

CUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

PhD THESIS

**Determining Physiological Characteristics of Sorghum
Accessions Under Drought Conditions**

Waqas LIAQAT

Field Crops Department

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PhD THESIS APPROVAL

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Determining Physiological Characteristics of Sorghum Accessions Under Drought Conditions

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ABSTRACT

Drought is one of the most devastating environmental stresses that significantly affect global agricultural production. Exploring and utilizing drought-resistant crops such as sorghum is crucial to compensate for yield losses caused by drought and ensure global food security. In this regard, a two-year experiment was conducted at the Mediterranean Agricultural Research Institute, Turkey, during 2022 and 2023. A total of 125 grain sorghum accessions, along with 6 commercial hybrids, were studied under full irrigation (100% field capacity) and deficit irrigation (50% field capacity). The experiment was laid out according to an augmented design. The results showed considerable variation in morpho-physiological and yield traits, such as plant height, grains per panicle, SPAD, stomatal conductance, canopy temperature, and NDVI. Traits such as tillers per plant, panicles per plant, panicle length, grain yield, and biological yield were similar under both deficit and full irrigation across both years, indicating that deficit irrigation does not significantly affect these traits. Under both full and deficit irrigation, grains per panicle showed a positive correlation with grain yield across both years. Similarly, SPAD (chlorophyll content) showed a positive correlation with stay green. Furthermore, 37 accessions (identified as ANZ_2, ANZ_10, ANZ_15, ANZ_17, ANZ_34, ANZ_39, ANZ_51, ANZ_58, ANZ_62, ANZ_84, ANZ_91, ANZ_105, ANZ_109, ANZ_112, ANZ_115, ANZ_123, ANZ_136, ANZ_156, ANZ_171, ANZ_180, ANZ_183, ANZ_191, ANZ_194, ANZ_195, ANZ_198, ANZ_199, ANZ_215, ANZ_218, ANZ_220, ANZ_225, ANZ_246, ANZ_343, ANZ_347, ANZ_351, ANZ_372, ANZ_418, ANZ_424) exhibited superior performance under deficit irrigation compared to full irrigation across both years, particularly in terms of grain yield. Cluster analysis revealed that most of these identified accessions clustered together, thereby confirming their consistently enhanced grain yield under deficit irrigation conditions over the two-year period. These accessions exhibit significant potential for drought tolerance and are thus prime candidates for incorporation into breeding programs focused on developing drought-resistant sorghum varieties. This experiment highlights the variability and adaptability of sorghum accessions to different irrigation treatments, offering valuable insights for future breeding and cultivation strategies.

Keywords: Sorghum, Deficit irrigation, Stay green, Grain yield, Stomatal conductance

Sorgum Genotiplerinin Kurak Koşullardaki Fizyolojik Özelliklerinin Belirlenmesi

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Tarla Bitkileri Anabilim Dalı

ÖZ

Kuraklık, küresel tarımsal üretimi önemli ölçüde etkileyen en yıkıcı çevresel streslerden birisidir. Kuraklık nedeniyle meydana gelen verim kayıplarını telafi etmek ve küresel gıda güvenliğini sağlamak için sorgum gibi kuraklığa dayanıklı bitkilerin araştırılması ve kullanılması çok önemlidir. Bu bağlamda, 2022 ve 2023 yıllarında Türkiye'de Doğu Akdeniz Tarımsal Araştırma Enstitüsü'nde iki yıllık bir deneme gerçekleştirilmiştir. Tam sulama (100% tarla kapasitesi) ve kısıntılı sulama (50% tarla kapasitesi) koşullarında toplam 125 dane sorgum hattı ve 6 ticari hibrit incelenmiştir. Deneme, augmented desenine göre düzenlenmiştir. Sonuçlar, bitki boyu, salkım başına tane sayısı, SPAD, stomatal iletkenliği, kanopi sıcaklığı ve NDVI gibi morfo-fizyolojik ve verim özelliklerinde önemli ölçüde varyasyon olduğunu göstermiştir. Bitki başına kardeş sayısı, bitki başına salkım sayısı, salkım uzunluğu, tane verimi ve biyolojik verim gibi özellikler her iki yıl boyunca hem kısıntılı hem de tam sulama koşullarında benzer bulunması kısıntılı sulamanın bu özellikleri önemli ölçüde etkilemediğini göstermektedir. Hem tam, hem de kısıntılı sulama koşullarında, salkımda tane sayısı her iki yıl boyunca tane verimi ile pozitif bir korelasyon göstermiştir. Benzer şekilde, SPAD (klorofil içeriği) yeşil kalma süresi ile pozitif bir korelasyon göstermiştir. Ayrıca, 37 hat (ANZ_2, ANZ_10, ANZ_15, ANZ_17, ANZ_34, ANZ_39, ANZ_51, ANZ_58, ANZ_62, ANZ_84, ANZ_91, ANZ_105, ANZ_109, ANZ_112, ANZ_115, ANZ_123, ANZ_136, ANZ_156, ANZ_171, ANZ_180, ANZ_183, ANZ_191, ANZ_194, ANZ_195, ANZ_198, ANZ_199, ANZ_215, ANZ_218, ANZ_220, ANZ_225, ANZ_246, ANZ_343, ANZ_347, ANZ_351, ANZ_372, ANZ_418, ANZ_424), özellikle tane verimi açısından her iki yıl boyunca kısıntılı sulama koşullarında tam sulamaya göre üstün performans sergilemiştir. Küme analizi, bu tanımlanan hatların çoğunun birlikte kümelendiğini ortaya koyarak, iki yıllık dönem boyunca kısıntılı sulama koşullarında yüksek tane verim potansiyeline sahip olduklarını doğrulamıştır. Bu hatlar, kuraklığa tolerans açısından önemli bir potansiyele sahiptir ve bu nedenle kuraklığa dayanıklı sorgum çeşitlerinin geliştirilmesine odaklanan ıslah programlarına dahil edilmek için birincil adaylardır. Bu denemede elde edilen sonuçlar, farklı sulama uygulamalarında sorgum genotiplerindeki değişimleri ve uyum yeteneklerini göstermekte ve gelecekteki ıslah ve yetiştirme stratejileri için değerli bilgiler sunmaktadır.

Anahtar Kelimeler: Sorgum, kısıntılı sulama, Yeşil kalma, Tane verimi, Stomatal iletkenliği

GENİŞLETİLMİŞ ÖZET

Sorgum, birçok az gelişmiş ülkede önemli bir temel gıda maddesi olarak kritik bir rol oynamakta ve gıda güvenliğine katkı sağlamada büyük önem taşımaktadır. Sorgum, dünya genelinde kurak ve yarı kurak bölgelerde yaşayan milyonlarca insan için temel bir tahıl ürünüdür. Bu bitki, kuraklık da dahil olmak üzere çeşitli abiyotik stres faktörlerine karşı olağanüstü bir dayanıklılık sergilemektedir. Ancak, sorgum üzerinde yapılan araştırmalar yeterli finansmanı almamıştır. Kuraklık, bitki gelişimini ve tarımsal verimliliği küresel ölçekte azaltan başlıca abiyotik stres faktörlerinden biridir. Şiddetli kuraklık, mahsul kalitesini ve miktarını önemli ölçüde düşürerek, gıda yetersizliği yaşayan bölgelerde potansiyel gıda kıtlığına yol açabilir. İklim değişikliği karşısında tarımsal üretimi optimize etmek amacıyla, kuraklık gibi aşırı hava koşullarına dayanıklı bitkilere olan talep giderek artmaktadır.

Sorgumun kuraklık koşullarına dayanabilme kapasitesi, hem genetik yapıya hem de çevresel koşullara bağlı olan çok yönlü bir özelliktir. Bu değişkenlik, çeşitli genotipler arasındaki morfo-fizyolojik özelliklerdeki farklılıklar tarafından etkilenmektedir. Bu çalışmada, toplamda 125 sorgum hat ve 6 ticari hibrit çişidi deneme materyali olarak kullanılmıştır. Deneme, 2022 ve 2023 yıllarında, Türkiye'nin Doğu Akdeniz Tarımsal Araştırma Enstitüsü'nde iki yıl süreyle gerçekleştirilmiştir. Denemeler, augmented densene göre yapılmış olup, sorgum hatları kısıntılı sulama (tarla kapasitesinin %50'si) ve tam sulama (tarla kapasitesinin %100'ü) koşullarında değerlendirilmiştir. Ekim işlemi, birinci yıl için 20 Nisan 2022 tarihinde, ikinci yıl için ise 24 Nisan 2023 tarihinde sıralara elle yapılmıştır.

Deneme sırasında, çiçeklenme süresi, fizyolojik olgunlaşma süresi, bitki boyu, gövde çapı, tane verimi, SPAD ve stomatal iletkenliği gibi çeşitli morfolojik ve fizyolojik parametreler değerlendirilmiştir. Varyans analizi, R Studio 4.3.2 kullanılarak ve "Augmented RCBBD" paketiyle gerçekleştirilmiştir. Hatlar arasındaki varyasyonu göstermek için Kutu grafiği, kullanılmıştır. Özellikler arasındaki ilişkileri belirlemek amacıyla korelasyon analizi yapılmıştır. Çalışılan özelliklerin anlamlı varyasyonlara genel katkısını değerlendirmek için temel bileşen analizi gerçekleştirilmiştir. Tüm özelliklerin hiyerarşik küme analizi, OriginPro 2024 yazılımı kullanılarak yapılmıştır.

Farklı sulama uygulamaları altında incelenen çoğu özelliğin varyans analizi (ANOVA), sorgum hatları arasında önemli bir çeşitliliğin mevcut olduğunu ortaya koymuştur. Fenolojik parametreler, başaklanma, çiçeklenme ve olgunlaşma gibi, her iki yıl boyunca tam sulama koşullarında daha yüksek bir değişkenlik göstermiştir. Aksine, tane sayısı ve bitki başına salkım sayısı gibi özellikler, her iki yıl boyunca eksik sulama koşullarında daha fazla değişkenlik sergilemiştir. Tane verimi, ilk yıl tam sulama koşullarında ve ikinci yıl kısıntılı sulama koşullarında artan bir değişkenlik göstermiştir. Biyolojik verim ise her iki yıl boyunca eksik sulama altında sürekli

olarak daha yüksek bir deęişkenlik göstermiştir. Fv/Fm özellikle ikinci yıl eksik sulama koşullarında artan bir deęişkenlik göstermiştir.

İki yıllık çalışma dönemi boyunca, tüm özelliklerin ortalama performansı, hem kısıntılı sulama hem de tam sulama uygulamaları altında deęişken tepkiler göstermiştir. Özellikle, salkım başına tane sayısı, bitki başına salkım sayısı, bitki başına kardeş sayısı, tane verimi, ekimden 45, 60 ve 75 gün sonra ölçülen SPAD deęerleri ve Fv/Fm oranı her iki yılda da kısıntılı sulama koşullarında tam sulamaya göre artış göstermiştir. Ayrıca, ikinci yıl kısıntılı sulama koşullarında, salkım uzunluğu, bitki boyu, sap çapı, biyolojik verim, ekimden 90 gün sonra ölçülen SPAD deęerleri ve stomatal iletkenliği deęerleri tam sulama koşullarına kıyasla artış göstermiştir.

Kısıntılı sulama ve tam sulama uygulamalarının karşılaştırılması, çeşitli büyüme parametrelerinde belirgin farklılıklar ortaya çıkmıştır. İkinci yıl kısıntılı sulama altında, başaklanma gün sayısı ve çiçeklenme gün sayısında net bir azalma gözlemlenmiştir. Her iki yılda da kısıntılı sulama altında fizyolojik olgunluk dönemine kadar geçen gün sayısı azalmıştır. Birinci yıl, kısıntılı sulama altında bitki boyunda bir azalma gözlemlenirken, ikinci yılda tam sulama altında bitki boyunda bir azalma yaşanmıştır. Gövde çapı, birinci yıl her iki sulama uygulamasında da aynı kalmış, ancak ikinci yıl tam sulama altında azalmıştır. Kardeş sayısı, bitki başına salkım sayısı, salkım uzunluğu, salkım başına tane sayısı, tane verimi, biyolojik verim ve hasat indeksi, her iki yıl boyunca her iki sulama uygulamasında da benzer kalmıştır.

İlk yıl kısıntılı sulama koşullarında yeşil kalma, tam sulama koşullarına göre daha düşükken, ikinci yıl her iki sulama uygulamasında da yeşil kalma benzer bulunmuştur. Bin tane ağırlığı ilk yıl her iki sulama koşulunda da tutarlı kalmış ancak ikinci yıl tam sulama koşulunda daha yüksek olmuştur. Ekimden 45 ve 60 gün sonra yapılan SPAD ölçümleri her iki yıl boyunca eksik sulama altında sürekli olarak daha yüksek bulunmuştur. Buna karşın, ekimden 75 ve 90 gün sonra yapılan SPAD ölçümleri, farklı sulama koşullarına deęişken tepkiler göstermiştir. Stomatal iletkenliği ilk yıl tam sulama koşullarında daha yüksekken, ikinci yıl kısıntılı sulama koşulunda artış göstermiştir.

Farklı gelişim evrelerinde ölçülen kanopi sıcaklıkları, sulama uygulamalarına farklı tepkiler göstermiştir. Birinci yıl kısıntılı sulama altında fotosistem II'nin kuantum verimi (Y(II)) daha yüksek bulunurken, ikinci yıl tam sulama altında daha yüksek olmuştur. Fotosistem II'nin maksimum kuantum verimliliği (Fv/Fm), birinci yıl her iki sulama uygulamasında da tutarlı kalmış, ancak ikinci yıl kısıntılı sulama altında anlamlı derecede yüksek bulunmuştur. Ekimden 65 gün sonra ölçülen NDVI, birinci yılda tam sulama koşullarında daha yüksek deęerler gösterirken, ikinci yılda kısıntılı sulama altında daha yüksek bulunmuştur. Öte yandan, ekimden 90 gün sonra ölçülen NDVI deęerleri her iki yılda da kısıntılı sulamaya kıyasla tam sulama altında sürekli olarak daha yüksek deęerler göstermiştir.

kısıntılı ve tam sulama uygulamalarında çeşitli morfolojik ve fizyolojik özellikler arasındaki korelasyonları deęerlendirmek amacıyla her bir sulama için ayrı analizler gerçekleştirilmiştir. 2022 büyüme sezonunda, kısıntılı sulama koşulları altında, bin tane ağırlığının hem fizyolojik olgunluk

günü hem de gövde çapı ile pozitif korelasyon gösterdiği belirlenmiştir. Ayrıca, salkım uzunluğu, yeşil kalma ve Fv/Fm ile pozitif korelasyon sergilemiştir. Bitki boyu ve gövde çapı biyolojik verim ile güçlü bir korelasyon gösterirken, bitki başına kardeş sayısı tane verimi ile pozitif bir korelasyon sergilemiştir. Yeşil kalma, farklı büyüme dönemlerinde yapılan SPAD ölçümleri ile anlamlı bir korelasyon göstermiştir. Ayrıca, tane verimi, salkım başına tane sayısı ile güçlü bir pozitif korelasyon sergilemekte olup, Fv/Fm de tane verimi ile pozitif bir korelasyon göstermektedir.

2022 yılında tam sulama koşulları altında çeşitli özellikler arasında birçok anlamlı korelasyon gözlemlenmiştir. Bin tane ağırlığı, hem tane verimi hem de biyolojik verim ile pozitif bir korelasyon sergilemiştir. Bitki boyu ve gövde çapı, biyolojik verim ile yüksek derecede pozitif bir korelasyon göstermiştir. Tane verimi, salkım başına tane sayısı ile güçlü bir şekilde pozitif korelasyon göstermektedir. Ayrıca, yeşil kalma özelliği SPAD değerleri ile pozitif bir korelasyon sergilemiş ve Fv/Fm, hem SPAD hem de yeşil kalma özelliği ile pozitif korelasyonlar göstermiştir.

2023 yılında kısıntılı sulama koşullarında, salkım başına tane sayısı SPAD değerleri ile pozitif bir korelasyon ve tane verimi ile güçlü bir pozitif korelasyon sergilemiştir. Bin tane ağırlığı, yeşil kalma özelliği, tane verimi, biyolojik verim, SPAD değerleri ve Fv/Fm ile pozitif korelasyonlar göstermiştir. Gövde çapı, biyolojik verim ile pozitif bir korelasyon göstermiştir. Yeşil kalma özelliği, hem biyolojik verim hem de SPAD değerleri ile güçlü bir pozitif korelasyon sergilemiştir. Ayrıca, tüm SPAD ölçümleri salkım başına tane sayısı ve bin tane ağırlığı ile pozitif bir korelasyon göstermiştir.

2023 yılında, tam sulama koşulları altında sorgumun farklı morfolojik ve fizyolojik özellikleri arasında birkaç önemli korelasyon gözlemlenmiştir. Özellikle, salkım başına tane sayısı, salkım uzunluğu ve SPAD değerleri ile güçlü bir pozitif korelasyon göstermekte olup, aynı zamanda tane verimi ile de pozitif bir korelasyon sergilemiştir. Ayrıca, salkım uzunluğu tane verimi ile pozitif bir korelasyon göstermiştir. Bitki başına kardeş sayısı ve bitki başına salkım sayısı arasında güçlü bir pozitif korelasyon gözlemlenmiştir. Ek olarak, gövde çapı ve bitki boyu biyolojik verim ile pozitif bir korelasyon göstermektedir. Tane verimi ise NDVI ile orta derecede pozitif bir korelasyon sergilemiştir.

Temel bileşen analizi 2022 yılında, veri setinin ilk iki temel bileşeni kısıntılı sulama ve tam sulama koşullarında sırasıyla toplam varyansın %33.44 ve %34.7'sini açıkladı. Benzer şekilde, 2023 yılında bu temel bileşenler kısıntılı sulama ve tam sulama koşullarında sırasıyla toplam varyansın %40.4 ve %35.8'ini açıkladı. Bu sonuçlar, farklı sulama uygulamaları altında çeşitli morfolojik ve fizyolojik özellikler arasındaki karmaşık ilişkileri vurgulamaktadır.

Ayrıca, 37 hat (ANZ_2, ANZ_10, ANZ_15, ANZ_17, ANZ_34, ANZ_39, ANZ_51, ANZ_58, ANZ_62, ANZ_84, ANZ_91, ANZ_105, ANZ_109, ANZ_112, ANZ_115, ANZ_123, ANZ_136, ANZ_156, ANZ_171, ANZ_180, ANZ_183, ANZ_191, ANZ_194, ANZ_195, ANZ_198, ANZ_199, ANZ_215, ANZ_218, ANZ_220, ANZ_225, ANZ_246, ANZ_343, ANZ_347, ANZ_351, ANZ_372, ANZ_418, ANZ_424) her iki yıl boyunca kısıntılı sulama

koşullarında tam sulama koşullarına kıyasla tane verimi açısından üstün performans sergilemiştir. Bu hatlar, kuraklık toleransı açısından önemli bir potansiyel göstermekte olup, bu nedenle kuraklığa dayanıklı sorgum çeşitlerinin geliştirilmesine yönelik ıslah programlarına entegre edilmek üzere ümitvar adaylar olarak değerlendirilmektedir.



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SYMBOLS AND ABBREVIATIONS

CF	:Chlorophyll fluorescence
CT	:Canopy temperature
DI	:Deficit irrigation
FI	:Full irrigation
Fm	:Maximum fluorescence of dark-adapted leaves
Fo	:Minimum fluorescence of dark-adapted leaves
Fv/m	:Maximum photochemical efficiency of PSII
NDVI	:Normalized difference vegetation index
PC	:Principle components
PRI	:Photochemical reflectance index
SC	:Stomatal conductance
Y(II)	:Quantum photosynthetic yield

1. INTRODUCTION

Sorghum (*Sorghum bicolor* L.) is a grass belonging to the Poaceae family and characterized by C4 photosynthesis. It initially emerged in Northeastern Africa between 8,000 and 5,000 B.C. (Mann et al. 1983). This region harbors the highest variety of cultivated and wild sorghum species (Baloch et al. 2023). Evidence of both domesticated and wild sorghum was discovered in burnt clay from the Kassala region, dating back to 3,500-1,500 B.C. (Beldados and Costantini, 2011; Beldados et al. 2018). The earliest evidence of domesticated sorghum was discovered in Sudan around 4,000 B.C. (Winchell et al. 2017). Sorghum ranks as the fifth most significant provider of carbohydrates globally, following wheat, maize, rice, and barley (Espitia-Hernández et al. 2022). Sorghum is highly valuable as a staple food crop for a big population living in semi-arid regions due to its high carbohydrate content (Hossain et al. 2022). It is frequently referred to as the "King of Millets," and is cultivated in several countries worldwide, such as India, China, Europe, Africa, the United States, and Mexico (Hossain et al. 2016; Popescu et al. 2018).

Sorghum has acquired substantial significance, particularly in Asia, Africa, and other regions characterized by a semi-arid climate (Hossain et al. 2022). Sorghum is predominantly utilized as a primary dietary source for humans, but in certain western nations like Brazil, Australia, and the USA, it is cultivated as a primary feed crop for animal consumption (Hossain et al. 2022). Nevertheless, researchers have extensively studied sorghum as a possible food source for humans due to its significant nutritional and physiological properties (Khoddami et al. 2023; Htet et al. 2022). It is a highly nutritious crop that is abundant in protein and fiber (Khalid et al. 2022) and is also a good source of calcium, phosphorus, and potassium, while having lower sodium levels compared to rice and wheat (Rao, 2019).

Sorghum is a vital staple crop in several underdeveloped countries globally and has a significant role in mitigating food insecurity (Motsi et al. 2022). Moreover, it is considered an essential primary cereal crop for millions of individuals residing in arid and semi-arid locations worldwide (Xin et al. 2021). Additionally, it finds application in many food products (Rashwan et al. 2021). Food items such as bread, biscuits, spaghetti, pastries, and porridge made from sorghum flour are appropriate for individuals following a gluten-free diet. It is also cultivated for the purpose of producing alcohol, bioethanol, and fuel (Tonapi et al. 2020; Mubarak Alqahtani, 2024). In order to meet the growing demand for food from the rapidly increasing global population, which is expected to surpass 9 billion by 2050, it is crucial to increase cereal production by around 70% (Neupane et al. 2022). According to Hadidi et al. (2023), there is an anticipated increase in food demand of 30% to 50% over the next three decades.

Sorghum is identified for its ability to self-pollinate and its possession of a C4 photosynthetic pathway, which contributes to its exceptional photosynthetic efficiency (Goyal et al. 2020). It demonstrates the effective use of nitrogen resources and the generation of significant biomass (Tu et

al. 2023). Sorghum and millets exhibit a wide range of variations, rendering them nutritionally superior and more adaptability to difficult ecological circumstances in comparison to maize (Hassan et al. 2021). Considering the impact of climate change, population expansion, and urbanization, it is essential for sorghum and millets to have a central position in the global food chain.

Sorghum demonstrates resilience to different abiotic stressors, including drought and salinity (Sharma and Joshi, 2022). However, it has not received significant research grant (George et al. 2022). According to Proietti et al. (2015), sorghum farming is mostly focused in areas where other food crops have difficulties in terms of performance. This crop exhibits resilience in regions where maize and other primary cereal crops struggle or are less compatible due to limitations imposed by changing climate and soil conditions (Rao et al. 2019). It can thrive in arid and hot areas with low resources (Prabhakar et al. 2022). Furthermore, it provides greater nutritional content, rendering it a more desirable option for consumption and exploitation (Khoddami et al. 2023). Due to its high tolerance to low input levels, this crop is well-suited for regions with limited rainfall. Sorghum may reach a height of up to 2 meters and is primarily cultivated in areas characterized by limited precipitation and high temperatures (Mundia et al. 2019). Sorghum can have a significant impact on feeding the world's most impoverished individuals, given the rising demand for scarce freshwater resources, the expanded utilization of marginal cropland, and the changing weather patterns (Hossain et al. 2022).

Climate change has a substantial impact on the earth's environment, which in turn affects the socioeconomic structure of human society. Climate change is a significant and pressing issue in the twenty-first century that has a profound impact on the well-being of the world and its natural resources (Arumugam et al. 2023). The recent escalation in climatic fluctuations and unfavorable meteorological circumstances has had a substantial impact on crop productivity in different places across the globe. Climate change is causing substantial disruptions to field agricultural production, making it imperative to enhance the ability of crops to withstand and recover from these challenges (Chandio et al. 2023). The decline in crop productivity can be ascribed to multiple factors including increasing temperatures, altered precipitation patterns, soil degradation, rise in sea levels, fluctuations in humidity levels, and changes in sunlight duration (Gershon and Mbajekwe 2020; Kumar et al. 2021; Chadalavada et al. 2021; Warsame et al. 2022). Without a doubt, these occurrences have a substantial impact on the worldwide agricultural system, leading to many aspects of food insecurity, such as the availability, stability, access, and usage of food (Naheed, 2023).

According to the IPCC (2018), it is estimated that approximately 122 million people could be forced into extreme poverty as a result of global warming by the year 2030. The combination of climate change's worldwide effects and the continuous reduction of cultivable and fertile land presents a complex situation in which meeting the global food demand with existing agricultural methods becomes progressively arduous, if not practically unattainable (Kumar et al. 2022). Introducing alternative crops into the global food network is crucial for effectively meeting the

nutritional requirements of persons affected by malnutrition, while also considering their ability to flourish in the face of climate change concerns (Feyisa, 2022). Currently, sorghum is considered a strong candidate because of its ability to withstand unfavorable weather conditions and thrive in less productive lands (Chadalavada et al. 2021). These beneficial characteristics allow it to be grown without taking up space that is designated for other main cereal crops. Furthermore, sorghum shows great potential in meeting the nutritional requirements of the world.

Drought is a major abiotic stress that globally diminishes plant development and agricultural productivity (Abreha et al. 2022). The prevalence of semi-arid regions has increased due to the scarcity, uneven distribution, and unpredictable nature of precipitation (Wang and Sun, 2023). Severe drought significantly diminishes the quality and quantity of crops, leading to potential food shortages in locations that already lack access to sufficient food (van Ginkel and Biradar, 2021). Drought stress hampers crop growth and development, leading to reduced yield due to decreased cell turgor, stomatal conductance, and carbon absorption (Prasad et al. 2019; Ghadirnezhad Shiade et al. 2023). In order to optimize agricultural production in the face of climate change, there is a growing demand for crops that possess the ability to endure severe weather conditions, such as drought. The primary challenges in agriculture at present are extreme environmental circumstances such as drought and high temperatures, among others (Zhao and Huang, 2023). Several places worldwide have become economically unviable for agricultural production as a result of environmental challenges and the impacts of climate change (Datta and Behera, 2022; Candau et al. 2022). According to the IPCC (2019), future climates are projected to experience an increase in the frequency of droughts.

Sorghum exhibits a low water requirement and is frequently seen as a crop that can withstand drought conditions. In addition, it has a higher tolerance for high temperatures compared to other cereals (Prasad et al. 2020; Mundia et al. 2019). It is mostly cultivated in regions with an annual rainfall ranging from 350 to 700 mm. Due to these characteristics, it is regarded as a valuable crop in regions characterized by unpredictable rainfall patterns and elevated air temperatures (Griebel et al. 2019). Sorghum has a reduced production cost, encompassing expenses for seed, fertilizer, pesticides, and other inputs, while yielding superior net profits in comparison to other prominent cereal crops like maize (Farré and Faci, 2006). The remarkable ability of sorghum to thrive in the face of ongoing climate change, which is marked by prolonged water scarcity and increasing temperatures, has been observed (Hossain et al. 2022).

Despite being a drought tolerant crop, sorghum output is also significantly affected by drought stress (Liaqat et al. 2024a). Drought stress affects every stage of plant development, with seed germination, early seedling growth, and reproductive phases being particularly vulnerable and crucial (Patanè et al. 2013; Prasad et al. 2019). Gano et al. (2021) revealed that early drought stress causes changes in sorghum physiology by reducing stomatal conductance, resulting in decreased CO₂ uptake and leaf transpiration rate. Additionally, it has been demonstrated that it decreases leaf water potential and the maximal quantum efficiency of photosystem II (Martínez-Goñi et al. 2023).

