UNIVERSIDAD AUTÓNOMA DE NUEVO LEÓN

FACULTAD DE AGRONOMÍA

THE VALUE OF PHENOLS IN QUALITY AND RESISTANCE TO ABIOTIC STRESS IN NATIVE PIGMENTED MAIZE

THESIS

DOCTORADO EN CIENCIAS AGRÍCOLAS

PRESENTA:
SABA YASIN

GENERAL ESCOBEDO, N.L. January 2026

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To my family:

Muhammad Yasin Khan (Father), Shakeela Yasmeen (Mother), Rizwan Yasin (Brother), Imran Yasin (Brother), and Usman Yasin (Brother)

ACKNOWLEDGEMENTS

I am fortunate enough to have the privilege and honor of working under the guidance of my director, Francisco Zavala García, who serves as the chair of my advisory committee. I express my sincere gratitude for his valuable guidance and encouragement throughout my investigation and in the preparation of the manuscript. I extend my cordial thanks to the thesis committee members, Dr. Guillermo Niño Medina, Dr. Pablo Alan Rodríguez Salinas, Dra. Adriana Gutiérrez Diez, Dra. Sugey Ramona Sinagawa García and Dr. Eleazar Lugo Cruz, for their cooperation and support.

I express my deep sense of gratitude to my beloved parents, Muhammad Yasin Khan and Shakeela Yasmeen, without whose blessings, I would not have grown to this level, and to my dear brothers, Rizwan Yasin, Imran Yasin, and Usman Yasin, and my sincere companion Muhammad Shafiq, for their intangible encouragement during my course of study.

Lastly, but not least, I am thankful to the Facultad de Agronomía for its support during my postgraduate studies and to SECITHI for financing this doctoral program.

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SUMMARY

Mexico, being the origin of cultivation and diversity of maize, maintains a broad array of grain colors and traits, including growth speed, height of both plants and ears, physiological attributes, chemical makeup of grains, nutritional benefits, and various uses. Pigmented maize contains high phenolic content, carotenoids, and anthocyanins, which make it a functional food with significant antioxidant properties and potential benefits for human health. Anthocyanins, which reduce oxidative damage in plants, are produced via the flavonoid pathway and can improve water regulation under stress conditions. Understanding how genes influence anthocyanins is essential for breeding initiatives aimed at increasing antioxidant levels. The complete study was divided into three phases, each with a specific objective: 1) to evaluate the morphophysiological responses of maize to drought during its reproductive stage; 2) to evaluate the physicochemical effects of seed heat treatment on drought tolerance; and 3) to estimate the genetic effects on anthocyanin expression levels in purple maize using generational mean analysis. The results of objective 1 showed that drought stress during the reproductive stage negatively affected photosynthetic parameters, resulting in damage to PSII. In objective 2, seed priming treatments improved drought tolerance by enhancing the antioxidant capacity in the plants grown from the treated seeds. In the case of objective 3, gene action estimation revealed significant genetic effects on the observed traits, with additive effects having a greater influence on total monomeric anthocyanins and cyanidin-3-glucoside, supporting the feasibility of early selection. Conversely, dominant effects were predominant only for total phenolic content, and dominance x dominance interactions played a crucial role in all parameters, suggesting potential for hybrid development strategies focused on dominance.

INTRODUCTION

Cereals are abundant in essential minerals, vitamins, proteins, lipids, and carbohydrates, which makes them crucial in addressing global malnutrition issues (Maphosa and Jideani, 2017). Maize (*Zea mays* L.), which originated and was first cultivated in Mexico, is significant to the country's economy, culture, and culinary practices. Mexico serves as the center for the origin and domestication of maize, possessing the highest genetic diversity of this crop (Ramírez-Vega *et al.,* 2022). In total, Mexico produced 27.5 million tons of maize annually, with 88% being white, 11% yellow, and 4% colored (Gobierno de México, 2024). Maize is consumed in Mexico in various forms, most commonly being tortillas (Ramírez-Vega *et al.,* 2022). The maize tortilla is the staple food in the Mexican diet, with an annual consumption rate of 75 kg per person (USDA, 2022). Noteworthy nutrients found in maize grains include vitamins (A, B1, B2, B5, B6, C, E, and K1), essential amino acids (such as tryptophan, threonine, isoleucine, lysine, methionine, phenylalanine, valine, and histidine), antioxidants (like lutein and zeaxanthin), along with carbohydrates, proteins, and lipids (Edelman and Colt, 2016; Yang *et al.,* 2018). Maize grains also contain several elements including manganese (Mn), molybdenum (Mo), magnesium (Mg), potassium (K), sodium (Na), barium (Ba), aluminum (Al), zinc (Zn), copper (Cu), iron (Fe), as well as calcium (Ca), cobalt (Co), and selenium (Se) (Qamar *et al.,* 2017; Jaradat and Goldstein, 2018). Minerals like calcium (Ca), iron (Fe), manganese (Mn), and zinc (Zn) are crucial for preserving the integrity of various physiological and metabolic functions within living tissues (Silva *et al.,* 2019). Maize grains are a significant source of energy for both livestock and humans. Starch constitutes about 68–74 % of the weight of the grains, serving as their primary nutritional energy source (Chen *et al.,* 2017). Additionally, maize grains are a notable source of protein; however, they are less nutritious than legume seeds because they lack two essential amino acids—tryptophan and lysine. The endosperm proteins are far more abundant than those from the germ, with an average lysine content of only 1.9 % compared to 5.4 % in the germ protein for the whole grain. This is because 60–70 % of the endosperm protein is made up of zeins, which have a low concentration of lysine residues (Nyakurwa *et al.,* 2017). Furthermore, oil extracted from maize grains is highly valued for its ability to lower cholesterol levels in the bloodstream (Bae *et al.,* 2022).

The global population is anticipated to rise swiftly until 2050; therefore, it is predicted that available arable land will be inadequate for producing cereal crops to satisfy future food demands. The reduction of agricultural land due to climate change, rising biotic and abiotic stressors, and escalating global climate change are significant barriers to agriculture and food supply (Hossain *et al.,* 2020). In the 1990s, the worldwide mean temperature rose by 0.3 °C and is projected to rise by 1 °C by 2025 and over 3°C by 2100 (Tracker, 2021). Among abiotic stressors, extreme temperatures and drought pose two of the most common abiotic challenges to crop yield, as well as to food security in a changing climate. In terms of extreme temperature and drought stress, maize is more vulnerable compared to other crops (Hussain *et al.,* 2019). Water scarcity diminishes production by leading to higher post-pollination embryo loss and lower seedling survival rates (Zhang *et al.,* 2018). Water stress can decrease potential yields by resulting in reduced leaf area, earlier blooming, and an extended anthesis-silking period (Sah *et al.,* 2020). In maize, drought triggers various critical physiological responses, including decreased cell turgor, leaf curling, restricted CO_2 exchange, and reduced chlorophyll and photosynthetic efficiency levels (Anjum *et al.,* 2017). One of the crucial metabolic processes impacted by drought is photosynthesis, and maintaining substantial photosynthetic activity could enhance plant resilience to drought (Razi *et al.,* 2021; Kalaji *et al.,* 2016). When photosynthesis is hindered, the plant may capture more light energy than it can utilize for carbon fixation through photosynthesis (Liu *et al.,* 2020). This surplus energy may significantly impede the electron transport chain between photosystem I (PSI) and photosystem II (PSII), leading to an increase in reactive oxygen species concentration within the plant (Foyer and Harbinson, 2019). Osmotic stress is primarily responsible for drought, as it disrupts normal developmental processes, boosts the generation of reactive oxygen species (ROS), and interferes with cell division and growth. These impacts detrimentally affect both the quality and quantity of crops (Bashir *et al.,* 2021). The reproductive phase of maize, from tassel formation to silking, is the most vulnerable to water scarcity, adversely affecting gas exchange, leaf water content, photosynthetic activity, chlorophyll a and b levels (Bheemanahalli *et al.,* 2022), and cell membrane flexibility

(Anjum *et al.,* 2017). Among the suggested strategies to mitigate drought stress, seed priming is a sustainable and cost-effective approach that can enhance crop tolerance to drought, potentially boosting crop productivity and food security amid changing climatic conditions. This technique is applied to seeds before planting to enhance germination and yield under stress by stimulating the plant's antioxidant defense system, creating a "memory" of stress and bolstering its resilience (Sher *et al.,* 2019). Studies done by Hussain *et al.* (2017), Samota *et al.* (2017), Günay *et al.* (2022), and Tabassum *et al.* (2018) indicate that plants developed from the pretreated seeds had reduced photosynthetic damage due to an increased accumulation of antioxidant compounds.

Pigmented maize is classified as containing both carotenoid (yellow) and anthocyanin (red, purple, pink, and other shades) pigments within its pericarp and aleuronic layer, or both, of the grain structure (Chatham *et al.,* 2019; Cassani *et al.,* 2017; Magaña Cerino *et al.,* 2020; Suriano *et al.,* 2021). Varieties of blue maize typically produce anthocyanins in the aleurone (Li *et al.,* 2017; Francavilla *et al.,* 2020). The concentration of anthocyanin in both the grain of the maize cob and the cob itself determines the vibrancy of these colors. Recent research suggests that drought-induced anthocyanin production may facilitate osmoregulation and the elimination of excess ROS (Dabravolski and Isayenkov, 2023). The production of anthocyanins (blue, purple, or red pigments) occurs through the flavonoid metabolic pathway; these pigments are easily soluble in water and are found in fruits, tubers, and flowers (Khoo *et al.,* 2017). Anthocyanins assist in seed dispersal, attract pollinators, protect from strong light, absorb UV radiation, scavenge reactive oxygen species, and help maintain osmotic balance. The six primary anthocyanidins present in plants are delphinidin, peonidin, malvidin, cyanidin, pelargonidin, and petunidin. Anthocyanins are derived from glycosylated or acylated anthocyanidins. Due to their antioxidant qualities, anthocyanins protect plants from oxidative stress caused by abiotic factors and support their responses to these stressors (Alappat and Alappat, 2020). While genetic factors play a major role, a few environmental conditions can also impact the expression of this pigment (Gil-Muñoz *et al.,* 2021). Thanks to the high levels of antioxidant compounds such as anthocyanins, carotenoids, phenolic acids, and flavonoids, pigmented maize varieties are particularly nutritious (Capocchi *et al.,* 2017). Generation mean analysis provides insight into the relative importance of the mean genetic effects (additive effects), dominance deviations, and non-allelic genetic interactions when calculating the genotypic values of individuals, as well as of families and generations (Kearsey and Pooni, 2020). It serves as a useful method for assessing gene effects related to quantitative traits like yield and yield components, as it can approximate epistatic effects: [aa], [ad], and [dd] (Mather and Jinks, 1977; Singh and Singh, 1992).

REVIEW OF LITERATURE

1.1. Maize (*Zea mays* L.)

1.1.1. Origin and Importance

Maize has been a quick food source since its use by early Americans. Teosinte (*Zea mays* subsp. *parviglumus*), a wild grass from the lower Balsas River Valley in southern Mexico, was domesticated around 9,000 years ago. Evidence indicates that maize was initially cultivated in Mexico before spreading to Argentina and Canada following the discovery of America by European explorers in the fifteenth century (Kennett *et al.,* 2020). As the most widely cultivated crop across temperate, tropical, and subtropical regions, it boasts a global distribution. Its adaptability allows it to thrive under a variety of production conditions. The maize grains are suitable for consumption by humans, can be fermented to create various foods and drinks, or can be fed to livestock. The leaves, stems, and tassels can be provided to cattle either fresh (as silage or fodder) or dry (as stover). The roots may be used for firewood, mulched, or combined with soil to enhance their physical properties (Lal *et al.,* 2019).

1.1.2. Production Statistics

The total global area devoted to maize cultivation for dry grain reached 197 million hectares, yielding 1,216.87 million tons (Erenstein *et al.,* 2022). In Mexico, maize is the principal source of basic food, often consumed in the form of tortillas. In 2025, a total of 209.86 million hectares were planted with maize, leading to a worldwide production of 1,286.23 million metric tons. The United States led the global maize production list with an output of 378.27 million metric tons, followed by China, which produces 294.92 million metric tons (USDA, 2025).

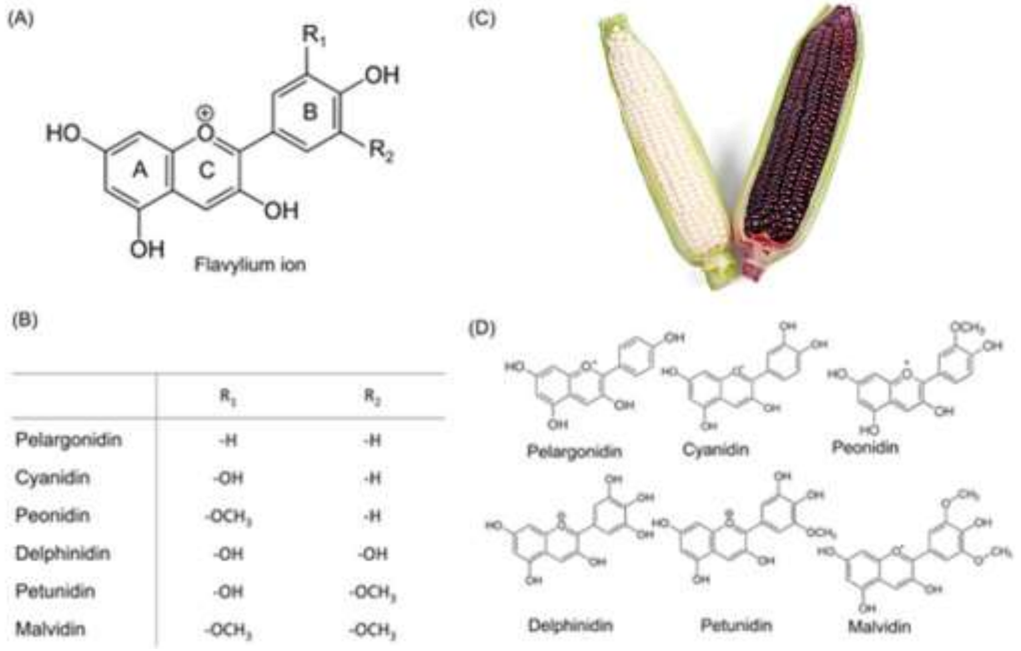
1.1.3. Nutritional and Nutraceutical Importance

Maize (*Zea mays* L.) is a fundamental food source in numerous cultures, including that of Mexico. While white and yellow maize are the most used varieties for nixtamalized foods, certain regions, particularly in central Mexico, also utilize colored types such as red, blue, purple, and black. In this area, these colorful maize varieties are favored for their unique flavor and texture, and are preferred for making "tortillas," "tamales," and "atole”. Research focusing on these pigmented maize types has indicated a lower digestibility of starch in tortillas (Bello-Pérez *et al.,* 2021), which might be attributed to the presence of anthocyanins. The elevated anthocyanin levels in colorful maize, resulting from its secondary metabolic compounds, attribute beneficial biological activity (as antioxidants) to this variety (Magaña Cerino *et al.,* 2020). These compounds are beneficial for health, as they exhibit antioxidant properties that help prevent mutagenesis, inhibit the proliferation of cancer cells, and promote the production of anti-inflammatory cells (Altemimi *et al.,* 2017). The pigmented native maize genotypes have been reported to exhibit a vast genetic variation in relation to phenolic compounds and antioxidant capacity levels, as well as nutraceutical properties (Rodríguez-Salinas *et al.*, 2020; Martínez-Martínez *et al.*, 2019).

1.1.4. Anthocyanins and Drought Stress Tolerance

1.1.4.1. Anthocyanins

Anthocyanins belong to the flavonoid category and are classified as secondary metabolites. They are a type of plant pigment that is soluble in water. All anthocyanins share a common structural framework known as the flavylium skeleton (2-phenylbenzopyrylium), which varies in hydroxylation and methoxylation based on the specific type of anthocyanin (Figure 1A). These pigments are primarily responsible for a variety of colors, such as orange, red, blue, and purple. They are present in several organs of vascular plants, particularly in flowers and fruits (Álvarez-Suárez *et al.,* 2021). The basic structure of anthocyanins can react with simple sugars like glucose, galactose, and rhamnose, depending on the substituents on the B-ring, leading to the formation of different anthocyanins such as pelargonidin, delphinidin, petunidin, cyanidin, and malvidin (Jezek *et al.,* 2023) (Figure 1D). By scavenging reactive oxygen species, anthocyanins help mitigate oxidative stress and prevent the degradation of biomolecules (Tena *et al.,* 2020). There is a growing body of research exploring the health benefits of anthocyanins for humans. These benefits encompass cancer prevention, enhancement of intestinal health, diabetes prevention, and supportive therapy for pancreatic islet transplantation (Verediano *et al.,* 2021; Chen *et al.,* 2022; Liu *et al.,* 2022).



	R_1	R_2
Pelargonidin	-H	-H
Cyanidin	-OH	-H
Peonidin	$-OCH_3$	-H
Delphinidin	-OH	-OH
Petunidin	-OH	$-OCH_3$
Malvidin	$-OCH_3$	$-OCH_3$

Figure 1. Chemical composition of anthocyanin. (A) Flavylium ion, (B) B-ring radical variations among six principal anthocyanins found in plant tissues, (C) Visual depiction of phenotypically varied maize cobs exhibiting different levels of anthocyanins, and (D) Configurations of six important anthocyanins.

1.1.4.2. Role of Anthocyanins in Drought Stress Tolerance

One of the major abiotic factors impacting agricultural yield is drought. Insufficient water disrupts the proliferation of root cells, the development of leaves and stems, and cell division; however, it also elevates ROS levels, adversely affecting plant growth (Yadav *et al.,* 2020). With global climate change, drought conditions are predicted to become more frequent and prolonged, potentially harming drought-sensitive plants and threatening the global food supply (Van Ginkel and Biradar, 2021). In response to drought stress, plants regulate the transcription of genes related to anthocyanin production, as anthocyanins can function as osmoregulatory compounds to maintain water balance (Naing and Kim, 2021). Kravic *et al.* (2021) also demonstrated that anthocyanin accumulation triggered by drought occurred in maize accessions, with these anthocyanins correlating to resistance against both oxidative and drought stress. This might be due to the accumulation of anthocyanins in vacuoles near ROS-generating components such as peroxisomes and chloroplasts. The ROS generated by drought stress that enters the vacuoles is neutralized by anthocyanins, preventing chain reactions and supporting resilience to drought. The link between an increase in anthocyanins and resistance to drought in maize was highlighted by Bheemanahalli *et al.* (2022), and an enhancement of anthocyanins in maize during drought conditions was also noted by Stefanov *et al.* (2023). Moreover, the ability of anthocyanins to lower stomatal conductance, which aids in maintaining the water balance by postponing the loss of water during drought, might be associated with the resilience observed in plants rich in anthocyanins (Naing and Kim, 2021). In transgenic plants rich in anthocyanins that overexpress regulatory factors (MYBs or bHLHs) activating anthocyanin gene synthesis, various studies have been conducted to analyze the influence of anthocyanin accumulation on drought stress tolerance (Li *et al.,* 2017; Naing *et al.,* 2017; Waseem *et al.,* 2019). These studies found a relationship between anthocyanin accumulation and drought resistance, as well as reduced water loss. When subjected to drought stress, transgenic plants may have utilized anthocyanins as antioxidants to maintain oxidative balance and water homeostasis, leading to enhanced relative water content and better stress resilience compared to wild-type plants. Notably, plants with higher anthocyanin levels exhibited greater drought resistance (Naing *et al.,* 2017). Some studies indicate that transgenic plants have elevated levels of genes responsible for proline biosynthesis and antioxidant production. While it remains unclear whether the overexpression of transcription factors directly influences gene expression, there is evidence that many plants exhibit increased gene expression under drought conditions (Khan *et al.,* 2019). Given that the products of these genes are recognized as signaling molecules (signal transduction pathway) and are involved in maintaining cellular water balance by scavenging ROS, one possible explanation for this situation might be the inadequacy of the anthocyanins to shield plants from extended periods of stress. This ultimately triggered the genes relating to proline and antioxidants, activating mechanisms for stress tolerance (Li *et al.,* 2017; Li *et al.,* 2018; Naing *et al.,* 2017; Waseem *et al.,* 2019).

1.1.4.3. Genetic Control of Anthocyanins in Pigmented Maize

Maize grains, featuring aleurone and/or pericarp, including varieties like purple, blue, and red maize, are commonly observed (Mahan *et al.,* 2013). The intensities of these colors vary due to the quantity of anthocyanins present in both the grains and cob. Gil-Muñoz *et al.* (2021) stated that while environmental conditions significantly influence this pigment, genetic factors also play a substantial role. Since investigations into the inheritance patterns of grain color supported classical genetics in the early 1900s, variations in maize grain color have captivated geneticists (Ford, 2000). Researchers focusing on colorful maize genetics aim to gain insights into how colored grains and cobs are quantitatively and qualitatively genetically regulated. Given that maize grain anthocyanin concentration is easily observable phenotypically, it has been a subject of research for a long time. However, most studies up to this point have concentrated on grains with colored aleurone, which constitutes the outermost single-cell layer of the triploid endosperm. Commonly referred to as purple maize (Chatham *et al.,* 2019), the pericarp, which is the outermost maternal layer of the grain in many varieties valued as food and beverage colorants, contains anthocyanin. Compared to aleurone, there is less variation in lines with anthocyanin in the pericarp, as they represent a smaller portion of available germplasm. Despite this limitation, the anthocyanin concentration in pericarp-pigmented maize is eight times greater than that in aleurone-pigmented maize (Paulsmeyer *et al.,* 2017). Depending on the plant system's genetic composition, naringenin can produce phlobaphenes, flavones, anthocyanins, flavans, and condensed tannins. Flavonoids are generated through the phenylpropanoid pathway. In maize, 3-hydroxyflavonoids create purple and red anthocyanins, and the transcription factors red1/booster1 (r1/b1) and colorless1/purple plant1 (c1/pl1) regulate the accumulation of anthocyanins to varying extents across different tissues. R1 and B1 are responsible for encoding bHLH transcription factors, while C1 and PL1 encode proteins with DNA-binding domains like MYB. While B1 and PL1 are essential to produce anthocyanins in vegetative plant parts, R1 and C1 must function together to promote anthocyanin accumulation in the aleurone (Lap *et al.,* 2021).

In relation to the mean genotypic values of individuals, families, and generations, generation mean analysis is an effective tool to understand the importance of mean gene effects (additive effects), dominance deviations, and effects resulting from non-allelic genetic interactions (Kearsey and Pooni, 2020). This analysis is a useful method for examining gene effects related to quantitative traits, as it can evaluate the epistatic gene effects: [aa], [ad], and [dd] (Singh and Singh, 1992; Mather and Jinks, 1977). As noted by Harakotr *et al.* (2016), additive genetic factors influenced the heritability of the total concentration of monomeric anthocyanins in purple maize cobs. The cyanidin-3-glucoside present in maize grain was particularly significant regarding the three epistatic effect types; on the maize cob, the dominant x dominant [dd] and additive x additive [aa] effects were most pronounced. Hackauf *et al.* (2017) published findings on the genetic mechanisms governing maize yield and traits, as well as other agronomic characteristics. However, studies on the anthocyanin content in maize grains and cobs are infrequent.

