving the genetic potential and performance of maize hybrids. For instance, a study by Sikirou et al. (2019) demonstrated that traits like ear length and kernel number per row had significant direct effects on grain yield, guiding the breeding strategy toward these critical yield determinants. The current study was planned to determine the association between grain yield and other related, agronomic traits under water stress conditions.

Materials and Methods

The research study was conducted during the spring growing season, in 2023 at the research farm of Maize and Millets Research Institute, Yusafwala-Sahiwal. The experimental material was comprised of ten maize hybrids including seven hybrids i.e., YH-5482, YH-5427, YH-5404, YH-1899, FH-949, YH-5577 and YH-5395 developed by the maize hybrid breeding teams, one local maize hybrid i.e., developed by Kissan Seed Corporation, Sahiwal (SB-9663) and two multinational maize hybrids i.e., P-1543 (Corteva Seeds Pvt Ltd.) and NK-8441 (Syngenta Seeds Pvt Ltd.). These hybrids were sown in RCBD design under four replications. The plant-to-plant distance was maintained at 20 cm while row-to-row was kept at 75 cm. These soil and irrigation water properties are given in Table 1. The sowing was done under water stress conditions i.e., at anthesis two irrigations were halted to create a water stress. All other production technology was kept the same as standard.

Several phonological, physiological, biochemical, and quality-related parameters were recorded/measured at their desired time frame. At the early flower stage, data was recorded for days

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to 50% tasselling and silking. Moreover, data for key morphological plant height, ear height, ear length, number of kernels per ear, thousand kernel weight, and kernel yield were also recorded. Plant physiological parameters i.e., SPAD value, transpiration rate, stomatal conductance, and chlorophyll contents recorded at the early reproductive stage of plant development. Seeds of all hybrids were subjected to chemical analysis through spectrometry to measure the accumulation of hydrogen peroxide (H_2O_2) and superoxide dismutase (SOD). Kernel quality traits including protein content percentage and starch content percentage were also calculated with the help of Near Infrared Spectroscopy (NIR).

Table 1: Properties of Irrigation water and Soil used for Maize sowing

Properties of Irrigation water used in the study		
Sr#	**Parameter**	**Value**
1	Total Soluble Salts (TSS)	1130 ppm
2	Sodium Adsorption Ratio (SAR	6.8
3	Residual Sodium Bicarbonate (RSB)	1.45
4	Chloride	4.3 meL
Properties of Soil on which the experiment was carried out		
1	Soil Texture	Sandy Loam
2	pH	7.9
3	EC	4.8 dSms
4	Organic Matter	0.73
5	Available P	7.5 ppm
6	Available K	85 ppm
7	Zinc	1.34 ppm

Statistical Data Analysis

The obtained data were subjected to analysis of variance (ANOVA), correlation coefficient analysis, and path coefficient analysis to observe the variations present in the data and to determine the relationship between different traits, respectively (Steel et al., 1997). The Statistix 8.1 and XLSTAT statistical packages were used to analyze the data.

Result and Discussion

The results obtained from the analyzed data showed the occurrence of significant variations among the maize hybrids under study (Table 2). The results revealed that variations among maize hybrids were highly significant for all the traits under study i.e., days to 50% tasselling (DT 50%), days to 50% silking (DS 50%), plant height (PH), ear height (EH), number of kernels per ear (NK/E), thousand kernel weight (TKW), kernel yield (KY), SPAD value (SPAD), transpiration rate (Tr), stomatal conductance (Ci), chlorophyll contents (Chl total), hydrogen peroxide (H_2O_2), superoxide dismutase (SOD), protein content percentage (Protein %) and starch content percentage (Starch %) except ear length (EL), for which the variations among maize hybrids were non-significant. The variations present in the data/germplasm are fundamental to the development of new plant varieties, including maize, as they provide the genetic diversity necessary for breeding programs to create improved cultivars. Genetic variation enables breeders to select traits that enhance yield, resilience to environmental stresses, disease resistance, and nutritional quality. Without sufficient variation, the potential for making significant genetic improvements is limited (Acquaah, 2012). For instance, phenotypic variations observed in traits such as drought tolerance, growth rate, and grain quality offer a rich resource for selecting superior genotypes that can thrive under various climatic conditions (Hallauer et al., 2010). Similar results were also reported by several researchers who described that variations present in the maize hybrids could be used to develop maize hybrids by selecting traits under water stress conditions (Shehzad et al., 2019; Ghani et al., 2020; Khalid et al., 2020; Yousaf et al., 2021; Yousaf et al., 2022; Ghani et al., 2023).