Drought stress can lead to damage to cell membranes, which in turn disrupts plant photosynthetic function (Zhang et al. 2019a). This stress greatly impacts the photosynthetic performance, which plays a crucial role in plant development and production during drought conditions (Zhang et al. 2022b).

The occurrence of drought has emerged as a significant determinant affecting sorghum output in semi-arid regions where sorghum plays a pivotal role as a primary crop. Eggen et al. (2019) found that certain areas in Africa that cultivate sorghum are prone to stress caused by drought. This stress has a remarkable impact on the physicochemical properties of sorghum, leading to a substantial decrease in both grain yield and quality (Queiroz et al. 2019; Liaqat et al. 2023). Insufficient water supply decreases the process of carbon absorption, cell turgor, and stomatal conductance, thus limiting the growth, development, and production of crops (Prasad et al. 2019). Consequently, plants respond to the severe effects of drought stress by exhibiting many reactions at the physiological, morphological, biochemical, and molecular levels.

The capacity of sorghum to endure drought is a multifaceted characteristic that relies on both genetic makeup and environmental conditions. The variability of this characteristic is influenced by differences in morpho-physiological properties among various genotypes (Gano et al. 2021). Sorghum's reaction to drought stress may encompass a range of mechanisms, including morphological, physiological, and anatomical adaptations. These adaptations help maintain an optimum water balance and enable the plant to tolerate drought, even when leaf water potential is low. This phenomenon has been recorded in prior studies (Badigannavar et al. 2018; Wagaw, 2019).

Sorghum's ability to withstand drought is due to a range of physiological adaptations, including leaf waxiness, leaf rolling, stomatal closure, stay-green, root morphological adjustments, solute accumulation, and osmotic adjustment (Badigannavar et al. 2018; Yahaya and Shimelis, 2022). As a result, sorghum is recognized as one of the most resilient crops when it comes to drought stress (Hadebe et al. 2017). However, crops vary in their ability to withstand drought, and even within a specific crop species, there is variance.

Comprehending the mechanisms by which plants respond to drought is crucial for the development of sorghum cultivars that can withstand drought conditions (Tovignan et al. 2023). Several plant species have developed enhanced resistance mechanisms to mitigate the adverse impacts of drought stress. However, the specific mechanisms vary depending on the plant species (Salehi-Lisar and Bakhshayeshan-Agdam, 2016).

Assessing the tolerance and adaptability of important agricultural crops to changing environmental conditions, such as drought, is of utmost importance. Similarly, studying the impact of drought on various plant species can aid in the advancement of breeding studies for the development of plant lines that are resilient to drought. The objective of this study was to thoroughly analyze the morpho-physiological responses of sorghum genotypes to both well-watered and water-stressed circumstances. In this study, the effects of drought on plant growth and development,

biomass and grain production were evaluated. Furthermore, the objective was also to gain a deeper understanding of the physiological reactions to drought stress conditions.





2. LITERATURE REVIEW

2.1. Main theme of the study

Sorghum is a genetically diverse grain crop cultivated in semiarid locations worldwide. It is an essential staple crop for millions of people in semi-arid regions of the world, where its production is severely limited by drought stress. Because of climate change, droughts are becoming more frequent and severe. As a result, there is a growing desire to comprehend the molecular and physiological mechanisms that contribute to sorghum's ability to withstand water deficient conditions. Therefore, to attain nutritional and food security, it is imperative to genetically boost the resistance of sorghum against water deficiency. Extensive study has been carried out regarding the processes that enhance drought tolerance, such as the ability to retain green foliage and maintain grain production in the presence of drought-induced stress. Nevertheless, it is imperative to thoroughly examine and utilize innovative drought-related characteristics including canopy temperature, reflectance indices, stomatal conductance, and chlorophyll fluorescence, in the process of breeding. Enhancing drought tolerance can be achieved by acquiring a more profound comprehension of the mechanisms behind tolerance, examining extensive sources of germplasm to identify resistant lines, and incorporating desirable features into superior breeding lines.

2.2. Role of climate change in increasing drought frequency and severity

Climate change presents a serious threat to worldwide agriculture, with drought emerging as one of its most catastrophic manifestations. The intricate interaction among climate change, drought, and agriculture gives rise to a multifaceted network of difficulties, necessitating immediate focus and viable remedies (Das and Ansari, 2021). Anthropogenic climate change is modifying the Earth's climate, resulting in changes in weather patterns, increasing temperatures, and erratic precipitation (Mulla et al. 2023). These alterations have a substantial effect on agriculture, causing disruptions to conventional growing seasons and posing increasing challenges for farmers in forecasting and organizing their crops.

Climate change significantly worsens drought situations (Zeng et al. 2023). There is an increasing occurrence of droughts, which are defined as extended periods of limited water availability, and they are growing more common and severe. Areas that were previously thought to be unaffected by water scarcity are now facing record periods of drought, putting crop production and food security at risk. The agricultural sector is highly susceptible to droughts since it heavily depends on water for irrigation and the growth of plants (Zaib et al. 2023). Inadequate water availability upsets the intricate equilibrium necessary for optimal agricultural growth, resulting in decreased yields, compromised crop quality, and financial losses for farmers (Cotrina Cabello et al. 2023). The ramifications of drought caused by changing climate on agriculture have far-reaching consequences that extend beyond the fields. Due to decreasing crop yields and rising costs,

vulnerable communities are experiencing a higher level of food insecurity (Christoforidou et al. 2023). Adopting measures to adjust and cope with the effects of climate change and drought on agriculture is of utmost importance (Grigorieva et al. 2023).

It is crucial to invest in water-efficient irrigation systems, create drought-resistant crop varieties, and promote sustainable farming methods. Moreover, aiding farmers in expanding their sources of income and promoting community resilience can improve their ability to adjust to a changing climate. Resilient crops have a crucial role in the context of climate change, and sorghum stands out as a notable candidate (Liaqat et al. 2024a). This resilient and water-efficient crop has attracted interest due to its capacity to endure difficult agricultural environments (Liaqat et al. 2024b). With the increasing occurrence of climate-induced drought, sorghum emerges as a valuable resource for sustaining agricultural output (Yahaya and Shimelis, 2022). Moreover, the crop's adaptability, as it may be utilized for nourishment, animal fodder, and bioenergy, enhances its importance in constructing a sustainable and diverse agricultural system (Nasidi et al. 2019). Encouraging the growth and use of sorghum, while also conducting ongoing research to improve its ability to withstand changing climate conditions, could make a substantial contribution to adapting agriculture to the more complex difficulties posed by climate change. Integrating these durable crops into farming methods is a proactive measure in establishing a food system that can withstand the effects of climate change.

2.3. Critical growth stages of sorghum in response to water deficiency

The impact of limited water availability is contingent upon the developmental stage of the plant at the commencement of the stress. Water shortage can occur at any stage of crop growth, from seedling emergence to physiological maturity in a natural environment (Verma et al. 2018). Sorghum is renowned for its capacity to withstand both intermittent and terminal water shortage (Hadebe et al. 2017). The main factors contributing to this phenomenon can be ascribed to the extensive root system of the plant, its ability to maintain relatively high levels of stomatal conductance, the preservation of internal tissue water potential through osmotic adjustment, and its phenological plasticity (Bejiga et al. 2021; Tsuji et al. 2003). Sorghum achieves higher yields than other major cereals in water-limited conditions because to its improved water usage efficiency (Bhattarai et al. 2020). This demonstrates that sorghum exhibits the capacity to endure water limited conditions and provide good yields in spite of the presence of stress caused by drought.

Sorghum has many reactions to drought stress, which might appear as changes in morphology, physiology, and phenology. Various sorghum genotypes have diverse levels of drought tolerance, particularly in relation to the timing of stress (Mutava et al. 2011; Yahaya et al. 2023; Rajarajan et al. 2021). According to Akram et al. (2011), certain genotypes of sorghum may exhibit significant tolerance to drought during one stage of growth but may be susceptible to drought during other stages. The variation in genetic sensitivity to water stress allows farmers to select sorghum

varieties that are best suited for specific local agricultural conditions, making it suitable for a wide range of locations.

The daily water requirements of sorghum fluctuate depending on the stage of crop growth, with the highest demand observed from booting to flowering (Abdel-Montagally, 2010). Consequently, sorghum is particularly vulnerable to water scarcity during this period. During the stages of grain filling, physiological maturity, and senescence, there is a steady decrease in the amount of water needed. To achieve optimal sorghum output, it is recommended to evenly distribute 450-650 mm of water throughout the growth season (Assefa et al. 2010). Reproductive phases are generally more vulnerable to ecological stressors compared to vegetative phases (Prasad et al. 2019; Djanaguiraman et al. 2020). Stages which are more vulnerable to water shortage in sorghum consist of panicle growth, flowering, and grain filling (Prasad et al. 2008).

2.4. Impact of drought on sorghum growth

Seedling mortality is a notable issue in arid areas where water scarcity is prevalent, and it is particularly severe during the primary stages of seedling establishment when both water deficiency and heat stress coincide (Ndlovu et al. 2021). Stand losses in sorghum may occur following full emergence as a result of limited water availability (Queiroz et al. 2019). Research conducted by Li et al. (2020), Bibi et al. (2012), and Tsago et al. (2014) has demonstrated that water scarcity during the seedling stage has negative effects on various physiological and morphological characteristics of sorghum. Considerable attention has been given to the influence of water scarcity on the early stages of sorghum development. However, the reaction of different sorghum genotypes differs under varying degrees of drought stress (Sarshad et al. 2021).

According to Bayu et al. (2005), seed germination was significantly reduced when there was a water shortage at soil water content levels of 40 and 60 percent of field capacity. The process of germination begins with imbibition, followed by the biochemical phase, active cellular division, and differentiation (Ali and Elozeiri, 2017). Limited availability of water, necessary for seed imbibition and the commencement of seed metabolic processes leads to a hindrance in seed germination. Consequently, this leads to a decrease in both the rate and total quantity of germination, even in crops capable of tolerating such conditions, like sorghum. Ndlovu et al. (2021) found that drought stress has the potential to significantly postpone the start of flower production and affect the growth of the panicle and the emergence of new leaves. The influence of water stress on shoot growth is more significant than on root growth (Bell et al. 2020; Bibi et al. 2010).

Following germination, a lack of moisture can greatly impede the development of radicle, hypocotyl, and plumule (Reiahi and Farahbakhsh, 2013; Queiroz et al. 2019). The inhibition of radicle development under water-limited conditions is likely caused by a reduction in turgor pressure in the radicle cells, which hampers cell division (Queiroz et al. 2019). This could significantly hinder the plant's development in the following stages. The influence of restricted water availability on the

growth of plants is more noticeable in sorghum cultivars that are vulnerable to drought stress, as opposed to those that demonstrate resilience to such circumstances (Rajarajan et al. 2021; Akman et al. 2021).

In a study conducted by Fadoul et al. (2018), it was shown that the cultivar that was susceptible to drought showed a decrease in both root and shoot length as compared to drought-resistant genotypes under water deficit conditions. These outcomes indicate that plant varieties with large and extended root systems have a higher chance of successfully establishing seedlings. This can be credited to the efficient root systems, which allow for rapid penetration of the topsoil and access to the moisture-laden layers below. Consequently, the plants can effectively uptake water and alleviate the negative consequences of water scarcity. Therefore, while evaluating sorghum genotypes for their ability to withstand drought, it is crucial to examine both the initial growth of the plants and other developmental factors such as seed vigor, germination rate, progression of the radicle and plumule, and the development of the root and shoot.

2.5. Impact of drought on sorghum productivity

Drought stress is a common source of food insecurity and yields gaps in many semi-arid environments (Mundia et al. 2019; Shayanmehr et al. 2024). On a global scale, drought reduces the maximum amount of grain that crop species can produce by as much as 70% (Kukul and Irmak, 2018). A significant portion of the sorghum cultivation area worldwide is primarily located in the semi-arid regions. These regions rely mostly on the cultivation of sorghum in arid areas, which are vulnerable to periodic droughts. According to Tack et al. (2017), an increase in temperature of one degree Celsius is predicted to result in a 10% decrease in sorghum output due to climate change. Drought leads to a reduction in chlorophyll content, photosynthesis, nutrient uptake, and translocation. This ultimately results in a loss in both grain yield and quality (Endris et al. 2021; Sehgal et al. 2018; Kapanigowda et al. 2013). Drought stress has a negative impact on grain size, weight, and other agronomic characteristics, resulting in a decrease in grain output (Impa et al. 2019; Sarshad et al. 2021; Sehgal et al. 2018).

Multiple studies have shown evidence that sorghum is an exceptionally resilient crop when it comes to drought, capable of flourishing in diverse agricultural environments and low-resource farming conditions (Mesfin and Girma, 2022; Dube et al. 2023). Nevertheless, even in plant varieties that demonstrate resistance to drought, the effect of drought stress can result in significant decreases in crop yield (Kapoor et al. 2020; Sabadin et al. 2012; Ray et al. 2018). In locations with limited water resources, the occurrence of unpredictable and reduced rainfall often has a substantial effect on crop production (Hattori et al. 2005; Nikolaou et al. 2020).

Gano et al. (2021) found that limited water availability can cause a decrease in agricultural output at any stage of crop growth, even the early stage of seedling development. Prior study has mostly concentrated on the consequences of stress encountered during a specific developmental

period, disregarding the reality that stress is consistently present across several stages in natural conditions. According to Assefa et al. (2010), drought stress during the vegetative stage leads to a 36% loss in yield, whereas during the reproductive stage, a reduction of 55% was found. Research has shown that the reproductive phases of plants are crucial and might adversely affect the amount of seeds produced (Sarshad et al. 2021). The grain filling stage is very vulnerable to water deficiency due to the involvement of enzymes, several metabolic processes, and transporters in the development of leaves and seeds (De Souza et al. 2015; Sehgal et al. 2018). The scarcity of water prior to and during the flowering phase has a negative influence on the productivity of sorghum, resulting in reduced grain output and lower quality in sorghum crops (Adugna and Tirfessa 2014; Kapanigowda et al. 2013). In a similar vein, Burke et al. (2018) found that drought stress after flowering led to nearly a 50% decrease in sorghum yield. This was mostly attributed to plant death prior to maturity and the production of smaller seeds.

Different sorghum genotypes exhibit varied responses to water scarcity, leading to diverse effects on their growth and development (Khalifa and Eltahir, 2023; Emendack et al. 2018). Sarshad et al. (2021) found that moisture deficiency during the pollination phase led to a significant decrease in crop output due to inadequate fertilization of the eggs in the ovary. The research indicates that drought stress has an impact on grain output, although the extent of this impact depends on several conditions. Factors such as the severity and duration of stress, the growth stage of the plant, the type of plant, changes in seasons, and other environmental stresses all add to the variation in the extent of damage caused by moisture deficiency.

2.6. Physiological characteristics for examining drought stress tolerance in sorghum

2.6.1. Stay green

Sorghum possesses the inherent quality of being drought-resistant, which allows it to maintain its greenness even under dry conditions. Stay green (SG) is the ability of the plants to maintain chlorophyll molecules during the post-flowering period, resist lodging, and produce seeds that are sufficiently filled (Jordan et al. 2012). Mahalakshmi and Bidinger (2002) stated that the sorghum homozygous line, B35, was created through the hybridization of Ethiopian durra and Nigerian landrace. This specific line is highly esteemed for its SG phenotype. Drought worsens the loss of chlorophyll in vulnerable genotypes, resulting in a reduction in the expression of SG trait (Begna et al. 2022).

Stay green is the practice of either postponing the start of leaf aging or reducing the rate at which leaf aging takes place (Jordan et al. 2012). There was a negative relationship between the rate at which leaves deteriorate and the crop yield when there is a lack of water both before and after flowering (Borrell et al. 2000a,b). The regulation of the SG trait is influenced by various mechanisms, including the nitrogen need of the seed, nitrogen supply from the leaf through translocation, and the nitrogen uptake assisted by the roots (Ostmeye et al. 2022). Researchers have identified four specific

QTLs, known as Stg1, Stg2, Stg3, and Stg4, that are linked to the SG phenotype. The QTLs account for roughly 54% of the observed phenotypic variance in sorghum genotypes with the SG feature. QTLs Stg1 and Stg2 have been discovered on the third chromosome using chromosome mapping techniques. Additionally, Stg3 and Stg4 have been found on the second and fifth sorghum chromosomes, respectively (Jaeggli et al. 2017; Borrell et al. 2014a).

Sorghum genotypes with QTLs for SG exhibit reduced canopy coverage and tiller number, resulting in decreased water usage during vegetative phase and increased water availability during post-anthesis moisture stress, which is crucial for seed filling (Jaeggli et al. 2017; Borrell et al. 2014a). In order to fully comprehend the communication between roots and shoots during drought conditions, it is imperative to carry out a meticulous examination of the root architecture of the SG genotype (Prasad et al. 2021). Research found that the correlation between canopy size before flowering and SG was not significant, but the correlation between canopy size after flowering and leaf withering was considerable (Liedtke et al. 2020). The preservation of chlorophyll content in sorghum plants plays a vital role in the formation of grains in conditions of sufficient irrigation as well as water deficient conditions (Prasad et al. 2021).

The feature of SG is widely recognized for its ability to help sorghum adapt to dry conditions after blooming by delaying leaf aging and enhancing grain production (Abeb et al. 2021; Begna et al. 2022). Preliminary studies have shown that during periods of drought, the ability to maintain green foliage is associated with increased levels of nitrogen concentration in leaves, as well as higher amounts of chlorophyll and cytokinin. For example, Borrell and Hammer (2000) found a correlation between the ability to SG and higher N concentrations in leaves, especially during flowering. Similarly, Thomas and Howarth (2000) revealed that sorghum genotypes with SG trait contain significant amounts of cytokinin, indicating a reduced rate of senescence. In addition, it was noted that the genotypes with the SG trait had a higher chlorophyll content compared to the senescent genotypes (Xu et al. 2000).

Research has shown that sorghum exhibits the SG phenomenon in response to drought. This is characterized by an increase in leaf chlorophyll content, a slower rate of leaf loss, reduced tillering, and smaller upper leaf size (Kassahun et al. 2010; Baloch et al. 2023; Borrell et al. 2014a; Jaeggli et al. 2017). In addition, Vadez et al. (2011) found that this characteristic is associated with increased water extraction and transpiration efficiency. The SG lines demonstrate accelerated age-related senescence of their lower leaves prior to blooming, resulting in the dropping of older leaves during the flowering phase. This tendency ultimately leads to a decrease in the size of the canopy.

According to Jaeggli et al. (2017), saving water before flowering can improve availability of water after flowering, which aids plants in maintaining their photosynthetic activity while keeping their green color during seed filling. Based on the research findings discussed earlier, it is reasonable to consider SG as a process that enhances drought tolerance after flowering. This mechanism ensures water availability, which is crucial for the overall plant growth and seed production.

2.6.2. Chlorophyll content

The chlorophyll content is a vital attribute that governs a plant's capacity to carry out photosynthesis. The spatial distribution of chlorophyll within the plant exhibits variation across different parts (Borsuk and Brodersen, 2019). Chlorophyll has a greater content in the central region of the plant as compared to the upper and lower regions (Mwamahonje et al. 2021). The content of chlorophyll varies according to the stage of the plant and is influenced by its genotype (Mwamahonje et al. 2021; Enyew et al. 2022).

The ability of a plant to sustain a stable chlorophyll content under drought-induced stress is a vital factor in its capacity to withstand drought conditions. The total chlorophyll concentration and the individual concentrations of chlorophyll a and b, directly affect the plant's capacity to absorb light for the process of photosynthesis. Multiple studies have seen a significant decline in chlorophyll levels in sorghum experiencing water deficiency (Fadoul et al. 2018; Djanaguiraman et al. 2020; Amoah and Antwi-Berko 2020; Fracasso et al. 2016a), leading to decreased grain production.

One drought adaptability method involves retaining a higher chlorophyll content when exposed to limited water availability, as opposed to genotypes that are vulnerable to drought (Abreha et al. 2022). Compared to the genotypes grown under normal conditions, the SG genotypes showed a 23% reduction in total chlorophyll levels, whereas the senescent genotypes had a 75% decrease upon exposed to drought stress (Xu et al. 2000). In contrast to the control group, Devnarain et al. (2016) reported a 4.3% decrease in the total chlorophyll concentration of plants subjected to stress.

Takele (2010) documented that sorghum's ability to withstand drought led to reduced chlorophyll and carotenoids levels in both the pre- and post-flowering stages. The decrease in carotenoids in genotypes that are sensitive to drought is probably due to the suppression of genes that are responsible for the production of carotenoids and terpenoids (Fracasso et al. 2016b). Drought stress leads to a decrease in the transcriptional activity of genes responsible for producing carotenoids and chlorophyll (Razi and Muneer, 2021). This phenomenon has a substantial impact on the processes of light response and carbon fixation. The study conducted by Reddy et al. (2014) found strong positive associations between the chlorophyll content at maturity and the number and area of green foliage during the flowering and maturity stages. Additionally, both of these leaf characteristics were strongly linked to grain output.

The high chlorophyll concentration in sorghum provides an advantage during periods of drought after flowering. This is because it enables the plant to carry out photosynthesis and sustain its physiological growth and grain yield. During periods of drought stress, the presence of a high concentration of chlorophyll in leaves slows down the natural aging process, known as leaf senescence. This helps to retain the photosynthetic capacity of the leaves, which in turn contributes to the overall yield of sorghum grains. The link observed between chlorophyll levels and SG expression in sorghum leaves during drought after flowering indicates that these features could be used to assess drought resistance and yield.

2.6.3. Reflectance indices

Spectral reflectance is the quantification of the wavelength of electromagnetic energy emitted by objects on Earth. The spectrum reflectance data are used to connect plant biochemical and biophysical parameters, such as biomass volume, crop evapotranspiration, and water content in the canopy, with their corresponding spectral features (Zhou et al. 2021; Virnodkar et al. 2020). Spectral indices are mathematical formulas that combine two or more spectral bands in order to assess agricultural water stress. The normalized difference water index (Xu et al. 2020; Zarco-Tejada et al. 2003; Rapaport et al. 2015), water index (Zhou et al. 2022; Zarco-Tejada et al. 2003), modified soil adjusted vegetation index (Shao et al. 2021; Rozenstein et al. 2018), photo-chemical reflectance index (Magney et al. 2016; Zarco-Tejada et al. 2013), normalized difference vegetation index (Ostmeyer et al. 2023; Rapaport et al. 2015; Baluja et al. 2012), and optimal soil adjusted vegetation index (Baluja et al. 2012; Romero et al. 2018) are commonly used spectral indices to assess water stress in crops. Photochemical reflectance index and NDVI are the most often used indicators for assessing crop water stress. These indices have been widely applied and thoroughly examined (Dembele et al. 2024; Katsoulas et al. 2016).

Normalized difference vegetation index

The NDVI is a widely recognized indicator that encompasses multiple reflectance ratios. The mathematical formula includes wavelengths in the green and near-infrared spectrum. Several studies have found a strong relationship between NDVI and biomass, chlorophyll, leaf area, and yield (Macedo et al. 2023; Maresma et al. 2020; Jones et al. 2007). However, Jones et al. (2004) suggest that $NDVI(800-640) = [(R800-R640)/(R800+R640)]$ may be a more accurate indicator of nitrogen content and biomass, although it provides only a moderate estimate of plant water content.

Investigations, conducted by Kim et al. (2010) and Genc et al. (2011), have found a robust correlation between NDVI (800-680) and the hydration condition of plants. Amatya et al. (2012) found that the relationship between soil moisture content and NDVI (800-640) in potatoes was stronger than the relationship between soil moisture content and NDVI (800-680). In contrast, Koksai (2011) and Jones et al. (2004) discovered a weak association between NDVI (800-640) and leaf water content in spinach, maize, and green beans. Nevertheless, a notable association was discovered between transpiration and enhanced crop productivity.

Marino et al. (2014) found that there is a direct relationship between the NDVI of the canopy and the rate at which leaves release water vapor through their stomata, known as stomatal conductance. A strong association between NDVI (490-620) and leaf water potential was reported by Shimada et al. (2012). In addition, Kittas et al. (2014) reported that NDVI (800-680) exhibited a more pronounced association with soil moisture levels in greenhouse tomatoes as compared to NDVI (490-620). Katsoulas et al. (2014) documented that the NDVI values at 800 and 680 nm did not vary even when the environmental variables, particularly the light intensity, changed.

Photochemical reflectance index

This index has been utilized in multiple investigations (Nakamura et al. 2024; Sarlikioti et al. 2010; Sukhov et al. 2021; Garbulsky et al. 2011; Magney et al. 2016). The formula for PRI is given by $PRI = (R531 - R570) / (R531 + R570)$. It is linked to rapid alterations in the de-epoxidation process of the xanthophylls cycle. The initiation of the xanthophylls cycle in a plant depends on its photosynthetic capability, which can change with light intensity (Magney et al. 2016; Sun et al. 2008).

Merlier et al. (2015) found that plants release excess absorbed energy as a protective measure against high leaf temperature, which in turn reduces photosynthetic activity during stressful situations. PRI exhibits enduring alterations in the carotenoids/chlorophyll ratio over a prolonged duration (Mänd et al. 2010). Thus, the impact of insufficient water on the efficiency of photosynthesis indirectly affects it (Garbulsky et al. 2011; Magney et al. 2016; Shimada et al. 2012).

PRI is affected by multiple leaf and canopy characteristics, including dry matter, chlorophyll content, leaf area index, leaf thickness, and leaf angle distribution. This was reported in multiple research (Kohzuma et al. 2021; Malenovský et al. 2009; Garbulsky et al. 2011; Mänd et al. 2010). According to Merlier et al. (2015), when chlorophyll content is not a limiting factor, PRI values are mostly linked to the moisture content of soil.

Water index

Water index (R970/R900) is a metric that quantifies the extent of water absorption in the mesophyll (Kakani et al. 2006; Hernandez et al. 2015). As the relative water content declines, the water index increases. The variability of WI is influenced by the leaf structure, which plays a crucial role in the absorption of radiation at wavelengths between 900 and 970 nm. Peñuelas and Inoue (1999) found that moisture stress led to an instantaneous decrease in water potential in monocotyledonous plants, particularly wheat. Nevertheless, in dicotyledonous plants, such as peanuts, which have a leaf structure that can hold twice as much water, the fall in WI only started when the leaf water content reached 60%.

Several investigations (Genc et al. 2011; Kittas et al. 2014; Amatya et al. 2012) have shown a correlation between the relative water content and the observed spectral relative changes in the range of 950 to 970 nm. The WI in olive trees exhibited a robust positive correlation with both stomatal conductance and water potential. Marino et al. (2014) observed that this correlation was more robust than other reflectance indicators like NDVI and PRI. Elsayed (2015) and Bandyopadhyay et al. (2014) used the normalized water index ($NWI = (R970 - R900) / (R970 + R900)$) to evaluate several spring wheat genotypes for seed yield. The performance of these genotypes was assessed under water-stressed and well-irrigated circumstances. The results of this study indicate a considerable negative correlation between NWI levels and grain yield, which can be attributed to

nutrient or water stress. This index can be effectively incorporated into techniques used to evaluate sorghum genotypes for their ability to withstand drought.

2.6.4. Canopy temperature

Canopy temperature (CT) measurements have been widely used to study how crops respond to drought conditions (Pradhan et al. 2022; Zhang et al. 2019b). This approach is based on the inverse correlation between leaf temperature and transpiration. In their study, Casari et al. (2019) found that there was an inverse correlation between grain yield and plant CT. However, it is important to note that simply keeping plant canopies cooler during drought conditions did not guarantee the maximum output. Many plants respond to drought conditions by closing their stomata to prevent water loss through transpiration. Exposure to drought stress leads to a reduction in leaf water potential and transpiration rate, resulting in an increase in CT (Turner et al. 2001).