1.1.5. Seed Priming and Drought Tolerance

Plants often face a variety of challenging environmental conditions, including high light intensity, extreme heat, ultraviolet radiation, drought, low temperatures, salinity, nutritional deficiency, and environmental pollutants (Banerjee and Roychoudhury, 2017; Martinez *et al.,* 2018; Gharechahi *et al.,* 2019). When exposed to these adversities, plants adjust their metabolic processes to sustain normal functions (Hussain *et al.,* 2019; Ansari *et al.,* 2021). Modifications in their morphological, physiochemical, and molecular traits enable plants to endure unfavorable conditions during a critical period when they may have the opportunity to recover from stress exposure (Song *et al.,* 2019).

Various strategies are being explored to develop plants with resistance to environmental stress, including traditional and modern techniques like genetic engineering and mutation breeding. However, these methods face challenges such as high resource requirements and ethical dilemmas (Jisha *et al.,* 2013). An alternative approach is seed priming, which enhances stress tolerance in plants by exposing seeds to mild stress through various treatments (Sen and Puthur, 2020; Thomas *et al.,* 2020). This enables plants with a more effective response to subsequent stressors by inducing a primed state that activates specific physiological responses and stress-responsive genes (Paparella *et al.,* 2015; Wojtyla *et al.,* 2016; Farooq *et al.,* 2020). Methods such as hydro-priming, osmo-priming, heat-priming, halo-priming, and chemical-priming potentially lead to improved drought stress tolerance by subjecting plants to mild stress that activates stress-responsive genes and proteins such as late embryogenesis abundant (LEAs), as well as establishing an epigenetic memory which may help plants respond more effectively to both similar and varied stresses (Sen *et al.,* 2020; Thomas *et al.,* 2020; Marcos *et al.,* 2018; Hossain *et al.,* 2018).

Priming in plants can be classified into cis- and trans- types based on the relationship between the stress signals. "Cis-priming" occurs when the stimulus and the resulting stress are similar, while "trans-priming" involves different stimuli that boost tolerance to subsequent stresses (Hilker and Schmülling, 2019). Trans-priming often arises from prior exposure to biotic or abiotic stresses and relates to shared signaling pathways involved in the perception and response to most abiotic stresses in plants (Zhu, 2016). Research indicates that stress-induced cross-tolerance from cold or heat priming is associated with elevated levels of reactive nitrogen species (RNS), ROS, and reactive carbonyl species (RCS, such as methylglyoxal, MG). These molecules function as stress signals, influencing the expression of genes that respond to stress, such as those that code for heat-shock proteins (HSPs) and enzymes that play a role in the metabolism of plant hormones, osmolytes, and/or compounds involved in redox signaling (Li *et al.,* 2017; Driedonks *et al.,* 2015; Faralli *et al.,* 2015). The levels of H_2O_2, NO, and MG in plant cells rise due to cold or heat priming, which enhances cross-stress tolerance when plants face additional abiotic challenges by activating various stress tolerance mechanisms (Ferreira-Silva *et al.,* 2011; Hossain *et al.,* 2013a, Hossain *et al.,* 2013b; Hoque *et al.,* 2016).

General Hypothesis

The phenolic compounds in pigmented native maize genotypes enhance abiotic stress tolerance, such as drought, and are controlled by both environmental as well as genetic factors.

Specific hypothesis

i. Drought stress applied during the reproductive stage in maize will negatively affect the plant's photosynthesis by causing significant damage to photosystem II.
ii. Seed thermopriming before planting will increase drought tolerance in native maize genotypes during the reproductive stage by increasing the phenolic content and antioxidant activity.
iii. The pigments (anthocyanins and phenols) in maize cob and grains will be genetically controlled by additive, dominance, and epistatic gene effects.

General Objective

To evaluate the morphophysiological and physicochemical responses of native maize to drought stress during the reproductive stage, to assess the effectiveness of the seed thermopriming in enhancing the drought tolerance, and to estimate the genetic effects controlling the inheritance of the phenolic compounds.

Specific Objectives

i. To evaluate the impact of drought stress on morpho-physiological parameters of maize during the reproductive stage.
ii. To evaluate the physiological and biochemical impacts of seed thermopriming on maize under drought stress during the reproductive stage.
iii. To estimate and compare genetic effects controlling the inheritance of phenolic compounds in pigmented maize grain and cob by generation mean analysis.

CHAPTER I. Morphological and Physiological Response of Maize (*Zea mays* L.) to Drought Stress during Reproductive Stage

1.1. Abstract

Maize is among the most significant crops in the world regarding production and yield, but it is highly sensitive to drought, which reduces the growth, photosynthetic efficiency, grain quality, and yield production of a plant. Quantum yield efficiency of photosystem II is a critical photosynthetic component that is susceptible to drought stress. This study intended to investigate the effects of drought stress on growth and morpho-physiological parameters using three maize hybrids (‘P-3011w’, ‘P-3092’, and ‘iku20’) with contrasting soil moisture contents (100 %, 40 %) at the pre-flowering stage. The stress treatment (40 %) was initiated at stage V7, for a period of 15 days; the experimental units were established in a completely randomized design with split-plot arrangement along with three replications in 42 L pots using a substrate of peat moss, black soil and poultry manure (1:2:1). The morphological, growth-related and physiological parameters were assessed, including chlorophyll fluorescence (Fv/Fm), which was measured using a LiCor-6400-40 fluorometer. The results showed that all morphological, growth-related, and physiological variables decreased under drought stress during the reproductive stage, with the exception of leaf temperature and intercellular CO_2 concentration, which increased by 12 % and 54 %, respectively. Drought stress significantly reduced photosynthetic chlorophyll fluorescence (43 %), due to damage to photosystem II. The lowest percentage of damage to photosystem II (34%) was observed in the ‘iku20’ genotype. In contrast, ‘P-3011w’ and ‘P-3092’ had the highest levels of significantly similar damage (49% and 46%, respectively). The correlation analysis showed a highly positive interaction of chlorophyll fluorescence (Fv/Fm) with net photosynthetic rate and stomatal conductance under drought conditions, and multiple regression analysis revealed that the maximum effect on net photosynthetic rate under drought was due to the damage it caused to photosystem II. Thus, ‘iku20’ might tend to be able to withstand drought stress in the dry northeast region of Mexico. Overall, we concluded that the photosystem II was negatively impacted by drought stress, thus causing a reduction in all physiological, morphological,

and growth-related variables.

1.2. Introduction

Maize is among the most extensively cultivated crops globally grown in tropical, sub-tropical, and temperate regions (Sahoo *et al.,* 2021). After rice and wheat, it was the third-most significant crop in the world with 1222.07 million metric tons production on a cultivated area of 201.98 million hectares (USDA, 2022). Maize is mostly consumed by the people in Mexico in a form of variety of tejuino, pinole, tortillas, tamales, tostadas, atole, and tortilla chips (Ramírez-Vega *et al.,* 2022). In 2022, the consumption of maize amounted to 1740 million bushels in Mexico (USDA, 2022). In the northeastern part of Mexico, most of the irrigated maize area is cultivated with hybrids (Ureta *et al.,* 2020). In addition to having a moisture level of less than 70 %, the grains have 18–20 % carbs, 5–6 % sugars, and 2.1–4.5 % proteins. Livestock is fed the leftover fodder (Revilla *et al.,* 2021). Różewicz (2019) has mentioned that when compared to other cereal grains, maize offers the best feed value for poultry. Due to its high fat content (44.0 g kg^{-1}) and starch content (64.5 % to 71.5 %), it has a significant quantity of metabolic energy (14.8 MJ kg^{-1}). Farmers who raise livestock view maize plants as a superior source of nourishment for their animals. Because of its high dry matter content and digestible elements in comparison to other crops, it is the perfect crop to utilize as silage or fodder. Even so, compared to colder regions, silage is less frequently used in tropical areas. Animals can also be fed the maize stover that is left in the field after the grain is harvested (Sánchez Hernández *et al.,* 2019). In Mexico, farmers use their maize production as a family food, feed for animals, for sales, and as a seed to grow in the following season; however, some of the production is lost due to crop losses. Framers that grow maize on a small scale use their production for food, feed, and sales in similar proportions (23–35 %); medium-scale farmers use only 5 % of their produce as food, 33 % for sales, and 25 % for feed. Only 0.1 % of the maize produced by large-scale farmers is used for food, 8 % for feed, and the majority, 89 %, is sold (Ibarrola-Rivas *et al.,* 2020).

In contrast to other grains such as sorghum and millet, maize is more vulnerable to water deficiency. For instance, compared to a 39 % yield loss in sorghum, drought stress can result in a 66 % loss in maize yield (Safian *et al.,* 2022; Hussain *et al.,* 2019). According to a multi-model approach (climate and crop), in the future, the heat will increase due to the current climate change scenarios caused by global warming, which will cause unexpected rainfall events and it would lead to a significant reduction in the yield of maize by the middle of the century (Yasin *et al.,* 2022) due to a decline in the favorable climate in the far future that makes maize farming less viable (Król-Badziak *et al.,* 2024). The local agricultural economies have been impacted directly by climate change. For instance, 87 % of maize farmers in the northeastern region of Mexico experienced adverse consequences from weather events and climate change (Harvey *et al.,* 2018). Król-Badziak *et al.* (2024) also projected that the future water shortages brought on by present climate change would reduce the climate's appropriateness for maize cultivation, perhaps rendering the regions now used for maize production unusable. The largest economic loss of USD 58 billion worldwide is thought to result from drought stress, which affected 124 million hectares of the maize-growing region by cutting 40 % of its production (Ndlovu *et al.,* 2019; Daryanto *et al.,* 2016). The photosynthetic process is majorly impacted by drought, which is among the most critical physiological processes (Kalaji *et al.,* 2016; Razi *et al.,* 2021). After photosynthesis inhibition, more light energy is absorbed by the plant as compared to the energy required for photosynthetic carbon fixation (Jedmowski *et al.,* 2013; Liu *et al.,* 2020); the electron transport chain between photosystem II and photosystem I is potentially triggered by this excess energy, leading to a rise in reactive oxygen species (Foyer and Harbinson, 2019). Since climate change has increased the likelihood of drought stress, soil cultivation techniques like strip-tillage and no-tillage have become more popular as protective measures against it (Różewicz *et al.,* 2024). According to studies by Stępień-Warda (2020), in water-deficient soil, maize production was greater when reduced tillage was combined with direct rather than full tillage. Furthermore, with no-tillage farming, photosystem II performed more effectively, as indicated by the maize yield. The lifecycle of maize has many stages, whereby water deficiency stress disrupts the crop’s growth and lowers its output. The two crucial maize stages for determining yield are vegetative flowering and grain filling (Ndlovu *et al.,* 2021; Liu *et al.,* 2018), and drought stress during these essential periods can cause a yield loss of up to 25 %, 50

% and 21 %, respectively (Wang *et al.,* 2019). Furthermore, plants under stress during blooming and early grain filling stages have hindered the supply of starch, which limits the grain's access to assimilates during seed formation (Liu *et al.,* 2022).

Water deficiency had a significantly negative impact on stomatal conductance in plants (Basal *et al.,* 2020). A notable decrease in transpiration rate and leaf water potential was seen because of water deficiency, leading to increased leaf and canopy temperatures. Because stomatal closure caused by drought stress decreases CO_2 accessibility, plants are more vulnerable to light damage (Iqbal *et al.,* 2020). Damage to chlorophyll content and thylakoid membrane is caused by drought stress (Wang *et al.,* 2019), whereas a decreased chlorophyll content reduces photosynthesis (Shin *et al.,* 2021). The quantum efficiency of photosystem II (PSII) is a fundamental component of photosynthetic processes, which is represented as a ratio between variable and maximum leaf chlorophyll fluorescence (Fv/Fm) (Badr and Brüggemann, 2020). Analysis of chlorophyll fluorescence is a very effective and widely used approach for estimating the effect of stress on photosynthetic equipment, as indicated by the availability of several non-invasive, easy-to-use, and portable fluorometers. Although the use of these fluorometers is rather easy, the science underlying it and interpreting the data received from these measurements remains challenging (Ruban, 2016; Stirbet *et al.,* 2018). The Fv/Fm ratio was the first significant parameter to be extracted from the Kautsky curve (Krause, 1991). Later on, it was used as a crucial parameter to identify photosystem II photoinhibition caused by stress. This ratio is calculated by measuring the yield of minimal fluorescence (Fo) in a leaf adapted to dark using a weakly modulated measuring beam and then inducing the maximum production of chlorophyll fluorescence (Fm) by superimposing a saturating flux. A measure of PSII's maximum photochemical efficiency is performed using the ratio Fv/Fm [(Fm − Fo)/Fm] to detect the photosystem II reaction center's loss of function. Fv/Fm values usually fall between 0.75 and 0.85, and this ratio is directly related to photochemistry's quantum yield (Öquist *et al.,* 1992). The damage to PSII by water deficiency stops the main photochemistry by interfering with the photosynthetic electron transport (Mehta *et al.,* 2010; Zushi and Matsuzoe, 2017). A decrease in the Fv/Fm value is indicative of reduced photosystem II efficiency, i.e., photoinhibition (Guidi *et al.,* 2019). Even though occasionally Fv/Fm is mistakenly interpreted as a sign of PS II photoinactivation, it ought to be mentioned that this ratio also decreases when competing processes, like the thermal dissipation of absorbed light, interfere with the separation of charge (Malnoë, 2018). The measurement of effective chlorophyll fluorescence in fields under natural light conditions has been supported by the advancements made in fluorometer technology. Due to a lack of dark adaptation, this parameter has been considered a useful indication of stress in plants. However, it is still challenging to measure the fluorescence under natural light; an advanced MONITORING-PAM would be a reliable instrument for measuring the effective quantum efficiency of photosystem II during daylight hours (He *et al.,* 2021).

We hypothesized that drought stress might induce negative impacts on morpho-physiological parameters among maize cultivars by causing damage to photosystem II. Therefore, the comparative study presented here was conducted to report the morpho-physiological responses to drought stress in maize hybrids during the reproductive stage and to evaluate if water deficiency results in statistically significant damage to photosystem II.

1.3. Materials and Methods

1.3.1. Plant Material and Site Description

The morphological, growth-related, and physiological response of three maize hybrids, ‘P-3011w’, ‘P-3092’, and ‘iku20’, under drought stress, was assessed. The seeds of ‘P-3011w’ and ‘P-3092’ were obtained from the local farmers in Miguel Aleman, Tamaulipas, Mexico, whereas the seeds of ‘iku20’ were obtained from AgroRivaz, Ocotlan, Jalisco, Mexico. Among these three hybrids, ‘P-3011w’ and ‘P-3092’ are the most used hybrids in the northeastern part of Mexico, and ‘iku20’, a newly developed single-cross hybrid, was used to see its behavior in the northeastern region of Mexico. The study was conducted at the Facultad de Agronomia of the Universidad Autonoma de Nuevo Leon in Marin, Nuevo Leon, Mexico (at 24°19′16.71″ N and 99°54′58.06″ W) from February to May 2023. Thirty seeds of each hybrid were sown in germination trays on 17 February 2023. Subsequently, the seedlings were transplanted into 42 L cylindrical pots (length = 56 cm and diameter = 30 cm) with a substrate mixture of peat moss, black soil, and poultry manure (1:2:1) having a field capacity of 45 %. Each pot contained 26 kg of substrate with one plant per pot. All pots were optimally watered (100 % irrigation) until the V7 (pre-tasseling) stage to avoid drought stress.

1.3.2. Stress Treatment

For the application of stress treatment, plants were divided into two irrigation levels, i.e., normal irrigation (100 % field capacity) and reduced irrigation (40 % field capacity) (Bheemanahalli *et al.,* 2022). The daytime and nighttime temperatures were recorded at 35 °C and 22 °C, respectively. When plants reached the V7 (Pre-tasseling) stage, drought stress treatment was initiated for fifteen days by providing enough water to maintain a humidity percentage of 40 %. The water quantity required to maintain the humidity levels at 100 % and 40 % was estimated by a weighing method (Imakumbili, 2019). After fifteen days of drought stress, when the plants were at the reproductive stage, physiological, morphological, and plant growth-related measurements were made.

1.3.3. Measurement of Morphological and Growth-Related Variables

Morphological (height of plant, diameter of the stem) and growth-related (area of leaf; length, fresh and dry weight of root) measurements were taken. To determine the plant height, measurement was taken in cm from the base to the apex of the plant by a meter scale. The total leaf area was computed using the following equation (Montgomery, 1911).

$$\mathrm{LA} = \mathrm{L} \times \mathrm{W} \times 0.75 \tag{1}$$

where LA = Leaf area, L = leaf length, W = leaf width, and 0.75 is the correction factor for maize leaf area estimation.

A Vernier caliper (14,388 6 PuLG, Truper, Mexico City, Mexico) was used to measure the stem diameter of the region 15 cm above the ground (Zhang *et al.,* 2018). In order to measure the length and fresh and dry weight of the roots, they were first sprayed with water to wash away any dirt particles. Then, they were placed on a level surface and, by using a meter scale, their length was measured in cm. An electronic weighing balance (Nimbus NBL 124E, AE Adam, CT, USA) was used to determine the fresh and dry weight of the roots. After measuring fresh weight, the roots were dried in an oven (Humboldt, H-30105, USA) at 70 °C until constant weight (Fang *et al.,* 2010) and were measured again for dry weight.

1.3.4. Measurement of Physiological Variables

1.3.4.1. Potential Quantum Yield Efficiency of PSII (Fv/Fm)

Measurements of Fv/Fm began 73 days following the date of sowing. A Li-6400-40 fluorometer (LI-COR Inc., Lincoln, NE, USA) was used on a flag leaf to measure the Fv/Fm (Galicia-Juárez *et al.,* 2021). Before making the measurement, each plant's flag leaf was covered with a black colored plastic bag for 20 min to open all the photosystem II reaction centers, which allowed the measurement of the minimal fluorescence (Fo) (Padhi *et al.,* 2021). In order to prevent any light from getting to the dark-acclimated leaf while taking the readings, the whole leaf and the fluorometer chamber were kept covered. Fv/Fm was calculated as follows:

$$\mathrm{Fv/Fm} = \frac{\mathrm{Fm} - \mathrm{Fo}}{\mathrm{Fm}} \tag{2}$$

where: Fo = Minimal chlorophyll fluorescence after dark adaptation; Fm = Maximum chlorophyll fluorescence after dark adaptation; Fv = Variable leaf chlorophyll fluorescence after dark adaptation.

1.3.4.2. Photosynthetic Parameters

Photosynthesis and gas exchange variables including net photosynthetic rate (P_N, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) transpiration rate (Tr, mmol H_2O m^{-2} s^{-1}), leaf temperature (T_{leaf}, °C) and intercellular CO_2 concentration (Ci, μmol CO_2 m^{-2} s^{-1}) were measured on the flag leaf with a Li-Cor 6400 apparatus using a 6400-02B LED light source (Hussain *et al.,* 2019). The calibration for taking the readings by the Li-Cor 6400 was: Rate of flow: 700 μmol s^{-1}, a constant CO_2 level: 400 μmol CO_2, and a 6400-02 LED light source: 1500 μmol m^{-2} s^{-1} (Galicia-Juárez *et al.,* 2021). The instantaneous water-use efficiency (WUEi) of the plant was estimated by P_N/Tr (Hasan *et al.,* 2017).

1.3.5. Statistical Analysis

This study was designed as a split-plot in a completely randomized design with three replications comprising a total of fifty-four pots. The replicated data normality was assessed using the Shapiro–Wilk test. The data that followed the normal distribution were then subjected to a two-way analysis of variance (ANOVA) by assuming the treatments to be the main plot factor and genotypes to be the subplot factor using the Statistix 10 software (Analytical Software, FL, USA). The significant differences among the treatments with regard to genotypes were calculated at a 5% level of significance, and the mean comparison through the Tukey test ($p \leq 0.05$). Correlation and stepwise multiple regression analyses were conducted using SPSS (IBM SPSS 22.0, IBM Corporation, New York, NY, USA).

1.4. Results

All the morphological, growth-related, and physiological variables varied significantly ($p \leq 0.05$) for the treatments and genotypes except for instantaneous water-use efficiency, which was significant for treatments only, whereas the genotype-treatment interaction was significant only in the quantum yield efficiency of photosystem II, leaf temperature, and intercellular CO_2 concentration (Table 1.1).

1.4.1. Morphological and Growth-Related Variables

Drought stress hampered the morphological growth of all genotypes studied. Compared with the control, drought stress significantly reduced the plant height, leaf area, stem diameter, root length, and root fresh and dry weight, as shown in Table 1.2. A reduction in the average values of plant height (13 %), leaf area (5 %), stem diameter (13 %), root length (21 %), and root fresh weight (12 %) and root dry weight (26 %) was recorded under drought compared to the control. This decrease in the average values of all variables indicates a decline in the plant's vegetative development and root attributes. Under stress conditions, all the morphological and growth-related parameters were least affected in 'iku20' compared to 'P-3011w' and 'P-3092'.

1.4.2. Physiological Parameters

1.4.2.1. Potential Quantum Yield Efficiency of PSII (Fv/Fm) under Drought

Drought reduced Fv/Fm compared to control (Table 1.3). It showed significant ($p < 0.05$) variations for treatments, genotypes, and genotype-treatment interaction. The Fv/Fm values recorded varied from 0.77 to 0.80 with an average of 0.78 in control conditions and 0.35 to 0.55 with an average of 0.44 in drought conditions. All the hybrids studied showed a significant decrease in Fv/Fm in drought compared to the control, with the maximum reduction recorded in genotype P-3011w (0.39 ± 0.03), followed by P-3092 (0.42 ± 0.05) and iku20 (0.52 ± 0.05).

A reduction of 43% in Fv/Fm was recorded in drought compared to the control. The genotype 'iku20' was least affected, showing a reduction of 34 % as compared to 'P-3011w' (49 %) and 'P-3092' (46 %) (Figure 1.1).

Table 1.1. Mean squares (M.S) of morphological, growth-related, and physiological parameters in maize hybrids.