Table 2: Mean Square (MS) of selected plant traits of maize hybrids under water stress conditions

Parameters/Source of Variations	**Replications**	**Genotypes**	**Error**
Degree of Freedom	3	9	27
Day to 50% tasselling (DT 50%)	340.75	6.32*	3.24
Days to 50% silking (DS 50%)	321.93	6.78**	2.93
Plant height (PH)	2903.14	375.79**	116.93
Ear height (EH)	1278.02	355.60**	94.27
Ear length (EL)	18.97	1.13NS	2.28
Number of kernels per ear (NK/E)	31890.40	7181.10**	1044.80
Thousand kernel weight (TKW)	12302.70	4796.20**	325.50

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SPAD Value (SPAD)	396.57	26.46**	5.93
Kernel Protein Contents Percentage (Protein %)	4.58	0.76**	0.18
Kernel Starch Contents Percentage (Starch %)	13.52	14.83**	2.26
Transpiration Rate (Tr)	2.10	0.15**	0.04
Stomatal Conductance (Ci)	23983.50	1892.60**	331.30
Total chlorophyll contents (Total Chl)	1.74	0.35**	0.06
Proline Contents (Proline)	532.65	12.02	2.18
Hydrogen Peroxide (H_2O_2)	31.21	0.45	0.17
Superoxide dismutase (SOD)	1688.97	57.96	26.02
Grain Yield (GY)	29170000	1218485	317799

*Significant changes are highlighted by an asterisk (*). * $P \leq 0.05$. ** $P \leq 0.01$.*

Correlation coefficient analysis is a pivotal statistical tool in maize evaluation, facilitating an understanding relationship between various agronomic traits. This analysis helps breeders and researchers determine how different traits, such as plant height, leaf area, kernel weight, and grain yield, interrelate. By identifying these relationships, breeders can decide which traits to select for improvement. For example, a positive correlation between kernel weight and grain yield indicates that selecting heavier kernels could lead to higher overall yields (Kumar et al., 2014). The current experimental study revealed the presence of both strong positive and negative correlations between different traits in maize hybrids under water stress conditions (Table 3 & Figure 1). The kernel yield was found to have a strong positive correlation with SPAD value (r = 0.859**), days to 50% tasselling (r = 0.83**), days to 50% silking (r = 0.827**), stomatal conductance (r = 0.798**), transpiration rate (r = 0.789**), total chlorophyll contents (r = 0.667**), thousand kernel weight (r = 0.640**), ear length (r = 0.637**), protein content percentage (r = 0.620**) and plant height (r = 0.531*) (Table 3 & Figure 1). However, a strong negative correlation of kernel yield was observed with hydrogen peroxide (r = -0.900**), Proline (r = -0.764**), and superoxide dismutase (r = -0.701**) (Table 3 & Figure 1). The highest positive correlation was observed between days to 50% tasselling and days to 50% silking ((r = 0.998**) followed by transpiration rate and stomatal conductance (r = 0.929**). On the other side, the highest negative correlation was found between days to 50% tasselling and proline contents (r = -0.908**) followed by days to 50% silking and proline contents (r = -0.908**) (Table 3 & Figure 1). Similar results were also reported by Kumar et al., 2014; Masood et al., 2020; Gazal et al., 2018; Erdal, 2016; Mason et al., 2019 who showed that kernel yield of maize hybrids had a significant correlation with SPAD value, days to 50% tasselling, days to 50% silking, stomatal conductance, transpiration rate, total chlorophyll contents, thousand kernel weight, ear length, protein content percentage, plant height, hydrogen peroxide, Proline and superoxide dismutase.