Saitoh et al. (1999) found that genotypes with the capacity to adapt to water-deficient condition by effectively managing soil moisture during early development through appropriate transpiration, and then effectively utilizing it to regulate CT until the seed filling stage, are more possible to succeed. Plants that have higher stomatal conductance usually have higher rates of transpiration, which leads to a decrease in CT. Canopy temperature depression is utilized to assess variations in transpiration, water utilization, and stomatal conductance among several crop genotypes (Rebetzke et al. 2013). Correlations were observed in sorghum between canopy temperatures, water use efficiency, and grain yield (Ajeigbe et al. 2018; Raphaël et al. 2024). These correlations can be harnessed to enhance drought tolerance (Mutava, 2012). Implementing water conservation practices during the early phases of crop development, especially in situations with high evaporation rates, can help improve the ability of crops to withstand drought. The phenomenon, referred to as restricted transpiration, occurs when there is a substantial vapor pressure deficit. This was found in sorghum genotypes and documented by Choudhary et al. (2013) and Gholipour et al. (2012). It has the potential to enhance drought tolerance in sorghum.

It is important to include slow wilting lines exhibiting warmer canopies in the drought selection approach, notwithstanding the presence of drought conditions (Prasad et al. 2019). Mutava et al. (2011) discovered that canopy depression was employed as a means of identifying sorghum genotypes that exhibited higher yields under conditions of limited moisture, namely genotypes that maintained a cooler canopy. However, genotypes with a cooler canopy tend to consume a greater amount of water and are able to avoid the negative effects of water deficiency. Meanwhile, genotypes that maintain a warmer canopy are able to withstand drought and conserve water.

The capability to maintain vital physiological processes like photosynthesis during limited moisture availability serves as a dependable predictor of the ability to sustain productivity in water-limited situations. Sorghum exhibits physiological processes that enable continuous growth even in the presence of water scarcity. The physiological traits that enhance sorghum's ability to withstand

drought include elevated chlorophyll levels, delayed aging, increased chlorophyll fluorescence, and reduced canopy temperature (Harris et al. 2007; Liaqat et al. 2024a; Kapanigowda et al. 2013).

2.6.5. Chlorophyll fluorescence

The determination of Chlorophyll fluorescence (CF) is a newly developed technique that assesses the impact of several stresses, such as salt, drought, and temperature, on the photosynthetic efficiency of leaves in natural and greenhouse settings (Wen et al. 2023). Researchers can assess a plant's ability to convert absorbed light energy into chemical energy by examining factors such as the maximum quantum yield of PSII. In the context of CF, the term, minimum fluorescence (F_o) denotes the initial level of fluorescence exhibited by the sample while it is in a state of darkness adaptation (Yue et al. 2023). The decrease in fluorescence due to photochemical processes is most noticeable during the F_o stage, as it takes advantage of the greater efficiency of photochemical reactions at higher energy levels.

When the light intensity is sufficient, the fluorescence of the F_o value will increase to its highest level, known as maximum fluorescence (F_m) (Keller et al. 2019). The transition occurs when the chlorophyll molecules are activated and reach their peak fluorescence capacity. It is crucial to highlight that the parameters F_o and F_m play a significant role in assessing the activity of photosynthesis and the well-being of plants. The observed increase indicates a gradual and continuous enhancement in fluorescence yield, accompanied by a decrease in the speed of photochemical reactions (Zhang et al. 2022a).

Chlorophyll fluorescence is used to study the movement of energy within the photosynthetic system and its associated processes. It is commonly used to assess how different environmental conditions affect the efficiency of photosynthesis (Stefanov et al. 2023). CF's capacity for rapidity, informativeness, and non-invasiveness has rendered it a highly esteemed method in the fields of plant physiology and ecophysiology (Brestic et al. 2023). The non-invasiveness of CF is particularly advantageous as it allows for continuous monitoring and provides immediate data on the photosynthetic efficiency of plants (Zhuang et al. 2023). Due to its rapid response time, this technique is valuable for studying the dynamic reactions to environmental changes and experimental treatments.

Severe drought causes a decrease in chlorophyll content and negatively impacts the functioning of PSII, leading to a decrease in maximal efficiency i.e., F_v/F_m ratio (Sommer et al. 2023; Pradhan et al. 2022). This ratio is an indicator of the efficiency of photosynthesis, which is dependent on the performance of photosystem II and other electron acceptors further down the process. The PSII is a vulnerable component of the photosynthetic system that quickly deteriorates when plants are exposed to light during periods of drought and heat-induced stress (Maxwell and Johnson, 2000; Killi et al. 2020). The PSII functions as a primary site of light energy absorption, facilitating the production of chemicals like NADPH and ATP within the thylakoids via non-cyclic

photophosphorylation. In addition, it is linked to the oxygen-evolving complex, which aids in the generation of O_2 and H^+ ions. A decrease in Fv/Fm ratio indicates a dysfunctional PSII system and photo inhibition.

The Fv/Fm ratio usually falls within the range of 0.75 to 0.80 and shows a direct relationship with quantum yield (Kitajima and Butler, 1975). Observations have shown that when heat and drought stress coincide, it leads to lower chlorophyll fluorescence values and subsequently reduces the yield of crops like tomato, wild barley, chickpea, and lentil (Nankishore and Farrell, 2016; Jedmowski et al. 2015; Sehgal et al. 2019; Awasthi et al. 2017). The cause of this impact is linked to reduced levels of photosynthetic pigments. The resistance of different genotypes in crops such as rice, wheat, and chickpea to combined heat and drought stress has been distinguished by analyzing chlorophyll fluorescence demonstrating that it is a reliable way to assess the inhibition of photosynthetic electron transport (Balla et al. 2006; Kumar et al. 2014; Awasthi et al. 2017).

Evaluating photosynthetic characteristics like chlorophyll levels and fluorescence can assist in evaluating the impact of stresses on crop development and productivity (Wang et al. 2018; Shahid et al. 2020). Research findings suggest that there are significant differences in the amounts of chlorophyll content and fluorescence among genotypes that are susceptible and those that are resistant to water constraint (Chen et al. 2016; Mutava et al. 2011). These characteristics offer valuable insights into the physiological responses of different genotypes to diverse stresses.

Mutava et al. (2011) utilized CF as a method to evaluate the drought tolerance of different sorghum genotypes. Pradhan et al. (2022) found that drought stress caused a decrease in Fv/Fm in sorghum in comparison to control. The researchers also observed that the decrease in PS-II efficiency, as evidenced by stress-induced changes in Fv/Fm, can mainly be attributed to an elevation in leaf temperature caused by limited water availability. The decrease in PS-II efficiency can be ascribed to the deterioration of the cell membrane, as well as a decrease in the concentration of chlorophyll. A reduction in Fv/Fm in sorghum when subjected to drought stress was recently documented by Asadi and Eshghizadeh (2021).

2.6.6. Stomatal conductance

The size of stomatal openings is essential for controlling the movement of gases and the rate of transpiration. Stomatal conductance (SC) facilitates the transfer of water vapor and carbon dioxide between the leaves and atmosphere. These parameters have a significant impact on agricultural yield, especially in environments with limited water availability (Assefa et al. 2010; Endris et al. 2021). Maintaining SC is crucial for efficient gas exchange, especially for carbon dioxide absorption.

Gano et al. (2021) demonstrated significant genetic diversity in sorghum varieties in terms of physiological plant functioning parameters such as photosynthetic rate, stomatal conductance, leaf transpiration, and leaf temperature. This variability was observed under both well-watered and drought circumstances. Drought stress reduces the photosynthetic rate in sorghum via decreasing SC

and transpiration rate (Zhang et al. 2019b). Moreover, researchers have noted that moisture stress leads to a decrease in chlorophyll and Rubisco levels, an increase in the rate of O₂ evolution, and a decline in PEPCase activity (Bao et al. 2017).

According to the findings of Zelalem Getnet et al. (2015), tolerant genotypes of sorghum rely on rise in photosynthetic rate as a major mechanism to maintain grain output. This augmentation in photosynthesis enhances the supply of vital raw materials and energy necessary for regular growth in situations of inadequate water. In their study, Fracasso et al. (2016b) observed that there was no significant difference in transpiration efficiency between drought-stressed plants and control plants in sorghum genotypes exhibiting drought tolerance. Nevertheless, there was a significant difference observed between plants that were affected by drought and those that were not in terms of their sensitivity to dryness in specific genetic types. In addition, it was noted that genotypes with a high tolerance to drought showed significantly higher water use efficiency than genotypes that are sensitive to drought when subjected to drought stress (Fracasso et al. 2016b).

Lopez et al. (2017) found that specific genotypes have the ability to save water by reducing SC and transpiration rates during the vegetative growth phase. This water-saving capacity can then be used during the grain filling stage when water is scarce. Consequently, these genotypes can be categorized as drought tolerant. Furthermore, they determined that there is a correlation between quantitative trait loci related to stomatal conductance and a decrease in transpiration rate.

Genotypes that exhibit a quick response to water scarcity by promptly closing their stomata and maintaining their leaves at an optimal water level are more likely to withstand drought-induced stress. Sorghum maintains a high level of photosynthetic and transpiration efficiency due to its very effective C₄ photosynthetic pathway, despite having a low stomatal conductance. However, Geetika et al. (2019) and Ghannoum (2009) have found that severe drought stress decreases the efficiency of transpiration and photosynthesis in sorghum.

Stomatal closure has been shown to be the cause of reduced photosynthesis in sorghum under drought, leading to an accumulation of abscisic acid. The rise in abscisic acid buildup has an impact on carbon absorption (Ghannoum, 2009). The genotype has been identified as a crucial factor in determining the recovery from drought (Goche et al. 2020). Different genotypes exhibit varying degrees of sensitivity in SC in response to soil moisture supply (Akman et al. 2021).

Sorghum exhibits adaptive responses to water stress, including partial stomatal closure, leaf rolling, and a narrow leaf angle. These adaptations serve to reduce the exposed area to solar radiation and minimize transpiration. Intermittent water stress causes sorghum to partially close its stomata, which helps to sustain reduced photosynthetic activity. This results in a greater and consistent water use efficiency compared to other cereals that are prone to drought (Takele and Farrant, 2013). A list of some physiological attributes used to evaluate drought stress has been presented in figure 2.1.

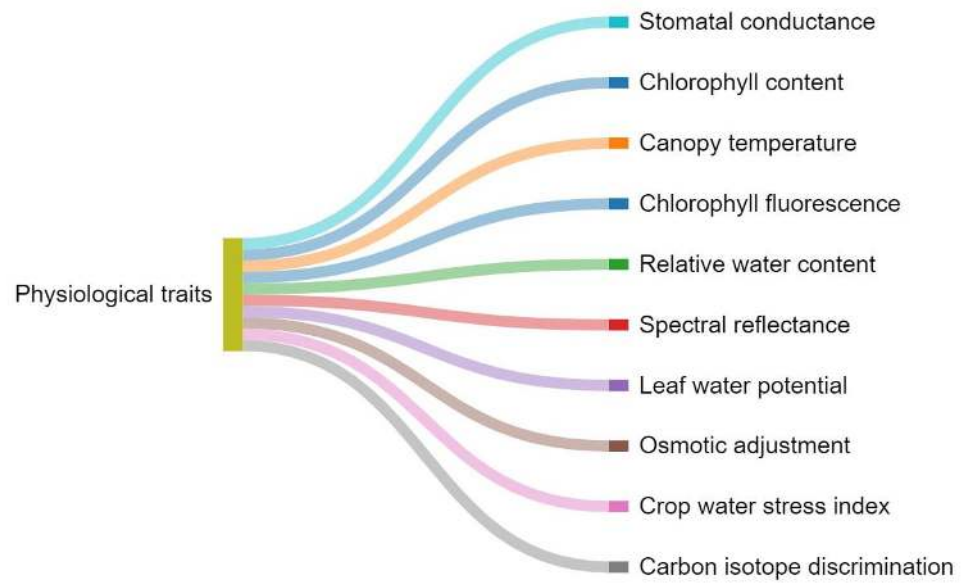


Figure 2.1 Some physiological traits utilized to evaluate drought stress in agricultural crops.

3. MATERIALS AND METHODS

3.1. Plant materials

A total of 125 sorghum accessions along with 6 commercial hybrids were used as the trial material in this study. These accessions were developed within the framework of the sorghum breeding program initiated 40 years ago (CREA-Italy) and are provided by Dr. Ephrem Habyarimana. Detailed information regarding the germplasm studied is provided in Table 3.1.

Table 3.1. Passport data of the studied sorghum germplasm.

S.No	Accession code	Pedigree	S.No	Accession code	Pedigree
1	ANZ_1	IS 20628	67	ANZ_163	IS 4717
2	ANZ_2	IS 3980	68	ANZ_165	76 BTP 52-2-1 x BTP-144
3	ANZ_8	IESV 94005 DL	69	ANZ_168	SDSL 98010
4	ANZ_10	IESV 92165 DL	70	ANZ_169	IESV 94027 SH
5	ANZ_12	IS 8315_bis	71	ANZ_170	IS 8193
6	ANZ_13	SDSL 98007	72	ANZ_171	IS 3681
7	ANZ_14	UMBRELLA (IDG 95893)	73	ANZ_172	Red Top Kandi
8	ANZ_15	BTX 623	74	ANZ_178	IESV 99091 DL
9	ANZ_17	KAOLIANG WAXY_gruppo_uno_2	75	ANZ_180	IS 2914
10	ANZ_18	IS 2134	76	ANZ_182	IESV 94027 DL
11	ANZ_25	ICSB 684	77	ANZ_183	K 1593
12	ANZ_27	IS 10422	78	ANZ_186	IS 205
13	ANZ_29	Gadam el Hamam	79	ANZ_188	IS 6253
14	ANZ_34	IS 7293	80	ANZ_189	HONEY (IDG 95901)
15	ANZ_39	IS 5098	81	ANZ_190	IESV 92011 DL
16	ANZ_44	BTP 28 (c)	82	ANZ_191	IS 12308
17	ANZ_45	PLAINS N-1 (SO 45)	83	ANZ_194	SDSL 90162-3
18	ANZ_50	IS 8563	84	ANZ_195	ICSB 686
19	ANZ_51	SDSL 96039	85	ANZ_198	IESV 92004 DL
20	ANZ_53	Early Amber	86	ANZ_199	ICSV-LM 87003
21	ANZ_58	IS 6278	87	ANZ_203	S. Durra (Stapf Redbine I-12-71)
22	ANZ_60	SDSL 90093	88	ANZ_204	SDSL 96040
23	ANZ_62	IS 3768	89	ANZ_209	SDSL 98002
24	ANZ_69	SDSL 90177-9	90	ANZ_211	IESV 92067 DL_gruppo_due
25	ANZ_74	SACCALINE	91	ANZ_212	IS 13118
26	ANZ_77	IS 1028	92	ANZ_215	IS 17917
27	ANZ_80	IS 8011	93	ANZ_218	IS 29139
28	ANZ_83	PI 250104	94	ANZ_220	SDSL 98014-1
29	ANZ_84	IS 23967	95	ANZ_223	ICSV - LM 90164

30	ANZ_85	IS 1220	96	ANZ_224	N98 (tall selection)
31	ANZ_86	IS 5124	97	ANZ_225	M36121
32	ANZ_88	IS 3517	98	ANZ_226	IESV 91110 DL
33	ANZ_91	Sever (Nord)	99	ANZ_229	SDSL 88298
34	ANZ_92	IS 9121	100	ANZ_230	L.S.803 x C.T.860
35	ANZ_95	Black Spanish Broomcorn	101	ANZ_232	DABAR
36	ANZ_96	SDSL 98013-1	102	ANZ_235	IS 2697
37	ANZ_105	SDSL 96036	103	ANZ_236	IS 12850
38	ANZ_106	KARI Mtama 1	104	ANZ_239	Town
39	ANZ_107	IESV 92021 DL_gruppo_uno	105	ANZ_241	SDSL 90143
40	ANZ_109	SDSL 96041	106	ANZ_246	IS 2118
41	ANZ_112	Mexico R-Line 5	107	ANZ_249	IS 17919
42	ANZ_114	VALLES ALTOS - 110 (d)	108	ANZ_253	S1477>R181
43	ANZ_115	Macia	109	ANZ_263	S1836-1-R89
44	ANZ_117	KSV 12	110	ANZ_266	X61-497-R4-R204C
45	ANZ_119	IS 23496	111	ANZ_272	S1468-4-R17LE
46	ANZ_120	IESV 92162 DL_gruppo_uno	112	ANZ_279	S1477-30-R51
47	ANZ_123	IS 1059	113	ANZ_290	X413-077-2
48	ANZ_129	LARSVYT 58-85	114	ANZ_297	S1479>R78C
49	ANZ_131	IS 6276	115	ANZ_309	H6-143-4
50	ANZ_133	S. sudanense (Sudangrain 2138)	116	ANZ_326	S1219-1-R166A
51	ANZ_135	IS 18493_gruppo_due_2	117	ANZ_343	S1264-1-14-R304
52	ANZ_136	IS 2867	118	ANZ_347	S1814-1-R220B
53	ANZ_138	IESV 99095 DL	119	ANZ_351	S1383-1-0-396D
54	ANZ_140	ICSV-LM 88110	120	ANZ_366	X1006-79-R5
55	ANZ_141	Da mangime	121	ANZ_372	X74-416-R4
56	ANZ_142	IESV 92053 DL_gruppo_due	122	ANZ_386	X61-497-R4
57			123	ANZ_387	S1479>R56B
58	ANZ_145	Zuccherino_gruppo_uno_1	124	ANZ_418	S1662-R33C
59	ANZ_146	IS 18209	125	ANZ_424	X61>R204A
60	ANZ_147	SDSL 90162- 2_gruppo_uno	C1	Gustav	-
61	ANZ_154	ENT 18-2 DTN_gruppo_uno	C2	Estyphon	-
62	ANZ_155	Sweet Bee	C3	Foehn	-
63	ANZ_156	IS 958	C4	Vegga	-
64	ANZ_157	IESV 91111 DL_gruppo_uno	C5	Aday1	-
65	ANZ_160	SDSL 90162-10	C6	Beydari	-
66	ANZ_161	IS 649	-	-	-

3.2. Experimental site and environmental conditions

The evaluation of all 125 sorghum accessions was conducted over two years, during the 2022 and 2023 sorghum growing seasons at the Eastern Mediterranean Agricultural Research Institute, Adana, Türkiye. This region is representative of the eastern Mediterranean climate, characterized by typically hot and humid summers. The experiments were performed on clay loam soil. The weather parameters recorded during the test seasons are depicted in Table 3.2.

Table 3.2. Weather data of the experimental location of two years i.e., 2022 and 2023 sorghum growing season.

Months	2022 growing season			
	Maximum Temperature (°C)	Minimum Temperature (°C)	Relative Humidity (%)	Total Rainfall (mm)
April	28.11	10.97	56.28	2.60
May	30.23	13.61	60.76	9.00
June	32.50	19.32	67.70	21.40
July	35.86	20.80	65.11	0.50
August	34.97	22.26	67.98	0.30
September	34.05	18.84	64.22	10.70
2023 growing season				
	Maximum Temperature (°C)	Minimum Temperature (°C)	Relative Humidity (%)	Total Rainfall (mm)
April	24.95	11.03	66.16	47.80
May	30.48	13.48	61.27	13.90
June	31.88	18.37	67.17	22.90
July	36.29	21.09	62.66	0.00
August	35.75	22.93	70.20	0.60
September	34.20	19.21	65.78	0.00

3.3. Crop husbandry/Experiment detail

3.3.1. Experimental design and field layout

Experiments were carried out according to Augmented design. The field was divided into two major sections: deficit irrigation (50% of field capacity) and full irrigation (100% of field capacity). In the full irrigation treatment, water was applied to replenish soil moisture to its maximum holding capacity when 50% of the available water was depleted in the top 90 cm of soil. In the deficit irrigation treatment, only half of this amount was applied (Alghory and Yazar, 2019). Soil moisture was measured using the gravimetric method, which is a standard technique for determining soil water content (Gardner, 1986).

The experimental field was subdivided into blocks and subplots according to an augmented design (Figure 3.1 and 3.2). Each experiment consisted of six blocks. Each block comprised 21

sorghum accessions and 6 commercial hybrids (C1 = Gustav, C2 = Estyphon, C3 = Foehn, C4 = Vegga, C5 = Aday1, C6 = Beydarı), which served as check or control genotypes. A distance of 2 meters was maintained between blocks, with a row-to-row spacing of 75 centimeters. Each accession was planted in a single row, 4 meters in length, with a plant-to-plant distance of 10 centimeters after final thinning. The first and last rows of each block were sown with the variety 'Erdurmuş' to serve as a border. The distance between the two major sections (deficit and full irrigation) was maintained at 7 meters. At the end of the two-year experiment, complete data was available for all the accessions, which were subsequently used for analysis.

2022

B-6	BC	BC	125	124	C3	123	122	121	C4	120	119	118	C1	117	116	115	C6	114	113	112	C2	111	110	109	C5	108	107	106	BC
B-5	BC	85	86	87	C4	88	89	90	C3	91	92	93	C6	94	95	96	C1	97	98	99	C5	100	101	102	C2	103	104	105	BC
B-4	BC	84	83	82	C2	81	80	79	C6	78	77	76	C4	75	74	73	C5	72	71	70	C3	69	68	67	C1	66	65	64	BC
B-3	BC	43	44	45	C6	46	47	48	C2	49	50	51	C5	52	53	54	C4	55	56	57	C1	58	59	60	C3	61	62	63	BC
B-2	BC	42	41	40	C1	39	38	37	C5	36	35	34	C3	33	32	31	C2	30	29	28	C4	27	26	25	C6	24	23	22	BC
B-1	BC	1	2	3	C5	4	5	6	C1	7	8	9	C2	10	11	12	C3	13	14	15	C6	16	17	18	C4	19	20	21	BC

Deficit irrigation

B-6	BC	BC	125	124	C3	123	122	121	C4	120	119	118	C1	117	116	115	C6	114	113	112	C2	111	110	109	C5	108	107	106	BC
B-5	BC	85	86	87	C4	88	89	90	C3	91	92	93	C6	94	95	96	C1	97	98	99	C5	100	101	102	C2	103	104	105	BC
B-4	BC	84	83	82	C2	81	80	79	C6	78	77	76	C4	75	74	73	C5	72	71	70	C3	69	68	67	C1	66	65	64	BC
B-3	BC	43	44	45	C6	46	47	48	C2	49	50	51	C5	52	53	54	C4	55	56	57	C1	58	59	60	C3	61	62	63	BC
B-2	BC	42	41	40	C1	39	38	37	C5	36	35	34	C3	33	32	31	C2	30	29	28	C4	27	26	25	C6	24	23	22	BC
B-1	BC	1	2	3	C5	4	5	6	C1	7	8	9	C2	10	11	12	C3	13	14	15	C6	16	17	18	C4	19	20	21	BC

Full irrigation

Figure 3.1. Field layout of the experiment in 2022. B= block, BC= border crop, C1= gustav, C2= estyphon, C3= foehn, C4= vegga, C5= aday1, C6= beydarı

2023

B-6	BC	BC	125	124	C3	123	122	121	C4	120	119	118	C1	117	116	115	C6	114	113	112	C2	111	110	109	C5	108	107	106	BC
B-5	BC	85	86	87	C4	88	89	90	C3	91	92	93	C6	94	95	96	C1	97	98	99	C5	100	101	102	C2	103	104	105	BC
B-4	BC	84	83	82	C2	81	80	79	C6	78	77	76	C4	75	74	73	C5	72	71	70	C3	69	68	67	C1	66	65	64	BC
B-3	BC	43	44	45	C6	46	47	48	C2	49	50	51	C5	52	53	54	C4	55	56	57	C1	58	59	60	C3	61	62	63	BC
B-2	BC	42	41	40	C1	39	38	37	C5	36	35	34	C3	33	32	31	C2	30	29	28	C4	27	26	25	C6	24	23	22	BC
B-1	BC	1	2	3	C5	4	5	6	C1	7	8	9	C2	10	11	12	C3	13	14	15	C6	16	17	18	C4	19	20	21	BC

Deficit irrigation

B-6	BC	BC	125	124	C3	123	122	121	C4	120	119	118	C1	117	116	115	C6	114	113	112	C2	111	110	109	C5	108	107	106	BC
B-5	BC	85	86	87	C4	88	89	90	C3	91	92	93	C6	94	95	96	C1	97	98	99	C5	100	101	102	C2	103	104	105	BC
B-4	BC	84	83	82	C2	81	80	79	C6	78	77	76	C4	75	74	73	C5	72	71	70	C3	69	68	67	C1	66	65	64	BC
B-3	BC	43	44	45	C6	46	47	48	C2	49	50	51	C5	52	53	54	C4	55	56	57	C1	58	59	60	C3	61	62	63	BC
B-2	BC	42	41	40	C1	39	38	37	C5	36	35	34	C3	33	32	31	C2	30	29	28	C4	27	26	25	C6	24	23	22	BC
B-1	BC	1	2	3	C5	4	5	6	C1	7	8	9	C2	10	11	12	C3	13	14	15	C6	16	17	18	C4	19	20	21	BC

Full irrigation

Figure 3. 2. Field layout of the experiment in 2023. B= block, BC= border crop, C1= gustav, C2= estyphon, C3= foehn, C4= vegga, C5= aday1, C6= beydari

Sowing and irrigation management

The seedbed for the experiment was prepared using a cultivator followed by a rotavator, and ridges were formed using a ridger. Sowing was conducted manually on the ridges on April 20, 2022, in the first year and April 24, 2023, in the second year on the soil to which no external fertilizer was applied before or during the experiment. Prior to sowing, composite soil samples were collected from three soil depths (0-30 cm, 30-60 cm, and 60-90 cm) to determine the soil's texture and chemical characteristics. The soil properties evaluated include organic matter content, total salt content, pH, lime content, electrical conductivity, texture, phosphorus, potassium, and nitrogen levels. The organic matter content, with values around 0.74% to 0.93%, indicates a moderately fertile soil that supports microbial activity and nutrient availability. The total salt content is low, between 0.03% to 0.04%, which is ideal for preventing salinity stress in plants. The soil pH is slightly alkaline, ranging from 7.81 to 7.93, which is generally suitable for most crops, allowing for good nutrient availability. Lime content is relatively high, around 14.78% to 15.41%, indicative of calcareous soil. Electrical conductivity values of 1.42 to 1.63 dS/m suggest slight salinity but is typically manageable for most crops. The texture of the soil is clay loam, providing a good balance of water retention and drainage, which is favorable for root growth and overall soil health. Phosphorus levels, approximately 9.93 ppm to 10.03 ppm, are adequate for promoting root development and plant growth. Potassium levels, ranging from 98.71 ppm to 101.4 ppm, are sufficient to support various plant functions. Nitrogen content, between 0.06% to 0.08%, indicates a moderate level of nitrogen availability, essential for

vegetative growth. Overall, these soil properties suggest a fertile and well-balanced soil environment, capable of supporting healthy plant growth and productivity. The results of the soil analyses are presented in Table 3.3.

Table 3.3. Chemical properties of experimental soil.

Soil properties	2022	2023
	Amount	
Organic matter content	0.74%	0.93%
Total salt content	0.04%	0.03%
pH	7.93	7.81
Lime	15.41%	14.78%
Electrical conductivity (dS m ⁻¹)	1.42	1.63
Texture	clay loam	clay loam
Phosphorus	9.93 ppm	10.03 ppm
Potassium	101.4 ppm	98.71 ppm
Nitrogen	0.06%	0.08%

After sowing, sprinkler irrigation system was installed in such a way (12m x 12m) to provide equal amount of water to each block for proper seed germination. The irrigation water properties indicate a slightly alkaline pH of 7.1 and moderate salinity with an electrical conductivity (EC) of 1 dS/m. The sodium adsorption ratio (SAR) of 0.82 and the irrigation water class of C2S1 suggest that the water is of medium salinity with low sodium hazard, suitable for most crops. Chemical properties of the water used for irrigation have been shown in table 3.4.