Parameters	M.S$_{\text{hum.}}$	Error (Main-plot)	M.S$_{\text{gen.}}$	M.S$_{\text{gen.} \times \text{hum.}}$	Error (Sub-plot)	C.V (%)
Plant height	404.42*	5.69	1703.77*	1.87	2.82	2.47
Leaf area	1017.46*	0.65	6692.83*	17.88	66.30	2.41
SD	21.49*	0.449	118.24*	0.99	3.426	5.13
RL	133.23*	3.518	447.04*	0.87	5.384	5.15
RFW	417.70*	7.54	3720.48*	0.87	21.73	6.74
RDW	100.77*	0.062	614.34*	0.29	2.918	6.89
Fv/Fm	0.51*	1.5×10^{-3}	0.01*	0.005*	4.2×10^{-4}	3.34
P_N	260.52*	0.055	11.50*	0.15	0.273	5.03
g_s	5.69×10^{-3}*	1.00×10^{-5}	9.70×10^{-4}*	4.00×10^{-5}	8.00×10^{-5}	6.24
Tr	8.52*	0.016	0.36*	0.02	0.028	7.70
WUEi	1.68*	0.04	0.28	0.16	0.10	6.91
T_{leaf}	59.29**	0.027	7.02**	3.45**	0.309	1.75
Ci	9842.51**	4.13	4.28**	68.85**	0.51	0.65

hum. =humidity; gen. = genotype; gen. x hum.= Interaction between genotype and humidity; SD: Stem diameter; RL: Root length; RFW: Root fresh weight; RDW: Root dry weight; P_N*: Net photosynthetic rate; gs: Stomatal conductance; Tr: Transpiration; WUEi: Instantaneous water use efficiency;* T_{leaf}*: Leaf temperature and Ci: Intercellular* CO_2 *concentration. * Significant at* $p \leq 0.05$*.*

Table 1.2. Effect of drought stress on maize growth and morphology-related attributes.

Maize Cultivars	Treatment	Plant Height (cm)	Leaf Area (cm^2)	Stem Diameter (mm)	Root Length (cm)	Root Fresh Weight (g)	Root Dry Weight (g)
P-3011w	Control	57.44 ± 1.55^{D}	321.81 ± 2.63^{BC}	12.41 ± 0.69^{BC}	17.12 ± 1.37^{DE}	51.32 ± 2.80^{DE}	10.99 ± 1.10^{CD}
	Drought	48.89 ± 1.52^{E}	302.86 ± 4.23^{C}	10.16 ± 0.90^{C}	13.00 ± 1.10^{E}	42.76 ± 1.60^{E}	7.04 ± 0.60^{E}
P-3092	Control	68.72 ± 1.51^{C}	331.51 ± 3.85^{B}	15.59 ± 0.91^{B}	23.67 ± 2.12^{C}	68.87 ± 3.06^{C}	12.50 ± 1.16^{C}
	Drought	59.56 ± 1.29^{D}	319.09 ± 2.67^{BC}	13.30 ± 0.77^{BC}	18.22 ± 1.47^{D}	59.78 ± 1.64^{D}	8.91 ± 0.92^{DE}
iku20	Control	91.61 ± 1.32^{A}	382.43 ± 4.23^{A}	21.05 ± 0.91^{A}	35.38 ± 2.65^{A}	101.75 ± 2.07^{A}	30.65 ± 1.33^{A}
	Drought	80.89 ± 1.69^{B}	368.70 ± 2.17^{A}	19.04 ± 0.79^{A}	29.22 ± 1.42^{B}	90.51 ± 2.23^{B}	24.00 ± 1.55^{B}
Genotype		*	*	*	*	*	*
Treatment		*	*	*	*	*	*
G × T		n.s	n.s	n.s	n.s	n.s	n.s

*Note: The values shown in the table are means ± SE. n.s indicates nonsignificant. Means sharing a common letter are not significantly different according to the Tukey test ($p \leq 0.05$). * Significance at $p \leq 0.05$.*

Table 1.3. Mean Fv/Fm under control and drought stress.

Variables	Control	Drought Stress
Fv/Fm	0.78^{A}	0.44^{B}

Note: Means sharing the same letters are not significantly different according to the Tukey test ($p \leq 0.05$).

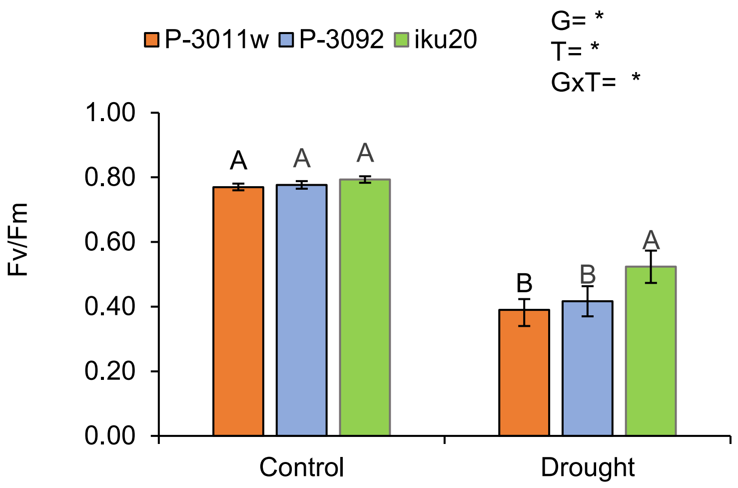


Figure 1.1. Effects of drought stress on maize hybrids (P-3011w, P-3092, iku20) during the reproductive stage on Fv/Fm. Means sharing the same letters are not significantly different according to the Tukey test ($p \leq 0.05$). * Significance at $p \leq 0.05$.

1.4.2.2. Photosynthetic Variables under Drought Stress

The P_N, g_s, Tr, and WUEi of all genotypes were negatively influenced by drought stress; therefore, they showed reduced values compared to the control, as shown in Table 1.4. All of these parameters were significantly different under control and drought stress for genotypes and treatments, except WUEi, which was statistically different only for treatments. All these parameters did not have significant differences for the genotype-treatment interaction. The P_N values ranged from 12.53 to 16.53 CO_2 μmol m^{-2} s^{-1} under control conditions, with an average of 14.18 CO_2 μmol m^{-2} s^{-1} recorded, whereas a range of 5.12–8.08 CO_2 μmol m^{-2} s^{-1} was observed under drought stress, with an average of 6.58 CO_2 μmol m^{-2} s^{-1} recorded. The g_s values varied from 0.07 to 0.10 H_2O mmol m^{-2} s^{-1} and 0.03 to 0.07 H_2O mmol m^{-2} s^{-1} under control and drought stress, respectively, with an average of 0.09 H_2O mmol m^{-2} s^{-1} recorded under control conditions and 0.05 H_2O mmol m^{-2} s^{-1} recorded in drought. The WUEi values ranged from 4.50 to 5.72 CO_2 μmol H_2O mmol with an average of 4.98 CO_2 μmol H_2O mmol, whereas under drought stress, the values varied from 4.05 to 4.96 CO_2 μmol H_2O mmol with an average of 4.36 CO_2 μmol H_2O mmol. The recorded values for Tr under control and drought stress were 2.32–3.56 H_2O mmol m^{-2} s^{-1} and 1.21–1.88 H_2O mmol m^{-2} s^{-1}, respectively. Mean values recorded for transpiration were 2.88 H_2O mmol m^{-2} s^{-1}

under control conditions and 1.50 H_2O mmol m^{-2} s^{-1} under drought stress. Among all maize hybrids, iku20 showed maximum values for photosynthesis (15.86), stomatal conductance (0.10), and transpiration (3.23) under the control conditions.

The drought stress decreased the P_N (54 %), g_s (42 %), Tr (48 %), and WUEi (12 %). Under stress conditions, genotype iku20 had reduced P_N by 50 %, g_s by 31 %, Tr by 47 % and WUEi by 5 %; genotype P-3092 had reduced P_N by 55 %, g_s by 44 %, Tr by 47 % and WUEi by 18 %; and genotype P-3011w had reduced P_N, g_s, Tr and WUEi by 57 %, 52 %, 50 % and 14 %, respectively (Figure 1.2a–d).

Table 1.4. Means of net photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration (Tr) under control and drought stress.

Variables	Control	Drought Stress
Net photosynthetic rate (P_N)	14.18^A	6.58^B
Stomatal conductance (g_s)	0.09^A	0.05^B
Transpiration (Tr)	2.88^A	1.50^B
Instantaneous water-use efficiency (WUEi)	4.98^A	4.36^B

Note: Means sharing the same letters are not significantly different according to the Tukey test ($p \leq 0.05$).

Leaf temperature and intercellular CO_2 concentration increased under drought conditions compared to the control, as shown in Table 1.5. These parameters were influenced significantly by treatments and genotypes and had a significant genotype–treatment interaction. The leaf temperature values measured varied from 28.80 to 30.87 °C with an average of 30.04 °C under control conditions, whereas, in the case of drought stress, the values varied from 31.62 to 36.24 °C with an average of 33.67 °C. In the case of intercellular CO_2 concentration, the values ranged from 80.52 to 91.07 CO_2 µmol m^{-2} s^{-1} under control conditions and 129.98 to 135.77 CO_2 µmol m^{-2} s^{-1} under drought stress, with an average of 89.09 CO_2 µmol m^{-2} s^{-1} and 132.32 CO_2 µmol m^{-2} s^{-1} recorded under control and drought stress, respectively.

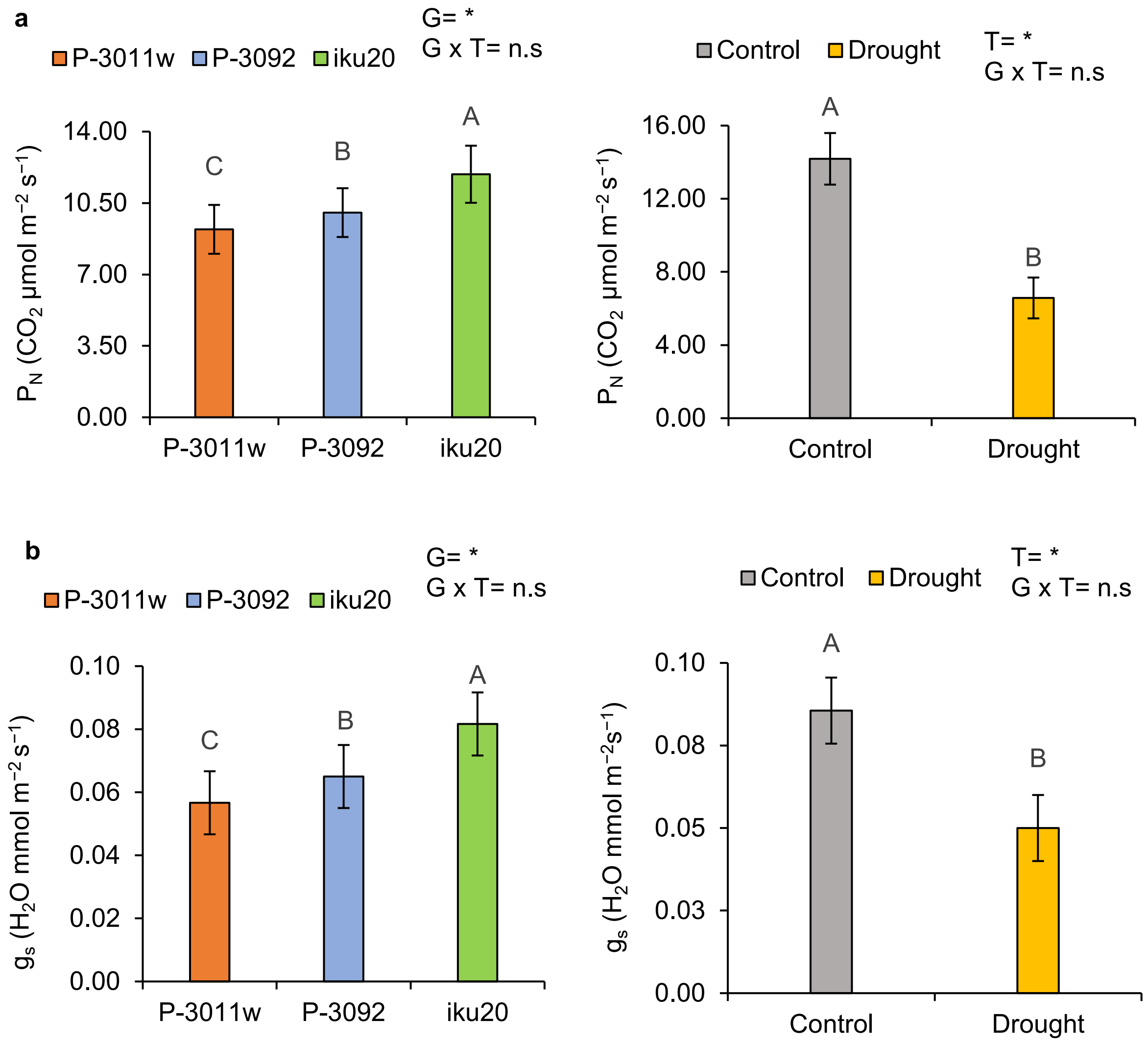
In all genotypes studied, stress conditions increased leaf temperature and intercellular CO_2 concentration. The leaf temperature and intercellular CO_2 concentration increased by 12 % and 54 %, respectively. The genotype ‘iku20’ had the lowest rise in leaf temperature (8 %) when compared to ‘P-3092’ (11 %) and ‘P-3011w’

(17 %). The greatest rise in intercellular CO_2 concentration during drought stress was observed in 'P-3011w' (64 %), followed by 'P-3092' (56 %), and 'iku20' (44 %) (Figure 1.2e,f).

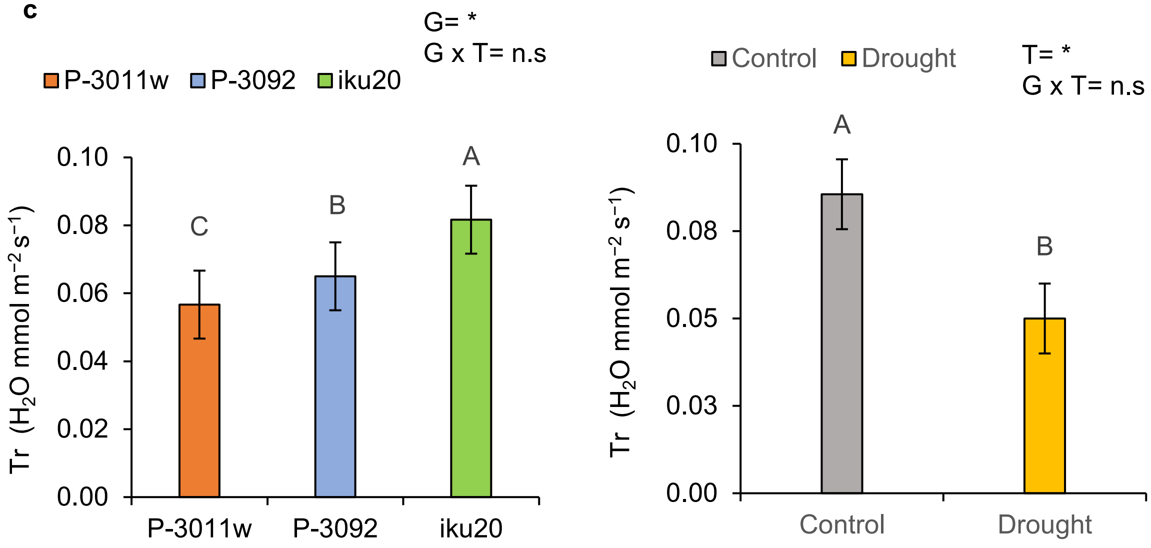
Table 1.5. Means of leaf temperature (T_{leaf}) and intercellular CO_2 concentration (Ci) under control and drought stress.

Variables	Control	Drought Stress
Leaf temperature	30.04^A	33.67^B
Intercellular CO_2 concentration	86.22^A	132.99^B

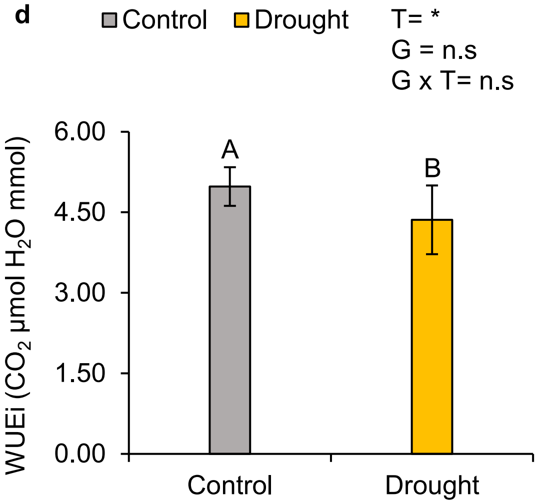
Note: Means sharing the same letters are not significantly different at 5% according to Tukey (p ≤ 0.05).



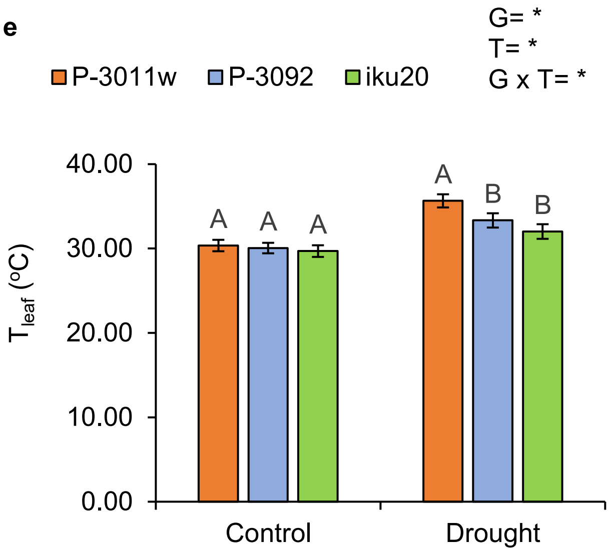
c


G= *
G x T= n.s
P-3011w
P-3092
iku20
Tr (H_2O mmol m^{-2} s^{-1})
0.10
0.08
0.06
0.04
0.02
0.00
C
B
A
P-3011w
P-3092
iku20
Control
Drought
T= *
G x T= n.s
A
B
0.10
0.08
0.05
0.03
0.00
Control
Drought

d


Control
Drought
T= *
G = n.s
G x T= n.s
WUEi (CO_2 µmol H_2O mmol)
6.00
4.50
3.00
1.50
0.00
A
B
Control
Drought

e


G= *
T= *
G x T= *
P-3011w
P-3092
iku20
T_{leaf} (°C)
40.00
30.00
20.00
10.00
0.00
A
A
A
A
B
B
Control
Drought

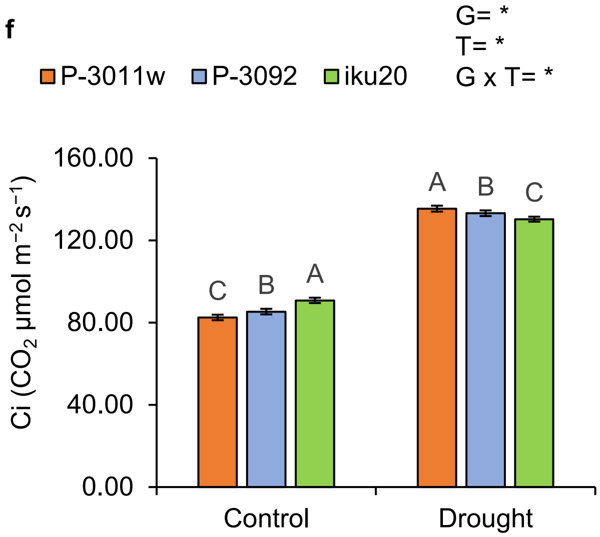


Figure 1.2. Effects of drought stress on maize hybrids (P-3011w, P-3092, iku20) during pre-flowering stage on (a) photosynthetic rate (P_N) (μmol CO_2 m^{-2} s^{-1}), (b) stomatal conductance (g_s) (mmol H_2O m^{-2} s^{-1}), (c) transpiration (Tr) (mmol H_2O m^{-2} s^{-1}), (d) instantaneous water-use efficiency (WUEi) (CO_2 μmol H_2O mmol), (e) leaf temperature (T_{leaf}) (°C) and (f) intercellular CO_2 concentration (Ci) (μmol CO_2 m^{-2} s^{-1}). Means sharing the same letters do not have significant differences according to the Tukey test (*$p \leq 0.05$*). * Significance at *$p \leq 0.05$*.

1.4.2.3. Correlation and Multiple Regression Analyses

The results of the correlation analysis of the selected morpho-physiological parameters under control conditions are shown in Table 1.6. All the parameters had significant correlation with Fv/Fm except for stem diameter and root dry weight. According to the thumb rule of correlation coefficient interpretation (Mukaka, 2012), under control conditions, the net photosynthetic rate showed a high positive correlation, and stomatal conductance, transpiration, plant height, and leaf area had a moderate positive correlation with Fv/Fm. The only parameter that had a significant negative correlation with photosystem II was intercellular CO_2 concentration (−0.422). Significantly high correlations were found between other variables, as can be seen in Table 1.6.

The correlation analysis of selected morpho-physiological parameters under drought stress showed that all variables had a significant correlation with Fv/Fm except for intercellular CO_2 concentration. Under stress conditions, stomatal conductance showed a remarkably high positive correlation with Fv/Fm, followed by P_N, whereas Tr, plant height, leaf area, and root dry weight had moderate positive correlation with Fv/Fm (Table 1.7). Significantly high correlations were also found between other variables, as can be seen in Table 1.7 (Mukaka, 2012).

The multiple regression analysis (stepwise method) of physiological parameters under control conditions showed that Fv/Fm and stomatal conductance had significant impacts on the net photosynthetic rate, as shown in Table 1.8. The analysis showed that almost 85 % of the variation in net photosynthetic rate was due to Fv/Fm, stomatal conductance, and WUEi (Table 1.9). Other variables had low contributions, so they were not included in the analysis. The predicted equation for the net photosynthetic rate under control conditions is also mentioned in Table 1.9.

Table 1.6. Pearson correlation analysis of selected growth and morpho-physiological parameters of maize hybrids under control conditions.

Traits	**Fv/Fm**	**P_N**	**g_s**	**Tr**	**WUEi**	**Ci**	**PH**	**LA**	**SD**	**RDW**
Fv/Fm	1	0.86*	0.68*	0.61*	0.53*	−0.42*	0.53*	0.62*	0.38	0.30
P_N	0.86*	1	0.78*	0.68*	0.60*	−0.50*	0.46*	0.43*	0.40*	0.26
g_s	0.68*	0.78*	1	0.76*	0.27	−0.61*	0.58*	0.49*	0.53**	0.39*
Tr	0.61*	0.68*	0.76*	1	−0.12	−0.56*	0.55*	0.50*	0.48*	0.42*
WUEi	0.53*	0.60*	0.27	−0.12	1	−0.13	0.06	0.01	0.06	0.02
Ci	−0.42*	−0.50*	−0.62*	−0.56*	−0.13	1	−0.67*	−0.63*	−0.66*	−0.58*
PH	0.53*	0.45*	0.58*	0.55*	0.07	−0.68*	1	0.92*	0.78*	0.76 *
LA	0.52**	0.30	0.28	0.53*	0.01	−0.39*	0.75*	1	0.57*	0.51*
SD	0.38	0.40*	0.53*	0.48*	0.06	−0.67*	0.78*	0.76*	1	0.77*
RDW	0.30	0.26	0.39*	0.42*	0.02	−0.58*	0.76*	0.73**	0.77*	1

*Note: Fv/Fm = Potential quantum yield efficiency of PSII; P_N = Photosynthetic rate; g_s = stomatal conductance; Tr = transpiration; Ci = Intercellular CO_2 concentration; WUEi = Instantaneous water-use efficiency; PH = Plant height; LA = Leaf area; SD = Stem diameter; and RDW = Root dry weight. * Correlation significance at 5% level (2-tailed).*

Table 1.7. Pearson correlation analysis of selected growth and morpho-physiological parameters of maize hybrids under drought stress.