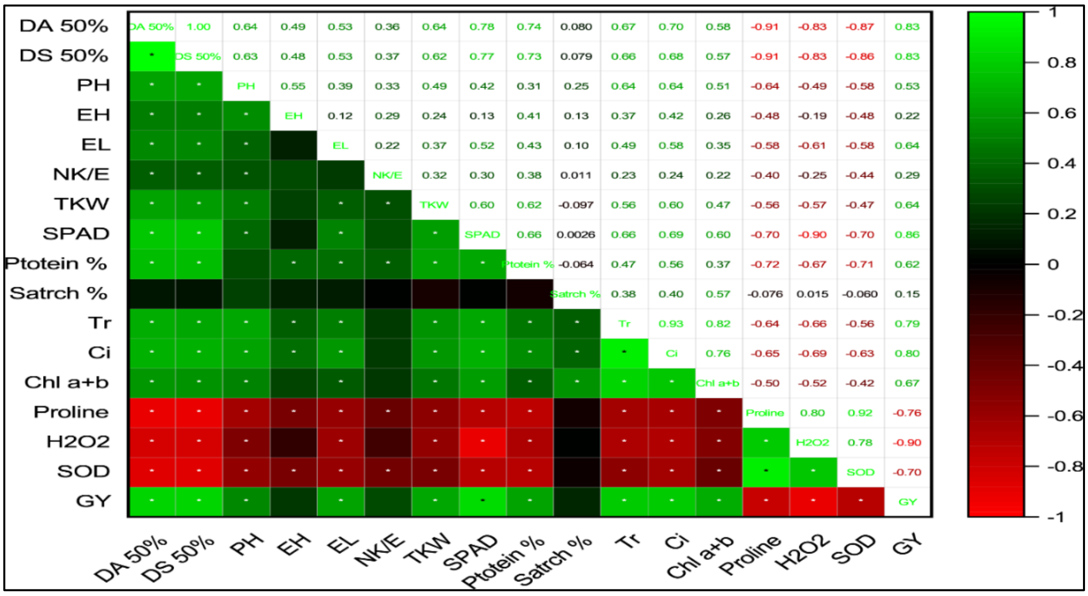

Fig 1: Correlation between different plant parameters in maize hybrids under water-deficient conditions

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Table 3: Correlation coefficients between different plant parameters in maize hybrids under water-deficient conditions

Variables	DA 50%	DS 50%	PH	EH	EL	NK/E	TKW	SPAD	Protein %	Starch %	Tr	Ci	Total Chl	Proline	H_2O_2	SOD
DS 50%	**0.998**															
PH	**0.635**	**0.633**														
EH	**0.487**	**0.484**	**0.555**													
EL	**0.532**	**0.528**	**0.392**	0.120												
NK/E	**0.360**	**0.369**	**0.328**	0.295	0.225											
TKW	**0.637**	**0.619**	**0.490**	0.241	**0.370**	**0.319**										
SPAD	**0.779**	**0.768**	**0.419**	0.129	**0.518**	0.301	**0.604**									
Protein %	**0.738**	**0.729**	0.307	**0.405**	**0.430**	**0.376**	**0.623**	**0.656**								
Starch %	0.080	0.079	0.255	0.128	0.104	0.011	-0.097	0.003	-0.064							
Tr	**0.666**	**0.655**	**0.641**	**0.372**	**0.488**	0.233	**0.562**	**0.657**	**0.466**	**0.379**						
Ci	**0.698**	**0.682**	**0.638**	**0.425**	**0.581**	0.239	**0.599**	**0.685**	**0.556**	**0.400**	**0.929**					
Total Chl	**0.581**	**0.567**	**0.506**	0.264	**0.350**	0.218	**0.473**	**0.604**	**0.372**	**0.567**	**0.823**	**0.762**				
Proline	**-0.908**	**-0.908**	**-0.639**	**-0.478**	**-0.582**	**-0.401**	**-0.557**	**-0.704**	**-0.721**	-0.076	**-0.639**	**-0.650**	**-0.498**			
H_2O_2	**-0.832**	**-0.827**	**-0.486**	-0.187	**-0.611**	-0.248	**-0.574**	**-0.903**	**-0.668**	0.015	**-0.665**	**-0.685**	**-0.518**	**0.800**		
SOD	**-0.866**	**-0.862**	**-0.579**	**-0.478**	**-0.584**	**-0.445**	**-0.474**	**-0.701**	**-0.712**	-0.060	**-0.558**	**-0.627**	**-0.418**	**0.924**	**0.780**	
KY	**0.831**	**0.827**	**0.531**	0.216	**0.637**	0.289	**0.640**	**0.859**	**0.620**	0.148	**0.789**	**0.798**	**0.667**	**-0.764**	**-0.900**	**-0.701**

Note: Values in bold are Significant

***DA 50%**: Days to 50% anthesis; **DS 50%:** Days to 50% silking; **PH**: Plant height (cm); **EH**: Ear height (cm); **EL**: Ear length (cm); **NK/E**: Number of kernels per ear; **SPAD** value; **TKW**: Thousand Kernel Weight (g); **Protein %**: Protein content percentage; **Starch %**: Starch content percentage; **Tr**: Transpiration rate (mmole m^{-2} s^{-1}); **Ci**: Stomatal Conductance (mmole m^{-2} s^{-1}); **Total Chl**: Total chlorophyll (mg g-1 FW); **Proline**: Proline contents (µg g^{-1} FW); **H_2O_2**: Hydrogen peroxide (µmole g-1 FW); **SOD**: Superoxide dismutase (U mg^{-1} Pro); **KY**, kernel yield per hectare (kg ha^{-1})*