Table 3.4. Chemical properties of water (irrigation).

pH	EC (dS m ⁻¹)	Na	K	Ca+Mg	Cl	SO ₄	SAR	Irrigation water class
		me/l						
7.1	1.00	1.07	0.04	3.38	1.48	0.72	0.82	C ₂ S ₁

EC= Electrical conductivity, SAR= Sodium adsorption ratio, C2S1= medium salinity with low sodium category

After emergence, drip irrigation system was established separately for each of the trial plots. Drip irrigation pipes (lateral) were installed in each plant row (Figure 3.3). In order to apply equal amounts of water to the plants, a pressure valve was installed in the irrigation system, and the system was enabled to operate at 1 atmospheric pressure.



Figure 3.3. Irrigation system installation.

3.3.2. Crop management

The field was regularly monitored for pests and disease attacks as well as weed infestations. Insecticide Genban 4 EC (active ingredients: 480 g/l Chlorpyrifos-Ethyl) was applied @ 180 ml/da during sowing and again 5 days after sowing to control insects such as ants. Following germination, the insecticide KarateZeon was applied at the rate of 30 ml/da after every 14 days till crop maturity to control stem borers. Fungicide Sakozeb M-45 was applied at the rate of 80g/da to the whole field 35 days after sowing. Weed control was initially managed through hand hoeing 25 days after germination. Thinning was performed 30 days after germination to maintain a 10 cm spacing between the plants. After thinning, earthing up was carried out using ridger to prevent lodging. For chemical weed control, the herbicide Arrat was applied @ 25 g/da 25 days after sowing to target broad leaf weeds. To ensure adequate micronutrient supply and mitigate any potential deficiencies, a foliar application of micronutrients (13% K₂O, 2% total Magnesiumoxide, 1.8% water soluble magnesiumoxide, 6% total-sulphur, 5.4% water soluble sulphur, 0.016% water soluble boron, 0.04% total copper, 0.4% total iron, 0.05% total Manganese, 0.01% total molybdenum, 0.01% total zinc chloridarm) was carried out uniformly for all the accessions. Field inspections were conducted daily. After the flowering stage, the panicles were covered with paper bags to protect them from bird damage (Figure 3.4).



Figure 3. 4. Field operations during the experiment.

3.4. Measurements

The details of the agronomical and physiological parameters taken during the experiment are given below.

3.4.1. Agronomical parameters

Days to 50% emergence: It was determined by counting the number of days from the date of sowing till 50% of plants emerged in each subplot.

Days to booting: Days to booting were determined by counting the number of days from the date of sowing till 50% of the sorghum panicle, also known as the head, is in the flag leaf sheath and can be seen as a bulge or swelling. Leaf collars of all leaves are visible, and the panicle is pushed up through the flag leaf collar by the upper stalk, known as the peduncle.

Days to heading: Days to heading were determined by counting the number of days from the date of sowing till 50% of the sorghum panicle becomes visible after it emerged from the flag leaf sheath.

Days to flowering: It was calculated as the number of days from the date of sowing till blooming has progressed halfway down the panicle in 50% of the plants in each subplot.

Days to Physiological maturity: It was determined by counting the number of days from the date of sowing till a dark spot or black layer on the bottom of the kernel appears in 50% of the plants in each subplot.

Plant height (cm): The length of the five selected plants from soil surface to the tip of the main panicle was measured at physiological maturity with a meter rod and averaged in each subplot.

Stem diameter (mm): Stem median diameter was calculated by measuring the diameter of five plants with the help of a digital vernier caliper in each subplot and then values were averaged.

Number of tillers per plant: Tillers in five representative plants in each subplot were counted and then averaged.

Number of panicles per plant: The number of panicles in five plants selected for determining tillers per plant was counted and then their average was taken.

Panicle length (cm): Panicle length was measured with a ruler by taking the length from the base to the tip of each panicle of five selected plants in each subplot. The data were then averaged for a single value.

Number of grains (grains panicle⁻¹): After harvesting, five main panicles were threshed, cleaned and the seeds in these panicles were counted with a seed counter and the average grains per main panicle was determined.

Biological yield (dry matter) (g plant⁻¹): Five plants in each subplot were harvested, cut into pieces and oven-dried at 60°C till constant weight. Each sample was separately weighed with electronic balance and then the average dry matter per plant was determined.

Grain yield (g plant⁻¹): Panicles were separated, threshed and cleaned from the plants harvested for determining biological yield. Seeds taken from each panicle were weighed with electronic balance and their average was taken to determine grain yield per plant.

Harvest index (%): Harvest index was determined by taking the ratio of grain yield to biological yield and then multiplying with hundred to get the result in percentage.

Stay green (%): Stay green was calculated by visual observations for each genotype. Genotypes were given percentages based on the green leaves present at physiological maturity (Xu et al. 2000; Jordan et al. 2012).

3.4.2. Physiological parameters

Chlorophyll content (SPAD unit): Chlorophyll content was determined in the youngest fully expanded leaf blade of 5 representative plants in each subplot using a hand-held chlorophyll meter (Model: SPAD-502, Minolta Co. JAPAN). The readings were taken 45, 60, 75, and 90 days after sowing. The leaves used for measurement were clean, dry, and green with no sign of disease or damage. The calibration disks provided with the chlorophyll meter were used to calibrate the device before taking the first measurement and then regularly used for the accuracy of the device.

Chlorophyll fluorescence: It was measured 65 days after sowing with a hand-held chlorophyll fluorescence meter (Model: OS1p, Modulated Chlorophyll Fluorometer, Opti-Sciences Inc., Hudson NH, USA) in the youngest, fully expanded, clean, dry, and green leaves with no sign of disease or damage. The measurements were made close to solar noon i.e. 11:00h to 14:00h. Dark adapted chlorophyll fluorescence measurements were taken from regions on the leaves that were dark adapted using clips. The clips were placed on the leaf and closed to prevent any light from

entering the clipped spot and the clips were left there for at least 30 minutes. Following dark adaptation, readings were taken by inserting the fluorometer tip, opening the clip shutter and then giving a flash of light from the fluorometer that activated the PS II reaction centers of the photosynthetic apparatus (Ristic et al. 2007).

Stomatal conductance ($\text{mmol m}^{-2}\text{s}^{-1}$): Stomatal conductance measurements were conducted between 11:00 and 14:00, when the sky was clear with no wind, using the portable leaf porometer instrument (Model: AP4, Delta-T Devices Ltd, Cambridge, United Kingdom. The measurements were made 60 days after sowing on the youngest fully emerged leaf receiving sunlight, clean, dry, intact, and green, with no sign of disease or damage.

Canopy temperature ($^{\circ}\text{C}$): Canopy temperature was measured with an infrared thermometer (Model: IRTS-P, Apogee Instrument, Inc., Logan, UT, USA) in each subplot. The readings were taken 60, 75, and 90 days after sowing when the sky was clear with little or no wind. It was ensured that the plant surfaces are dry and not wet from dew, irrigation, and rain. The measurements were taken close to solar noon i.e. 11:00h to 14:00h.

Vegetation indices: The crop reflectance was measured using a Handheld spectroradiometer (Model: BLK-CXR-SR, StellarNet Inc., USA). This instrument measures reflectance with a wavelength range of 223–1102 nm. The spectrum of a white (BaSO_4) reference panel with known reflectance properties was acquired to derive the reflectance of the target. The measurements were made 65 and 90 days after sowing on a clear sunny day with no wind. The measurements were taken close to solar noon i.e. 11:00h to 14:00h. The following formulas were used for measurements.

Normalized Difference Vegetation Index (NDVI) = $(R_{860} - R_{650}) / (R_{860} + R_{650})$ (Rouse et al. 1974)

Photochemical reflectance index (PRI) = $(R_{531} - R_{570}) / (R_{531} + R_{570})$ (Peñuelas et al. 1994)

where R represents the reflectance value at specified wavelengths in nm.

3.5. Data analysis

The analysis of variance was carried out using the statistical software R studio 4.3.2 (R Core Team, 2023). The package used for analysis was “Augmented RCBD” package (Aravind et al. 2023). Box and whisker plots were used to decipher the variation in germplasm. Correlation was performed to determine the relationship among the traits. Principal component analysis was performed to determine the overall contribution of studied traits to significant variations. The hierarchical cluster analysis of all the studied traits was conducted using the sum of squares of distances with OriginPro 2024 (Origin Lab Corporation, Northampton, MA, USA). All the figures were created using OriginPro 2024. The numerical data were also subjected to basic descriptive statistics using MS Office Excel Software.

4. RESULTS

4.1. Phenological traits of sorghum during 2022 and 2023 growing season

The analysis of variance (ANOVA) for the phenological traits of sorghum accessions under different irrigation treatments reveals significant diversity among 125 sorghum accessions with six check genotypes (checks). By utilizing block-adjusted ANOVA, one-way heterogeneity was effectively eliminated. The mean sum of squares for most studied traits exhibited significance at both the 5% and 1% levels of significance. In the years 2022 and 2023, significant differences were observed for days to emergence, days to booting, days to heading, days to flowering, and days to maturity among the test accessions, checks, and the comparison between test accessions and checks under both deficit and full irrigation treatments. Notably, in the second year, no significant differences were detected among the checks for days to emergence under either irrigation treatment (Tables 4.1 and 4.2). This comprehensive analysis underscores the variability and adaptability of sorghum accessions to varying irrigation treatments, providing valuable insights for future breeding and cultivation strategies.

Table 4.1 Analysis of variance (mean squares) of days to emergence, days to booting, and days to heading of sorghum accessions under different irrigation treatments.

Source of variation	Mean squares 2022						
	Deficit irrigation			Full irrigation			DTH
	df	DTE	DTB	DTH	DTE	DTB	
Treatment (ignoring Blocks)	130	3.15**	343.97**	345.3**	3.31**	395.04**	404.62**
Treatment: Check	5	3.56**	700.78**	675.92**	5.13**	675.07**	685.76**
Treatment: Test	124	2.51**	321.69**	322.7**	2.44**	376.61**	383.77**
Treatment: Test vs. Check	1	80.63**	1322.16**	1494.1**	102.18**	1279.78**	1583.87**
Block (eliminating Treatments)	5	1.83	3.71	3.98	0.47	4.87	4.36
Residuals	25	0.27	6.67	5.17	0.48	14.13	13.45
2023							
Source of variation	Deficit irrigation			Full irrigation			DTH
	df	DTE	DTB	DTH	DTE	DTB	
Treatment (ignoring Blocks)	130	2.3**	253.47**	262.64**	0.91**	365.02**	383.27**
Treatment: Check	5	1.71 ^{ns}	511.91**	577.49**	0.56 ^{ns}	652.23**	669.92**
Treatment: Test	124	2.15**	239.21**	245.02**	0.82**	344**	361.18**
Treatment: Test vs. Check	1	23.13**	730.54**	872.7**	14.71**	1535.43**	1689.18**
Block (eliminating Treatments)	5	3.18	120.44	129.03	0.09	21.16	21.98
Residuals	25	0.67	31.92	34.73	0.25	42.32	45.01

df, degrees of freedom; DTE, days to 50% emergence; DTB, days to booting; DTH, days to heading; ns, non-significant ($P > 0.05$); * $P \leq 0.05$; ** $P \leq 0.01$

Table 4.2. Analysis of variance (mean squares) of days to flowering, days to physiological maturity, and plant height of sorghum accessions under different irrigation treatments.

Source of variation	df	2022					
		Deficit irrigation			Full irrigation		
		DTF	DTM	PH	DTF	DTM	PH
Treatment (ignoring Blocks)	130	350.24**	313.37**	3244.4**	416.82**	332.44**	4248.92**
Treatment: Check	5	666.24**	529.11**	4977.49**	704.89**	454.89**	7780.27**
Treatment: Test	124	324.58**	293.47**	2462.84**	393.44**	316.49**	3244.41**
Treatment: Test vs. Check	1	1953.02**	1702.63**	91492.44**	1875.2**	1698.66**	111151.2**
Block (eliminating Treatments)	5	5.78	11.11	592.01	6.43	14.29	176.9
Residuals	25	6.7	13.9	147.83	13.59	20.6	300.83
2023							
Source of variation	df	Deficit irrigation			Full irrigation		
		DTF	DTM	PH	DTF	DTM	PH
Treatment (ignoring Blocks)	130	277.08**	269.69**	1642.53**	412.74**	421.41**	1135.56**
Treatment: Check	5	591.6**	477.11**	473.85**	721.23**	500.93**	295.74 ^{ns}
Treatment: Test	124	257.95**	249.71**	1350.32**	388.23**	399.87**	892.4**
Treatment: Test vs. Check	1	1076.26**	1710.2**	43719.95**	1908.93**	2694.58**	35485.93**
Block (eliminating Treatments)	5	131.4	174.31	362.81	23.23	37.53	408.74
Residuals	25	31.56	39.72	122.88	47.55	67.27	131.95

df, degrees of freedom; DTF, days to flowering; DTM, days to physiological maturity; PH, plant height (cm); ns, non-significant ($P > 0.05$); * $P \leq 0.05$; ** $P \leq 0.01$

4.2. Morphological traits of sorghum during 2022 and 2023 growing season

The analysis of variance demonstrated that irrigation treatments significantly impacted the morphological traits of sorghum accessions, including checks, across both years. Significant differences were also observed between the test accessions and checks for morphological traits. However, plant height among the checks was found to be non-significant in the second year. Similarly, tillers per plant and panicles per plant were non-significant among the checks in both years. Additionally, no significant differences in stem diameter were recorded among the checks in the second year (Tables 4.2 and 4.3). This analysis highlights the influence of irrigation treatments on sorghum morphology and indicates specific traits that exhibit variability or stability under varying irrigation conditions, offering valuable information for breeding programs and agronomic practices.



Table 4.3 Analysis of variance (mean squares) of stem diameter, tillers per plant, and panicles per plant of sorghum accessions under different irrigation treatments.

Source of variation	df	Mean squares 2022					
		Deficit irrigation			Full irrigation		
		SD	TPP	PPP	SD	TPP	PPP
Treatment (ignoring Blocks)	130	19.36**	0.54**	4.63**	22.58**	0.32**	2.14**
Treatment: Check	5	10*	0.02 ^{ns}	0.53**	20.43**	0*	0.27 ^{ns}
Treatment: Test	124	17.26**	0.53**	4.79**	18.28**	0.29**	2.23**
Treatment: Test vs. Check	1	326.41**	4.82**	4.52**	566.55**	5.12**	0.88*
Block (eliminating Treatments)	5	4.93	0.02	0.17	4.56	0	0.11
Residuals	25	2.78	0.02	0.13	2.89	0	0.14
2023							
Source of variation	df	Deficit irrigation			Full irrigation		
		SD	TPP	PPP	SD	TPP	PPP
Treatment (ignoring Blocks)	130	24.86**	0.2**	1.78**	16.44*	0.05**	0.42**
Treatment: Check	5	14.16**	0 ^{ns}	0.17**	11.46ns	0*	0.02 ^{ns}
Treatment: Test	124	24.87**	0.19**	1.81**	15.77*	0.05**	0.42**
Treatment: Test vs. Check	1	76.95**	1.6**	6.53**	124.94**	0.66**	2.54**
Block (eliminating Treatments)	5	2.12	0	0.02	14.08	0	0.02
Residuals	25	0.96	0	0.03	7.3	0	0.02

df, degrees of freedom; SD, stem diameter (mm); TPP, tillers per plant; PPP, panicles per plant; ns, non-significant ($P > 0.05$); * $P \leq 0.05$; ** $P \leq 0.01$

4.3. Yield and yield traits of sorghum during 2022 and 2023 growing season

In both years, under deficit and full irrigation treatments, significant differences were observed for panicle length, grains per panicle, thousand grain weight, grain yield, biological yield, and harvest index among the test accessions and checks (Tables 4.4, 4.5, and 4.6). The comparison between test accessions and checks was also significant for these traits. However, the stay-green trait was non-significant among the checks under full irrigation in the first year and under both irrigation treatments in the second year. This analysis highlights the significant impact of irrigation treatments on important yield traits of sorghum and provides valuable insights into genotype performance, aiding in the selection and breeding of sorghum for varying irrigation conditions.



Table 4.4. Analysis of variance (mean squares) of stay green, panicle length, and grains per panicle of sorghum accessions under different irrigation treatments.

Source of variation	df	Mean squares 2022					
		Deficit irrigation			Full irrigation		
		SG	PL	GPP	SG	PL	GPP
Treatment (ignoring Blocks)	130	523.31**	51.48**	313857.94**	464.69**	47.74**	275132.16**
Treatment: Check	5	1057.78**	24.47**	382556.72**	457.78 ^{ns}	27.1*	133178.15**
Treatment: Test	124	407.42**	52.21**	309180.76**	414.3*	48.42**	281986.83**
Treatment: Test vs. Check	1	12221.96**	96.43**	550333.91**	6747.83**	66.28*	134923.18**
Block (eliminating Treatments)	5	17.78	11	567.17	171.11	13.78	16401.46
Residuals	25	99.11	4.2	7527.53	203.11	8.8	11006.14
2023							
Source of variation	df	Deficit irrigation			Full irrigation		
		SG	PL	GPP	SG	PL	GPP
Treatment (ignoring Blocks)	130	477.73**	37.52**	381714.19**	353.38**	32.41**	190779.63**
Treatment: Check	5	238.33 ^{ns}	54.01**	81474.26**	49.44 ^{ns}	23.41**	105350.45**
Treatment: Test	124	397.74**	36.49**	380507.09**	319.12**	33.03**	192775.79**
Treatment: Test vs. Check	1	11593.82**	83.34**	2032595.02**	6120.4**	0.1 ^{ns}	370401.52**
Block (eliminating Treatments)	5	758.33	14.23	80307.86	209.44	1.63	3659.71
Residuals	25	139.67	5.69	17841.09	80.11	1.8	10956.73

df, degrees of freedom; SG, stay green (%); PL, panicle length (cm); GPP, grains per panicle; ns, non-significant ($P > 0.05$); * $P \leq 0.05$; ** $P \leq 0.01$

Table 4.5 Analysis of variance (mean squares) of thousand grain weight, grain yield, and biological yield of sorghum accessions under different irrigation treatments.

Source of variation	df	Mean squares 2022					
		Deficit irrigation			Full irrigation		
		TGW	GY	BY	TGW	GY	BY
Treatment (ignoring Blocks)	130	29.63**	168.51**	14292.13**	33.28**	233.07**	15212.75**
Treatment: Check	5	127.72**	96.34**	2642.73**	102.79**	152.22**	2595.4**
Treatment: Test	124	14.73**	159.5**	12892.35**	19.07**	237.71**	13248**
Treatment: Test vs. Check	1	1386.4**	1646.35**	246111.34**	1448.18**	61.74*	321929.25**
Block (eliminating Treatments)	5	2.97	24.9	533.74	3.03	15.11	511.58
Residuals	25	4.84	12	476.81	1.51	14.35	308.74
2023							
Source of variation	df	Deficit irrigation			Full irrigation		
		TGW	GY	BY	TGW	GY	BY
Treatment (ignoring Blocks)	130	14.34**	64.12**	2663.53**	17.24**	30.39**	1961.4**
Treatment: Check	5	13.27**	47.49**	1585.79**	26.84**	7.62**	474.72 ^{ns}
Treatment: Test	124	13.51**	60.6**	2402.75**	14.22**	29.84**	1872.1**
Treatment: Test vs. Check	1	121.91**	584.64**	40388.32**	343.85**	212.19**	20467.41**
Block (eliminating Treatments)	5	11.85	9.78	849.58	3.87	1.86	252.34
Residuals	25	2.7	4.04	268.09	1.5	1.38	226.12

df, degrees of freedom; TGW, thousand grain weight (g); GY, grain yield (g/plant); BY, biological yield (g/plant); ns, non-significant ($P > 0.05$); * $P \leq 0.05$;

** $P \leq 0.01$

4.4. Physiological parameters of sorghum during 2022 and 2023 growing season

4.4.1. SPAD (chlorophyll content) and stomatal conductance

In both years of the experiment, significant variations were observed in SPAD measurements taken at different stages, as well as in stomatal conductance across both irrigation treatments and accessions. Notably, the comparisons among checks, test accessions, and test vs check were statistically significant for these traits. However, the comparison of test vs check was non-significant only for SPAD-1 under full irrigation in the first year of the experiment (Tables 4.6 and 4.7).



Table 4.6 Analysis of variance (mean squares) of harvest index and SPAD of sorghum accessions under different irrigation treatments.

Source of variation	df	Mean squares 2022					
		Deficit irrigation			Full irrigation		
		HI	SPAD-1	SPAD-2	HI	SPAD-1	SPAD-2
Treatment (ignoring Blocks)	130	206.04**	24.23*	37.54**	169.29**	22.54**	37.99**
Treatment: Check	5	558.14**	59.46**	93.19**	515.95**	27.62**	96.5**
Treatment: Test	124	78.81**	21.95*	29.63**	66.9**	22.47**	32.86**
Treatment: Test vs. Check	1	14222.66**	130.87**	741.15**	11132.68**	6.3 ^{ns}	381.94**
Block (eliminating Treatments)	5	22.71	39.41	3.78	7.13	8.5	8.43
Residuals	25	12.26	10.9	6.28	12.43	6.51	10.7
2023							
Source of variation	df	Deficit irrigation			Full irrigation		
		HI	SPAD-1	SPAD-2	HI	SPAD-1	SPAD-2
Treatment (ignoring Blocks)	130	153.77**	32.21**	56.8**	90.11**	33.15**	29.49**
Treatment: Check	5	415.18**	38.21**	45.46**	183.56**	49.06**	33.52**
Treatment: Test	124	93.61**	31.81**	53.95**	59.97**	32.29**	28.58**
Treatment: Test vs. Check	1	6306.99**	52.17**	467.41**	3359.17**	61.32**	121.98**
Block (eliminating Treatments)	5	19.68	1	3.15	2.85	3.71	8.94
Residuals	25	7.18	1.63	2.42	8.8	2.92	3.28

df, degrees of freedom; HI, harvest index (%); SPAD-1, SPAD taken at 45 days after sowing (DAS); SPAD-2, SPAD taken at 60 DAS; ns, non-significant ($P > 0.05$); * $P \leq 0.05$; ** $P \leq 0.01$

Table 4.7 Analysis of variance (mean squares) of SPAD and stomatal conductance of sorghum accessions under different irrigation treatments.

Source of variation	df	Mean squares 2022					
		Deficit irrigation			Full irrigation		
		SPAD-3	SPAD-4	SC	SPAD-3	SPAD-4	SC
Treatment (ignoring Blocks)	130	43.83**	53.2**	25406.81**	40.7**	35.12**	27323.08**
Treatment: Check	5	120.48**	61.18**	30723.87**	144.22**	45.2**	74082.03**
Treatment: Test	124	34.35**	41.84**	25217.02**	33.21**	31.87**	25565.48**
Treatment: Test vs. Check	1	835.54**	1421.59**	22355.6**	451.46**	386.8**	11470.94*
Block (eliminating Treatments)	5	1.24	0.53	1422.73	22.71	6.52	981.96
Residuals	25	1.4	0.79	1281.6	9.83	10.61	1965.83
2023							
Source of variation	df	Deficit irrigation			Full irrigation		
		SPAD-3	SPAD-4	SC	SPAD-3	SPAD-4	SC
Treatment (ignoring Blocks)	130	74.67**	61.32**	27239.97**	27.46**	28.63**	26802.09**
Treatment: Check	5	84.75**	19.37**	17010.23**	14.13**	17.57**	17875.58**
Treatment: Test	124	66.16**	55.09**	27256.21**	27.39**	24.3**	26074.93**
Treatment: Test vs. Check	1	1079.81**	1043.78**	76374.91**	103.63**	621.87**	161602.48**
Block (eliminating Treatments)	5	2.14	7.2	1846.36	3.91	0.93	6638.58
Residuals	25	2.9	3.75	3046.91	3.6	4.17	3923.6

df, degrees of freedom; SPAD-3, SPAD taken at 75 DAS; SPAD-4, SPAD taken at 90 DAS; SC, stomatal conductance ($\text{mmol m}^{-2}\text{s}^{-1}$); ns, non-significant ($P > 0.05$); * $P \leq 0.05$; ** $P \leq 0.01$

4.4.2. Canopy temperature

The analysis of variance revealed a significant effect of both irrigation treatments and accessions on canopy temperature (CT) measured at various stages. However, no significant differences were observed in canopy temperature (1st CT) among the checks under both deficit and full irrigation treatments in the first year. Similarly, in the second year, the checks exhibited no significant variation in 1st CT under deficit irrigation, and in 1st CT and 2nd CT under full irrigation. Additionally, test vs checks showed no significant difference for 1st CT under full irrigation in the first year, as well as for 1st CT and 3rd CT under full irrigation in the second year of the experiment (Table 4.8).



Table 4.8 Analysis of variance (mean squares) of canopy temperature of sorghum accessions under different irrigation treatments.

Source of variation	df	Mean squares 2022					
		Deficit irrigation			Full irrigation		
		1 st CT	2 nd CT	3 rd CT	1 st CT	2 nd CT	3 rd CT
Treatment (ignoring Blocks)	130	2.3*	1.82**	2**	1.26*	0.95**	0.51**
Treatment: Check	5	0.76 ^{ns}	5.46**	8.48**	0.44 ^{ns}	4.29**	3.5**
Treatment: Test	124	2.33*	1.65**	1.61**	1.31*	0.78**	0.38*
Treatment: Test vs. Check	1	6.74*	5.21**	17.91**	0.01 ^{ns}	5.48**	1.33**
Block (eliminating Treatments)	5	2.32	3.96	2.44	0.31	0.14	0.17
Residuals	25	1.14	0.49	0.39	0.66	0.32	0.17
2023							
Source of variation	df	Deficit irrigation			Full irrigation		
		1 st CT	2 nd CT	3 rd CT	1 st CT	2 nd CT	3 rd CT
Treatment (ignoring Blocks)	130	3.55**	1.47**	7.11**	3.52**	1.94*	1.13**
Treatment: Check	5	0.68 ^{ns}	5.28**	2.98**	1.16 ^{ns}	2.65 ^{ns}	1.35**
Treatment: Test	124	3.69**	1.31**	7.2**	3.62**	1.85*	1.13**
Treatment: Test vs. Check	1	0.25 ^{ns}	2.42**	16.21**	2.62 ^{ns}	8.66**	0.19 ^{ns}
Block (eliminating Treatments)	5	0.3	1.04	1.17	1.38	3.28	0.26
Residuals	25	0.26	0.23	0.31	0.96	1.03	0.33

df, degrees of freedom; 1st CT, canopy temperature (°C) taken at 60 DAS; 2nd CT, canopy temperature (°C) taken at 75 DAS; 3rd CT, canopy temperature (°C) taken at 90 DAS; ns, non-significant ($P > 0.05$); * $P \leq 0.05$; ** $P \leq 0.01$

4.4.3. Chlorophyll fluorescence parameters

Chlorophyll fluorescence parameters of sorghum accessions revealed significant variations in response to different irrigation treatments. However, the checks exhibited no significant variation in quantum photosynthetic yield i.e., $Y(II)$ under full irrigation conditions during the first year, and under both deficit and full irrigation conditions in the second year. Additionally, test vs checks showed no significant differences in F_v/m and F_v/o under deficit irrigation in the first year, as well as under both deficit and full irrigation treatments in the second year of the experiment. Specifically, the checks demonstrated no significant variation in F_v/m under full irrigation during the second year of the study (Tables 4.9, 4.10 and 4.11).

These findings suggest that while the genotypic response to varying irrigation treatments was notable, the control checks maintained consistent chlorophyll fluorescence parameters under the specified irrigation conditions. This consistency in the control checks underscores the robustness of the experimental design and the reliability of the observed genotype-specific responses.