Traits	Fv/Fm	P_N	Gs	Tr	WUEi	Ci	PH	LA	SD	RDW
Fv/Fm	1	0.90*	0.91*	0.65*	0.41*	−0.31	0.57*	0.64*	0.47*	0.65*
P_N	0.90*	1	0.82*	0.54*	0.60*	−0.26	0.48*	0.56*	0.47*	0.55*
g_s	0.91*	0.82*	1	0.65*	0.34	−0.52*	0.74*	0.77*	0.69*	0.77*
Tr	0.65*	0.54*	0.65*	1	−0.30	−0.25	0.39*	0.34	0.41*	0.47*
WUEi	0.41*	0.60*	−0.34	0.30	1	−0.13	0.25	0.30	0.18	0.20
Ci	−0.30	−0.26	−0.52*	−0.25	−0.13	1	−0.71*	−0.64*	−0.64*	−0.70*
PH	0.57*	0.48*	0.74*	0.39*	0.25	−0.71*	1	0.87*	0.89*	0.89*
LA	0.61*	0.45*	0.72*	0.40*	0.30	−0.64*	0.87*	1	0.74*	0.91*
SD	0.47*	0.47*	0.69*	0.41*	0.18	−0.64*	0.89*	0.78*	1	0.81*
RDW	0.65*	0.55*	0.77*	0.47*	0.20	−0.70*	0.90*	0.93*	0.82*	1

*Note: Fv/Fm = Potential quantum yield efficiency of PSII; P_N = Photosynthetic rate; g_s = stomatal conductance; Tr = transpiration; Ci = Intercellular CO_2 concentration; WUEi = Instantaneous water-use efficiency; PH = Plant height; LA = Leaf area; SD = Stem diameter; and RDW = Root dry weight. * Significant correlation at 5% level (2-tailed).*

Table 1.8. Analysis of variance of stepwise multiple regression of physiological variables under control conditions.

Model	Sum of Squares	Df	Mean Square	F	Sig.
Regression	137.989	3	45.996	44.511	<0.001 [a]
Residual	23.768	23	1.033		
Total	161.757	26			

Note: Fv/Fm = Quantum yield efficiency of photosystem II; g_s = stomatal conductance; WUEi = instantaneous water-use efficiency. [a]. Predictors: (Constant), Fv/Fm, g_s, WUEi.

Table 1.9. The regression coefficients (b), standard error, and R-squared values of the estimated variables in predicting maize net photosynthetic rate under control conditions by stepwise multiple regression analysis.

Model	**Unstandardized Coefficients**		**R Square**
	B	**Std. Error**	
(Constant)	−43.533*	11.206	
Fv/Fm	60.544*	16.652	0.741
g_s	65.689*	18.319	0.067
WUEi	0.980*	0.368	0.045
$P_N = -43.533 + 60.544\ (Fv/Fm) + 65.689\ (g_s) + 0.980\ (WUEi)$			

*Note: P_N = Photosynthetic rate; Fv/Fm = Quantum yield efficiency of photosystem II; g_s = stomatal conductance; WUEi = instantaneous water-use efficiency. * Significance at $p \leq 0.05$.*

The multiple regression analysis of physiological parameters under drought stress using net photosynthetic rate as a dependent variable showed that Fv/Fm and WUEi had a significant impact on net photosynthetic rate, as shown in Table 1.10. According to the results, 84 % of the variation in net photosynthetic rate was attributed to Fv/Fm and WUEi (Table 1.11). The other variables that had low relative contributions were not included in the analysis. The predicted equation for the net photosynthetic rate under drought stress is also mentioned in Table 1.11.

***Table 1.10*.** Analysis of variance of stepwise multiple regression of physiological variables under drought stress.

Model	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Regression	92.567	3	30.856	64.979	<0.001 [a]
Residual	5.554	23	0.241		
Total	98.121	26			

[a] Predictors: (Constant), Fv/Fm, WUEi, Tr.

Table 1.11. The regression coefficients (b), standard error, and R-squared values of the estimated variables in predicting maize net photosynthetic rate under drought stress by stepwise multiple regression analysis.

Model	**Unstandardized Coefficients**		**R Square**
	B	**Std. Error**	
(Constant)	−5.265*	0.690	
Fv/Fm	4.641*	2.430	0.805
WUEi	1.216*	0.164	0.068
Tr	2.974*	0.556	0.071

$P_N = -5.265 + 4.641\,(Fv/Fm) + 1.216\,(WUEi) + 2.974\,(Tr)$

*Note: P_N = Photosynthetic rate; Fv/Fm = Quantum yield efficiency of photosystem II; WUEi = instantaneous water-use efficiency; Tr = Transpiration. * Significance at $p \leq 0.05$.*

1.4.2.4 Discussion

1.4.2.4.1 Morphological and Growth-Related Variables

As one of the abiotic factors, drought limits the production and development of many field crops (Fahad *et al.,* 2017). Lack of water causes changes in morphology and anatomy, as well as in physicochemical processes, changing many functions in a plant (Devi *et al.,* 2022). Under such conditions, the plant loses its turgidity causing a reduction in the growth of the plant, which is dependent on cell expansion and division and involves intricate interactions among processes of physiology, morphology and genetics (Wach and Skowron, 2022), elongation of the stem, leaf expansion, and opening of stomata, thus changing the sink–source relationship, which has an impact on the translocation of photosynthates (Salgado-Aguilar *et al.,* 2020). The plant's metabolic activity is also limited by water deficiency in soil, causing a reduction in biomass and leaf area, and reduced photosynthesis due to the loss of chlorophyll in the leaves, all of which contribute to a low maize yield (Laskari *et al.,* 2022). The height reduction in plants might be associated with inefficient mitosis, high leaf abscission rate under drought, and reduced development of a cell (Wahab *et al.,*2022). In the current study, plant height, leaf area, stem diameter, root length, root fresh and dry weight were reduced by drought as shown in Table 1.1. This stress treatment has more severe negative effects on ‘P-3011w’ as compared to ‘P-3092’ and ‘iku20’. Previously, it has been reported that a significant decline occurred in the plant height, leaf area, and stem diameter of maize hybrids subjected to drought than control conditions (Hussain *et al.,* 2019; Anjum *et al.,* 2017). In another study, a significant decrease in the plant height, leaf area, root length, and root fresh and dry weight in maize genotypes under drought stress in comparison to the control has been reported (Sah *et al.,* 2020). Laskari *et al.* (2020) also showed a significant difference in the height of the maize plants under different irrigation levels, with the tallest being observed under normal irrigation and the shortest under reduced irrigation.

1.4.2.4.2. Physiological Variables

1.4.2.4.2.1. Potential Quantum Yield Efficiency of PSII (Fv/Fm)

Drought affected all the physiological parameters of maize, such as Fv/Fm, P_N, g_s, Tr, leaf temperature, and intercellular CO_2 concentration. Under control conditions, iku20 had the maximum Fv/Fm value (0.79 ± 0.01) followed closely by P-3092 (0.78 ± 0.01) and P-3011w (0.77 ± 0.01), which indicates an undamaged photosystem II and these results are similar to the ones reported earlier (Krause, 1988), where they registered a value range of 0.76–0.80 for Fv/Fm under normal irrigation. Under drought stress, all of our genotypes showed values below 0.75, which is considered a cutoff point for completely functional PSII reaction centers (Wada *et al.,* 2019). Changes in stomatal conductance, leaf temperature, chlorophyll, and respiration in a plant are caused by drought, and it also causes damage to photosystem II (Qaseem *et al.,* 2019). The reduced values under drought stress show damage to PSII, which is probably the result of damage to thylakoid membranes; increased permeability of the membrane or increased oxidative stress response, which probably led to proton leakage and a drop in the production of ATP and NADPH; and decreased photochemical efficiency (Kohzuma *et al.,* 2009). Results found in our study were also reported previously (Chiango *et al.,* 2021), where a significant reduction in Fv/Fm under drought conditions than the control was observed. Other studies also reported a significantly decreased Fv/Fm in severe drought stress conditions (Badr and Brüggemann, 2020; Liu *et al.,* 2012; Ghassemi-Golezani *et al.,* 2018).

Based on the assumption that the damage caused by drought stress to photosystem II is less in tolerant genotypes, the presence of a significant genotype–treatment interaction allowed us to identify the tolerant genotype, which was ‘iku20’.

1.4.2.4.2.2. Photosynthetic Parameters

Certain elements, such as leaf water potential, canopy and leaf temperature, transpiration, and stomatal conductance, have an influence on plant–water relations. During drought, the stomata are closed, reducing transpiration and ultimately impairing photosynthesis (Fahad *et al.,* 2017). Drought tolerance and water-use efficiency are often used as synonymous terms. Water usage efficiency is frequently used as a direct indicator of the fundamental physiological response of a plant to water deficiency (Hasan *et al.,* 2017). In the present study, the genotypes showed a negative response to drought stress for photosynthetic parameters. The drought decreased the stomatal conductance and transpiration to prevent excessive water loss, which resulted in an increased leaf temperature and water-use efficiency, and reduced photosynthesis because of less availability of CO_2. The intercellular CO_2 concentration increased due to damage caused by drought stress to photosynthetic activity. Temperatures above 35 °C are known to reduce the RuBisCO activity, reducing transpiration and photosynthesis processes (Salgado-Aguilar *et al.,* 2020). The results recorded in this study are in accordance with several studies conducted in the past. A study conducted on the effects of drought stress during the reproductive stage of maize hybrids found that drought stress reduced stomatal conductance (52 %) and transpiration (47 %) (Bheemanahalli *et al.,* 2022). Another study observed higher P_N, g_s, and Tr reduction by drought stress compared to control conditions (Cai *et al.,* 2020). In a study about drought influence on the physiological parameters of maize, stomatal conductance values of 0.05–0.07 H_2O mmolm^{-2} s^{-1} have been observed under drought stress due to reduced relative water content, along with a significant increase in the intercellular CO_2 concentration compared to control conditions (Hussain *et al.,* 2019). A significant increase has been recorded in the leaf temperature of maize when subjected to drought stress (Ghassemi-Golezani *et al.,* 2018). Yousaf *et al.* (2023) reported a significant reduction in instantaneous water-use efficiency in maize hybrids under drought stress during the reproductive stage. Salgado-Aguilar *et al.* (2020) observed a significant elevation in the leaf temperature of maize hybrids, and even after restoring the irrigation, they did not return to their initial temperature values. Stomatal regulation maintains an ideal internal CO_2 concentration level under water availability conditions, enabling the Calvin cycle to proceed. In case of water deficiency, the plant will make a compromise between its need to produce carbohydrates and maintain a proper water balance (Cornic, 2000; Lawlor and Cornic, 2002).

1.4.2.4.2.3. Correlation and Multiple Regression Analyses

In this study, the Pearson correlation analysis of selected morphological and physiological parameters showed significant positive and negative correlations under both water regimes. Intercellular CO_2 concentration had a significant negative correlation with Fv/Fm under control conditions because, when the photosystem II is functioning properly, plants are using the intercellular CO_2 for photosynthesis, and thus the concentration will be low and vice versa (Hussain *et al.,* 2019). Under both water regimes, the correlations between P_N and g_s, P_N and Tr, and g_s and Tr were significantly positive, indicating a positive linear relationship among them (Miner and Bauerle, 2017; Wang *et al.,* 2022). Additionally, a significant positive correlation was found among Fv/Fm, plant height, and stem diameter. These findings were also reported previously (Holá *et al.,* 2010). These results therefore demonstrated the importance of these characteristics in choosing drought-tolerant genotypes. When combined, the strong positive and negative correlations between the many variables under study and the water stress further support the use of these correlations to find prospective maize lines that are tolerant to drought. On the other hand, distinct patterns of correlations between the same traits under various water regimes ought to be heavily employed, as certain interrelationships among certain influential parameters under particular water conditions might serve as selection criteria for genotypes that have promising traits responsive to drought. The stepwise multiple regression analysis showed that the maximum variation in net photosynthetic rate under both water regimes was attributed to the Fv/Fm, which indicates the importance of this variable. A properly functioning photosystem II increases photosynthesis, which will ultimately enhance the overall plant yield (Djemel *et al.,* 2019).

1.5. Conclusion

In this study, we screened three maize hybrids under different water regimes for various traits. Overall, drought stress brought a remarkable negative impact on morphological and physiological attributes and caused statistically significant damage to photosystem II as well. The genotypes ‘P-3011w’, ‘P-3092’, and ‘iku20’ varied in their responses to drought stress treatment. Overall, the morpho-physiological performance of ‘iku20’ was better than that of ‘P-3011w’ and ‘P-3092’. As a result, we conclude that ‘iku20’ might tend to tolerate drought stress in the arid northeast of Mexico.

CHAPTER II. Physicochemical Response of Maize (*Zea mays* L.) to Seed Thermopriming Under Drought Stress

2.1. Abstract

Extreme temperatures and water deficits pose a significant threat to crop growth and food security in changing climates. Maize, a widely distributed crop, is susceptible to water deficiency. Seed priming, a low-cost and sustainable technology, can enhance drought tolerance, potentially improving crop productivity and food security. The current study aimed to investigate the impacts of seed thermopriming at physiological and biochemical levels under drought stress during the reproductive stage using three maize genotypes (Red, White, and P-3057w). The experiment was split into six treatments (control, drought, thermopriming at 40 °C, thermopriming at 40 °C + drought, thermopriming at 50 °C, and thermopriming at 50 °C + drought). Drought stress was induced at the pre-tasseling stage by withholding irrigation for 20 days in a split-plot under a completely randomized design. Our results showed that the drought stress significantly reduced photosynthetic rate (51 %), stomatal conductance (65 %), transpiration (69 %), cell membrane injury (55 %) chlorophyll a (85 %), chlorophyll b (74 %), total chlorophyll (81 %) and carotenoids (66 %) while increasing anthocyanins (7 %), phenols (8 %), DPPH (6 %) and ABTS (11 %) activity in non-primed seeds whereas thermopriming at 40 ^{o}C and 50 ^{o}C reduced significantly the negative effects of drought on photosynthesis (18 %; 21 %), stomatal conductance (16 %), transpiration (15 %; 16 %) and cell membrane damage (36 %; 37 %) through increased anthocyanin content (25 %; 24 %), total phenols (30 %; 29 %), DPPH (10 %; 11 %) and ABTS activity (16 %; 17 %). However, there were no significant differences between thermopriming at 40 °C and 50 °C. Consequently, both thermopriming treatments were found to be effective for increasing the drought stress tolerance during the reproductive stage in maize.

Keywords: Anthocyanins; Cross stress tolerance; Photosynthesis; Reproductive stage

2.2. Introduction

The world's population is expected to increase significantly by 2050. As a result, it is believed that there will not be enough land available to cultivate cereal crops and meet the growing global demand for food. The loss of agricultural land as a result of climate change, the increase in biotic and abiotic stressors, and the acceleration of global climate change are some of the primary challenges facing agriculture and food production (Hossain *et al.,* 2020). Drought, heat, salinity, and cold are examples of environmental stressors that can hinder crop quality and yield. Global climate change is predicted to increase the frequency and severity of severe weather events, such as extreme temperatures and decreased precipitation (Ncama *et al.,* 2021). In the final decade of the 20th century, the average global temperature increased by 0.3 °C, and by 2100, it is expected to have increased by nearly 3 °C (Valone, 2021). Two of the most prevalent abiotic stressors that can impact crop growth and productivity, and ultimately food security in a changing climate, are extreme heat and water scarcity (Hussain *et al.,* 2019). Additionally, they have the potential to significantly alter the composition of the germplasm (He and Li, 2020; Atlin *et al.,* 2017).

Maize (*Zea mays* L.), the most widely distributed crop in the world, is grown in temperate, tropical, and subtropical regions (Revilla *et al.,* 2021). It ranks as the third most significant crop globally after rice and wheat, with a production of 1241.56 million metric tons over 208.23 million hectares of cultivated land (FAOSTAT, 2025). Maize is more susceptible to drought than crops like sorghum, resulting in a 66% decrease in maize yield and a 33% decrease in sorghum yield (Safian *et al.,* 2022).

Drought stress is known to be the most harmful abiotic stressor to crops, affecting the growth, development, and production of a wide range of crops. It changes the physiology, biochemistry, and morphology of a plant (Yadav *et al.,* 2020). Plant photosynthesis is severely hampered by water deprivation stress because it greatly reduces the concentrations of vital photosynthetic pigments in the majority of plants, which slows down the rate at which CO_2 is absorbed (Bhattacharya and Bhattacharya, 2021). Plants have evolved several defensive mechanisms, such as an increase in antioxidant compounds, to fend off oxidative damage from drought. Polyphenolic compounds are essential for mitigating the negative impact of stress (Grcia-Caparros *et al.,* 2021). The phenolics production in plants is a complicated web of reactions that are either endogenously controlled or triggered by external stimuli like such as moisture (Marchiosi *et al.,* 2020). In reaction to environmental stresses, phenols accumulate to protect tissues from oxidative stress by free radicals (Meulmeester *et al.,* 2022). The reproductive period of maize plants, which occurs between tassel development and silking, is the most susceptible to water scarcity. Gas exchange, leaf water content, photosynthetic activity, chlorophyll a and b content, and cell membrane flexibility are all negatively impacted by this stage (Bheemanahalli *et al.,* 2022; Anjum *et al.,* 2017).

Given the world's expanding population, new effective plant breeding methods are needed to improve crop quality, boost yield output, raise stress tolerance, and develop more sustainable and adaptable germplasm pools for future climatic issues (Liu *et al.,* 2022). Numerous studies have been conducted on the intricate networks of stress response and adaptation mechanisms of high-value crops, like soybean (*Glycine max*), wheat (*Triticum aestivum*), rice (*Oryza sativa*), and maize (*Zea mays*) (Abhinandan *et al.,* 2018; Liu *et al.,* 2016; Naikoo *et al.,* 2019; Tiwari and Yadav, 2019). One of the recommended management techniques to treat drought stress is stress priming, often referred to as stress hardening, training, or conditioning. It is a low-cost and environmentally friendly technology. Plants can develop either short-term or long-term stress memory, which increases their resistance to stressors in the present or even in subsequent generations (Fan *et al.,* 2018; Wang *et al.,* 2016). Reports of the phenomenon known as "plant stress memory," which characterizes the enduring effects and stress imprint where past exposures to biotic and/or abiotic stress have significantly influenced future stress responses, have increased in recent years (Liu *et al.,* 2022). The capacity of a plant to grow more resistant to a range of abiotic stressors and, in some cases, biotic stressors following exposure to a single stressor is known as cross-stress tolerance (Llorens *et al.,* 2020). One possible outcome of stress exposure to plants is the establishment of stress memory, which makes them more resistant to future stressors. This process is also referred to as priming, acclimatization, conditioning, or hardening (Pissolato *et al.,* 2024). There is a lag or memory phase between the priming event and the second stress event during priming (Galviz *et al.,* 2022). During the priming phase, physiological, metabolic, molecular, and epigenetic changes occur. Throughout a plant's life, these alterations might be transient or longlasting, and in some cases, they can even be inherited by subsequent generations (Mauch-Mani *et al.,* 2017). Stress memory depends on the imprints left on chromatin, which subsequently affect the transcription of genes upon the initial stress event, or on the regulators that control the post-transcriptional expression of the gene, to either stimulate or inhibit the buildup of transcription factors, signaling metabolites, and proteins produced in response to stress through activating or silencing genes (Gallusci *et al.,* 2023; Liu and He, 2020). These regulators interact to fine-tune their molecular activities in a variety of abiotic stress scenarios, including heat, cold, drought, and floods. Small noncoding RNAs (sRNAs), including small-interfering RNAs (siRNAs) and miRNAs, are commonly responsible for mediating the feedback loop (Liu and He, 2020; Yakovlev and Fossdal, 2017). Many types of plants have been seen to exhibit stress cross-tolerance to a wide range of abiotic stressors brought on by heat priming. Thanks to previous studies on cross-stress tolerance, researchers have been able to link various individual stress responses and begin to clarify the molecular and physiological mechanisms involved in signal initiation and transduction that impart stress tolerance (Hossain *et al.,* 2018). Pre-sowing priming compounds improve seed germination ability under demanding conditions. Thus, by pre-stimulating the plant's antioxidant defense mechanism, it is possible to create a stress memory and increase stress tolerance (Sher *et al.,* 2019). The effects of preconditioning vary according to plant species and concentration (Guo *et al.,* 2022). Using the heat priming method, seeds are kept in a dark environment at a high temperature for certain periods. Germination rates are positively impacted by treatments with either hot or cold temperatures before planting (Hardegree, 1996). Heat priming has a positive effect on seed germination and seedling emergence while also encouraging plant growth and development. It has also been demonstrated to enhance enzyme performance, plant growth, and metabolism. Uncertainty surrounds the effect of seed thermal priming on maize's resistance to drought stress throughout maturity. Understanding the physiological and biochemical impacts of seed thermopriming and drought stress on maize was the aim of this investigation.

2.3. Materials and Methods

2.3.1. Plant material and site description

This study examined the physicochemical response of maize throughout the reproductive stage to seed thermopriming under drought stress. For this investigation, two native genotypes of maize (white and red) were gathered from farmers in Aramberri, Nuevo Leon, Mexico, and one hybrid (P-3057w) was gathered from farmers in Miguel Aleman, Tamaulipas, Mexico. From February to April of 2024, the experiment was carried out at the Facultad de Agronomia, Universidad Autonoma de Nuevo Leon in Marin, Nuevo Leon, Mexico (located at 24°19'16.71"N and 99°54'58.06" W).

2.3.2. Seed thermopriming treatments

Three different heat stress treatments, i.e., control, 40 °C, and 50 °C, were used. Before being sown, seeds of the three genotypes of maize were exposed to high temperatures of 40 °C and 50 °C for 72 h in an oven (Humboldt, H-30105, USA).