Table 4: Direct and Indirect effects of different plant parameters in maize hybrids under water-deficient conditions

Traits	DA 50%	DS 50%	PH	EH	EL	NK/E	TKW	SPAD	Protein %	Starch %	Tr	Ci	Total Chl	Proline	H_2O_2	SOD
DA 50%	**-2.1661**	2.2509	0.1968	0.8428	0.0005	-0.0493	0.0656	-0.0139	-0.9746	-0.1360	0.0003	-0.0013	-0.0011	-0.1017	-0.0573	0.1052
DS 50%	-2.1445	**2.2736**	0.0529	0.8067	0.0135	-0.0402	-0.0613	-0.0288	-0.7701	-0.1754	-0.0326	0.0154	-0.0017	-0.1032	-0.0540	0.1103
PH	0.8331	-0.2350	**-0.5118**	-0.3949	0.1344	0.1150	-0.5156	-0.0370	1.2227	0.0941	-0.0533	0.0565	-0.0027	0.0153	-0.1617	-0.1702
EH	-2.6044	2.6165	0.2883	**0.7009**	0.0303	0.0627	-0.0661	-0.0543	-0.6911	-0.2260	-0.1445	0.0545	-0.0024	-0.2515	-0.7921	0.3159
EL	0.0092	-0.2463	0.5539	-0.1712	**0.1241**	-0.1765	-0.0467	-0.0073	-0.2742	0.1878	0.0199	-0.0111	0.0007	0.0248	-0.0226	-0.0112
NK/E	0.8785	-0.7512	-0.4842	0.3614	0.1802	**-0.1216**	-0.1592	-0.0997	0.5931	-0.2602	-0.0963	0.0791	-0.0016	-0.1990	-0.7158	-0.0943
TKW	-0.1260	-0.1235	0.2339	-0.0411	0.0051	-0.0172	**1.1285**	0.0533	-0.5929	-0.2605	0.0796	-0.0448	0.0005	0.0833	0.4263	-0.1746
SPAD	0.2784	-0.6082	0.1755	-0.3532	0.0084	-0.1125	0.5583	**0.1078**	-0.2007	0.1742	0.2209	-0.0945	0.0038	0.1749	0.6706	-0.1726
Protein %	-1.9870	1.6482	0.5890	0.4560	-0.0320	-0.0679	0.6298	0.0204	**-1.0624**	-0.1409	-0.0235	-0.0192	0.0009	-0.0615	-0.2038	0.1171
Starch %	0.4361	-0.5900	-0.0713	-0.2344	-0.0345	-0.0468	-0.4350	0.0278	0.2216	**0.6757**	0.0857	-0.0502	0.0030	0.0876	0.1102	0.0199
Tr	-0.0028	-0.3297	0.1212	-0.4501	-0.0110	-0.0521	0.3991	0.1059	0.1110	0.2575	**0.2249**	-0.0967	0.0042	0.2663	0.8032	-0.2625
Ci	-0.0298	-0.3672	0.3036	-0.4010	-0.0145	-0.1009	0.5303	0.1069	-0.2143	0.3559	0.2284	**-0.0953**	0.0046	0.2244	0.6464	-0.2191
Total Chl	0.5506	-0.8703	0.3092	-0.3808	-0.0189	-0.0431	0.1179	0.0909	-0.2161	0.4619	0.2125	-0.0979	**0.0044**	0.2043	0.4819	-0.1409
Proline	0.8669	-0.9232	-0.0307	-0.6940	-0.0121	-0.0953	0.3702	0.0742	0.2572	0.2331	0.2358	-0.0842	0.0036	**0.2540**	0.9329	-0.3552
H_2O_2	-0.1416	0.1403	-0.0945	0.6341	-0.0032	0.0994	-0.5494	-0.0826	-0.2473	-0.0850	-0.2063	0.0703	-0.0024	-0.2707	**-0.8756**	0.3447
SOD	0.7475	-0.8226	-0.2858	-0.7264	-0.0046	0.0376	0.6465	0.0610	0.4081	-0.0440	0.1937	-0.0685	0.0021	0.2961	0.9900	**-0.3048**