Table 4.9 Analysis of variance (mean squares) of chlorophyll fluorescence parameters of sorghum accessions under different irrigation treatments.

Source of variation	df	2022					
		Mean squares					
		Deficit irrigation			Full irrigation		
		F'	Fm'	Y(II)	F'	Fm'	Y(II)
Treatment (ignoring Blocks)	130	19940.37**	89062.79**	0.01**	6870.66**	64561.71**	0.01**
Treatment: Check	5	33403.78**	100367.13**	0.01**	7624.51**	40002.36**	0.01 ^{ns}
Treatment: Test	124	18689.47**	87371.82**	0.01**	6633.51**	65651.47**	0.01**
Treatment: Test vs. Check	1	107735.41**	242220.76**	0**	32507.68**	52229.09*	0.06**
Block (eliminating Treatments)	5	1874.84	8476.13	0.01	4533.11	2066.43	0.01
Residuals	25	1162.94	1426.43	0.01	1914.63	7925.48	0.01
2023							
Source of variation	df	Deficit irrigation			Full irrigation		
		F'	Fm'	Y(II)	F'	Fm'	Y(II)
Treatment (ignoring Blocks)	130	19905.02**	104242.71**	0.01**	5088.7**	68705.77**	0.01**
Treatment: Check	5	14056.71**	96312.83**	0 ^{ns}	4278.65*	9725.49 ^{ns}	0 ^{ns}
Treatment: Test	124	19725.77**	104947.03**	0.01**	5110.29**	71546.44**	0.01**
Treatment: Test vs. Check	1	71373.15**	56556.34**	0.12**	6461.61*	11363.98 ^{ns}	0 ^{ns}
Block (eliminating Treatments)	5	2602.91	12918.23	0	783.92	8682.36	0.01
Residuals	25	1199.14	6873	0	1127.76	8201.43	0

df, degrees of freedom; F', minimal fluorescence of light adapted leaves; Fm', maximal fluorescence of light adapted leaves; Y(II), quantum photosynthetic yield; ns, non-significant ($P > 0.05$); * $P \leq 0.05$; ** $P \leq 0.01$

Table 4. 10 Analysis of variance (mean squares) of chlorophyll fluorescence parameters of sorghum accessions under different irrigation treatments.

Source of variation	df	Mean squares 2022					
		Deficit irrigation			Full irrigation		
		Fo	Fm	Fv	Fo	Fm	Fv
Treatment (ignoring Blocks)	130	173.65**	9453.97**	8440.65**	431.03**	17609.27**	14049.09**
Treatment: Check	5	55.89 ^{ns}	5069.44**	5242.56**	344.31**	11926.11*	8777.18*
Treatment: Test	124	179.68**	9704.13**	8633.65**	435.94**	17665.64**	14108.22**
Treatment: Test vs. Check	1	14.13 ^{ns}	356.27 ^{ns}	499.06 ^{ns}	255.97 ^{ns}	39036.33**	33076.99**
Block (eliminating Treatments)	5	43.96	958.04	2935.96	837.44	10754.91	2936.91
Residuals	25	42.68	1093.43	999.16	86.02	3219.83	2513.64
2023							
Source of variation	df	Deficit irrigation			Full irrigation		
		Fo	Fm	Fv	Fo	Fm	Fv
Treatment (ignoring Blocks)	130	470.19**	12812.73**	10917.43**	826.87**	14854.53**	10895.15**
Treatment: Check	5	256.49 ^{ns}	7624.96*	4678.78*	257.18 ^{ns}	2904.23 ^{ns}	1714.84 ^{ns}
Treatment: Test	124	482.06**	13101.15**	11253.58**	856.02**	15435.33**	11338.73**
Treatment: Test vs. Check	1	67.65 ^{ns}	2986.65 ^{ns}	428.23 ^{ns}	60.71 ^{ns}	2587.96 ^{ns}	1793.2 ^{ns}
Block (eliminating Treatments)	5	503.43	2025.16	575.78	900.24	6171.89	2454.71
Residuals	25	190.61	2241.67	1571.86	113.88	3730.28	2751.06

df, degrees of freedom; Fo, minimal fluorescence of dark-adapted leaves; Fm, maximal fluorescence of dark-adapted leaves; Fv, variable fluorescence; ns, non-significant ($P > 0.05$); * $P \leq 0.05$; ** $P \leq 0.01$

Table 4. 11 Analysis of variance (mean squares) of chlorophyll fluorescence parameters and normalized difference vegetation index (NDVI) of sorghum accessions under different irrigation treatments.

Source of variation	df	2022					
		Deficit irrigation			Full irrigation		
		Fv/m	Fv/o	NDVI-1	Fv/m	Fv/o	NDVI-1
Treatment (ignoring Blocks)	130	0**	0.25**	0.02**	0**	0.25**	0.02**
Treatment: Check	5	0**	0.25**	0.02**	0**	0.11ns	0.01*
Treatment: Test	124	0**	0.25**	0.02**	0**	0.25**	0.02**
Treatment: Test vs. Check	1	0 ^{ns}	0.05 ^{ns}	0.19**	0**	0.61*	0 ^{ns}
Block (eliminating Treatments)	5	0	0	0	0	0.26	0
Residuals	25	0	0.03	0	0	0.08	0
2023							
Source of variation	df	Deficit irrigation			Full irrigation		
		Fv/m	Fv/o	NDVI-1	Fv/m	Fv/o	NDVI-1
Treatment (ignoring Blocks)	130	0**	0.29*	0.03**	0**	0.24*	0.04**
Treatment: Check	5	0 ^{ns}	0.23 ^{ns}	0.02**	0 ^{ns}	0.02 ^{ns}	0.02**
Treatment: Test	124	0**	0.29*	0.03**	0**	0.25*	0.04**
Treatment: Test vs. Check	1	0 ^{ns}	0.01 ^{ns}	0.06**	0 ^{ns}	0.02 ^{ns}	0.02*
Block (eliminating Treatments)	5	0	0.32	0.01	0	0.07	0.01
Residuals	25	0	0.16	0	0	0.13	0

df, degrees of freedom; Fv/m, maximum photochemical efficiency of PSII; Fv/o, more sensitive stress detector than Fv/Fm, but does not measure plant efficiency; NDVI-1, normalized difference vegetation index taken at 65 DAS; ns, non-significant ($P > 0.05$); * $P \leq 0.05$; ** $P \leq 0.01$

4.4.4. Vegetation indices

The analysis of variance indicated that both irrigation treatments and sorghum accessions significantly affected vegetation indices measured at different growth stages. However, certain indices did not exhibit significant changes under specific conditions. Specifically, no significant changes were observed for normalized difference vegetation index-2 (NDVI-2) and photochemical reflectance index-2 (PRI-2) under deficit irrigation, and PRI-1 under full irrigation in the first year. Additionally, checks revealed no significant variations in PRI-1 under deficit irrigation and PRI-2 under full irrigation during the second year. Test vs check treatments were also found to be non-significant for NDVI-1 and PRI-1 under full irrigation in the first year (Tables 4.11 and 4.12).

These findings suggest that while irrigation and genotype play crucial roles in influencing vegetation indices, certain indices remain stable under specific irrigation regimes across different years. This stability can provide insights into the resilience of particular sorghum accessions to varying irrigation levels and inform irrigation management practices. Further research may be needed to explore the underlying mechanisms driving these responses and to confirm these results across diverse environmental conditions.

Table 4.12 Analysis of variance (mean squares) of normalized difference vegetation index and photochemical reflectance index of sorghum accessions under different irrigation treatments.

Source of variation	df	Mean squares 2022					
		Deficit irrigation			Full irrigation		
		PRI-1	NDVI-2	PRI-2	PRI-1	NDVI-2	PRI-2
Treatment (ignoring Blocks)	130	0**	0.04**	0**	0**	0.04**	0**
Treatment: Check	5	0**	0.01 ^{ns}	0 ^{ns}	0 ^{ns}	0.02*	0**
Treatment: Test	124	0**	0.04**	0**	0**	0.04**	0**
Treatment: Test vs. Check	1	0**	0.03**	0.01**	0 ^{ns}	0.06**	0.01**
Block (eliminating Treatments)	5	0	0	0	0	0	0
Residuals	25	0	0	0	0	0.01	0
2023							
Source of variation	df	Deficit irrigation			Full irrigation		
		PRI-1	NDVI-2	PRI-2	PRI-1	NDVI-2	PRI-2
Treatment (ignoring Blocks)	130	0**	0.05**	0**	0**	0.04**	0**
Treatment: Check	5	0 ^{ns}	0.06**	0*	0*	0.02*	0 ^{ns}
Treatment: Test	124	0**	0.05**	0**	0**	0.04**	0**
Treatment: Test vs. Check	1	0**	0.09**	0.09**	0.02**	0.04**	0.01**
Block (eliminating Treatments)	5	0	0.01	0	0	0	0
Residuals	25	0	0	0	0	0.01	0

df, degrees of freedom; NDVI-2, normalized difference vegetation index taken at 90 DAS; PRI-1, photochemical reflectance index taken at 65 DAS; PRI-2, photochemical reflectance index taken at 90 DAS; ns, non-significant ($P > 0.05$); * $P \leq 0.05$; ** $P \leq 0.01$

4.5. Ranges of morpho-physiological traits of sorghum accessions under deficit and full irrigation treatments

The ranges of the studied traits for all accessions, including checks, under both deficit and full irrigation treatments across both years, are presented in Table 4.13. The phenological parameters, such as days to booting, heading, flowering, and maturity, exhibited higher variability under full irrigation in both years. Conversely, traits such as grains per panicle and panicles per plant demonstrated greater variability under deficit irrigation conditions in both years. Grain yield displayed increased variability under full irrigation in the first year and under deficit irrigation in the second year. Biological yield consistently showed higher variability under deficit irrigation across both years. Additionally, SPAD-4 values revealed higher variability under both irrigation treatments and in both years. Y(II) exhibited greater variability under both irrigation treatments in the second year. Fv/m showed increased variability specifically under deficit irrigation in the second year. These observations highlight the differential responses of different accessions to irrigation treatments, emphasizing the need for tailored irrigation strategies to optimize phenological and yield-related traits.

Table 4.13 Range for various traits of sorghum accessions under deficit and full irrigation.

Traits	2022		2023	
	Deficit irrigation	Full irrigation	Deficit irrigation	Full irrigation
Days to emergence	5-12	5-12	7-14	8-14
Days to booting	45-115	48-134	49-109	48-148
Days to heading	49-120	55-139	52-114	53-155
Days to flowering	57-124	60-143	55-120	57-161
Days to physiological maturity	93-161	96-173	83-154	85-193
Grains/panicle	463-3423	89-2834	67-3435	90-2237
1000-grain weight (g)	8.14-29.61	8.04-36.07	2.86-31.3	6.81-26.64
Panicle length (cm)	11.66-56.87	13.11-55.99	10.24-48.5	9.71-47.6
No. of panicles/plant	1-18	1-10	1-13	1-5
Plant height (cm)	84.03-324.37	85.08-373.11	87.92-321.42	75.68-225.74
Stem diameter (cm)	5.83-31.99	6.16-26.47	3.97-27.01	3.52-21.82
No. of tillers/plant	0-5	0-2	0-3	0-1
Stay green (%)	10-90	10-90	10-90	10-80
Grain yield/plant (g)	6.22-85	4.95-98.82	0.38-33.94	1.61-25
Biological yield/plant (g)	38.62-735.37	38.94-631.71	18.11-290	10.04-228.51
Harvest index (%)	1.89-54.41	2.51-57.06	0.34-48.14	1.56-38.05
SPAD-1	24.63-50.4	24.76-51.62	26.92-54.69	19.96-46.51
SPAD-2	31-57.31	26.79-54.56	25.48-56.93	17.84-45.42
SPAD-3	31-58.24	30.66-58.71	16.42-55.47	15.38-41.18
SPAD-4	22.14-52.1	27.53-56.21	19.91-51.91	13.44-41.74
Stomatal conductance (mmol m ⁻² s ⁻¹)	94.33-760.17	130.86-811.86	79.81-739.64	66.58-808.75
1 st Canopy temperature (°C)	37.94-47.59	38.28-44.3	35.76-43.92	37.06-45.38
2 nd Canopy Temperature (°C)	35.52-42.53	35.08-40.82	36.8-42.38	35.65-42.21
3 rd Canopy Temperature (°C)	35.25-43.92	36.86-40.46	37.7-47.44	44.35-49.61
F'	355.56-1220.06	336.39-756.06	261.11-984.11	274.42-687.42
Fm'	754.33-2101.83	641.14-1908.81	312.64-1874.47	489.53-1678.69
Y(II)	0.31-0.7	0.26-0.69	0.14-0.7	0.13-0.71
Fo	147.97-212.64	149.22-233.72	139.64-240.64	133.78-282.78
Fm	534.61-1053.61	512.22-1081.56	464.97-986.47	446.53-1060.53
Fv	370.03-859.19	350.56-883.72	229.94-781.11	268.61-845.94
Fv/m	0.68-0.82	0.69-0.81	0.49-0.8	0.61-0.79
Fv/o	2.14-4.47	1.99-4.29	1.24-3.94	1.65-3.76
NDVI-1	0.12-0.84	0.16-0.95	0.08-0.89	0.12-0.87
PRI-1	-0.04-0.06	-0.03-0.08	-0.04-0.06	-0.04-0.06
NDVI-2	0.2-0.94	0.18-0.96	0.1-0.94	0.15-0.94
PRI-2	-0.06-0.07	-0.06-0.09	-0.08-0.09	-0.07-0.09

SPAD-1, SPAD taken at 45 days after sowing (DAS); SPAD-2, SPAD taken at 60 DAS; SPAD-3, SPAD taken at 75 DAS; SPAD-4, SPAD taken at 90 DAS; 1st CT, canopy temperature (°C) taken at 60 DAS; 2nd CT, canopy temperature (°C) taken at 75 DAS; 3rd CT, canopy temperature (°C) taken at 90 DAS; F', minimal fluorescence of light adapted leaves; Fm', maximal fluorescence of light adapted leaves; Y(II), quantum photosynthetic yield; Fo, minimal fluorescence of dark-adapted leaves; Fm, maximal fluorescence of dark-adapted leaves; Fv, variable fluorescence; Fv/m, maximum photochemical efficiency of PSII; Fv/o, more sensitive stress detector than Fv/Fm, but does not measure plant efficiency; NDVI-1, normalized difference vegetation index taken at 65 DAS; NDVI-2, normalized difference vegetation index taken at 90 DAS; PRI-1, photochemical reflectance index taken at 65 DAS; PRI-2, photochemical reflectance index taken at 90 DAS

4.6. Means of morpho-physiological traits of sorghum accessions under deficit and full irrigation treatments

The mean performance of all traits demonstrated variable responses under both deficit and full irrigation treatments across the two years of study. Traits such as number of grains per panicle, the number of panicles per plant, the number of tillers per plant, grain yield, SPAD-1, SPAD-2, SPAD-3, and Fv/m exhibited an increase under deficit irrigation compared to full irrigation in both years. Additionally, panicle length, plant height, stem diameter, biological yield, SPAD-4, and stomatal conductance showed an increase under deficit irrigation as opposed to full irrigation in the second year of the experiment (Table 4.14).



Table 4.14. Means and average percentage increase or decrease under drought stress as compared to normal irrigation for morpho-physiological and yield traits in sorghum accessions

Traits	2022			2023		
	Full irrigation	Deficit irrigation	Average % decrease (-) or increase (+) in deficit irrigation as compared to full irrigation	Full irrigation	Deficit irrigation	Average % decrease (-) or increase (+) in deficit irrigation as compared to full irrigation
Days to emergence	8.8	8.4	-4.5	9.9	9.6	-2.9
Days to booting	79.3	76.3	-3.8	85.1	76.0	-10.7
Days to heading	83.7	80.4	-3.9	89.7	80.4	-10.4
Days to flowering	88.2	84.9	-3.8	94.4	84.6	-10.4
Days to physiological maturity	121.4	117.1	-3.6	125.7	115.3	-8.3
Grains/panicle	1103.5	1201.4	8.9	655.6	885.0	35.0
1000-grain weight (g)	18.3	17.7	-3.3	13.9	12.4	-11.0
Panicle length (cm)	24.6	24.4	-0.7	19.6	20.7	5.5
No. of panicles/plant	2.0	2.2	13.8	1.4	1.7	24.2
Plant height (cm)	226.5	202.6	-10.6	157.7	170.9	8.4
Stem diameter (cm)	13.2	12.8	-2.8	11.0	12.6	14.9
No. of tillers/plant	0.4	0.4	4.8	0.1	0.2	59.4
Stay green (%)	63.0	56.2	-10.8	51.2	49.6	-3.1
Grain yield/plant (g)	24.4	24.8	1.4	9.5	11.4	19.9
Dry biological yield/plant (g)	177.1	174.9	-1.2	77.7	94.5	21.6
Harvest index (%)	16.2	17.4	6.9	14.0	14.4	3.1
SPAD-1	36.1	37.6	4.3	30.6	40.2	31.3
SPAD-2	39.0	42.5	9.0	31.1	37.4	20.4
SPAD-3	42.7	43.1	1.0	27.8	31.4	12.9
SPAD-4	39.8	37.9	-4.8	26.4	31.1	17.6
Stomatal conductance (mmol m ⁻² s ⁻¹)	456.0	386.9	-15.1	249.1	295.4	18.6

1 st Canopy temperature (°C)	41.5	41.8	0.9	41.0	39.1	-4.7
2 nd Canopy temperature (°C)	37.5	37.5	-0.1	40.2	39.5	-1.6
3 rd Canopy temperature (°C)	38.5	37.7	-1.9	46.4	41.7	-10.0
F'	502.7	562.3	11.8	433.2	512.8	18.4
Fm'	1095.4	1268.2	15.8	1007.6	1065.6	5.7
Y(II)	0.5	0.5	5.2	0.6	0.5	-10.0
Fo	185.9	173.0	-6.9	182.5	180.4	-1.1
Fm	781.3	730.8	-6.5	671.5	702.6	4.6
Fv	595.4	557.8	-6.3	489.1	523.1	7.0
Fv/m	0.8	0.8	0.2	0.7	0.7	1.9
Fv/o	3.2	3.2	0.9	2.7	2.9	7.8
NDVI-1	0.7	0.5	-23.0	0.4	0.5	25.5
PRI-1	0.0	0.0	-42.9	0.0	0.0	59.7
NDVI-2	0.7	0.6	-16.1	0.6	0.5	-18.9
PRI-2	0.0	0.0	-69.9	0.0	0.0	-41.5

SPAD-1, SPAD taken at 45 days after sowing (DAS); SPAD-2, SPAD taken at 60 DAS; SPAD-3, SPAD taken at 75 DAS; SPAD-4, SPAD taken at 90 DAS; 1st CT, canopy temperature (°C) taken at 60 DAS; 2nd CT, canopy temperature (°C) taken at 75 DAS; 3rd CT, canopy temperature (°C) taken at 90 DAS; F', minimal fluorescence of light adapted leaves; Fm', maximal fluorescence of light adapted leaves; Y(II), quantum photosynthetic yield; Fo, minimal fluorescence of dark-adapted leaves; Fm, maximal fluorescence of dark-adapted leaves; Fv, variable fluorescence; Fv/m, maximum photochemical efficiency of PSII; Fv/o, more sensitive stress detector than Fv/Fm, but does not measure plant efficiency; NDVI-1, normalized difference vegetation index taken at 65 DAS; NDVI-2, normalized difference vegetation index taken at 90 DAS; PRI-1, photochemical reflectance index taken at 65 DAS; PRI-2, photochemical reflectance index taken at 90 DAS

4.7. Phenotypic variation of morpho-physiological traits among sorghum accessions

When evaluating the effects of irrigation treatments across all accessions, several key observations were made regarding plant development and physiological responses which are described below (figures 4.1-4.6).

Plant phenology

There was a clear reduction in the number of days to booting, days to heading, and days to flowering under deficit irrigation in the second year. Days to physiological maturity decreased under deficit irrigation in both years.

Plant morphology and yield

Plant height decreased under deficit irrigation in the first year. Conversely, plant height decreased under full irrigation in the second year. Stem diameter remained the same under both irrigation treatments in the first year but decreased under full irrigation in the second year. Tillers per plant, panicles per plant, panicle length, grains per panicle, grain yield, biological yield, and harvest index remained consistent across both irrigation treatments in both years.

Stay green

Stay green was lower under deficit irrigation in the first year compared to full irrigation. In the second year, stay green was similar for both irrigation treatments.

Thousand grain weight

Thousand grains weight was consistent under both irrigation treatments in the first year but was higher under full irrigation in the second year.

Chlorophyll content (SPAD values)

SPAD-1 and SPAD-2 values were higher under deficit irrigation in both years. SPAD-3 and SPAD-4 values showed variable responses to the irrigation treatments.

Stomatal conductance

Stomatal conductance was higher under full irrigation in the first year, but higher under deficit irrigation in the second year.

Canopy temperature

Canopy temperatures measured at different growth stages exhibited variable responses to the irrigation treatments.

Photosynthetic Efficiency

Quantum yield of photosystem II (Y(II)) was higher under deficit irrigation in the first year but higher under full irrigation in the second year. Maximum quantum efficiency of PSII (Fv/Fm) remained the same under both irrigation treatments in the first year but was higher under deficit irrigation in the second year.

Normalized difference vegetation index (NDVI)

NDVI-1 was higher under full irrigation in the first year but higher under deficit irrigation in the second year. NDVI-2 was consistently higher under full irrigation in both years compared to deficit irrigation.

These observations underscore the complex interactions between irrigation treatments and plant physiological and morphological responses, highlighting the importance of tailored irrigation strategies for optimizing crop performance.

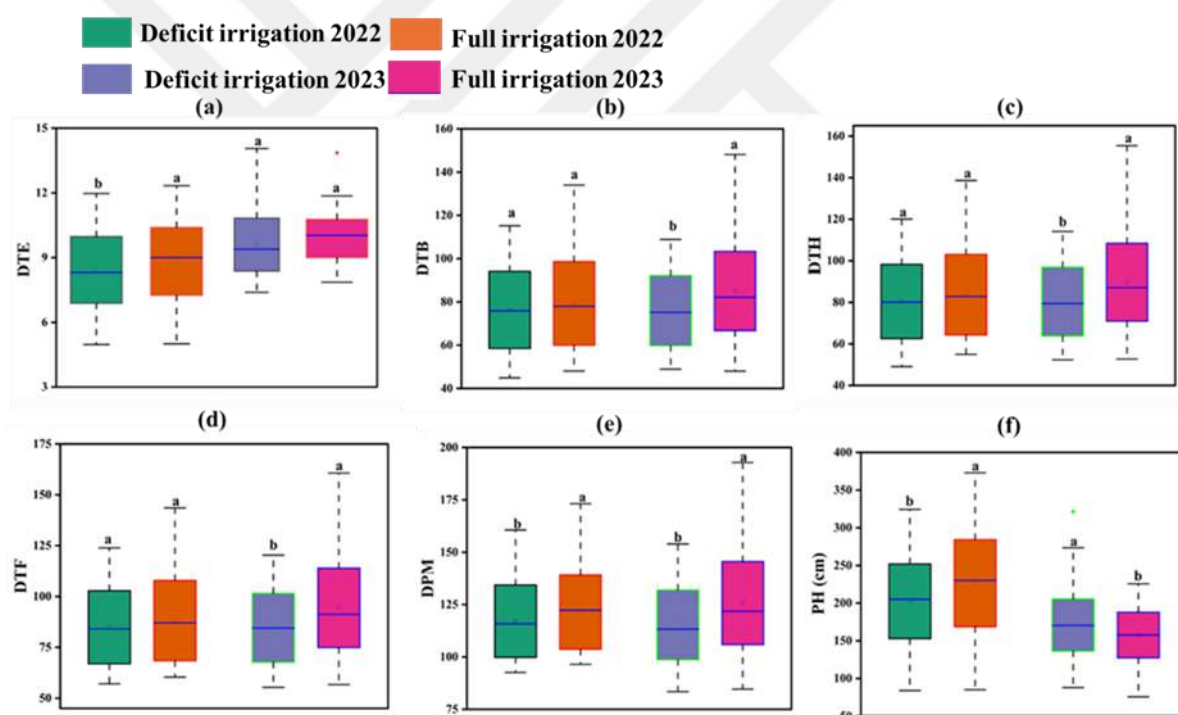


Figure 4.1 Box plots of the studied traits (a) DTE, days to emergence (b) DTB, days to booting (c) DTH, days to heading (d) DTF, days to flowering (e) DPM, days to physiological maturity (f) PH, plant height, showing the mean performance (LSD analysis at a 0.05 level of probability) of all the 131 sorghum accessions under deficit irrigation (green and purple) and full irrigation (orange and dark pink) in 2022 and 2023, respectively.

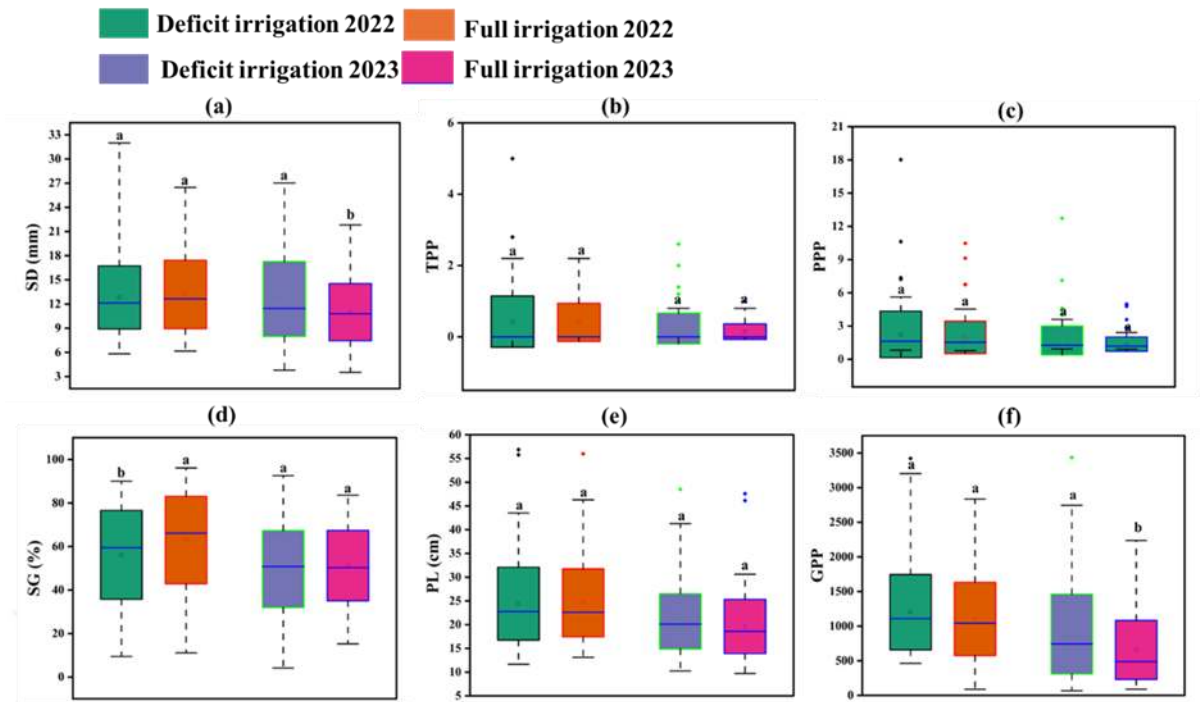


Figure 4.2 Box plots of the studied traits (a) SD, stem diameter (b) TPP, tillers per plant (c) PPP, panicles per plant (d) SG, stay green (%) (e) PL, panicle length (cm) (f) GPP, grains per panicle, showing the mean performance (LSD analysis at a 0.05 level of probability) of all the 131 sorghum accessions under deficit irrigation (green and purple) and full irrigation (orange and dark pink) in 2022 and 2023, respectively.