2.3.3. Drought treatment

Each genotype's seeds were planted in germination trays, and when the plants had three leaves, they were moved into 42-liter pots filled with a mixture of peat moss, chicken manure, and black soil (1:1:1). Six treatments were used in the experiment: control, drought, heat priming at 40 °C, heat priming at 40 °C + drought, heat priming at 50 °C, and heat priming at 50 °C + drought. There was one plant per pot in a split-plot with a randomized design, and each pot had 26 kg of substrate. To avoid drought stress, all of the pots received regular irrigation until stage V7 (pre-heading). All the pots were irrigated to saturation the afternoon before the drought stress initiation, following an overnight drainage. A small hole was made to re-irrigate the pots of the control treatment as frequently as needed. After that, the pots were enclosed around the stem to avoid direct evaporation of soil water. In the drought treatment, the irrigation was withheld for twenty days. After twenty days, when the plants showed signs of wilting or leaf rolling, particularly in the morning, data collection was made.

2.3.4. Data collection

Measurements of photosynthetic and biochemical parameters were taken when the plants attained the reproductive phase (VT).

2.3.4.1. Photosynthetic parameters

The Li-Cor 6400 (LI-COR Inc., Lincoln, NE, USA) with a 6400-02B LED light source was used to quantify the photosynthesis variables using the flag leaf, including the net photosynthetic rate (Pn, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs, mmol H_2O m^{-2} s^{-1}), and transpiration rate (Tr, mmol H_2O m^{-2} s^{-1}). During the measurements, the following calibration conditions were used: 700 µmol s^{-1} of flow, 400 µmol CO_2 at constant levels, and a 6400-02 LED light source at 1500 µmol m^{-2} s^{-1} (Galicia-Juárez *et al.,* 2021).

2.3.4.2. Cell membrane damage

The protocol of Tas (2022) was used to quantify cell membrane damage. A 100 mg sample was taken from each randomly chosen leaf, weighed, and then rinsed three times with distilled water. After that, the plant samples were put into test tubes with 10 mL of deionized water. After being sealed, the tubes were placed in a water bath (Lab-Line Instruments Inc., 18002, USA) at 32 °C for 2 h. To find the initial electrical conductivity (EC1) value, the solution's electrical conductivity (EC) was measured with an electrical conductivity meter (Thermo Scientific, Orion Star A215, USA). To eliminate all cells and allow organic and inorganic ions to enter the solution, the samples were autoclaved for 20 min at 120 °C in the second phase. After the samples were brought to room temperature, the electrical conductivity of the solutions was again measured to determine a second electrical conductivity (EC2) value. The cell membrane damage (CMD, %) value was calculated using the following equation:

$$CMD = [1 - \frac{(EC1)}{(EC2)}]\ x\ 100$$

2.3.4.3. Photosynthetic pigments

Carotenoids and chlorophyll were measured using the methodology of Rodríguez-Salinas *et al.* (2020). 10 mL of 80 % acetone was added to 100 mg of leaf material in test tubes, which were then agitated for half an hour at room temperature. Following the solution's filtration, absorbance was measured at 663, 645, 480, and 510 nm and reported in mg g^{-1} of fresh material. Using the following formulae, the amount of carotenoids and chlorophyll was determined:

$$Chlorophyll\ a = \frac{12.7(\text{A663}) - 2.69\ (\text{A645}) x\ V}{1000}\ x\ W$$

$$Chlorophyll\ b = \frac{22.9\ (\text{A645})\ -\ 4.68\ (\text{A663})\ \text{x}\ V}{1000}\ x\ W$$

$$Chlorophyll\ total = \frac{20.2\ (\text{A645}) +\ 8.02\ (\text{A663})\ \text{x}\ V}{1000}\ x\ W$$

$$Carotenoides = \frac{7.6(\text{A480}) -\ 1.49\ (\text{A510}) x\ V}{1000}\ x\ W$$

Where:

W = Weight of the sample.

V= Volume of the solution.

2.3.4.4. Total Anthocyanins

The methodology described by Rodríguez-Salinas *et al.* (2020) was used to extract total anthocyanins. A volume of 5 mL of an acidified ethanol solution (ethanol and 1 N HCl, 85:15 v/v) adjusted to pH = 1 was added to 200 mg of leaf sample that had been weighed in a test tube. The mixture was then purged with nitrogen flow for 30 seconds. After that, it was kept out of the light at 4 °C for 30 min at 200 rpm on a stirrer plate (Corning, 6795-220, Mexico). The supernatant was then extracted and examined at a wavelength of 535 nm after it had been centrifuged for 20 min at 4 °C at 6000 rpm (HERMLE Labortechnik GmbH, Z400k, Germany). A sample's anthocyanin concentration was expressed as milligrams of cyanidin-3 glucoside equivalent (C3GE) per one hundred grams of sample (mg C3GE $100g^{-1}$) as follows:

$$C = (A/\mathcal{E}) \times (V/1000) \times \text{MW} \times (1/\text{weight of sample}) \times 10\text{\^{}}6$$

Where:

C = anthocyanin concentration (mg C3GE L^{-1}).

A = sample absorbance.

Ɛ = molar extinction coefficient of cyanidin-3-glucoside (25,965 cm^{-1} M^{-1}).

V = total volume of the extract.

MW = molecular weight of cyanidin-3-glucoside (449 g mol^{-1}).

2.3.4.5. Extraction of total phenols

The extraction of total phenols was done using the protocol of Rodríguez-Salinas *et al.* (2020), which consisted of weighing 200 mg of maize flour into a test tube. Then, 3 mL of 80 % methanol (80:20 methanol: water v/v) was added, and the mixture was purged with a nitrogen flow for 30 s. It was then shaken for 1 h under light protection on a stirrer plate (Corning, 6795-220, Mexico) at 200 rpm. Finally, it was centrifuged (HERMLE Labortechnik GmbH, Z 400 k, Germany) at 6000 rpm for 5 min. The supernatant was recovered and stored at -20 °C under light protection until further analysis.

2.3.4.6. Total phenols and antioxidant activities

Using the protocol of Rodríguez-Salinas *et al.* (2020), a colorimetric method based on the Folin-Ciocalteau reagent reaction was used to quantify the total phenolic content. 2 mL of phenolic extract was mixed with 2.6 mL of distilled water, oxidized with 0.2 mL of Folin-Ciocalteau reagent, and neutralized with 2 mL of a 7 % Na_2CO_3 solution after five min. After 90 min, the reaction was stopped, and the samples' absorbance at 750 nm was finally measured. Results were reported as milligrams of gallic acid equivalent per hundred grams of sample, with gallic acid serving as a reference for the calibration curve (0, 40, 80, 120, 160, and 200 mg L^{-1}) (mg GAE $100g^{-1}$).

Antioxidant activity was assessed using DPPH and ABTS, following the methodology of Rodríguez-Salinas *et al.* (2020). A 60 µM working solution with an absorbance set to 1.0 at 517 nm was used to assess DPPH. To perform the test, 0.2 mL of phenolic extract and 3 mL of DPPH working solution were combined. The reaction was then allowed to sit in the dark for 30 min, and the amount of DDPH that was reduced was measured. 1 mL of 7.4 mM ABTS and 1 mL of 2.6 mM $K_2S_2O_8$ were combined to create a working solution, which was then allowed to react for 12 h in the dark. The working solution's absorbance was then adjusted to 1.0 at 734 nm by diluting it with methanol. To perform the ABTS assay, 0.2 mL of phenolic extract and 3 mL of ABTS working solution were combined. The reaction was then allowed to sit in the dark for 2 h, and the amount of reduced ABTS was quantified.

2.3.5. Statistical Analysis

This study used a split-plot design with three replications, totaling fifty-four pots, and a randomized design. The normality of the repeated data was evaluated by the Shapiro-Wilk test. Following the normal distribution, the data were then submitted to a two-way analysis of variance (ANOVA) using Statistix 10 software (Analytical Software, FL, USA), where genotypes were assumed as a subplot component and treatments as a main plot factor. The mean comparison was determined using the Tukey test ($p \leq 0.05$).

2.4. Results

As indicated in Table 2.1, every physiological and biochemical parameter examined for priming treatments under drought stress had significant outcomes ($p \leq 0.05$).

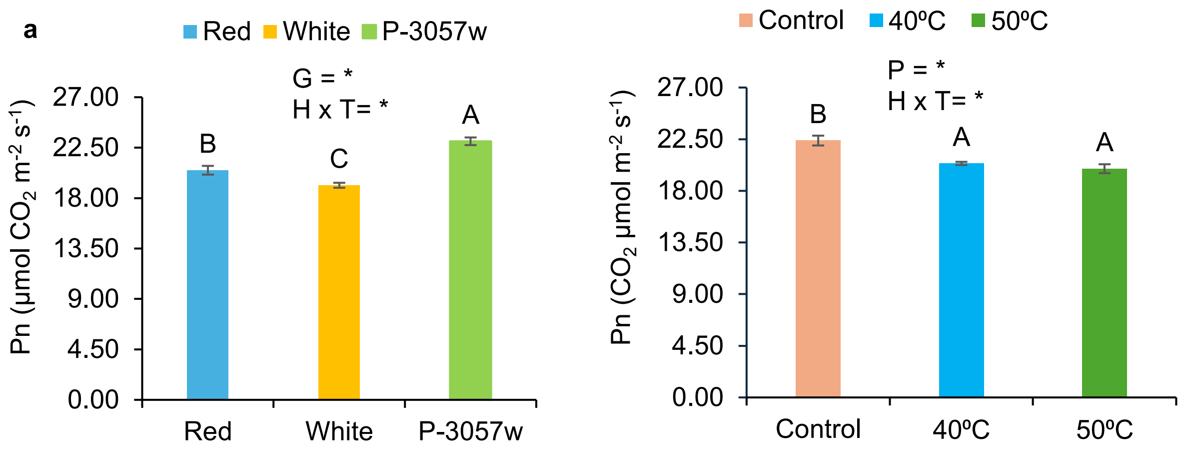
2.4.1. Physiological parameters and cell membrane damage

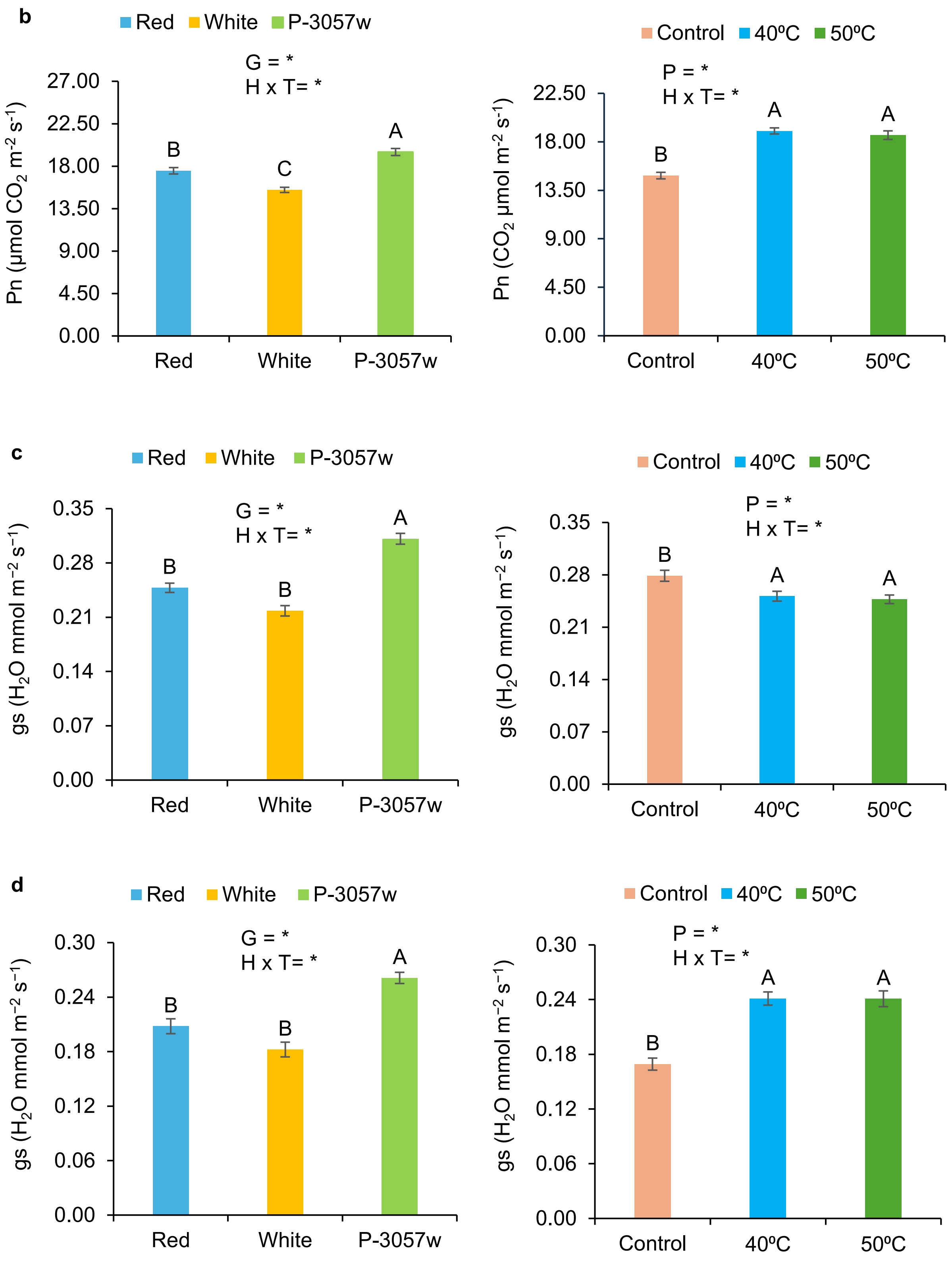
Drought caused a notable decrease in stomatal conductance (65 %), transpiration (69 %), and photosynthetic rate (51 %), as well as a 55 % increase in cell membrane damage, in unprimed seeds of all genotypes. With a 58 % decrease in photosynthetic rate, a 71 % decrease in stomatal conductance, a 78 % decrease in transpiration, and a 54 % rise in cell membrane damage, the white genotype had the most damage in physiological measures. 51 % less photosynthetic rate, 67 % less stomatal conductance, 61 % less transpiration, and 56 % less cell membrane damage were seen in P-3057w, whereas 48 % less photosynthetic rate, 57 % less stomatal conductance, 66 % less transpiration, and 53 % less cell membrane damage were observed in the red genotype. The physiological damage caused by drought stress was significantly reduced by heat priming at 40 °C and 50 °C. In plants, 40 °C priming decreased photosynthetic rate, stomatal conductance, transpiration, and cell membrane damage to 18 %, 16 %, 15 %, and 36 %, respectively, whereas 50 °C priming decreased drought damage to 21 % in photosynthetic rate, 16 % in stomatal conductance and transpiration, and 37 % in cell membrane injury (Figure 2.1a-g).

Table 2.1. Mean squares of physiological and biochemical variables in maize genotypes.

Variables	**M.S$_{hum.}$**	**Error hum.**	**M.S$_{trt.}$ P**	**G**	**P × G**	**M.S hum. × trt.**	**Error hum. × trt.**	**C.V (%)**
P_n	155.82*	0.98	19.26*	5.29*	$71.46^{n.s}$	14.63*	0.49	3.63
g_s	0.02*	0.00	0.00*	0.03*	$0.00^{n.s}$	0.00*	0.00	6.96
Tr	10.01*	0.09	1.48*	0.93*	$0.00^{n.s}$	1.09*	0.03	4.02
CMD	1120.3*	20.44	72.77*	335.67*	$0.77^{n.s}$	$37.93^{n.s}$	22.13	7.49
Chl-a	7.20*	0.30	1.10*	0.55*	$0.01^{n.s}$	0.18*	0.04	9.59
Chl-b	$2.57^{n.s}$	0.71	0.36*	1.84*	$0.05^{n.s}$	$0.07^{n.s}$	0.17	12.56
Chl total	$18.35^{n.s}$	1.91	2.69*	4.27*	$0.10^{n.s}$	$0.35^{n.s}$	0.29	13.41
Carot.	$1.78^{n.s}$	0.12	0.17*	0.35*	$0.00^{n.s}$	$0.03^{n.s}$	0.03	10.91
Anth.	269.30*	5.73	133.47*	366.32*	$2.85^{n.s}$	$8.08^{n.s}$	8.94	9.74
TPH	3476.83*	45.30	4355.25*	3954.25*	$67.65^{n.s}$	622.63*	50.56	5.93
DPPH	12719.00*	229.90	1271.05*	28490.50*	$70.78^{n.s}$	353.80*	104.80	2.54
ABTS	20471.50*	76.60	1117.55*	9968.90*	$35.13^{n.s}$	364.20*	70.00	2.96

*M.S$_{hum.}$ =Mean square of humidity; M.S$_{trt.}$ = Mean square of treatment; M.S$_{hum.\ x\ trt.}$= Mean square of interaction between humidity and treatment; Pn = photosynthetic rate; gs = stomatal conductance; Tr = transpiration; CMD = cell membrane damage; Chl-a = chlorophyll a; Chl-b = chlorophyll b; and Chl Total = total chlorophyll; Carot. = carotenoides; Anth. = anthocyanins and TPH = total phenols. Note: * indicates significance levels at $p \leq 0.05$.*




b
Red
White
P-3057w
G = *
H x T= *
Pn (µmol CO$_2$ m^{-2} s^{-1})
27.00
22.50
18.00
13.50
9.00
4.50
0.00
B
C
A
Red
White
P-3057w
Control
40ºC
50ºC
P = *
H x T= *
Pn (CO$_2$ µmol m^{-2} s^{-1})
22.50
18.00
13.50
9.00
4.50
0.00
B
A
A
Control
40ºC
50ºC
c
Red
White
P-3057w
G = *
H x T= *
gs (H$_2$O mmol m^{-2} s^{-1})
0.35
0.28
0.21
0.14
0.07
0.00
B
B
A
Red
White
P-3057w
Control
40ºC
50ºC
P = *
H x T= *
gs (H$_2$O mmol m^{-2} s^{-1})
0.35
0.28
0.21
0.14
0.07
0.00
B
A
A
Control
40ºC
50ºC
d
Red
White
P-3057w
G = *
H x T= *
gs (H$_2$O mmol m^{-2} s^{-1})
0.30
0.24
0.18
0.12
0.06
0.00
B
B
A
Red
White
P-3057w
Control
40ºC
50ºC
P = *
H x T= *
gs (H$_2$O mmol m^{-2} s^{-1})
0.30
0.24
0.18
0.12
0.06
0.00
B
A
A
Control
40ºC
50ºC

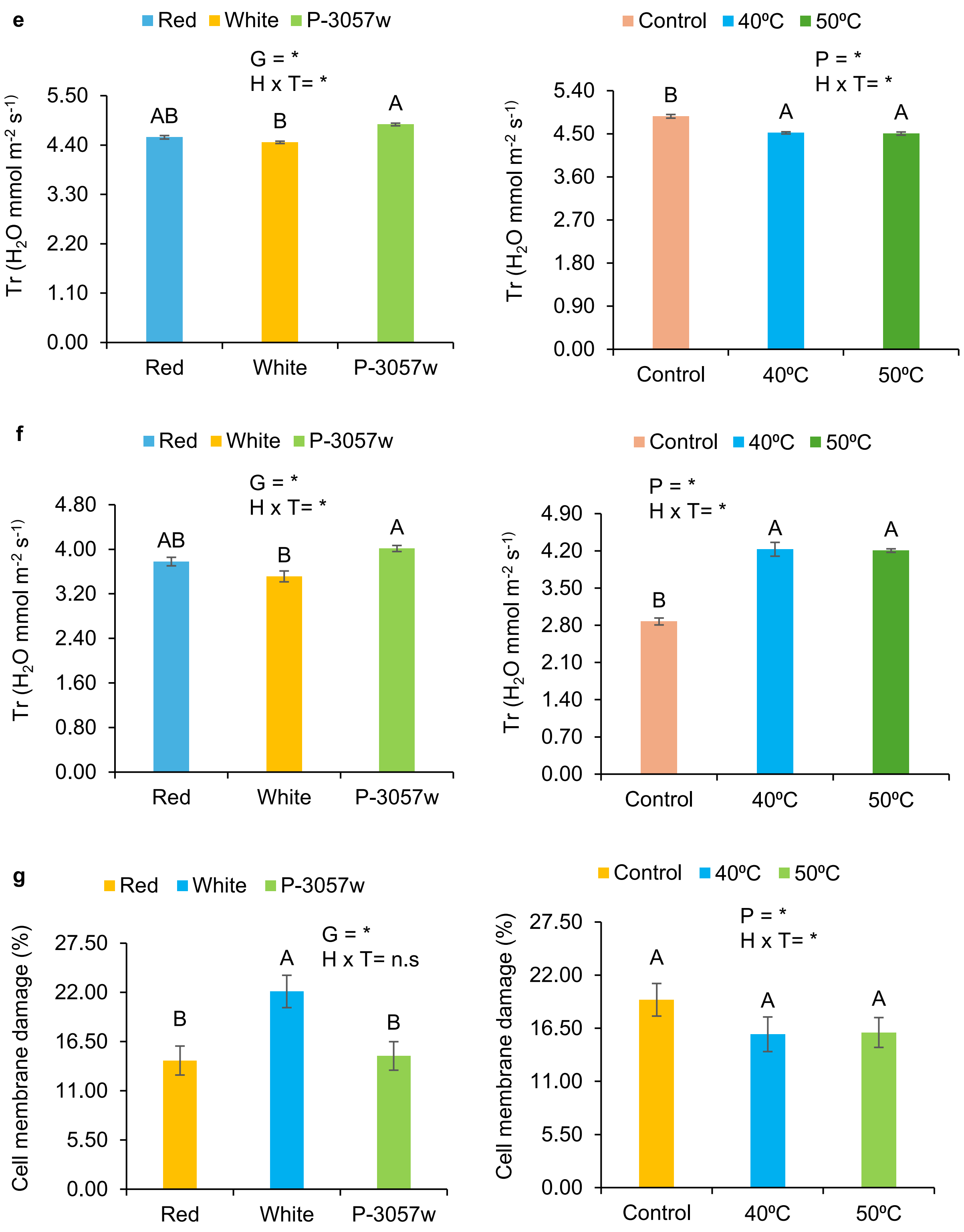
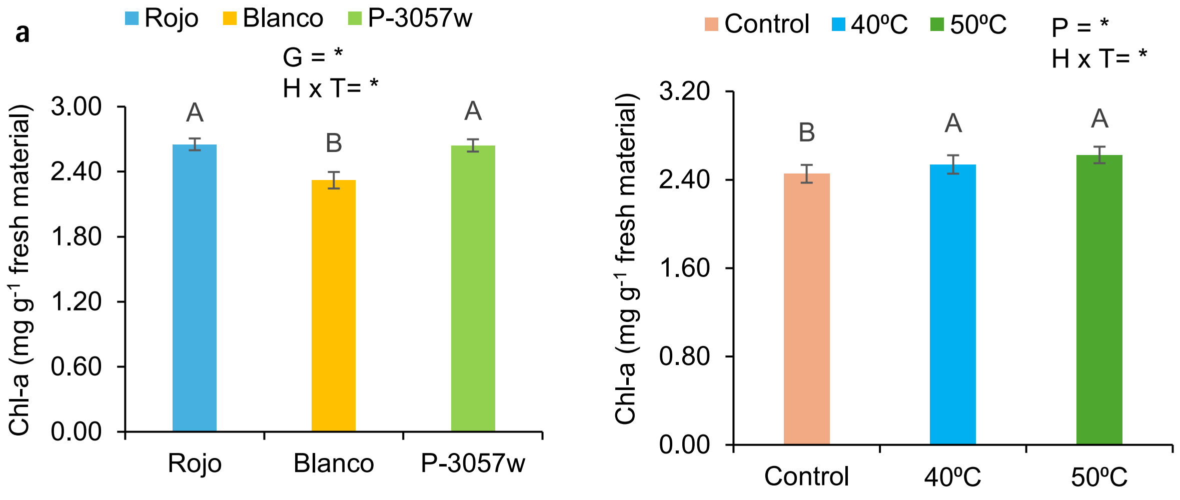