*Note: Values in bold are Direct effects of the corresponding traits **DA 50%**: Days to 50% anthesis; **DS 50%:** Days to 50% silking; **PH**: Plant height (cm); **EH**: Ear height (cm); **EL**: Ear length (cm); **NK/E**: Number of kernels per ear; **SPAD** value; **TKW**: Thousand Kernel Weight (g); **Protein %**: Protein content percentage; **Starch %**: Starch content percentage; **Tr**: Transpiration rate (mmole m^{-2} s^{-1}); **Ci**: Stomatal Conductance (mmole m^{-2} s^{-1}); **Total Chl**: Total chlorophyll (mg g-1 FW); **Proline**: Proline contents (µg g^{-1} FW); **H_2O_2**: Hydrogen peroxide (µmole g-1 FW); **SOD**: Superoxide dismutase (U mg^{-1} Pro); **KY**, kernel yield per hectare (kg ha^{-1})*

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Path coefficient analysis is an important biometrical approach that divides the correlation into its direct and indirect effects and it could be usefully implemented to identify the major contributing traits. The path coefficient analysis showed that several traits including days to 50% silking (2.2736), thousand kernel weight (1.1285), ear height (0.7009), and starch contents percentage (0.6757) had the highest positive direct effects on kernel yield (Table 4). However, days to 50% tasselling (-2.1661), protein contents percentage (-1.0624), hydrogen peroxide (-0.8756) and plant height (-0.5118) had the highest negative direct effects on kernel yield (Table 4).

Days to 50% tasselling had a positive indirect effect on kernel yield through days to 50% silking (2.25.9), ear height (0.8428), and plant height (0.1968). Ear height exhibited a positive indirect effect on maize kernel yield through days to 50% silking (2.6165), superoxide dismutase (0.3159), and plant height (0.2883) (Table 4). Ear length had a positive direct effect on kernel yield through plant height (0.5539), and starch contents percentage (0.1878). The number of kernels per ear had an indirect positive effect on maize kernel yield through days to 50% tasselling (0.8785), protein contents percentage (0.5931), and ear height (0.3614). Thousand kernel weight had a positive indirect effect on kernel yield via hydrogen peroxide (0.4263) and plant height (0.2339). Proline contents had a positive indirect effect on kernel yield via hydrogen peroxide (0.9329), days to 50% tasselling (0.8689), thousand kernel weight (0.3702), protein contents percentage (0.2572), transpiration rate (0.2358) and starch contents percentage (0.2331) (Table 4). Similarly, superoxide dismutase showed a significantly positive effect on kernel yield via hydrogen peroxide (0.9900), days to 50% tasselling (0.7475), thousand kernel weight (0.6465), protein contents percentage (0.4081) and proline contents (0.2961). The path coefficient analysis is an efficient analysis to divide the correlative effects of different parameters into their direct and indirect influences. Several researchers found it helpful in the characterization and selection of maize parents under water-deficient conditions (Garko et al., 2023; Ali et al.,2011; Aman et al., 2020; Mohanapriya et al., 2022; Ayalew et al 2018; Beiragi et al., 2011; Bankole et al., 2019; Rafiq et al., 2010; Krishna et al., 2021). Kernel yield depends upon several independent traits including morphological, physiological, biochemical, and quality-related parameters. Therefore, selection should not be solely based on yield alone but on the contributing or associated traits that must be included in the selection processes.

Conclusion

The current study revealed the presence of significant variation among maize hybrids under water-deficient conditions for studied morphological, physiological, biochemical, and quality-related plant traits. The correlation coefficient analysis showed that kernel yield had a positive and significant correlation with days to 50% silking, days to 50% tasselling, stomatal conductance, transpiration rate, total chlorophyll contents, 1000-kernel weight, ear length, and plant height. However, a strong negative correlation of kernel yield was observed with hydrogen peroxide, superoxide dismutase, and proline contents. Similarly, the path coefficient analysis showed that days to 50% silking had the highest positive direct effects on kernel yield followed by thousand-grain weight, starch contents percentage, and ear height. While days to 50% tasselling, hydrogen peroxide, superoxide dismutase, protein contents percentage, and plant height had strong negative direct effects on kernel yield in maize hybrid under water-deficient conditions. These results showed that any breeding program that aimed to develop a drought-tolerant maize hybrid must include these traits for the selection of parental material.

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Declaration

Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate

Approved by the department concerned.

Consent for Publication

The study was approved by authors.

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Conflict of Interest

There is no conflict of interest among the authors regarding this case study.

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All authors contributed equally.

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