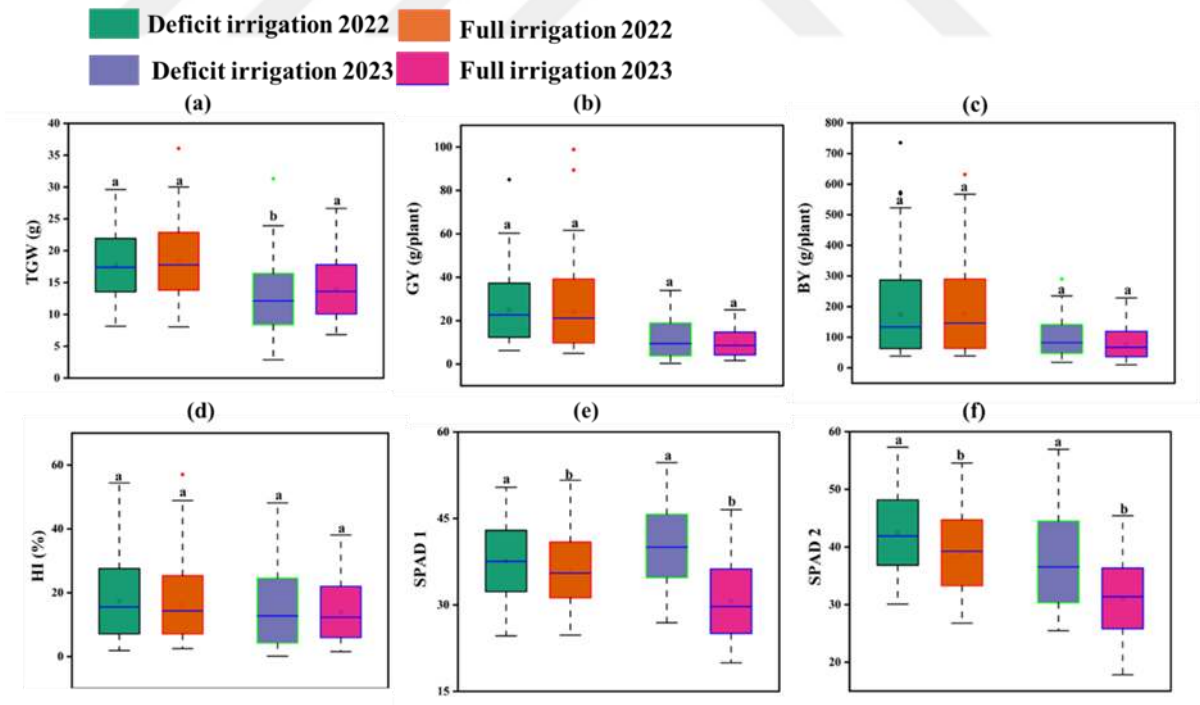


Figure 4.3 Box plots of the studied traits (a) TGW, thousand grains weight (g) (b) GY, grain yield (g/plant) (c) BY, biological yield (g/plant) (d) HI, harvest index (%) (e) SPAD 1, SPAD taken at 45 DAS (f) SPAD 2, SPAD taken at 60 DAS, showing the mean performance (LSD analysis at a 0.05 level of probability) of all the 131 sorghum accessions under deficit irrigation (green and purple) and full irrigation (orange and dark pink) in 2022 and 2023, respectively.

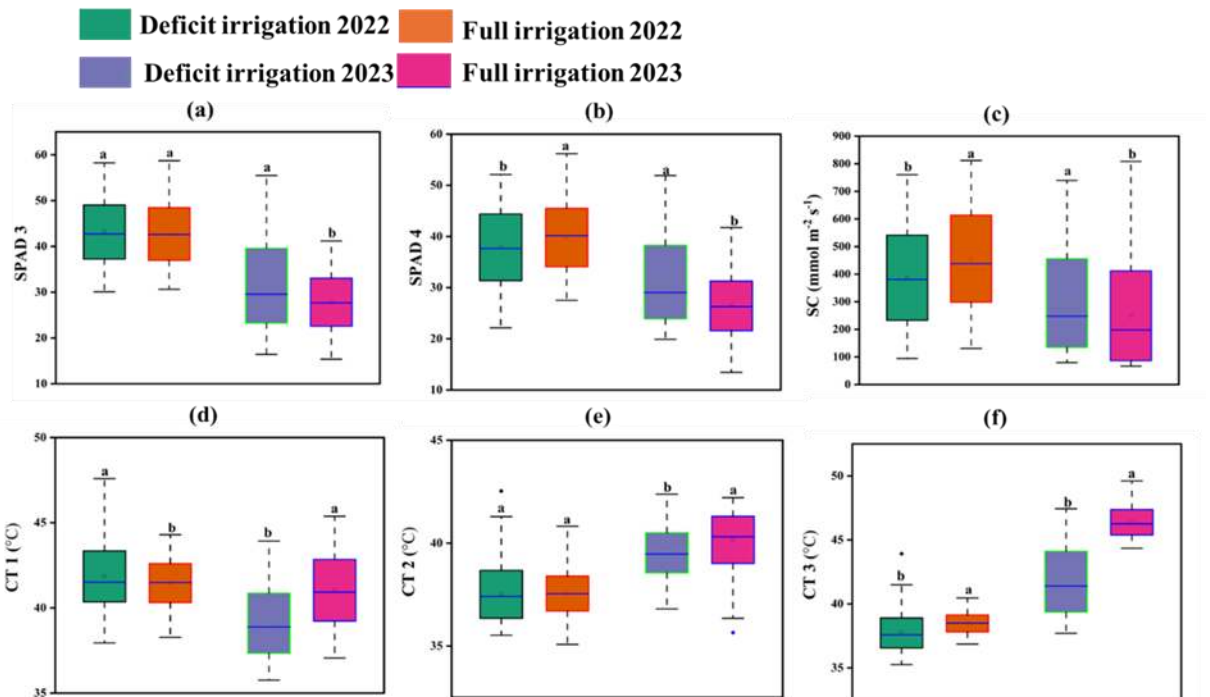


Figure 4.4 Box plots of the studied traits (a) SPAD 3, SPAD taken at 75 DAS (b) SPAD 4, SPAD taken at 90 DAS (c) SC, stomatal conductance ($\text{mmol m}^{-2}\text{s}^{-1}$) (d) CT 1, canopy temperature ($^{\circ}\text{C}$) taken at 60 DAS (e) CT 2, canopy temperature ($^{\circ}\text{C}$) taken at 75 DAS (f) CT 3, canopy temperature ($^{\circ}\text{C}$) taken at 90 DAS, showing the mean performance (LSD analysis at a 0.05 level of probability) of all the 131 sorghum accessions under deficit irrigation (green and purple) and full irrigation (orange and dark pink) in 2022 and 2023, respectively.

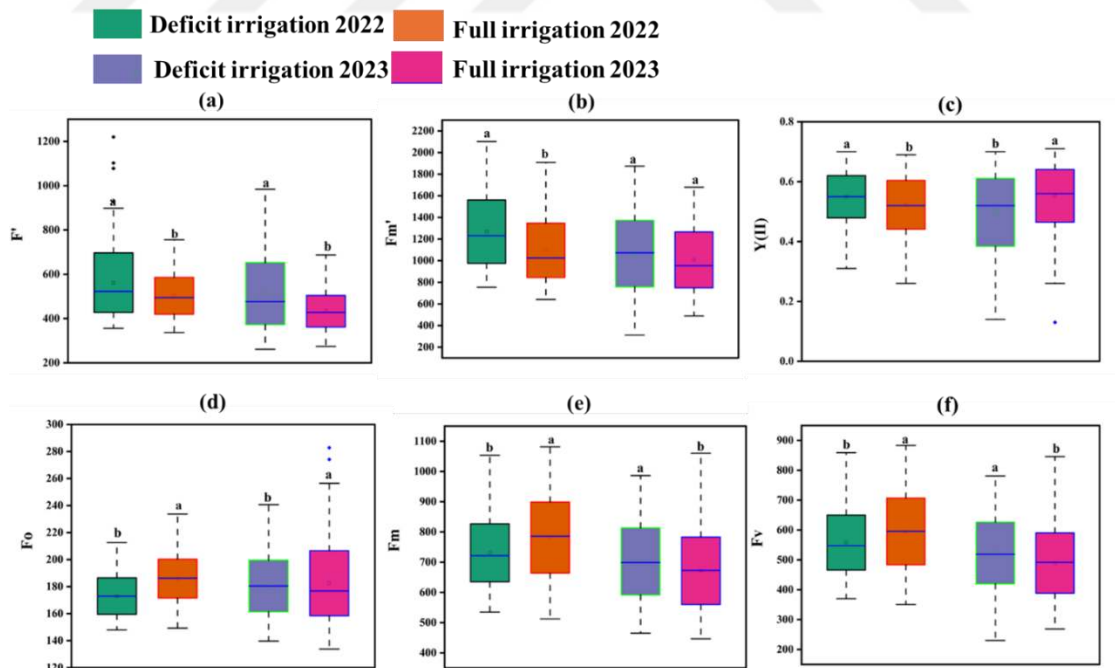


Figure 4.5 Box plots of the studied traits (a) F' , minimal fluorescence of light adapted leaves (b) F_m' , maximal fluorescence of light adapted leaves (c) $Y(II)$, quantum photosynthetic yield (d) F_o , minimal fluorescence of dark-adapted leaves (e) F_m , maximal fluorescence of dark adapted leaves (f) F_v , variable fluorescence; showing the mean performance (LSD analysis at a 0.05 level of probability) of all the 131 sorghum accessions under deficit irrigation (green and purple) and full irrigation (orange and dark pink) in 2022 and 2023, respectively.

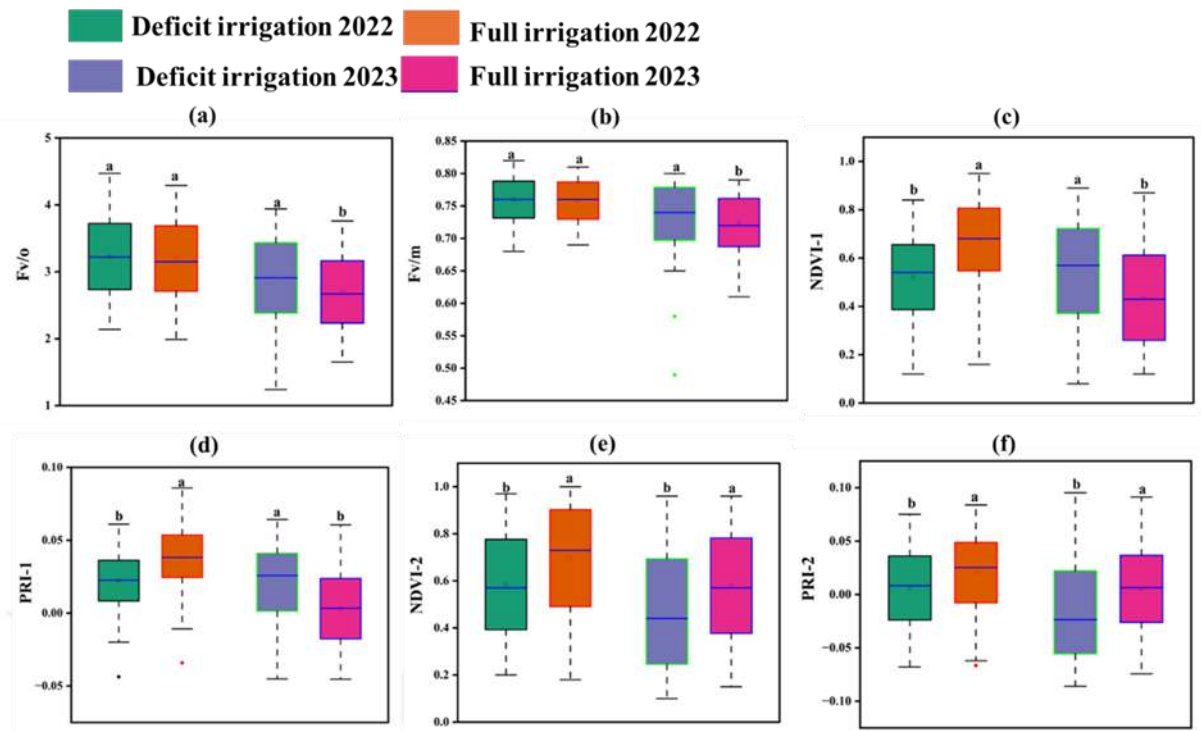


Figure 4.6 Box plots of the studied traits (a) F_v/o , more sensitive stress detector than F_v/F_m , but does not measure plant efficiency; (b) F_v/m , maximum photochemical efficiency of PSII (c) NDVI-1, normalized difference vegetation index taken at 65 DAS (d) PRI-1, photochemical reflectance index taken at 65 DAS (e) NDVI-2, normalized difference vegetation index taken at 90 DAS (f) PRI-2, photochemical reflectance index taken at 90 DAS; showing the mean performance (LSD analysis at a 0.05 level of probability) of all the 131 sorghum accessions under deficit irrigation (green and purple) and full irrigation (orange and dark pink) in 2022 and 2023, respectively.

To more clearly determine the response of various physiological traits measured at different stages, the data were plotted together. The SPAD readings (SPAD-1 and SPAD-2) exhibited consistent responses under both deficit and full irrigation treatments across both years, with higher values recorded under deficit irrigation compared to full irrigation. In contrast, SPAD-3 showed no difference between irrigation treatments in the first year, but in the second year, it recorded higher values under deficit irrigation. SPAD-4 showed differing responses over the two years: higher values were recorded under full irrigation in the first year, whereas higher values were recorded under deficit irrigation treatment in the second year (figure 4.7).

Canopy temperature (CT) also showed variable responses to the irrigation treatments across the two years. CT-1 was higher under deficit irrigation in the first year, but this trend reversed in the second year, with higher values under full irrigation. CT-2 showed no difference between irrigation treatments in the first year, but in the second year, higher values were recorded under full irrigation. CT-3 consistently showed higher values under full irrigation in both years (figure 4.7).

For NDVI measurements, NDVI-1 was higher under full irrigation in the first year, but higher under deficit irrigation in the second year. NDVI-2 consistently recorded higher values under full irrigation in both years. Regarding PRI, both PRI-1 and PRI-2 were lower under deficit irrigation

in the first year. However, in the second year, PRI-1 was higher under deficit irrigation, while PRI-2 remained higher under full irrigation (figure 4.8).

These findings indicate that physiological responses of sorghum to irrigation treatments can vary significantly depending on the growth stages and across years, highlighting the significance of considering temporal variability in agronomic studies.

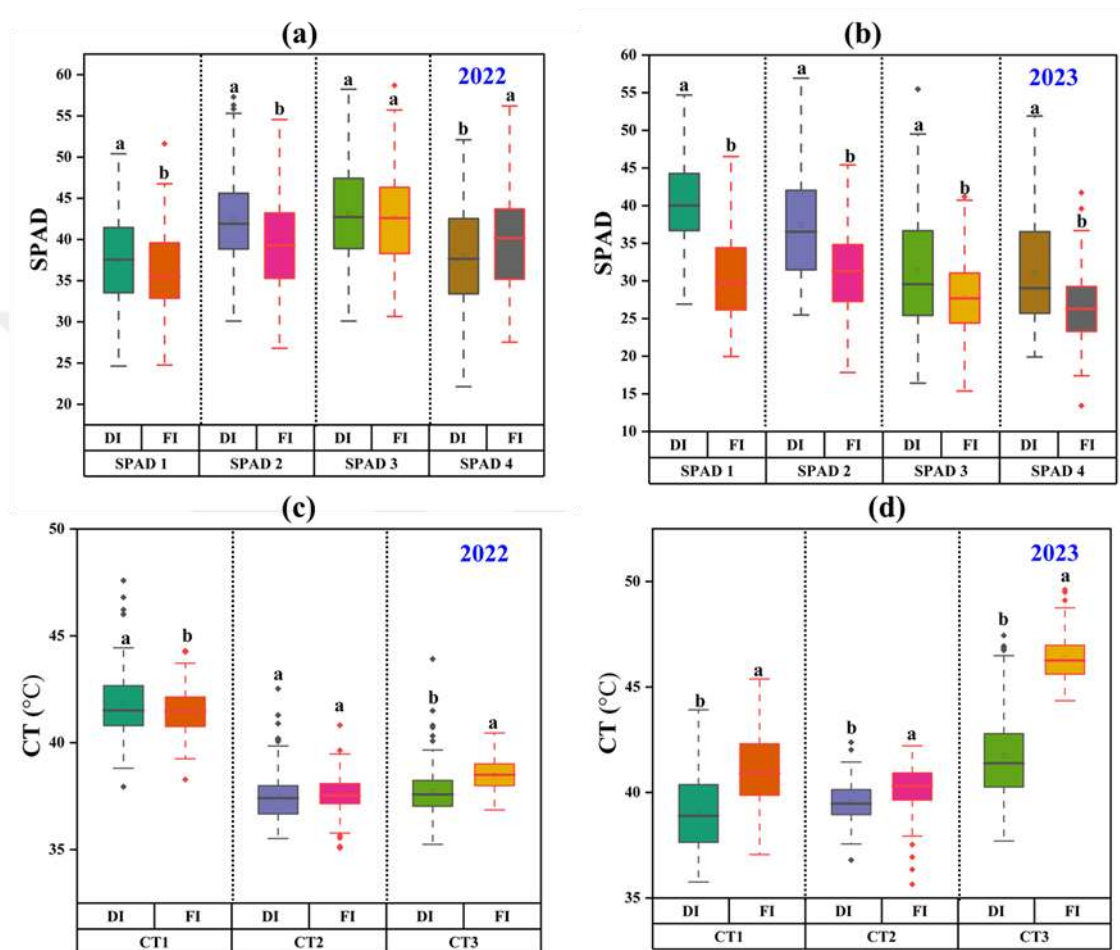


Figure 4.7 Box plots of the studied traits i.e., SPAD (a and b), CT, canopy temperature (c and d), showing the mean performance (LSD analysis at a 0.05 level of probability) of all the 131 sorghum accessions under deficit irrigation (DI) and full irrigation (FI) in 2022 and 2023.

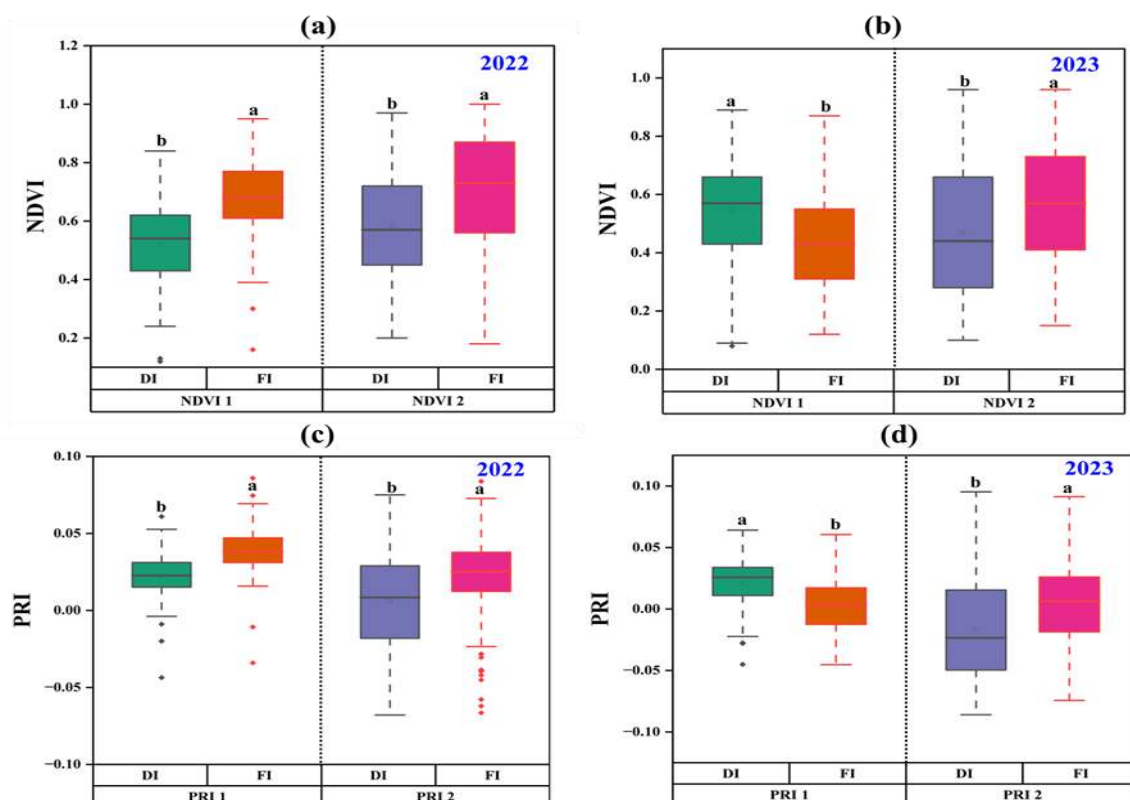


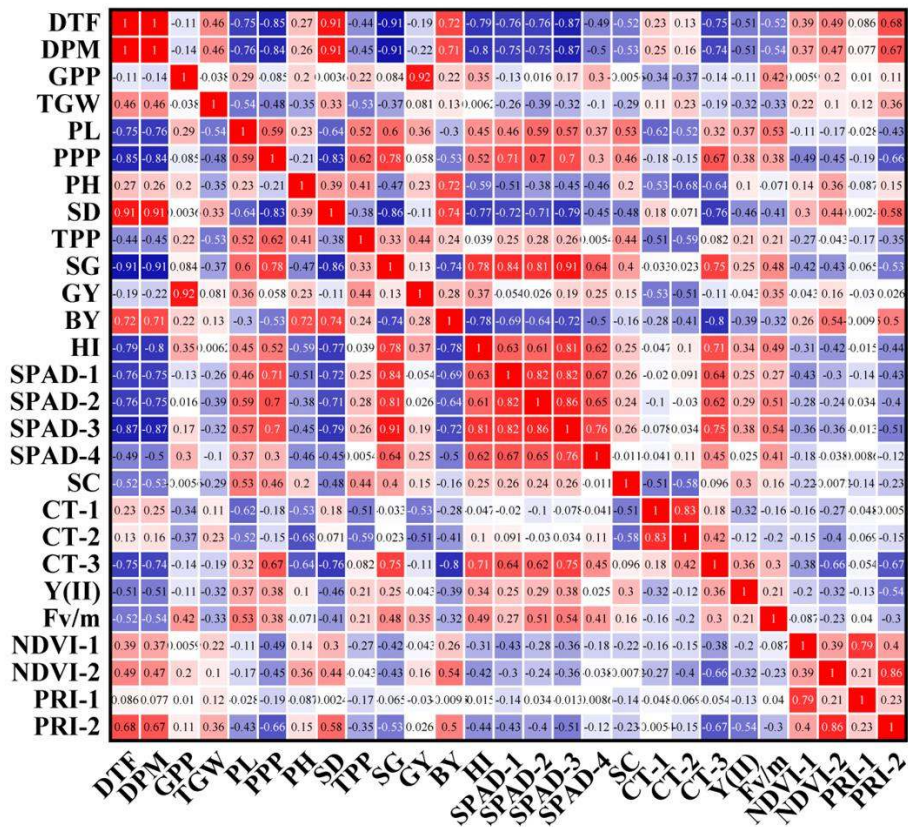
Figure 4.8 Box plots of the studied traits i.e., NDVI (a and b) and PRI (c and d), showing the mean performance (LSD analysis at a 0.05 level of probability) of all the 131 sorghum accessions under deficit irrigation (DI) and full irrigation (FI) in 2022 and 2023.

4.8. Correlation analysis

To evaluate the correlation among various morphological and physiological traits under both deficit and full irrigation treatments, we conducted a separate analysis for each irrigation treatment. In 2022, under deficit irrigation, we observed several significant positive correlations among the traits: Thousand grain weight was positively correlated with days to physiological maturity and stem diameter. Panicle length showed a positive correlation with stay green and Fv/m. Plant height and stem diameter were highly correlated with biological yield. Tillers per plant were positively correlated with grain yield per plant. Stay green demonstrated a strong correlation with SPAD measurements taken at different stages. Grain yield exhibited a strong positive correlation with grains per panicle. Fv/m was positively correlated with grain yield (figure 4.9).

Under full irrigation in 2022, thousand grain weight was positively correlated with grain yield and biological yield. Plant height and stem diameter showed a highly positive correlation with biological yield. Grain yield exhibited a strong positive correlation with grains per panicle. Stay green was positively correlated with SPAD. Grain yield was positively correlated with NDVI-2. Fv/m showed a positive correlation with SPAD and stay green. These findings highlight the intricate relationships between various morphological and physiological traits under different irrigation treatments (figure 4.9).

Deficit Irrigation (2022)



Full Irrigation (2022)

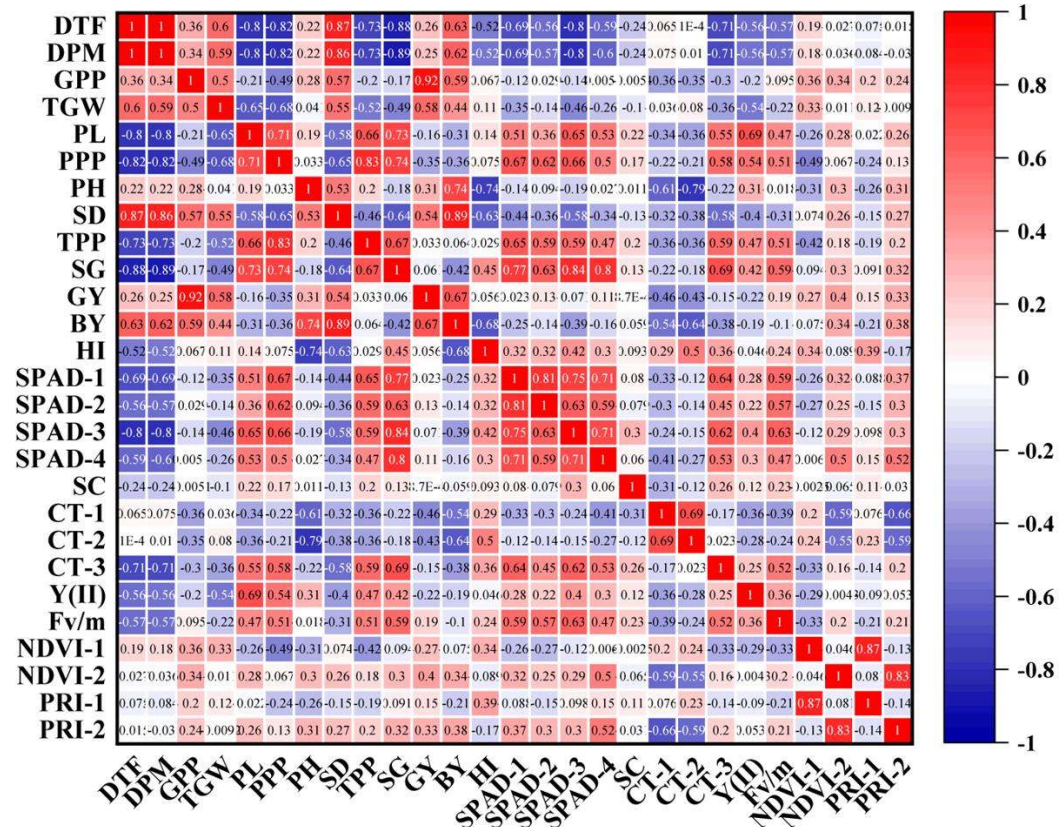


Figure 4.9. Correlation among morphological and physiological traits of sorghum accessions during 2022.