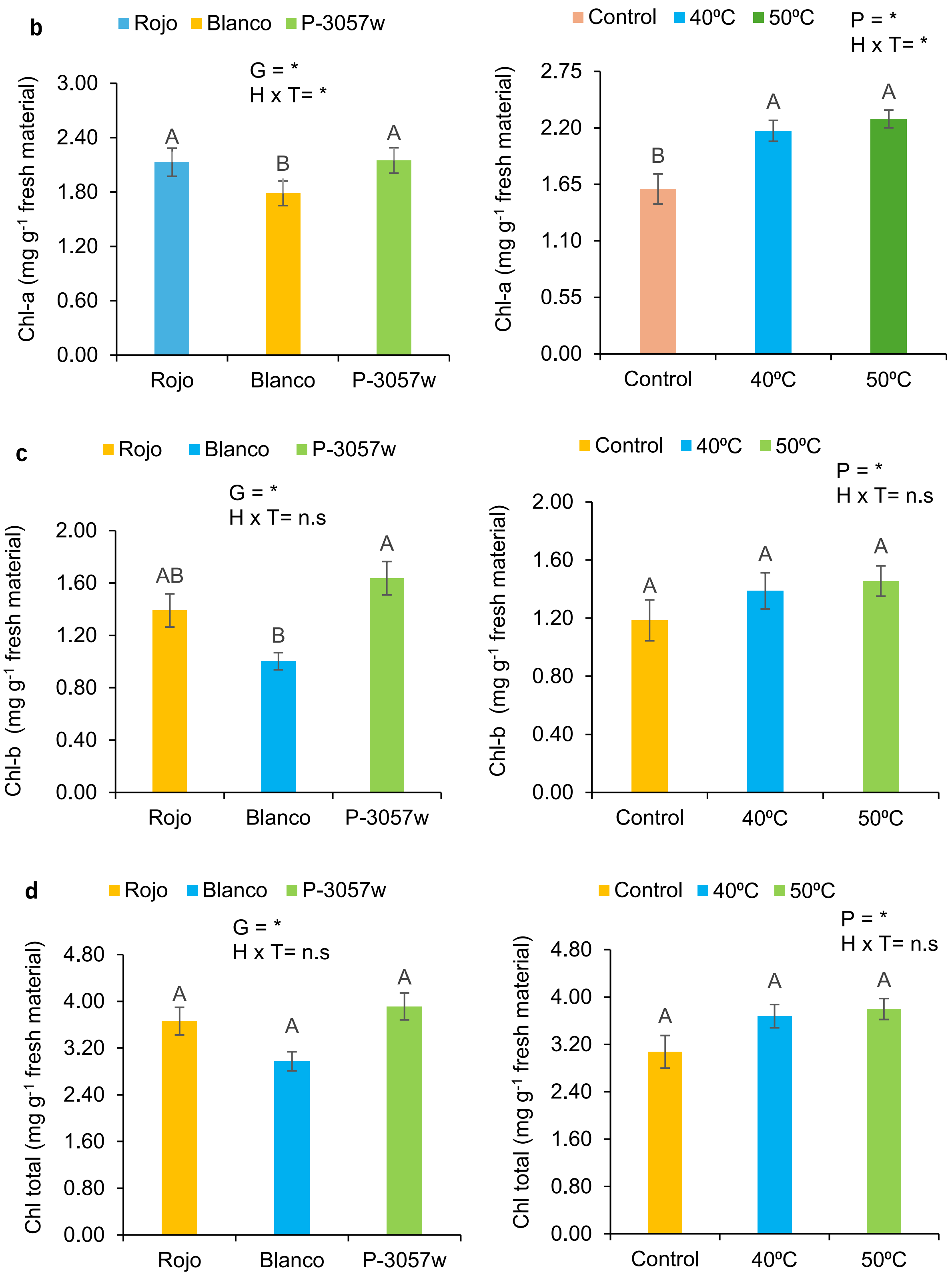
Figure 2.1. Effect of heat priming on maize genotypes (Red, White, P-3057w) during reproductive stage on (a) photosynthetic rate (P_n) (μmol CO_2 m^{-2} s^{-1}) under contol, (b) photosynthetic rate (P_n) (μmol CO_2 m^{-2} s^{-1}) under drought, (c) stomatal conductance (g_s) (mmol H_2O m^{-2} s^{-1}) under control, (d) stomatal

conductance (g_s) (mmol H_2O m^{-2} s^{-1}) under drought, (e) transpiration (Tr) (mmol H_2O m^{-2} s^{-1}) under control, (f) transpiration (Tr) (mmol H_2O m^{-2} s^{-1}) under drought, (g) cell membrane damage (%) under drought. Means sharing the same letters do not vary significantly using the Tukey test ($p \leq 0.05$). * Significance at $p \leq 0.05$.

2.5.2. Photosynthetic pigments

Plants cultivated from unprimed seeds under drought stress showed a significant decrease in chlorophyll a (85 %), chlorophyll b (74 %), total chlorophyll (81 %), and carotenoids (66 %). The red genotype showed the greatest damage to photosynthetic pigments, with reductions of 90 % in chlorophyll a, 103 % in chlorophyll b, 95 % in total chlorophyll, and 57 % in total carotenoids. The white genotype showed reductions of 89 % in chlorophyll a, 58 % in chlorophyll b, 78 % in total chlorophyll, and 86 % in total carotenoids, while P-3057w showed reductions of 77% in chlorophyll. Heat priming at 40 °C and 50 °C considerably reduced the physiological harm brought on by drought stress. Chlorophyll a, chlorophyll b, total chlorophyll, and total carotenoids dropped to 20 %, 26 %, 23 %, and 28 % in plants primed with 40 °C, whereas drought damage dropped to 19 % in chlorophyll a, 14 % in chlorophyll b, 17 % in total chlorophyll, and 18 % in total carotenoids in plants primed with 50 °C (Figure 2.2a-e).




b
🟦 Rojo 🟨 Blanco 🟩 P-3057w
G = *
H x T= *
Chl-a (mg g^{-1} fresh material)
3.00
2.40
1.80
1.20
0.60
0.00
A
B
A
Rojo
Blanco
P-3057w
🟧 Control 🟦 40ºC 🟩 50ºC
P = *
H x T= *
Chl-a (mg g^{-1} fresh material)
2.75
2.20
1.65
1.10
0.55
0.00
B
A
A
Control
40ºC
50ºC
c
🟨 Rojo 🟦 Blanco 🟩 P-3057w
G = *
H x T= n.s
Chl-b (mg g^{-1} fresh material)
2.00
1.60
1.20
0.80
0.40
0.00
AB
B
A
Rojo
Blanco
P-3057w
🟨 Control 🟦 40ºC 🟩 50ºC
P = *
H x T= n.s
Chl-b (mg g^{-1} fresh material)
2.00
1.60
1.20
0.80
0.40
0.00
A
A
A
Control
40ºC
50ºC
d
🟨 Rojo 🟦 Blanco 🟩 P-3057w
G = *
H x T= n.s
Chl total (mg g^{-1} fresh material)
4.80
4.00
3.20
2.40
1.60
0.80
0.00
A
A
A
Rojo
Blanco
P-3057w
🟨 Control 🟦 40ºC 🟩 50ºC
P = *
H x T= n.s
Chl total (mg g^{-1} fresh material)
4.80
4.00
3.20
2.40
1.60
0.80
0.00
A
A
A
Control
40ºC
50ºC

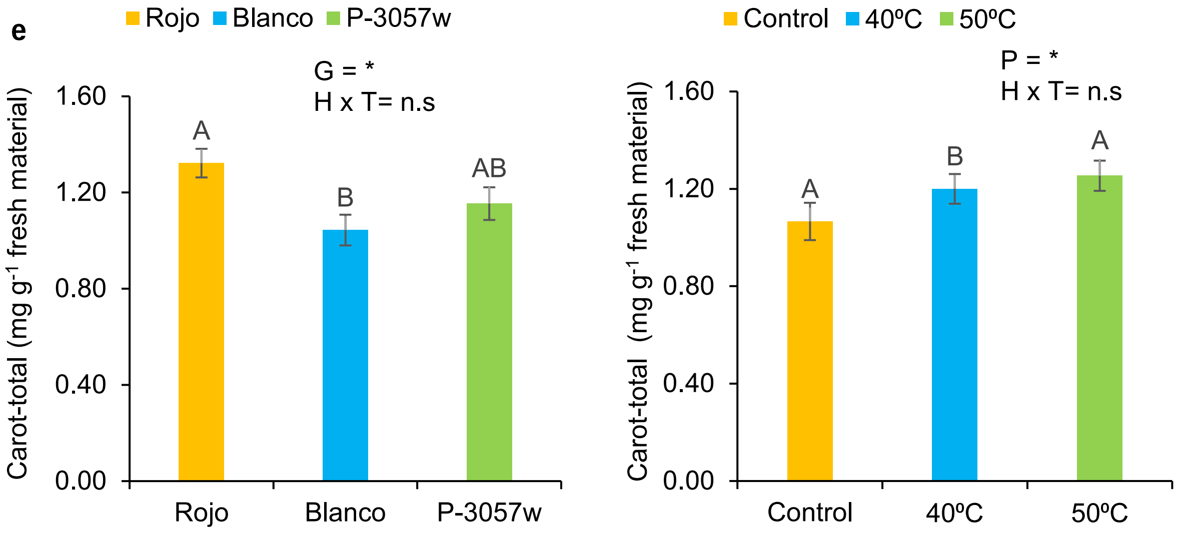
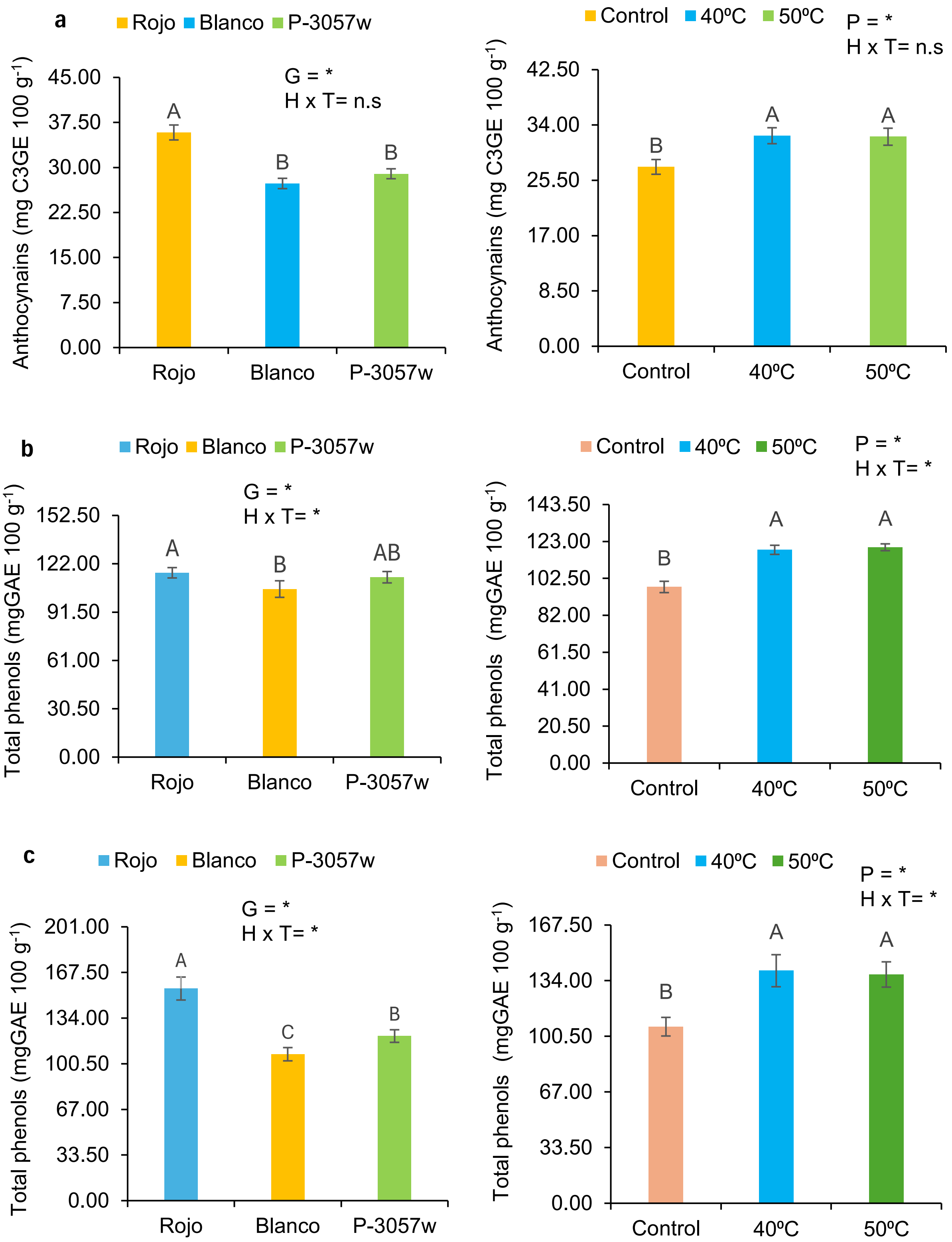
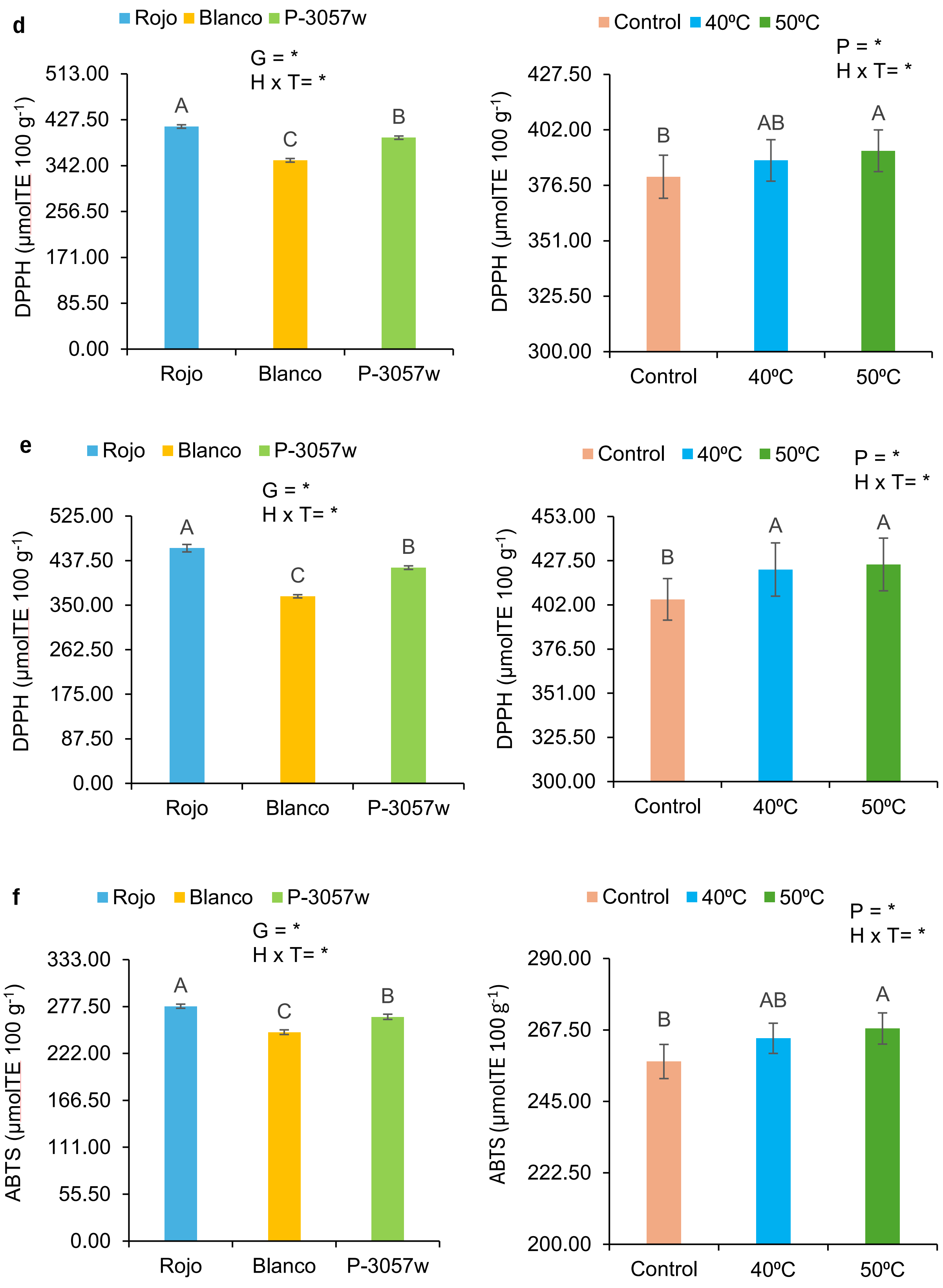


Figure 2.2. Effect of heat priming on maize genotypes (Red, White, P-3057w) during reproductive stage on (a) chlorophyll a (Chl-a) (mg g^{-1} fresh material) under contol, (b) chlorophyll a (Chl-a) (mg g^{-1} fresh material) under drought, (c) chlorophyll b (Chl-b) (mg g^{-1} fresh material) under drought, (d) total chlorophyll b (Chl-total) (mg g^{-1} fresh material) under drought, (e) total carotenoids (Chl-total) (mg g^{-1} fresh material) under drought. Means sharing the same letters do not vary significantly using the Tukey test ($p \leq 0.05$). * Significance at $p \leq 0.05$.

2.5.3. Anthocyanins, Phenols, and Antioxidant Activities

In plants cultivated from unprimed seeds, drought stress raised the levels of anthocyanins (7 %), total phenols (8 %), and antioxidant activities by DPPH (6 %) and ABTS (11 %). P-3057w had an 11 % increase in anthocyanins, 2 % in total phenols, 7 % and 11 % in DPPH and ABTS activities, and a 1 % increase in anthocyanins, 6 % in total phenols, 4 % and 9 % in DPPH and ABTS activities. The red genotype had a greater increase in anthocyanins (8 %), total phenols (15 %), and antioxidant activities by DPPH (7 %) and ABTS (14 %). Heat priming, on the other hand, improved the anthocyanins, total phenols, and antioxidant activities, which lessened the damage brought on by drought. Plants primed with anthocyanins at 40 °C showed increases in total phenols, DPPH, and ABTS activity of 25 %, 30 %, 10 %, and 16 %, respectively. In contrast, plants primed at 50 °C showed increases in anthocyanin content of 24 %, total phenols of 29 %, DPPH activity of 11 %, and ABS activity of 17 %. Heat priming at 40 °C and 50 °C increased anthocyanins (26 %, 27 %), total phenols (40 %, 36 %), and antioxidant activities by DPPH (13 %, 14 %) and ABTS (20 %, 21 %), the most in pigmented red genotypes (Figure 2.3a-g).




d
Rojo
Blanco
P-3057w
G = *
H x T= *
DPPH (µmolTE 100 g^{-1})
513.00
427.50
342.00
256.50
171.00
85.50
0.00
A
C
B
Rojo
Blanco
P-3057w
Control
40ºC
50ºC
P = *
H x T= *
DPPH (µmolTE 100 g^{-1})
427.50
402.00
376.50
351.00
325.50
300.00
B
AB
A
Control
40ºC
50ºC
e
Rojo
Blanco
P-3057w
G = *
H x T= *
DPPH (µmolTE 100 g^{-1})
525.00
437.50
350.00
262.50
175.00
87.50
0.00
A
C
B
Rojo
Blanco
P-3057w
Control
40ºC
50ºC
P = *
H x T= *
DPPH (µmolTE 100 g^{-1})
453.00
427.50
402.00
376.50
351.00
325.50
300.00
B
A
A
Control
40ºC
50ºC
f
Rojo
Blanco
P-3057w
G = *
H x T= *
ABTS (µmolTE 100 g^{-1})
333.00
277.50
222.00
166.50
111.00
55.50
0.00
A
C
B
Rojo
Blanco
P-3057w
Control
40ºC
50ºC
P = *
H x T= *
ABTS (µmolTE 100 g^{-1})
290.00
267.50
245.00
222.50
200.00
B
AB
A
Control
40ºC
50ºC

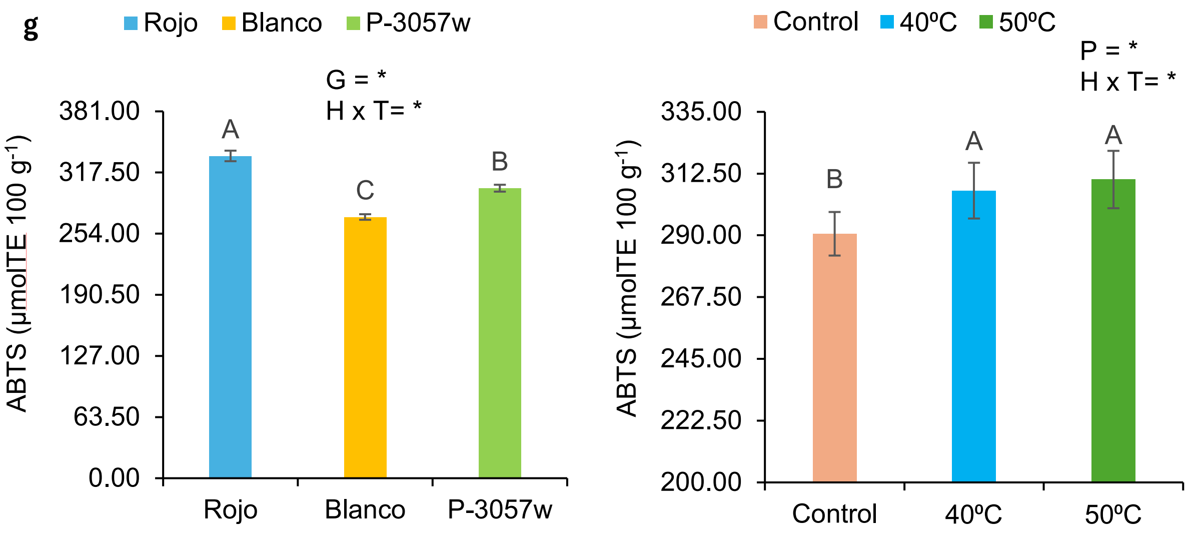


Figure 2.3. Effect of heat priming on maize genotypes (Red, White, P-3057w) during reproductive stage on (a) anthocyanins (mg C3GE 100 g^{-1}) under drought, (b) total phenols (mgGAE 100 g^{-1}) under control, (c) total phenols (mgGAE 100 g^{-1}) under drought, (d) DPPH activity (µmolTE 100 g^{-1}) under control, (e) DPPH activity (µmolTE 100 g^{-1}) under drought, (f) ABTS activity (µmolTE 100 g^{-1}) under control, (g) ABTS activity (µmolTE 100 g^{-1}) under drought Means sharing the same letters are not significantly different according to Tukey test ($p \leq 0.05$). * Significance at $p \leq 0.05$.