Similarly, in 2023, under deficit irrigation, grains per panicle demonstrated a positive correlation with SPAD values and a strong positive correlation with grain yield. Thousand grain weight exhibited positive correlations with stay-green, grain yield, biological yield, SPAD values, Fv/m, NDVI, and PRI. Stem diameter showed a positive correlation with biological yield. Stay-green trait exhibited a strong and positive correlation with both biological yield and SPAD values. All SPAD measurements demonstrated positive correlations with grains per panicle and thousand grain weight. NDVI and PRI were positively correlated with thousand grain weight, stay-green trait, grain yield, biological yield, and SPAD values (figure 4.10).

In 2023, under full irrigation, grains per panicle showed positive correlations with panicle length and SPAD values, along with a strong positive correlation with grain yield. Panicle length was positively correlated with grain yield. Tillers per plant and panicles per plant exhibited a strong and positive correlation with each other. Both stem diameter and plant height showed positive correlations with biological yield. Grain yield demonstrated a positive correlation with NDVI, though this correlation was moderate (figure 4.10).

Overall, these findings underscore the interconnected relationships between various plant traits and yield metrics under different irrigation treatments, highlighting the importance of SPAD values, stay-green, and NDVI as indicators of grain yield and other important agronomic traits.

Deficit Irrigation (2023)

Full Irrigation (2023)

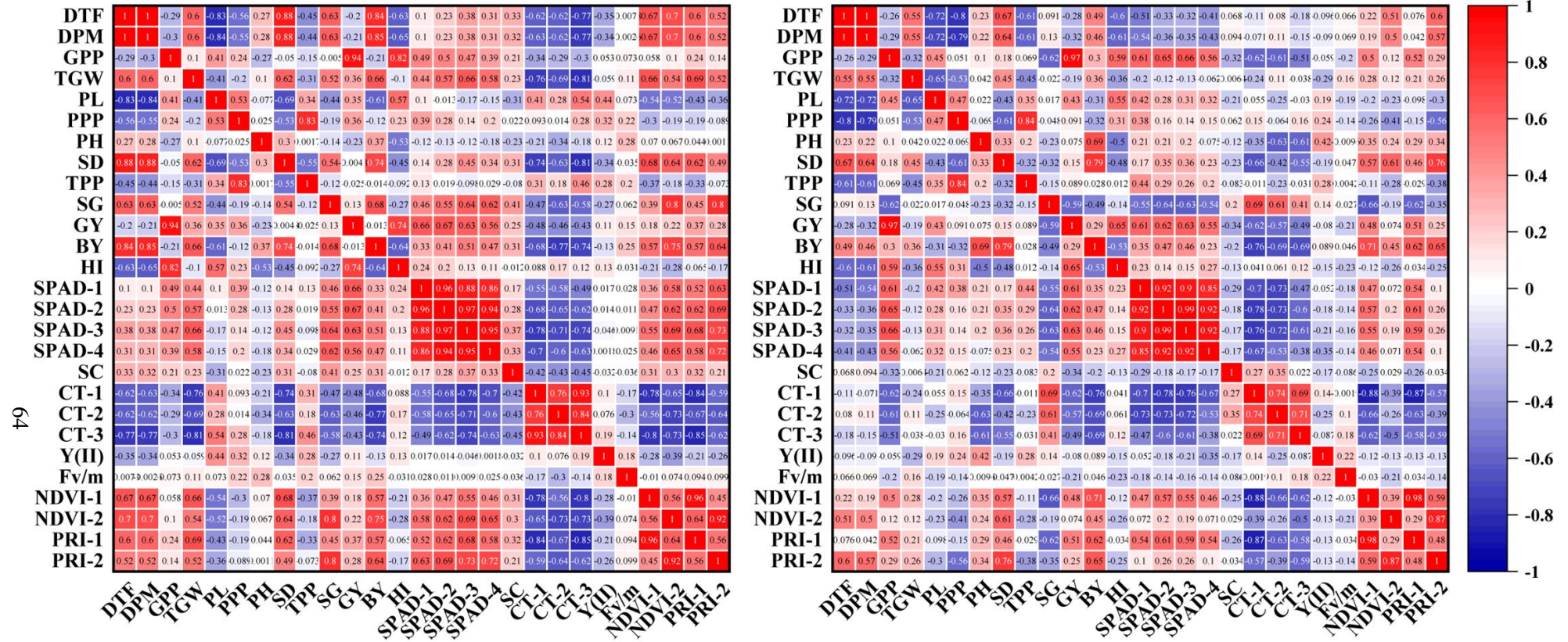


Figure 4.10. Correlation among morphological and physiological traits of sorghum accessions during 2023.

4.9. Principal component analysis

The eigenvalues for the corresponding principal components (PC) describe the proportion of variance each component explains. Principal components with eigenvalues greater than 1 are considered the main contributors to the variance. In 2022, the first two principal components of the given data set accounted for 33.44% and 34.7% of the total variance under deficit and full irrigation, respectively. Similarly, in 2023, the first two principal components accounted for 40.4% and 35.8% of the total variance under deficit and full irrigation. PC1 has the highest eigenvalue and contributes the most to the total variance, followed by PC2.

Table 4.15 and 4.16 summarize the eigenvalues and the percentage variance explained by each principal component. The variables under study account for a certain percentage of variability in the given principal components. Figures 4.11 and 4.12 visualize the contribution and representation of each variable on the factor map. The analysis underscores the significance of PC1 and PC2 as primary contributors to the observed variance in both deficit and full irrigation conditions across the two years. Variables that are not correlated with any major principal component or are correlated only with the last PC contribute less to the variance of principal components with eigenvalues greater than one.

In 2022 study conducted under deficit irrigation, the primary traits demonstrating greater contributions in PC1 include stay green, SPAD, and panicles per plant. Conversely, in PC2, the major contributors are identified as plant height, grain yield, and biological yield. In contrast, under full irrigation, the major traits exhibiting higher contributions in PC1 encompass stay green, panicles per plant, and panicle length. Meanwhile, in PC2, the predominant contributors are biological yield, grain yield, and stem diameter (table 4.15). These findings highlight the differential impact of irrigation on the key agronomic traits, delineating distinct patterns of trait association under deficit and full irrigation conditions.

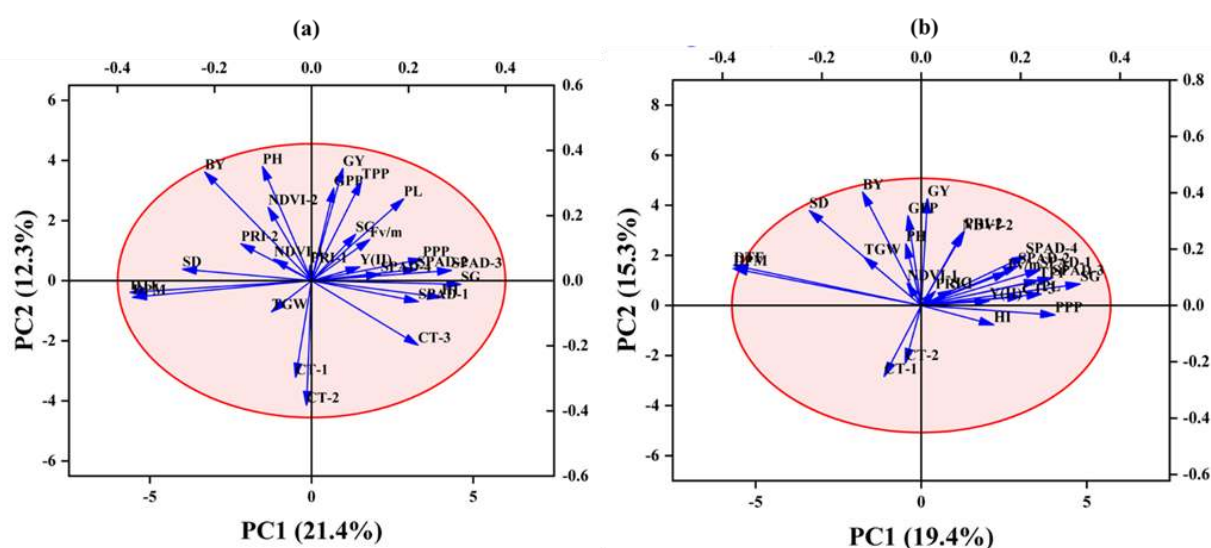


Figure 4.11 Biplot of morphophysiological traits of 131 sorghum accessions (a) under deficit irrigation (b) under full irrigation in 2022.

In 2023, under deficit irrigation, the principal components analysis reveals that the primary traits contributing significantly to the variance in PC1 include SPAD and biological yield. Meanwhile, in PC2, the key contributors are grain yield, harvest index, and grains per panicle. Conversely, under full irrigation, the principal traits influencing PC1 are SPAD, grain yield, biological yield, and thousand grain weight. In PC2, the major contributors are days to flowering and days to maturity (table 4.16). This analysis suggests that the impact of irrigation treatments on crop traits can be delineated through PCA, with distinct trait associations observed under deficit irrigation versus full irrigation conditions.

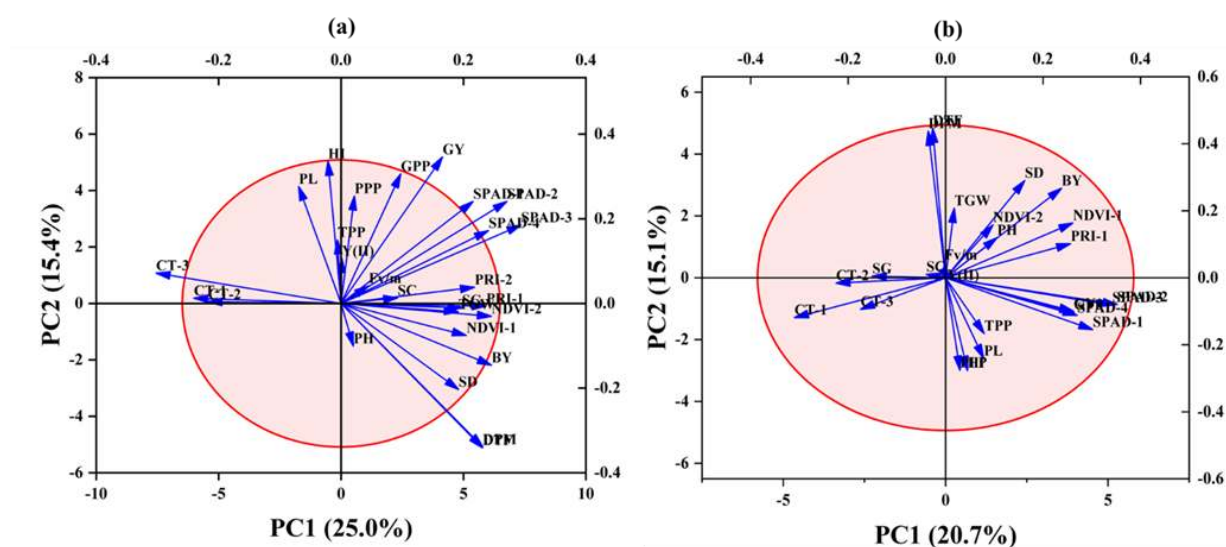


Figure 4.12 Biplot of morphophysiological traits of 131 sorghum accessions (a) under deficit irrigation (b) under full irrigation in 2023.

Table 4.15 Contribution of the variables to the principle components in 2022.

Traits	Deficit Irrigation		Full Irrigation	
	PC1	PC2	PC1	PC2
DTF	-0.37	-0.04	-0.38	0.14
DPM	-0.37	-0.05	-0.38	0.13
GPP	0.05	0.28	-0.03	0.32
TGW	-0.08	-0.10	-0.12	0.17
PL	0.19	0.25	0.24	0.04
PPP	0.23	0.07	0.27	-0.03
PH	-0.10	0.35	-0.03	0.22
SD	-0.27	0.04	-0.23	0.34
TPP	0.10	0.31	0.24	0.09
SG	0.31	-0.01	0.32	0.08
GY	0.06	0.34	0.01	0.38
BY	-0.22	0.33	-0.12	0.40
HI	0.27	-0.05	0.15	-0.07
SPAD-1	0.22	-0.06	0.24	0.13
SPAD-2	0.22	0.04	0.19	0.14
SPAD-3	0.29	0.03	0.26	0.10
SPAD-4	0.14	0.02	0.21	0.18
SC	0.09	0.14	0.06	0.05
CT-1	-0.03	-0.30	-0.07	-0.25
CT-2	-0.01	-0.38	-0.03	-0.20
CT-3	0.22	-0.20	0.20	0.03
Y(II)	0.10	0.04	0.14	0.02
Fv/m	0.12	0.13	0.17	0.12
NDVI-1	-0.08	0.07	-0.03	0.08
NDVI-2	-0.09	0.23	0.08	0.26
PRI-1	0.00	0.05	0.03	0.05
PRI-2	-0.15	0.11	0.09	0.26
Eigenvalue	5.76	3.32	5.24	4.13
Percentage of variance (%)	21.36	12.32	19.43	15.31
Cumulative (%)	21.36	33.69	19.43	34.75

Table 4. 16 Contribution of the variables to the principle components in 2023.

Traits	Deficit Irrigation		Full Irrigation	
	PC1	PC2	PC1	PC2
DTF	0.23	-0.34	-0.03	0.45
DPM	0.23	-0.34	-0.04	0.44
GPP	0.10	0.31	0.26	-0.10
TGW	0.19	-0.02	0.02	0.21
PL	-0.07	0.28	0.08	-0.24
PPP	0.02	0.25	0.03	-0.28
PH	0.02	-0.10	0.11	0.12
SD	0.19	-0.20	0.16	0.29
TPP	-0.01	0.15	0.08	-0.17
SG	0.20	-0.01	-0.15	0.00
GY	0.17	0.35	0.26	-0.10
BY	0.25	-0.15	0.24	0.27
HI	-0.02	0.34	0.04	-0.28
SPAD-1	0.22	0.24	0.30	-0.15
SPAD-2	0.27	0.24	0.35	-0.08
SPAD-3	0.29	0.19	0.34	-0.08
SPAD-4	0.24	0.17	0.27	-0.11
SC	0.09	0.01	-0.04	0.01
CT-1	-0.24	0.01	-0.31	-0.12
CT-2	-0.22	0.00	-0.22	-0.02
CT-3	-0.30	0.07	-0.17	-0.09
Y(II)	0.00	0.11	0.00	-0.01
Fv/m	0.05	0.04	0.00	0.05
NDVI-1	0.20	-0.08	0.26	0.16
NDVI-2	0.25	-0.03	0.10	0.16
PRI-1	0.24	-0.01	0.26	0.10
PRI-2	0.22	0.04	0.09	0.26
Eigenvalue	6.75	4.15	5.38	3.91
Percentage of variance (%)	25.01	15.37	20.7	15.05
Cumulative (%)	25.01	40.38	20.7	35.76

4.10. Cluster analysis

To optimize the production of high-quality crossings, breeding programs require genetically distinct and desirable accessions suitable for hybridization. Utilizing cluster analysis is a common approach to assess the extent of genetic variation and classify accessions based on their similarities into distinct clusters. This understanding of genetic relationships among accessions provides valuable insights for selective breeding and management of germplasm resources. The results of the cluster analysis for both deficit and full irrigation experiments are displayed in figures 4.13 and 4.14. Utilizing the means of all studied traits across two irrigation levels, the analysis grouped the 131 sorghum accessions (including checks) into six distinct clusters.

Furthermore, 37 accessions (identified as 2, 4, 8, 9, 14, 15, 19, 21, 23, 29, 33, 37, 40, 41, 43, 47, 52, 63, 72, 75, 77, 82, 83, 84, 85, 86, 92, 93, 94, 97, 106, 117, 118, 119, 121, 124, 125) exhibited superior performance under deficit irrigation compared to full irrigation across both years, particularly in terms of grain yield. Notably, cluster analysis revealed that most of these identified accessions were grouped within the same cluster, corroborating their enhanced grain yield under deficit irrigation conditions consistently over the years. These accessions demonstrate potential drought tolerance and are therefore prime candidates for integration into breeding programs aimed at developing drought-resistant sorghum varieties. Their inclusion could significantly contribute to the enhancement of sorghum cultivars with improved resilience to water stress, thereby bolstering agricultural sustainability and productivity.

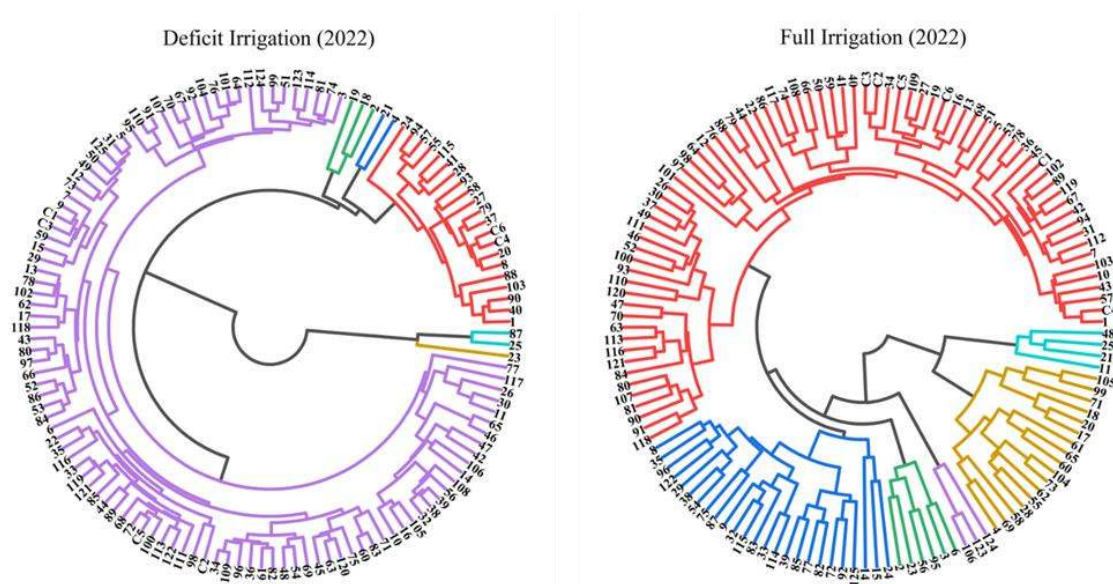


Figure 4.13 Cluster analysis of 131 sorghum accessions under deficit and full irrigation in 2022 growing season.

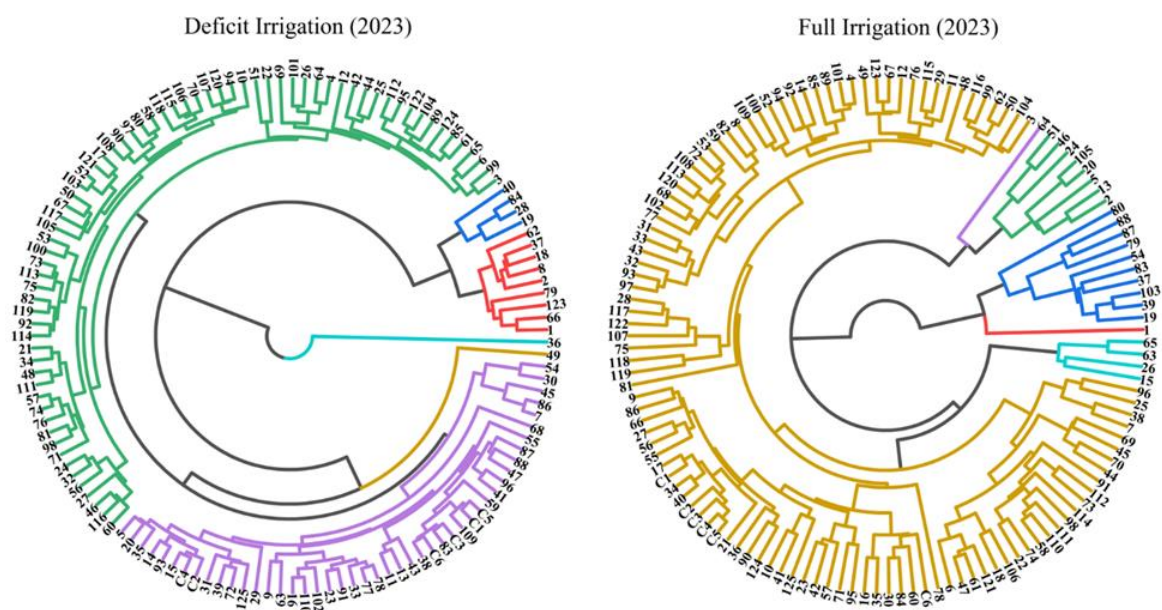


Figure 4.14 Cluster analysis of 131 sorghum accessions under deficit and full irrigation in 2023 growing season.

5. DISCUSSION

The two-year experiment on 131 sorghum accessions under deficit irrigation (50% of field capacity) and full irrigation (100% of field capacity) revealed significant variability in most of the studied morpho-physiological traits measured, both at the genotype level and across the irrigation treatments. This genetic variability, as expressed phenotypically, is a key aspect of screening and breeding strategies. Phenotypic differences are largely dictated by environmental conditions, complicating the variability as accessions react differently to environmental changes since no two environments are exactly the same. Differential genotypic responses across environments often pose challenges in breeding, testing, and selecting superior accessions. Therefore, it is crucial to identify accessions that are either adapted or stable across different environments. Our results indicated that 37 accessions were more stable across the varying environments i.e., across the two years of the experiment.

Drought stress is a major factor limiting the growth of field crops globally (Hadebe et al. 2020; Kamatchi et al. 2024). Understanding drought tolerance mechanisms in plants is crucial for reducing the negative effects of stress on yield (Sanchita et al. 2015). Therefore, this study evaluated the response of various sorghum accessions to water shortage stress compared to control conditions. Effective screening of germplasm for agronomical and physiological traits, especially under drought stress, is a valuable approach for selecting material to develop new varieties and hybrids. The highly significant differences observed between the accessions for all physiological and agronomical traits indicate that the sorghum accessions has high variability, which can be effectively utilized for breeding purposes.

5.1. Phenological traits of sorghum under deficit and full irrigation

The observed differences in days to 50% emergence under deficit irrigation between the two years, despite identical initial water applications, could be attributed to several factors. In the first year, the delay in emergence under deficit irrigation could be due to soil moisture retention and distribution differences, with soils in the deficit irrigation treatment drying out faster, thus delaying germination (Passioura, 2006). Additionally, microclimatic variations such as humidity and temperature between the two years could have impacted seedling emergence rates (Bewley et al. 2012). The vigor and health of seeds, affected by storage conditions or aging, might have also played a role (Finch-Savage and Bassel, 2016). Moreover, the similarity in emergence rates in the second year suggests a potential physiological adaptation of the genotypes to deficit irrigation conditions, enhancing their resilience and efficient use of available water (Bruce et al. 2007). These factors when combined explain the initial delay and subsequent alignment in emergence times under different irrigation treatments.

In the first year, accessions responded similarly for days to booting under both deficit and full irrigation. However, in the second year, days to booting were shorter under deficit irrigation. This accelerated development under water stress aligns with findings that crop plants often exhibit a shorter growth cycle to escape drought (Mabhaudhi and Modi, 2013). This drought avoidance mechanism allows sorghum to complete its life cycle before severe stress periods, mitigating yield loss (Manavalan and Nguyen, 2017). The observed genotypic variability in days to booting highlights the genetic diversity crucial for breeding drought-tolerant sorghum varieties (Haussmann et al. 2012).

In the first year, accessions exhibited similar responses under both deficit and full irrigation for days to heading. However, in the second year, days to heading were shorter under deficit irrigation. This observation is consistent with findings by Sakhi et al. (2013), who reported that sorghum genotypes exhibit earliness in days to heading under drought stress compared to control conditions. This accelerated development under water stress is a known drought avoidance mechanism, allowing crops to complete their life cycle before the onset of severe stress periods (Manavalan and Nguyen, 2017; Seleiman et al. 2021). The variability in days to heading among accessions underscores the genetic diversity within the sorghum accessions, essential for breeding drought-tolerant varieties.

Initially, sorghum accessions exhibited comparable responses under deficit and full irrigation in the first year for days to flowering. However, in the second year, there was a noticeable reduction in days to flowering under deficit irrigation compared to full irrigation. This variability in days to flowering can be attributed to genotype-specific responses to the evaluated stress conditions.

Rakshit et al. (2016) reported differential behavior of sorghum genotypes under drought stress, with some genotypes flowering earlier and others later. This variability underscores the importance of selecting genotypes based on their flowering behavior in environments prone to pre- and post-flowering drought stress. Early flowering can confer advantages by allowing plants to escape drought during reproductive stages, while late flowering can be beneficial under conditions where drought occurs early in development (Sabadin et al. 2012). Conversely, Abraha et al. (2015) noted a delay in average days to flowering of sorghum genotypes under water stress compared to control conditions, highlighting sensitivity to stress conditions.

Across both years, the box plots indicated that days to maturity were consistently shorter under deficit irrigation compared to full irrigation. Derese et al. (2018) reported early maturity in sorghum genotypes under water-limited conditions compared to normal irrigation. This variation underscores the genetic diversity among sorghum genotypes in their response to moisture availability. Rakshit et al. (2016) noted variation among sorghum genotypes for days to maturity; however, the average days to maturity were similar under both well-watered and water stress conditions. This suggests that while individual genotypes may vary, overall, sorghum exhibits a consistent response in maturity across irrigation regimes. Abraha et al. (2015) documented that under drought stress conditions, days to maturity ranged from 95 to 115 days after sowing, whereas under

control conditions, the range was narrower at 93 to 107 days. This broader range under stress conditions indicates the influence of water deficit on delaying maturity across most sorghum genotypes. They reported a delay in average days to maturity compared to control conditions. Understanding genotype-specific responses to stress conditions is crucial for developing drought-tolerant varieties that can maintain stable maturity dates across varying environmental conditions.

Year-to-year differences in plant phenological responses highlight the significance of multi-year trials for evaluating genotype performance under stress (Cooper et al. 2014). Selecting genotypes that consistently perform well under water stress will enhance the development of drought-resilient sorghum varieties.

5.2. Morphological traits of sorghum under deficit and full irrigation

In the first year, plant height was lower under deficit irrigation compared to full irrigation, whereas in the second year, plant height was higher under deficit irrigation. Drought stress is known to reduce plant height in sorghum, as reported by Shehab and Guo (2021) and Gano et al. (2021). Increasing severity of drought stress exacerbates these reductions (Fardin et al. 2023). Conversely, under well-watered conditions, sorghum genotypes tend to exhibit taller plants (Gano et al. 2021). Sarshad et al. (2021) observed variation in sorghum genotypes for plant height, highlighting genetic diversity in response to irrigation levels. Similarly, Abraha et al. (2015) and Derese et al. (2018) reported lower plant height under drought stress compared to irrigated conditions.

Ahmed et al. (2017) also noted significant variation in plant height among sorghum genotypes under different irrigation treatments, emphasizing the importance of genotype selection for specific growth conditions. Additionally, producers prefer cultivars with shorter plant height due to sturdier stems and ease of mechanical harvesting (Mutava et al. 2011).

In the first year, accessions showed similar responses for stem diameter under deficit and full irrigation, while in the second year, stem diameter was lower under full irrigation compared to deficit irrigation. Drought stress significantly reduces stem diameter in sorghum, as reported by Shehab and Guo (2021). This reduction is attributed to decreased cell expansion due to limited water availability, which affects overall plant growth (Rad et al. 2021). Alderfasi et al. (2016) found that irrigation frequency had no significant impact on sorghum stem diameter. However, Ahmed et al. (2017) observed significant variation in stem diameter among sorghum genotypes under different irrigation regimes, highlighting genotype-specific responses. Mubarik et al. (2022) demonstrated that increasing irrigation frequency enhances stem diameter in sorghum, indicating the sensitivity of this trait to water availability.

Among sorghum morphological traits, stem diameter is particularly sensitive to reductions in irrigation levels, reflecting the direct impact of water stress on cell expansion and growth (Rad et al. 2021). In the second year, the observed decrease in stem diameter under full irrigation compared to deficit irrigation could be attributed to physiological responses of plants to water availability.