2.5.4. Discussion

2.5.4.1. Photosynthetic pigments and gas exchange attributes

In a water-limited environment, plants experience reduced photosynthesis, slowed leaf expansion, stomatal narrowing and blockage, early leaf senescence, decreased translocation, and decreased crop yield (Nikolaeva *et al.,* 2010; Khan *et al.,* 2019; Rafiq *et al.,* 2023; Zafar *et al.,* 2023). Drought also affects plant-water relations, such as transpiration rate, stomatal opening, leaf water capacity, comparative water accumulation, efficient water utilization, and plant canopy temperature (Kosar *et al.,* 2015; Saud *et al.,* 2017). Plants grown under drought have much lower levels of total chlorophyll and carotenoid content than plants cultivated in normal irrigation (Figure 2d,e). Similarly, the water deficit environment significantly impacted several gaseous exchange parameters and stomatal behavior, which significantly decreased when compared to the plants growing in the control treatment (Figure 1a-f). Among other regulatory mechanisms that reduce net photosynthesis, plants that experience drought have decreased stomatal behavior and photosynthetic pigments (Liang *et al.,* 2018). We found that plants grown from unprimed seeds under drought stress had reduced rates of photosynthesis, photosynthetic pigments, and stomatal conductance in comparison to plants grown in a control condition. It was previously demonstrated that plants with adequate water supplementation had much higher levels of photosynthetic pigments, gaseous exchange parameters, and stomatal apertures than plants grown in water-limited conditions (Khan *et al.,* 2019; Bashir *et al.,* 2020; Sharma *et al.,* 2020). A similar trend was also seen in maize plants under drought stress circumstances (Bheemanahalli *et al.,* 2022; Hussain *et al.,* 2019; Anjum *et al.,* 2017). Additionally, the decline in transpiration rate might be due to the plants' inability to maintain field water capacity, which was most likely caused by high canopy transpiration (Rivas *et al.,* 2016). We also saw an electrolyte leak due to cell membrane degradation in unprimed plants under drought stress. Heat-induced seed priming significantly reduced the damage to photosynthetic pigments, gaseous exchange parameters, and cell membrane damage in this study. In this work, seed priming at 40 °C and 50 °C significantly increased photosynthetic rate (28 %, 24 %), stomatal conductance (42 %, 42 %), and transpiration (47 %, 46 %), while the cell membrane damage index during drought significantly decreased by 28 %. In plants cultivated from seeds primed at 40 °C and 50 °C, we found a considerable rise in chlorophyll a of 54 % and 55 %, respectively. Under dryness, there was no discernible change in chlorophyll b, total chlorophyll, or carotenoids between heat-primed and control plants. Similar results were reported by Luqman *et al.* (2023), who discovered that seed priming treatments significantly boosted photosynthetic rate, stomatal conductance, and transpiration in maize hybrids under dry conditions as compared to the control. According to Zhao *et al.* (2023), drought stress in unprimed wheat seedlings severely compromised cell membrane integrity and increased electrolyte leakage by 33 %, whereas priming reduced it by 23 %. Ru *et al.* (2023), Hussain *et al.* (2017), and Sen and Puthur (2020) have found that seed priming significantly reduced the damage that drought caused to photosynthetic pigments such as carotenoids and chlorophyll. They also found that priming had improved the integrity of the cell membrane by reducing electrolyte leakage during drought stress.

2.5.4.2. Anthocyanins, Total phenols, and Antioxidant capacities

Abiotic stresses cause plant cells to release reactive oxygen species (ROS). These ROS can lead to cellular dysfunction and death because of their high reactivity with a variety of biological components, including proteins, lipids, nucleic acids, and cell membranes (Choudhury *et al.,* 2017). Plants respond to abiotic stresses by encouraging the production of secondary metabolites that are antioxidants, particularly polyphenolic compounds, as a means of defense and adaptation (Sachdev *et al.,* 2021). Plant resistance to a range of abiotic stresses, including salt, heavy metal toxicity, drought, heat stress, chilling damage, UV radiation, and others, is significantly increased by these chemicals (Syvertsen and Garcia-Sanchez, 2014; Nawaz *et al.,* 2023). The enhanced production and rapid accumulation of these molecules, which have potent antioxidative properties and may efficiently quench ROS to prevent cellular membrane damage, are essential markers of a plant's tolerance and resistance to oxidative stress (Birben *et al.,* 2012). Research indicates that plants under stress start the synthesis of polyphenolic compounds more quickly than plants developing normally (Scagel *et al.,* 2019; Gharibi *et al.,* 2019; Rao *et al.,* 2018; Rao *et al.,* 2019; Rao *et al.,* 2021). Important enzymes like PAL and CHS are crucial for regulating phenolic synthesis, and complex enzymatic pathways are involved in the regulation of phenolic compound production under stress. Plants control several genes that encode vital enzymes under various abiotic stressors, such as PAL, C4H, C3H, 4CL, COMT, CHS, CHI, F3H, DFR, F3′M, FLS, ANR, and ANS (Ahmed *et al.,* 2021). The enhanced synthesis of a range of bioactive polyphenolic compounds under adverse climatic circumstances results from the increased expression of these genes, which in turn enhances plant resilience through intricate stress tolerance pathways. Plants use several survival strategies, such as the production of secondary metabolites like flavonoids and phenolics, when they are water-limited. The buildup of polyphenolic compounds, such as anthocyanins and other flavonoids, is impacted by drought stress (Park *et al.,* 2023). In this study, heat-induced seed priming dramatically raised the levels of anthocyanins and total phenols. The antioxidant activity in the genotypes under investigation was demonstrated to increase with seed thermopriming at 40 °C and 50 °C. This greatly reduced the physiological damage caused by drought stress and may have improved the genotypes' ability to tolerate drought. When seeds were thermoprimed at 40 °C and 50 °C, the red genotype showed the highest increase in antioxidant capacity by DPPH (13 %, 14 %) and ABTS (20 %, 21 %), respectively, following the P-3057w and white genotypes. Our results are consistent with those of Samota *et al.* (2017), who discovered that plants grown from primed seeds exhibited noticeably greater levels of DPPH antioxidant capacity and total phenols. Hussain *et al.* (2017) reported that seed priming enhanced the total phenolic contents and antioxidant activity in rice genotypes under drought stress; however, the benefits of priming were more pronounced in rice that was pigmented during drought. Another study of the grain by Zhao *et al.* (2023) found that the ABTS and DPPH radical scavenging activities of wheat seedlings increased in response to drought stress. It has been shown that the concentration of phenolic chemicals is the reason for this increase in antioxidant capability.

2.6. Conclusion

By boosting antioxidant activity in every genotype examined, seed thermopriming in this study demonstrated a decrease in physiological damage caused by drought stress. Nonetheless, no discernible variations were found between pretreatments conducted at 40 °C and 50 °C. Thus, both treatments were shown to be successful in improving drought tolerance in maize throughout the reproductive stage.

CHAPTER III. Generation mean analysis of phenolic content in pigmented maize (*Zea mays* L.) grain and cob

3.1. Abstract

Pigmented maize is rich in phenolic compounds, carotenoids, and anthocyanins, which are linked to health-promoting and nutraceutical properties. Consequently, this type of grain is categorized as a functional food due to its high antioxidant activity and its potential preventive roles against cancer, diabetes, obesity, and neurodegenerative diseases. The numerous health advantages of anthocyanins in pigmented maize have recently drawn more attention from consumers. A better understanding of how genes influence anthocyanins is crucial for breeding initiatives aimed at increasing antioxidant levels. This study aimed to analyze the genetic influence on anthocyanin levels in the grain and cob of purple maize utilizing generation mean analysis, for which six generations (P_1, P_2, F_1, F_2, BC_{P1}, and BC_{P2}) were developed and planted in a randomized complete block design with three replications at the experimental station of the Facultad de Agronomia, Universidad Autonoma de Nuevo Leon in Marin, Mexico. A six-parameter model was used to estimate the genetic effects controlling the inheritance of anthocyanins, revealing the significance of all genetic effects for the traits studied in grain and cob, up to varying extents. Additive gene effects appeared to have a greater contribution in the inheritance of total monomeric anthocyanins and cyanidin-3-glucoside in grain and cob, indicating the possibility of making an early generation or recurrent selection, whereas dominant effects had a major contribution to total phenolic content in grain and cob. Moreover, among epistatic effects, the dominance x dominance gene interaction was important for all parameters in grain and cob, suggesting that the selection should focus on a strategy harnessing dominance, such as hybrid development.

Keywords: Additive and dominance effects; Epistatic gene effects; Cyanidin-3-glucoside; Monomeric anthocyanins.

3.2. Introduction

Mexico is the original center for the domestication and diversification of maize, maintaining a broad array of grain colors and traits, including growth speed, height of both plants and ears, physiological attributes, chemical makeup of grains, nutritional benefits, and various uses (Amanjyoti *et al.,* 2024; Li, 2024). Consequently, the diversity of maize is primarily found among indigenous communities or ethnolinguistic groups (Curry, 2021). There is a research bias concerning maize due to its commercial significance, with most investigations on maize grain composition concentrating on yellow and white varieties (Kaul *et al.,* 2019); studies focusing on pigmented maize have only recently begun to receive attention over the last ten years (Magaña-Cerino *et al.,* 2020).

Pigmented maize is rich in phenolic compounds, carotenoids, and anthocyanins, which are linked to health-promoting and nutraceutical properties. Consequently, this type of grain is categorized as a functional food due to its high antioxidant activity and its potential preventive roles against cancer, diabetes, obesity, and neurodegenerative diseases (Sánchez-Nuño *et al.,* 2024; Navarro *et al.,* 2018). Although lutein, zeaxanthin, and α and β cryptoxanthin are the most frequently studied carotenoids in yellow maize (Prasanthi *et al.,* 2017), cyanidin-3-glucoside (C3G), cyanidin-3,5-diglucoside, pelargonidin, and peonidin-3-glucoside, along with their malonyl derivatives, are the main anthocyanins present in blue, red, and purple grains (Colombo *et al.,* 2021). Anthocyanins, a kind of naturally occurring phenolic phytochemical found in many food sources, particularly fruits and vegetables, which have a well-established presence in the diet, are produced via the flavonoid pathway in plant tissues (Pervaiz *et al.,* 2017). These compounds serve as pigments that display a spectrum of colors, including red, orange, purple, and blue, which depend on pH levels and their chemical structure. The potency of these colors is reliant on the concentration of anthocyanins found in maize grain and cob. Although environmental variables also have a role, genetic factors are the main determinant of this pigment's occurrence (Enaru *et al.,* 2021). Reports state that water-soluble anthocyanins, reddish-purple pigments found in a range of fruits and vegetables (Chatham and Juvik, 2021; Nawaz *et al.,* 2018), as well as phytochemicals and antioxidants, are abundant in purple maize and its byproducts, including cobs, grains, silk, and husks (Zhu, 2018). Growing consumer demands may be met by maize, a scalable crop that might provide a natural supply of colorant (Chatham *et al.,* 2019). However, large-scale commercial production will require breeding lines with the stability, color, and maximal anthocyanin production while maintaining yield and other agronomic qualities (Chatham and Juvik, 2021). Finding lines that produce the most anthocyanins and have ideal anthocyanin profiles to backcross into existing elite inbreds will be necessary to achieve these objectives. Selections need to be made with every backcrossing cycle to minimize linkage drag without compromising any elements linked to anthocyanin synthesis. Backcrossing with non-pigmented lines may cause a variety of structural genes and regulatory systems necessary for anthocyanin production to become unfixed. The advantage of creating genetic resources to aid in breeding is demonstrated by the fact that losing any one of these elements might have varied effects on anthocyanin output.

To develop the best breeding practices for enhancing the desired trait, plant breeders are interested in assessing gene effects (Carvalho *et al.,* 2018). Thus, breeders require knowledge of heritability, heterosis, inbreeding depression, predictability of genetic gain from selection for yield, and the mechanism of gene action (Begna, 2021). Designing a suitable breeding process for genetic improvement requires an understanding of genetic behavior and the type of gene activity that governs nutraceutical properties (Benavente and Giménez, 2021; Manimurugan *et al.,* 2023). Because quantitative characteristics are influenced by interactions between genes as well as between genotype and environment, in addition to the minor individual impacts of numerous genes, the inheritance of these traits has been characterized as a shifting target (Mackay and Anholt, 2024). To study how certain features are inherited, genetic statistical models have been developed. Among these models, generation means analysis is a helpful method for estimating the effects of genes, variance components, and heritability-regulating characteristics of interest (Mather and Jinks, 1982; Kearsey and Pooni, 1996). It provides information on the relative significance of dominance deviations, effects from non-allelic genetic interactions, and the average impact of genes (additive effects) in determining the genotypic values of individuals and, in turn, the mean genotypic values of families and generations (Said, 2014). A helpful method for estimating the impact of genes on quantitative variables, such as yield and yield components, is generation mean analysis. Estimating the three different forms of epistatic gene effects—additive × additive, additive × dominance, and dominance × dominance—has several advantages (Pujar *et al.,* 2022). This approach facilitates understanding of the performance of selected parents and the potential of their progeny for use in pedigree selection or heterosis exploitation (Abd El-Aty *et al.,* 2023). Numerous studies have documented the genetic pathways that control maize production, yield characteristics, and agronomic qualities (Zhang *et al.,* 2021; Baye *et al.,* 2022; Dong *et al.,* 2023). On the other hand, data on the amounts of anthocyanins in maize cobs and grains are quite rare. This research was conducted to evaluate the relative importance of additive and non-additive gene effects in regulating the inheritance of anthocyanin and its derivatives in purple maize grain and cob.

3.3. Materials and Methods

3.3.1. Plant material and site description

This study utilized two native genotypes of maize (purple and white) gathered from farmers in Ciudad del Maiz, San Luis Potosi, and Hualahuises, Nuevo Leon, Mexico. The experiment was conducted at the experimental field of the Facultad de Agronomia, Universidad Autonoma de Nuevo Leon, in Marin, Mexico (located at 24°19'16.71"N and 99°54'58.06" W).

3.3.2. Field management

The original native populations, Morado San Luis Potosi (purple grain and cob) and Blanco Hualahuises (white grain and cob), served as the genetic basis for the creation of backcrosses (BC_{P1}, BC_{P2}), F_1 hybrids, and inbred lines. The parents were the inbred lines of purple maize (P_1) and white maize (P_2). Both parents were crossed to develop the F_1 generation, which was then self-pollinated to produce the F_2 generation. The F_1 seeds were subsequently backcrossed with both parental lines to generate backcrosses (BC_{P1} and BC_{P2}). Therefore, in March 2025, the six populations were set up in the field using a random complete block design with three replications. The genetic homogeneity of each generation determined the size of the experimental units. The experimental units were two rows for the non-segregating generations (P_1, P_2, and F_1) and five rows for the F_2 generation. Four rows were utilized for backcrosses.

The row length was 5 m, with 0.8 m and 0.25 m between rows and plants, respectively. For the parents, F_1, and backcross generations, adjacent plants in each plot were manually pollinated to prevent contamination from stray pollen. For the F_2 generation, individual self-pollination was performed. When they reached maturity, cobs were hand-picked. Maize ears with a moisture level of less than 14% were allowed to air dry. Before being ground into whole-grain flour and cob powder, samples of cob from the parental and F_1 generations were first hand-shelled into grain and cob. Samples were then bulked within replications in each generation. Additionally, the F_2 and first backcross cob samples were hand-shelled and ground into a fine powder. Before examination, all ground materials were thoroughly mixed, sieved through a 30-mesh screen, and stored at -20 °C.

3.3.3. Data collection

3.3.3.1. Extraction of total phenols

The extraction of total phenols was done using the protocol of Rodríguez-Salinas *et al.* (2020), which consisted of weighing 200 mg of maize flour into a test tube. Then, 3 mL of 80 % methanol (80:20 methanol: water v/v) was added, and the mixture was purged with a nitrogen flow for 30 s. It was then shaken for 1 h under light protection on a stirrer plate (Corning, 6795-220, Mexico) at 200 rpm. Finally, it was centrifuged (HERMLE Labortechnik GmbH, Z400k, Germany) at 6000 rpm for 5 min. The supernatant was recovered and stored at -20 °C under light protection until further analysis.

3.3.3.2. Total phenolic content

A colorimetric technique based on the Folin-Ciocalteau reagent reaction was employed to measure the total phenolic content, following the methodology of Rodríguez-Salinas *et al*. (2020). After oxidizing with 0.2 mL of Folin-Ciocalteau reagent and neutralizing with 2 mL of a 7 % Na_2CO_3 solution for 5 min, 2 mL of the phenolic extract was combined with 2.6 mL of distilled water. The samples' absorbance at 750 nm was ultimately measured when the process was halted after 90 min. The results were expressed as milligrams of gallic acid equivalent per 100 grams of sample, using

0, 40, 80, 120, 160, and 200 mg L^{-1} of gallic acid as a reference for the calibration curve (mg GAE 100 g^{-1}).

3.3.3.3. Total monomeric anthocyanin content

3.3.3.3.1. Sample extraction

Anthocyanins extraction was performed using the protocol of Lao and Giusti (2016) with some modifications. A 200 mg sample of powdered maize grains was taken, and 10 mL of 70 % aqueous acetone acidified with 0.01% (v/v) 6-N HCl was added. The samples were purged with nitrogen at 4 °C for 30 seconds and stirred for 30 min. The solution was filtered using Whatman No. 4 filter paper. 10 mL of chloroform was added, and the samples were allowed to stand overnight. The colored upper phase was collected the next day and placed on a rotary evaporator at 40 °C under vacuum to remove any remaining acetone. Finally, the remaining extract was topped up to the known volume with water acidified with 0.01 % HCl.

3.3.3.3.2. Quantification by the pH differential method

For the pH differential method, a protocol by Yang and Zhai (2010) was followed. In this method, the absorbance of purple maize pigments diluted in buffer at pH = 1.0 (0.025 M potassium chloride) and pH = 4.5 (0.4 M sodium acetate) was measured at a wavelength of maximum absorbance (around 520 nm) and 700 nm using a DLAB SP-UV1100 spectrophotometer (DLAB Scientific, Beining, China). The total monomeric anthocyanin was calculated using the molecular weight of cyanidin-3-glucoside (449.2) and its molecular absorptivity of 26,900 in an aqueous buffer solution. The measurement was performed in triplicate. The anthocyanin content of each sample was calculated using the following equation:

$$C(mg/kg) = \frac{A\ x\ MW\ x\ DF\ x\ V\ x\ 1000}{\varepsilon\ x\ l\ x\ Sample\ weight}$$

Where,

C = Anthocyanin concentration.

A = (A520nm – A700nm) pH 1.0 – (A520nm – A700nm) pH 4.5.

MW = Molecular weight of cyanidin 3-glucoside (449 g M^{-1}).

DF = Dilution factor.
V = Final Volume.
ε = Molar absorptivity of cyanidin 3-glucoside (25,965 $cm^{-1}M^{-1}$).
l = 1 cm path length

3.3.3.4. Quantification of anthocyanins by HPLC

Anthocyanins (Cyanidin-3-glucoside) quantification was performed based on the methodology described by Rodríguez-Salinas *et al.* (2020), on an Agilent Technologies 1260 Infinity HPLC with an Agilent 1260 diode array detector (DAD) (G4212B) and an Agilent 1260 quaternary pump (G1311B), with a ZORBAX Eclipse Plus C-18 reversed-phase analytical column (100 mm x 3 mm i.d., 5 µm). The mobile phase was 4.5 % acidified water with formic acid (solvent A) and acetonitrile (solvent B). The gradient used was as follows: 0-1 min, 97 % A and 3 % B; 1-51 min, 60 % A and 40 % B; 51-53 min, 50 % A and 50 % B; 53-60 min, 97 % A and 3 % B. The post-run time was 5 min. The flow rate used was 0.8 mL min^{-1} with a 50 µL injection, and the wavelength was monitored at 520 nm. The spectrum of the compounds was obtained in the UV region (200-600 nm). Cyanidin-3-glucoside in the samples was identified by comparing its relative retention time with that of the standard compound.

3.3.4. Statistical Analysis

This study employed a randomized complete block design. The normality of the repeated data was evaluated by the Shapiro-Wilk test. Following the normal distribution, the data were then submitted to a two-way analysis of variance (ANOVA) using Statistix 10 software (Analytical Software, FL, USA). The mean comparison was determined using the Tukey test ($p \leq 0.05$).

Generation mean analysis (GMA) was carried out individually for each trait to identify the type of gene action influencing their expression. To assess the suitability of the additive-dominance model and determine the existence of epistatic effects, scaling tests A, B, C, and D were employed, as outlined by Hayman and Mather (1995). Epistasis was present if one or more of the scales were significant. The genetic parameters, namely mean [m], additive gene effects [a], dominance gene effects [d], and three types of non-allelic gene interactions, namely additive x additive [aa], additive x dominance [ad], and dominance x dominance [dd], were thus estimated using the six-parameter genetic model proposed by Mather and Jinks (1982). The significance of the above genetic parameters was tested using the t-test. First, the standard error was worked out for each component separately by taking the square root of the variance of the respective element. The significance of the genetic effect was tested similarly using the t-test as in the case of the scaling test. All computations for the generation mean analysis were conducted using the Microsoft Excel software.

3.4. Results

The analysis of variance (ANOVA) revealed that the impact of different generations on all parameters studied was statistically significant ($p \leq 0.05$), as shown in Table 3.1.

Table 3.1. Mean squares of different maize generations for total phenolic content (TPC), monomeric anthocyanin content (MAC), and cyanidin 3 glucoside (C3G) in grains and cobs.