Studies have shown that moderate water stress can stimulate cell expansion and thicker stem development (Farooq et al. 2009).

Interestingly, the box plots revealed consistent responses of tillers per plant of sorghum accessions under both deficit and full irrigation across both years. Previous studies have found that water stress did not influence tiller numbers, genotypic variation was observed (Santana et al. 2020; Sarshad et al. 2021). This variation underscores the genetic control of tillering, influenced by the internal carbon balance and assimilate availability (Bos and Neuteboom, 1998; Lafarge et al. 2002). Tillering in sorghum serves as a mechanism for adapting to environmental stresses like water scarcity (Hadebe et al. 2017), enabling plants to optimize panicle production and regulate yield under varying conditions (Bell et al. 2018). Sakhi et al. (2013) reported increased tiller numbers in sorghum genotypes under drought stress, highlighting the adaptive nature of tillering in response to stress conditions. There were not any significant differences between tillers in sorghum under different irrigation treatments (Bell et al. 2018). However, genetic differences play a crucial role in determining tillering responses to environmental stresses like drought.

The box plots showed consistent responses of sorghum accessions for the number of panicles per plant under both deficit and full irrigation across both years. Sakhi et al. (2013) reported an increase in the number of panicles per plant in sorghum genotypes under drought stress conditions compared to control conditions, highlighting the adaptive response of sorghum to water stress. Fardin et al. (2023) found significant differences in the number of panicles per plant among sorghum genotypes under various irrigation treatments, with fewer panicles recorded under extreme water stress conditions. This suggests that water availability influences panicle development through its impact on photosynthetic activity and assimilate transport (Kamali and Mehraban, 2020). Contrary to some findings (Berenguer and Faci, 2001), where irrigation intervals did not significantly affect panicle number in sorghum at certain planting densities, Fardin et al. (2023) emphasized that the number of panicles per plant is a critical yield component influenced by both genotype and agronomic practices. Water depletion affects plant morphological traits by reducing cell elongation and division, which are critical for growth processes such as photosynthesis and carbohydrate metabolism (Farooq et al. 2008; Jaleel et al. 2008). This reduction in growth under stress conditions is influenced by genotype-specific responses. Understanding the genetic variability in sorghum for morphological traits under varying water conditions is crucial for developing drought-tolerant cultivars that maintain robust growth under limited water availability. These findings underscore the importance of irrigation management in optimizing overall plant growth in sorghum production systems.

In the first year, stay green was lower under deficit irrigation compared to full irrigation, while in the second year, stay green was higher under deficit irrigation. Stay green genotypes are crucial targets for drought tolerance breeding due to their ability to sustain prolonged photo-assimilation, contributing to better yield potential (Mahalakshmi and Bidinger, 2002). This trait

enhances drought resistance by delaying senescence, allowing green leaves to persist longer and support grain production (Borrell et al. 2003).

Under severe drought stress, cultivars with a higher stay-green maintain an active transport system in the stem, crucial for nutrient uptake and grain filling (Xu et al. 2000; Awala and Wilson, 2005). The expression of stay green throughout drought periods, particularly before and after flowering, serves as an indicator of drought tolerance (Mwamahonje et al. 2021). This tolerance is essential for maintaining photosynthesis and nutrient assimilation during water scarcity, critical for grain development (Mwamahonje et al. 2021).

Nitrogen availability plays a significant role in stay green expression, influencing the delay of leaf senescence and ensuring sustained photosynthesis for grain filling (Borrell et al. 2000a,b). Chlorophyll content and stay green are pivotal traits in evaluating drought-tolerant sorghum varieties under water-limited environments, highlighting their importance in sustainable agriculture (Mwamahonje et al. 2021). Understanding the genetic and physiological mechanisms underlying stay green in sorghum is essential for developing cultivars resilient to drought, thereby enhancing grain yield stability in variable water conditions.

5.3. Yield and yield-related traits of sorghum under deficit and full irrigation

The box plots showed no significant difference between deficit irrigation and full irrigation for panicle length in both years of the study. Alderfasi et al. (2016) demonstrated that panicle length in sorghum increases with higher irrigation levels, indicating the sensitivity of this trait to water availability. In contrast, Fardin et al. (2023) found no significant variation in panicle length across different levels of water stress, suggesting that sorghum may prioritize other physiological responses under varying water conditions. Panicle length plays a critical role in determining the number of grains per panicle and ultimately influences overall plant yield (Kamali and Mehraban, 2020).

Under drought stress, reductions in panicle length are attributed to compromised cell elongation rather than cell division, impacting key physiological processes like photosynthesis and nutrient uptake (Kamali and Mehraban, 2020). Consistent with these findings, studies by Jabereldar et al. (2017) and Abraha et al (2015) reported decreased panicle length in sorghum under drought stress conditions, highlighting the physiological constraints imposed by water scarcity on panicle development. Moreover, Derese et al. (2018) observed significant reductions in panicle-related traits including panicle length under rainfed conditions, underscoring the impact of water availability on sorghum yield components.

In the first year, grains per panicle showed similar responses under deficit irrigation and full irrigation, while in the second year, there was a higher number of grains per panicle under deficit irrigation compared to full irrigation. Grains per panicle in sorghum are sensitive to water stress conditions, as observed by Fardin et al. (2023), who noted a decrease in grain numbers under stress. Similarly, studies by Azarinasrabad et al. (2016) and Alderfasi et al. (2016) reported that irrigation

levels significantly influence grain production per panicle, with higher irrigation promoting greater grain numbers.

Under water stress, the reduction in grains per panicle is attributed to compromised physiological processes such as reduced leaf surface area, accelerated leaf senescence, and impaired nutrient assimilation, affecting both source-sink dynamics and overall yield (Kamali and Mehraban, 2020; Ahmad et al. 2020). Fardin et al. (2023) highlighted the significant impact of drought stress on grains per panicle, emphasizing the role of genotype variability in sorghum's response to water scarcity. Nasrabad et al. (2017) further supported these findings by documenting a decrease in grain numbers per panicle under water stress conditions. While the first year showed no significant difference between deficit and full irrigation for grains per panicle, the second year's results underscored the influence of water availability on this critical yield component in sorghum.

In the first year, thousand grain weight was similar under both deficit and full irrigation, while in the second year, thousand grain weight was higher under full irrigation compared to deficit irrigation. Ochieng et al. (2021) reported no observable difference in the means of hundred seed weight among sorghum genotypes under stress and well-watered conditions. This aligns with our first-year findings where no significant difference was observed. However, our second-year results showing higher thousand grain weight under full irrigation are consistent with findings by Fardin et al. (2023) and Azarinasrabad et al. (2016), who reported a decrease in thousand grain weight under increasing water stress conditions.

Alderfasi et al. (2016) and Mubarik et al. (2022) also observed that increasing irrigation frequency significantly increases thousand grain weight in sorghum, which supports our second-year results. The observed variation in thousand grain weight among different genotypes under varying irrigation treatments might be attributed to the genetic diversity of the sorghum accessions studied, as reported by Kamal et al. (2023). Water stress significantly decreases the thousand grain weight due to its adverse effects on grain filling and overall plant metabolism (Nasrabad et al. 2017; Prasad et al. 2008). The yield loss under water stress conditions is often driven by reductions in both grain number per panicle and thousand seed weight. These findings corroborate the general understanding that water stress negatively impacts thousand grain weight, although the extent can vary annually due to environmental conditions and genotype-specific responses. Further research focusing on the genetic mechanisms underlying these variations could provide deeper insights into improving sorghum yield under water-limited conditions.

Grain yield, a critical indicator of sorghum productivity, was found to be significantly influenced by both irrigation treatments and genotype variability in our two-year study. Our results align with previous findings indicating that grain yield is markedly affected by water availability (Rakshit et al. 2016; Kamali and Mehraban, 2020). In our study, despite significant differences observed in grain yield across genotypes and irrigation treatments according to ANOVA, the box plots revealed similar grain yields between deficit irrigation and full irrigation in both years of

experimentation. This suggests that under our experimental conditions, the irrigation treatments did not lead to statistically significant differences in grain yield, contrasting with findings by Kazemi et al. (2022) who reported higher yields under full irrigation.

According to Mubarik's (2021) findings, a higher irrigation level did not result in a significant increase in yield and yield-related parameters in sorghum which is in line with our findings. The consistency in grain yield between deficit and full irrigation treatments in our study could be attributed to several factors, including the genetic makeup of the sorghum accessions studied and possibly the adaptive responses of these accessions to water stress conditions. This observation resonates with the notion that genotypic variation plays a crucial role in determining yield stability under varying environmental stresses (Mubarik et al. 2022).

The complexity of grain yield determination involves a combination of morphological and physiological traits influenced by water availability (Mutava et al. 2011). This aligns with the broader literature, which consistently reports yield reductions under drought conditions (Mutava et al. 2011; Safian et al. 2022). Specifically, Prasad et al. (2008) highlighted that yield loss is driven by reductions in both grain number per panicle and thousand seed weight, with the critical period being the grain filling stage. Furthermore, our results reflect the broader understanding that while water stress generally reduces grain yield in sorghum (Yahya et al. 2023; Nasrabad et al. 2017), the specific responses can vary depending on the interaction between genotype and environmental conditions. This variability underscores the complexity of managing water resources for optimizing sorghum productivity, especially in regions prone to water scarcity.

Based on the findings of this study, biological yield in sorghum did not differ significantly between deficit irrigation and full irrigation treatments over two consecutive years. This contrasts with previous studies indicating that increasing irrigation levels, as shown by Mubarik et al. (2022), can notably enhance biological yield. Similarly, Habyarimana et al. (2004) highlighted inferior aboveground biomass performance in rain-fed conditions compared to fully irrigated conditions, underscoring the critical role of adequate water supply. Moreover, Khazaei and Nazari (2022) observed that while biological yield increased under well-watered conditions, it significantly decreased under mild and severe water stress scenarios. Our study suggests that while sorghum shows resilience to moderate water stress in terms of biological yield, optimal irrigation management remains pivotal for maximizing yield potential under varying environmental conditions.

Harvest index (HI) reflects the efficiency of a plant in converting biomass into grain yield, making it a crucial trait in evaluating genotype performance under varying irrigation conditions. Mubarik et al. (2022) noted that increasing irrigation frequency from 2 to 8 did not significantly increase HI, with the maximum HI observed at four irrigation levels, indicating an optimal irrigation level for maximizing this trait. Under drought stress, Jeevitha et al. (2022) reported a reduction in HI compared to well-watered conditions, with values ranging between 10.75 to 38.92. This reduction underscores the sensitivity of HI to water scarcity, highlighting its role as a reliable indicator of crop

performance under stress. The ability of a genotype to maintain a high HI under stress conditions is critical for sustaining yield stability and ensuring efficient resource utilization in agricultural production systems (Kusalkar et al. 2003).

5.4. Physiological traits of sorghum under deficit and full irrigation

In the first year of the experiment, SPAD-1 and SPAD-2 values were higher under deficit irrigation compared to full irrigation, whereas SPAD-3 showed no significant difference between irrigation treatments. However, SPAD-4 was lower under deficit irrigation than full irrigation. This contrasts with the second-year results, where all SPAD values were higher under deficit irrigation compared to full irrigation conditions. These findings are consistent with previous studies indicating that chlorophyll content, as measured by SPAD values, is influenced by water availability and developmental stage (Gonulal, 2022). The observed variations in SPAD values under different irrigation regimes reflect the plant's response to water stress, where reduced water availability generally leads to decreased chlorophyll content due to impaired nitrogen assimilation and reduced photosynthetic activity (Abd El-Mageed et al. 2018). However, the specific response varies across growth stages, as noted by Vasilakoglou et al. (2011), who found that chlorophyll contents varied with the growth stages under water stress conditions. The non-significant effect of irrigation treatments on SPAD values aligns with previous findings suggesting that early growth stages may not exhibit pronounced differences in chlorophyll content under varying water availability (Ahmed and Khurshid, 2011). Nonetheless, significant reductions in SPAD values under drought stress emphasize the sensitivity of chlorophyll content to water stress (Akman et al. 2021). This sensitivity underscores the importance of maintaining adequate water availability to sustain chlorophyll levels critical for photosynthetic efficiency and subsequent yield (Allamine et al. 2023).

The correlation between chlorophyll content and drought tolerance, as highlighted by Djanaguiraman et al. (2023), suggests that genotypes with higher chlorophyll retention under stress conditions may exhibit improved drought resilience and yield stability. Mechanistically, the decrease in chlorophyll content under drought stress is linked to oxidative stress and chloroplast damage induced by reactive oxygen species (Zhang et al. 2023a; Impa et al. 2004). This reduction in chlorophyll content compromises photosynthetic capacity and overall plant productivity, consistent with findings that drought stress reduces total chlorophyll content in sorghum (Shehab and Guo, 2021; Nasrabad et al. 2017). Moreover, the delay in leaf senescence associated with higher chlorophyll content during drought stress underscores its role in maintaining green leaf area and enhancing grain yield (Mwamahonje et al. 2021; Xu et al. 2000).

The variability observed in chlorophyll content among sorghum genotypes under different irrigation conditions highlights the genetic basis and adaptability of this trait in response to water availability (Goche et al. 2020; Chutia and Borah, 2012). Understanding these responses is crucial

for selecting drought-tolerant genotypes capable of maintaining chlorophyll levels and sustaining productivity under varying environmental stresses.

In the second year of the experiment, all SPAD values were higher under deficit irrigation compared to full irrigation, contrasting with the first year's findings. This observation can be attributed to several physiological responses of sorghum to water stress. Firstly, under deficit irrigation, plants often exhibit mechanisms to maintain higher chlorophyll content as a strategy to cope with reduced water availability. This phenomenon is supported by studies indicating that moderate water stress can induce physiological adjustments that enhance chlorophyll stability and photosynthetic efficiency in sorghum (Harris et al. 2007; Mwamahonje et al. 2021). Additionally, the delay in leaf senescence observed under water stress conditions contributes to sustained chlorophyll content, as senescence processes are often associated with chlorophyll degradation (Mwamahonje et al. 2021).

Furthermore, the genetic makeup of sorghum genotypes plays a crucial role in determining their response to water stress. Genotypes with inherent drought tolerance mechanisms, such as efficient water use, enhanced root architecture, and osmotic adjustment, may exhibit higher chlorophyll retention under deficit irrigation conditions (Mwamahonje et al. 2021; Djanaguiraman et al. 2023). Therefore, the higher SPAD values observed under deficit irrigation in the second year likely reflect a combination of physiological acclimatization responses and genetic adaptations that enhance chlorophyll stability and maintain photosynthetic efficiency under water-limited conditions in sorghum.

Stomatal conductance plays a crucial role in the physiological response of sorghum to varying water availability, as evidenced by the significant differences observed between deficit irrigation and full irrigation treatments across the two years of this experiment. In the first year, stomatal conductance was lower under deficit irrigation compared to full irrigation, indicating a typical response to water stress where plants close their stomata to conserve water (Gonulal, 2022). This reduction in stomatal conductance under water stress aligns with findings that sorghum adjusts its stomatal openings to limit water loss during drought conditions (Barbosa et al. 2020). Conversely, in the second year, stomatal conductance was higher under deficit irrigation than full irrigation, suggesting a different physiological response possibly influenced by genotype-specific adaptations or environmental conditions (Gano et al. 2021; Akman et al. 2021). The ability of sorghum to maintain relatively higher stomatal conductance under deficit irrigation in the second year could be indicative of inherent drought tolerance mechanisms, such as delayed stomatal closure to sustain photosynthesis and growth (Sinclair et al. 2005). This resilience in stomatal conductance under varying water availability underscores the complex interplay between genetic traits and environmental factors in determining drought response strategies in sorghum (Gano et al. 2021; Akman et al. 2021). Moreover, the literature supports that stomatal conductance is closely linked to

CO₂ assimilation and water use efficiency, making it a critical trait for understanding plant water relations and adaptation to drought stress in sorghum (Messina et al. 2015; Dahmardeh et al. 2015).

The study investigated canopy temperature (CT) as a physiological response indicator in sorghum under varying irrigation regimes across two consecutive years. In the first year, 1st CT and 2nd CT were observed to be higher under deficit irrigation compared to full irrigation, while 3rd CT showed lower values under deficit irrigation. This trend suggests that early growth stages (1st CT and 2nd CT) were more susceptible to water stress, impacting canopy temperature differently than later stages (3rd CT). Conversely, in the second year, all CT values were higher under full irrigation, indicating enhanced water stress compared to deficit irrigation conditions.

Gonulal (2022) highlighted variable responses of sorghum canopy temperature to different irrigation levels, i.e., in the first year of the experiment, decreasing irrigation levels increased canopy temperatures however, full irrigation and 75% of full irrigation resulted in similar canopy temperatures. Similarly, 50% and 25% of full irrigation resulted in similar canopy temperature. While in the second year, decreasing irrigation levels significantly increased canopy temperatures, affirming that reduced irrigation significantly elevated CT levels. Pradhan et al. (2022) reported consistently higher CT under drought stress across sorghum cultivars. Azarinasrabad et al. (2016) also observed increased CT in water-stressed sorghum compared to normal irrigation conditions, underscoring the sensitivity of CT to water availability.

The cooler CT observed under controlled conditions are indicative of efficient water use and stress adaptation mechanisms in sorghum (Mutava et al. 2011). This adaptation allows for sustained transpiration and photosynthesis rates, critical for biomass accumulation and drought tolerance (Kimball and Bernacchi, 2006). While CT is influenced by stomatal conductance and transpiration rates, it is also subject to ambient conditions like air temperature and solar radiation (Sinclair et al. 2005). The significant genotype-specific responses underscore the importance of selecting cultivars with lower CT as a marker for drought tolerance (Lopes et al. 2011). The study reinforces the utility of CT as an effective indicator to assess sorghum responses under drought stress, aligning with its application in evaluating drought-resistant genotypes in other cereal crops (Golestani and Assad, 1998; Han et al. 2016).

The chlorophyll fluorescence parameters are vital indicators of photosynthetic performance and plant stress responses. Y(II) was higher under deficit irrigation in the first year compared to full irrigation, whereas it was lower under deficit irrigation in the second year. Similarly, Fv/Fo and Fv/Fm were consistent across irrigation treatments in the first year but were higher under deficit irrigation compared to full irrigation in the second year. The large range of variation in chlorophyll fluorescence variables observed in our study agrees with previous reports (Balota et al. 2008; Kiani et al. 2008; Salas Fernandez et al. 2015; Ortiz et al. 2017).

Drought stress generally has a deleterious impact on the photosynthetic apparatus, particularly on chlorophyll fluorescence parameters such as Fv/Fm, which reflects the maximum

quantum efficiency of Photosystem II. The decrease in Fv/Fm ratio under drought conditions can be attributed to ultrastructural damage to the chloroplast and photoinhibition (Liu et al. 2022; Badr and Brüggemann, 2020). Azarinasrabad et al. (2016) showed no significant variation in Fv/Fm under different irrigation treatments, though genotypic variations were present.

Severe water stress typically leads to an increase in minimal fluorescence (Fo) and a decrease in maximal fluorescence (Fm), which subsequently reduces Fv/Fm (Nematpour and Eshghizadeh, 2024; Asadi and Eshghizadeh, 2021). This reduction signifies impaired photosynthetic efficiency, often due to the closure of stomata, which limits CO₂ uptake and ultimately reduces photosynthetic activity (Haworth et al. 2016). In our study, the higher Fv/Fo and Fv/Fm under deficit irrigation in the second year suggest an adaptive response, possibly due to enhanced efficiency in utilizing available water for photosynthesis and maintaining cellular functions under stress. Lashkari et al. (2022) have reported reductions in Fv/Fm and Fv/Fo under water deficit stress due to the reduction in leaf photosynthetic pigments required for photosynthesis.

Y(II), the effective quantum yield of PSII, also exhibited variable responses under different irrigation treatments. The higher Y(II) under deficit irrigation in the first year indicates a potential adaptive mechanism where plants optimize light energy use under limited water availability. However, the reduction of Y(II) under deficit irrigation in the second year aligns with findings by Bashir et al. (2021), where drought stress led to decreased Y(II) in maize, highlighting the stress-induced limitations on photosynthetic efficiency.

Previous studies have shown that chlorophyll fluorescence parameters, such as Fv/Fm and Y(II), are sensitive indicators of water stress in plants (Kalaji et al. 2016). These parameters reflect the intrinsic properties of the photosynthetic system and are widely used in water stress physiology research (Raines, 2011; Ashraf and Harris, 2013). These parameters serve as crucial indicators for assessing drought tolerance and can guide the selection of resilient genotypes in sorghum breeding programs (Lashkari et al. 2022; Tovignan et al. 2023). The observed variations in chlorophyll fluorescence parameters in our study are consistent with findings in other crops under drought stress (Dannehl et al. 1996; Elsheery and Cao, 2008). The adaptive increase in Fv/Fm and Y(II) under deficit irrigation conditions in certain cases suggests that specific genotypes may possess inherent mechanisms to maintain photosynthetic activity and efficiency under water-limited conditions.

The normalized difference vegetation index (NDVI) and the photochemical reflectance index (PRI) are valuable indicators of plant health, growth status, and physiological conditions under various irrigation treatments. These indices provide insight into the vegetation coverage and photosynthetic efficiency of crops, especially under stressful conditions. As water stress advances, chlorophyll breakdown and stomatal closure may occur, impacting photosynthesis and other biochemical reactions that, in turn, affect leaf reflectance (Sun et al. 2018). Leaf structural properties, including thickness and cell breakage or shrinkage, are closely associated with leaf reflectance in the 750 nm to 1300 nm range (Cao et al. 2015).

The NDVI is commonly used to reflect plant growth status and coverage. A higher NDVI value indicates greater plant coverage and better growth conditions. In the first year of this study, NDVI-1 was higher under full irrigation compared to deficit irrigation, indicating better growth conditions with sufficient water. This observation is supported by Zhang et al. (2023b), who reported that increasing water stress decreases the NDVI in maize. Similarly, Song et al. (2023) reported a reduction in NDVI under drought compared to well-watered conditions.

Bheemanahalli et al. (2022) reported different responses of genotypes to NDVI under drought and control conditions. The authors reported that NDVI remained relatively stable in response to different stressors including drought, heat and their combination for most of the genotypes. However, in the second year, NDVI-1 was higher under deficit irrigation, suggesting an adaptive response of the plants to water stress, possibly due to improved drought tolerance mechanisms developed over time or differences in environmental conditions between the two years.

Similarly, NDVI-2 was higher under full irrigation in both years, indicating that prolonged water availability supports sustained plant growth and higher vegetation coverage. This finding aligns with the results reported by Pipatsitee et al. (2023), who observed significant variation in genotypes for NDVI, with some genotypes showing stable NDVI values under both well-watered and water-deficit conditions. The consistency of NDVI-2 being higher under full irrigation in both years suggests that sustained water availability is crucial for maintaining higher vegetation coverage and growth throughout the growing season.

The photochemical reflectance index (PRI) is used to assess photosynthetic efficiency and stress levels in plants (Song et al. 2023). In the first year, PRI-1 was lower under deficit irrigation, indicating a higher stress level and reduced photosynthetic efficiency due to water limitation. This observation is in line with Alordzinu et al. (2019), who reported that PRI tends to decrease under soil moisture stress. Conversely, in the second year, PRI-1 was higher under deficit irrigation, which could indicate an adaptation to water stress or improved efficiency in the xanthophyll cycle under these conditions.

PRI-2 was consistently lower under deficit irrigation in both years, suggesting that prolonged water stress reduces photosynthetic efficiency and increases stress levels in the plants. This finding is supported by studies such as those by Chou et al. (2017) and Song et al. (2023), which reported reductions in PRI under drought conditions in maize.

Overall, the findings of this study highlight the dynamic nature of different morphological and physiological parameters including SPAD, stomatal conductance, chlorophyll fluorescence, and vegetation indices in sorghum under different irrigation regimes, reflecting the complexity of plant-water interactions and the importance of understanding these dynamics for optimizing agricultural productivity under variable environmental conditions. These traits are valuable tools for monitoring crop health and performance, providing insights into the effectiveness of irrigation strategies and the resilience of sorghum genotypes under water stress conditions.

6. CONCLUSIONS AND RECOMMENDATIONS

In this experiment, 125 sorghum accessions along with 6 commercial hybrids were studied under full irrigation (100% field capacity) and deficit irrigation (50% field capacity) for two consecutive years, 2022 and 2023, during the sorghum growing season. The experiment was conducted using an augmented design at the Eastern Mediterranean Agricultural Research Institute, Turkey. The analysis of variance revealed significant effects of both genotypes and irrigation treatments on the studied morpho-physiological and yield traits. The main conclusions drawn from the experiment are as follows:

- i. The average days to various phenological stages, including emergence, booting, heading, flowering, and maturity, decreased under deficit irrigation compared to full irrigation in both years of the study.
- ii. The average number of grains per panicle increased by 8.9% under deficit irrigation in the first year and by 35% in the second year compared to full irrigation.
- iii. The average panicle length remained almost similar under both irrigation treatments in the first year, whereas in the second year, it increased by 5.5% under deficit irrigation compared to full irrigation.
- iv. The average number of panicles per plant increased by 13.8% under deficit irrigation in the first year and by 24.2% in the second year compared to full irrigation.
- v. The average thousand grain weight decreased by 3.3% under deficit irrigation in the first year and by 11% in the second year compared to full irrigation.
- vi. The average grain yield was similar under both irrigation treatments in the first year; however, in the second year, grain yield increased by 19.9% under deficit irrigation compared to full irrigation.
- vii. Both stem diameter and plant height showed positive correlations with biological yield under both deficit and full irrigation across both years.
- viii. Stay green exhibited a positive correlation with SPAD values under both deficit and full irrigation across both years.
- ix. Grains per panicle demonstrated a positive correlation with grain yield under both deficit and full irrigation across both years.
- x. Thirty-seven sorghum accessions (ANZ_2, ANZ_10, ANZ_15, ANZ_17, ANZ_34, ANZ_39, ANZ_51, ANZ_58, ANZ_62, ANZ_84, ANZ_91, ANZ_105, ANZ_109, ANZ_112, ANZ_115, ANZ_123, ANZ_136, ANZ_156, ANZ_171, ANZ_180, ANZ_183, ANZ_191, ANZ_194, ANZ_195, ANZ_198, ANZ_199, ANZ_215, ANZ_218, ANZ_220, ANZ_225, ANZ_246, ANZ_343, ANZ_347, ANZ_351, ANZ_372, ANZ_418, ANZ_424) exhibited superior performance under deficit

irrigation compared to full irrigation, particularly in terms of grain yield across both year.

Furthermore, physiological parameters showed variable responses under both irrigation treatments across the two years. This experiment underscores the variability and adaptability of sorghum accessions to different irrigation regimes, providing valuable insights for future breeding and cultivation strategies aimed at optimizing water use efficiency and improving yield under varying water availability conditions.

These findings highlight the potential for selecting and breeding sorghum varieties that are resilient to water stress, which is increasingly critical in the context of climate change, water scarcity and food security. The superior performance of certain accessions under deficit irrigation suggests promising avenues for developing drought-tolerant sorghum cultivars that can sustain higher yields even under limited water supply, thereby contributing to food security and sustainable agriculture. Based on the conclusions of this study, the following recommendations can be made:

- Focus on breeding and selecting sorghum accessions that exhibit superior performance under deficit irrigation, particularly those that demonstrated increased grain yield.
- Implement deficit irrigation strategies to optimize water use efficiency without compromising yield, especially in regions prone to water scarcity.
- Conduct additional research to explore the underlying genetic and physiological mechanisms that enable certain sorghum accessions to thrive under water-limited conditions, thereby enhancing breeding efforts for drought tolerance.
- Utilize advanced artificial intelligence models, such as Random Forest and LightGBM, for predicting and enhancing sorghum traits, enabling more precise and efficient breeding decisions.

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