Variables	Mean Square (Grains)	Error	C.V (%)	Mean Square (Cob)	Error	C.V (%)
TPC	1827.89*	16.63	5.47	1150.03*	2.04	2.42
MAC	3905.63*	1.63	2.21	2463.83*	1.90	3.11
C3G	297.71*	0.18	2.96	91.01*	0.02	1.93

*Note: * indicates significance levels at p ≤ 0.05.*

The maximum total phenolic content of 115.41 ± 2.46 in grain and 89.71 ± 2.34 in cob was recorded in purple maize. In contrast, the white maize genotype had the minimum total phenolic content of 54.74 ± 2.85 and 36.08 ± 2.91 in grain and cob, respectively (Figure 3.1a, 2a). The average total phenolic content for the F_1 generation of maize grain and cob was 91.31 ± 2.64 and 70.41 ± 3.88 mg GAE $100g^{-1}$ DW, respectively, representing increases of 7 % and 11 % over the mid-parent values. In contrast, the average total phenolic content for the F_2 generation of maize grain and cob, measured at 64.38 ± 8.51 and 54.84 ± 9.25 mg GAE $100g^{-1}$ DW, respectively, represented reductions of 32 % and 15 % compared to the mid-parent averages. The average values for the backcrosses were either situated between those of the F_1 generation and the first backcross generation with a white parent, or they fell below the averages of the F_1 and F_2 generations for both grain and cob. Conversely, the backcross that included the F_1 and the first backcross generation with a purple parent surpassed the average values of the F_2 generation for both grain and cob (Table 3.2). These findings suggest that dominance deviations have a lesser influence. In contrast, additive effects have a more significant impact on the total phenolic content in the purple maize grain and cob.

The maximum total monomeric anthocyanin content of 103.53 ± 2.02 in grain and 82.83 ± 2.94 in cob was recorded in purple maize; meanwhile, the white maize genotype had the minimum total monomeric anthocyanin content of 0.39 ± 0.58 and 0.22 ± 0.58 in grain and cob, respectively (Figure 3.1b, 3.2b). The average total monomeric anthocyanin content in the F_1 generation of maize grain (85.83 ± 3.67 mg CGE $100g^{-1}$ DW) exceeded the mid-parent value by 39 %; similarly, the maize cob (62.01 ± 2.65 mg CGE 100 g^{-1} DW) was 33 % higher than the mid-parent figure. In the F_2 generation, the average total monomeric anthocyanin content for maize grain and cob (48.43 ± 8.53 and 39.35 ± 8.76 mg CGE $100g^{-1}$ DW, respectively) showed a 7 % and 6 % decrease compared to the mid-parent values. The means of the backcrosses fell between those of the F_1 and the recurrent parents or were lower than the means for both the F_1 and F_2 generations in terms of grain and cob (Table 3.2). These findings suggest that the effects of additive variance and dominance deviations on total monomeric anthocyanin content in purple maize grain and cob vary in their relative significance.

The maximum cyanidin-3-glucoside of 30.92 ± 1.43 in grain and 16.35 ± 1.59 in cob was recorded in purple maize (Figure 3.1c, 3.2c). The average concentration of cyanidin-3-glucoside in the F_1 generation of maize grain (16.38 ± 1.21 mg $100g^{-1}$ DW) was 6% greater than the mid-parent value, while in the maize cob (11.37 ± 2.02 mg $100g^{-1}$ DW), it was 28 % above the mid-parent value. For the F_2 generation, the average cyanidin-3-glucoside in maize grain (12.96 ± 5.25 mg $100g^{-1}$ DW) was 19 % lower than the mid-parent value; likewise, in the maize cob (7.56 ± 3.75 mg $100g^{-1}$ DW), it was 8

% lower than the mid-parent value. The means of both backcrosses fell between the values of the F_1 and the recurrent parents or were lower than the means recorded for the F_1 and F_2 generations in both grain and cob (Table 3.2).

Table 3.2. Mean values and standard errors of six developed generations for total phenolic content, monomeric anthocyanin content, and cyanidin-3-glucoside.

Generations	TPC	MAC	C3G
Grain			
P_1	115.41 ± 2.46	103.53 ± 2.02	30.92 ± 1.43
P_2	54.74 ± 2.85	0.39 ± 0.58	0.00 ± 0.00
F_1	91.31 ± 2.64	85.83 ± 3.67	16.38 ± 1.21
F_2	64.38 ± 8.51	48.43 ± 8.53	12.96 ± 5.25
BC_{P1}	71.48 ± 7.82	64.68 ± 10.14	13.78 ± 6.45
BC_{P2}	50.54 ± 8.82	43.64 ± 7.82	11.26 ± 5.82
MPV	**85.08**	**51.96**	**15.46**
Cob			
P_1	89.71 ± 2.34	82.83 ± 2.94	16.35 ± 1.59
P_2	36.08 ± 2.91	0.22 ± 0.58	0.00 ± 0.00
F_1	70.41 ± 3.88	62.01 ± 2.65	11.37 ± 2.02
F_2	54.84 ± 9.25	39.35 ± 8.76	7.56 ± 3.75
BC_{P1}	61.74 ± 7.18	53.10 ± 7.52	6.98 ± 3.13
BC_{P2}	41.88 ± 9.18	28.05 ± 7.78	5.70 ± 3.36
MPV	**62.89**	**41.52**	**8.18**

Note: P_1= Morado San Luis Potosí; P_2= Blanco Hualahuises; F_1= First filial generation of crossing between parents; F_2= Second filial generation of crossing; BC_{P1}= First backcross between F_1 and P_1; BC_{P2}= First backcross between F_1 and P_2; TPC= Total phenolic content; MAC= Monomeric anthocyanin content; C3G= Cyanidin-3-Glucoside; MPV= Mean parent value. Values within the same column sharing different letters are indicated as significantly different ($p \leq 0.05$).

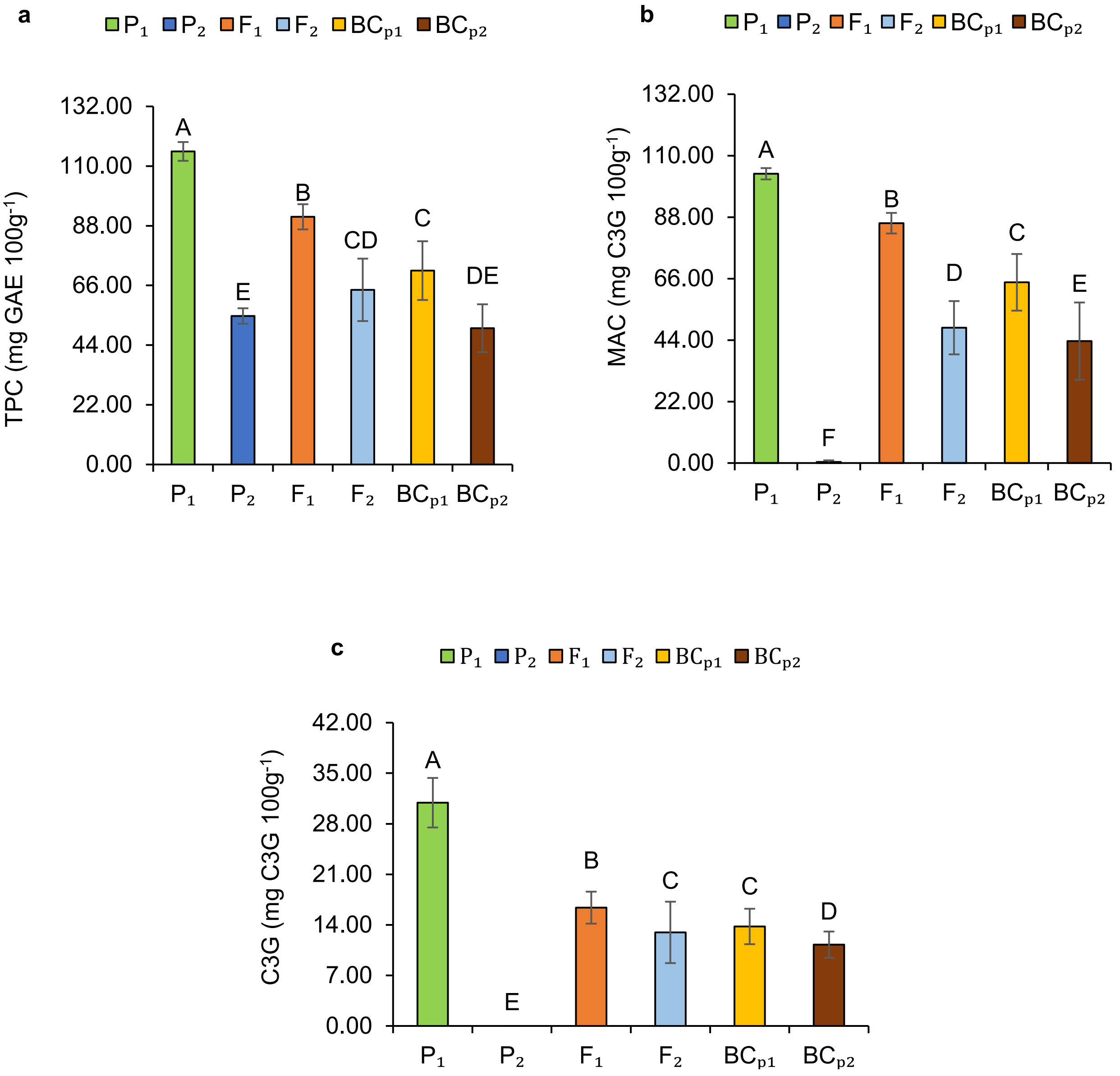


Figure 3.1. Mean values of studied traits in the maize grain of six developed generations (P_1, P_2, F_1, F_2, BC_{P1}, BC_{P2}). a) Total phenolic content (mg GAE $100g^{-1}$); b) Total monomeric anthocyanin content (mg C3GE $100g^{-1}$) and c) Cyanidin-3-glucoside (mg C3GE $100g^{-1}$). The different letters on the bars show significant differences ($p \leq 0.05$). The P_2 generation recorded a value of 0.00 ± 0.00 for cyanidin-3-glucoside (C3G).

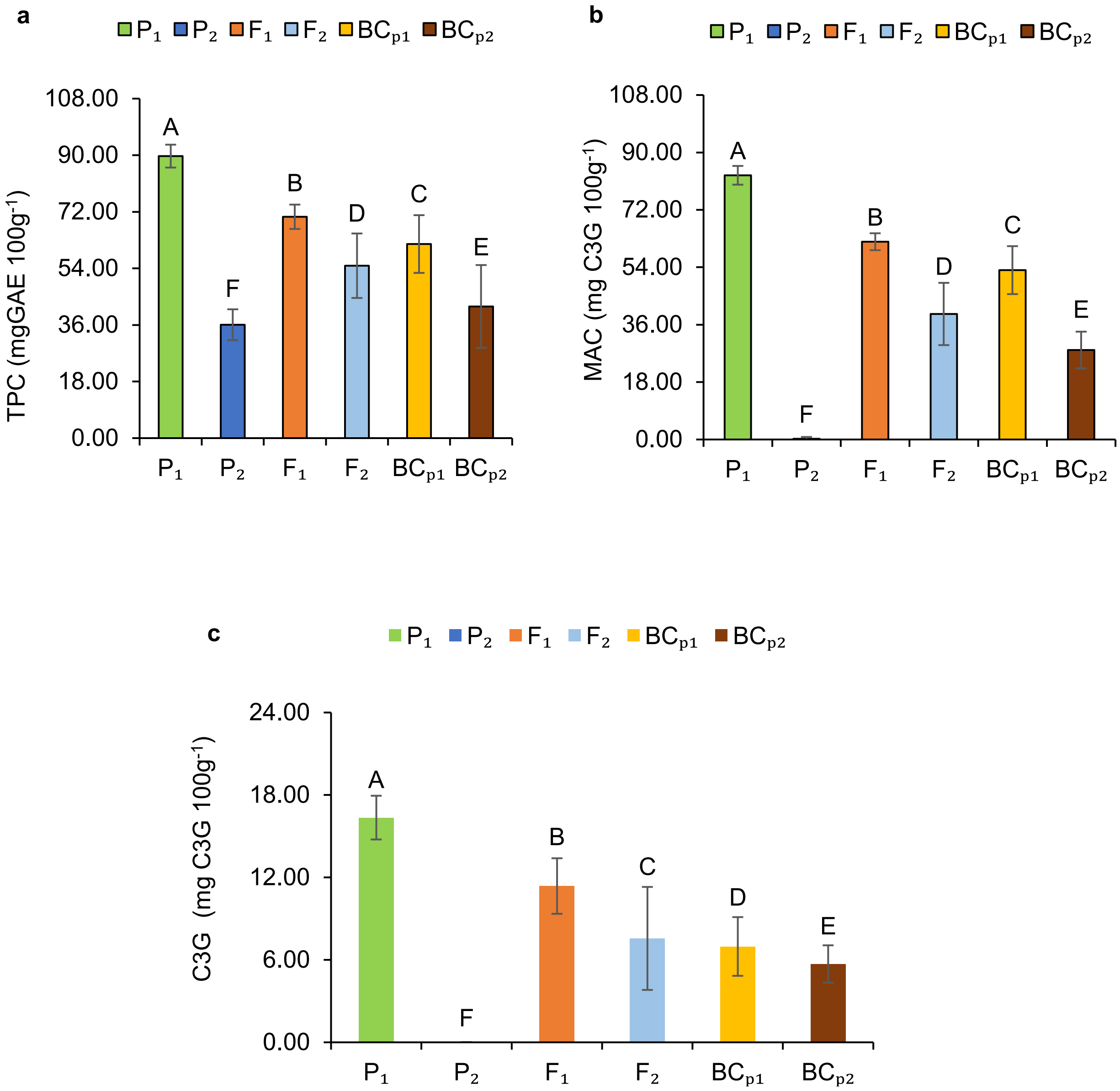


Figure 3.2. Mean values of studied traits in the maize cob of six developed generations (P_1, P_2, F_1, F_2, BC_{P1}, BC_{P2}). a) Total phenolic content (mg GAE $100g^{-1}$); b) Total monomeric anthocyanin content (mg C3GE $100g^{-1}$) and c) Cyanidin-3-glucoside (mg C3GE $100g^{-1}$). The different letters on the bars show significant differences ($p \leq 0.05$). The P_2 generation recorded a value of 0.00 ± 0.00 for cyanidin-3-glucoside (C3G).

3.4.1. Genetic Effects

In the F_1 generation, utilizing purple-colored grains and cobs from a female parent and white-colored grains and cobs from a male parent resulted in progeny that all exhibited purple-colored grains and cobs. This outcome implies that the inheritance of purple maize coloration is primarily maternal. The color of the grains and cobs in the F_2 generation varied from white to purple, displaying various intensities, which made it challenging to classify them into specific categories. The observed segregation pattern did not align with a single-gene or two-gene model, but rather displayed continuous variation, indicating that it follows a quantitative inheritance pattern. Additionally, the grain color within backcross generations leaned towards one of the parents. This suggested that the changes (whether an increase or a decrease) in color tendency depend on the selection of the P_1 parent.

There is a strong interest in breeding maize with higher levels of anthocyanins due to their potent antioxidant properties and associated health benefits. To enhance anthocyanin levels through traditional hybrid breeding approaches, it is essential to comprehend the genetic influences associated with anthocyanins. One implication of the varying gene influences on selecting a breeding methodology is that the resulting hybrid, from crossing with a high parent, is anticipated to increase anthocyanin levels in maize due to prevailing additive gene effects. The model was expanded to include six parameters, demonstrating a good fit for the data.

The inheritance of various traits was significantly influenced by additive [a], dominance [d], and epistasis, highlighting the importance of both additive and dominance effects (Table 3.3). For total phenolic content in grain, the significant gene effects included additive [a] (21.23 ± 2.22), dominance [d] (46.91 ± 8.18), additive × additive [aa] (-14.07 ± 7.90), additive × dominance [ad] (-9.10 ± 8.82), and dominance × dominance [dd] (525.35 ± 11.82). In the case of cob, the significant gene effects were additive [a] (19.87 ± 2.44), dominance [d] (34.46 ± 8.65), and dominance × dominance [dd] (406.51 ± 13.12). Regarding monomeric anthocyanin content, all gene effects, including additive [a], dominance [d], and the three epistatic effects, were significant for total monomeric anthocyanin content in cob. In contrast, the additive x additive [aa] gene effect was found to be non-significant in grain.

Notable epistatic gene influences were detected for all traits in both maize grain and cob, although to varying extents (Table 3.3). For cyanidin-3-glucoside in the maize grain, all three types of epistatic effects—additive × additive [aa] (-12.95 ± 7.08), additive × dominance [ad] (-3.01 ± 8.12), and dominance × dominance [dd] (105.47 ± 10.49)—were significant. In contrast, in the maize cob, the dominance × dominance [dd] [64.23 ± 11.58] epistatic effect was the most prominent.

Table 3.3. Estimates of different gene effects with standard errors for total phenolic content (TPC), total monomeric anthocyanin content (MAC), and cyanidin-3-glucoside (C3G) in maize grain and cob.

Parameters	TPC	MAC	C3G
Grain			
M	53.65 ± 3.64*	19.37 ± 2.64*	14.64 ± 1.43*
[a]	21.23 ± 2.22*	21.04 ± 2.56*	2.51 ± 2.08*
[d]	46.91 ± 8.18*	-57.19 ± 8.58*	-0.82 ± 7.23*
[aa]	-14.07 ± 7.90*	N.S	-12.95 ± 7.08*
[ad]	-9.10± 8.82*	-30.53 ± 6.76*	-3.01 ± 6.12*
[dd]	525.35 ± 11.82*	385.13 ± 12.88*	105.47 ± 10.49*
Cob			
M	43.48 ± 4.63*	10.82 ± 1.56*	7.56 ± 1.54*
[a]	19.87 ± 2.44*	25.05 ± 2.41*	1.28 ± 2.42*
[d]	31.46 ± 8.65*	-25.60 ± 7.95*	-1.69 ± 7.88*
[aa]	N.S	4.90 ± 7.87*	N.S
[ad]	N.S	-16.25 ± 9.57*	N.S
[dd]	406.51 ± 13.12*	264.29 ± 11.69*	64.23 ± 11.58*

*Note: m: mean effect; [a]: additive effect; [d]: dominance effect; [aa]: additive x additive effect; [ad]: additive x dominance effect; [dd]: dominance x dominance effect. * Significance from zero at $p \leq 0.05$. N.S: non-significant.*

3.4.2. Identification of anthocyanins by HPLC-DAD

In the analysis of anthocyanin extraction and purification, chromatograms recorded at 520 nm revealed the presence of six glycosylated anthocyanins in the grain and cob of the pigmented maize genotype, as shown in Figure 3.3. These compounds were quantified and expressed in mg C3GE 100g^{-1} DW for genotype Morado San Luis Potosi based on the peak area (Table 3.4) as well as for each generation in both grain and cob (Table 3.5).

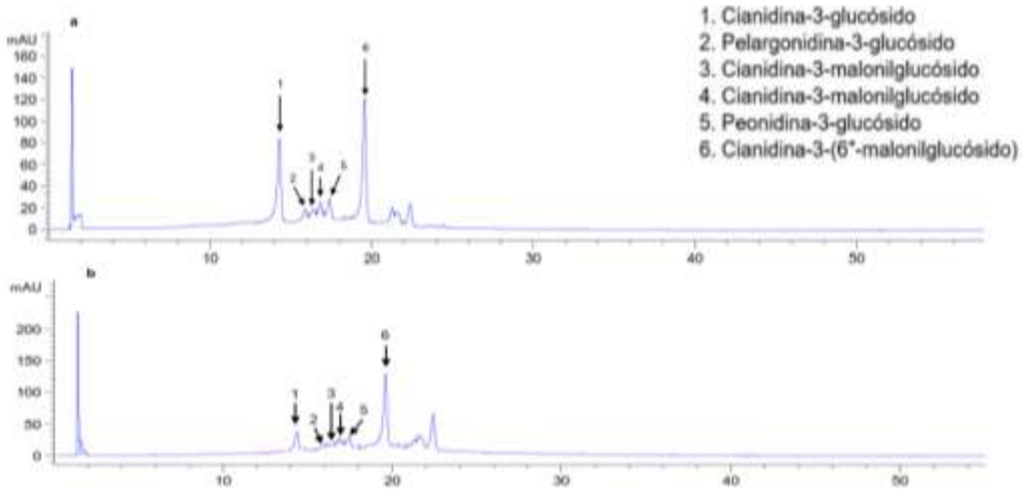


Figure 3.3: HPLC-DAD chromatogram of genotype Morado San Luis Potosi (P_1) with six identified anthocyanins present at a wavelength of 520nm. a) maize grain; b) maize cob.

Table 3.4. Anthocyanins detected in HPLC-DAD in genotype Morado San Luis Potosi (P_1) with quantification expressed in mg C3GE $100g^{-1}$ DW.

Peak	Compound	Peak area	Quantification
Grain			
1	Cyanidin-3-glucoside (C3G)	1680.43	30.92
2	Pelargonidin-3-glucoside (Pg3G)	193.19	8.13
3	Cyanidin-3-malonylglucoside (C3MG)	135.65	7.25
4	Cyanidin-3-malonylglucoside (C3GM)	149.47	7.46
5	Peonidin-3-glucoside (Pn3G)	152.64	7.51
6	Cyanidin-3-(6”-malonylglucoside) (C3-6’MG)	1695.1	31.14
Total		**4006.48**	**92.40**
Cob			
1	Cyanidin-3-glucoside (C3G)	30.92	16.35
2	Pelargonidin-3-glucoside (Pg3G)	8.13	7.07
3	Cyanidin-3-malonylglucoside (C3MG)	7.25	5.53
4	Cyanidin-3-malonylglucoside (C3GM)	7.46	6.64
5	Peonidin-3-glucoside (Pn3G)	7.51	6.46
6	Cyanidin-3-(6”-malonylglucoside) (C3-6’MG)	31.14	10.96
Total		**1436.86**	**53.02**

Table 3.5. Quantification of detected anthocyanins in HPLC-DAD from maize grain and cob in six generations (P_1, P_2, F_1, F_2, BC_{P1}, and BC_{P2}) expressed in mg C3GE 100g^{-1} DW.

Generations	Quantification	Detected anthocyanins
Grain		
P_1	92.40	All
F_1	59.34	All
F_2	42.38	All
BC_{P1}	47.36	All
BC_{P2}	34.62	1-3, 5, and 6
Cob		
P_1	53.02	All
F1	51.63	All
F_2	33.40	1-3, 5, and 6
BC_{P1}	31.08	1, 2, 5, and 6
BC_{P2}	22.41	1,2,5 and 6

Note: 1: Cyanidin-3-glucoside (C3G); 2: Pelargonidin-3-glucoside (Pg3G); 3: Cyanidin-3-malonylglucoside (C3MG); 4: Cyanidin-3-malonylglucoside (C3GM); 5: Peonidin-3-glucoside (Pn3G); 6: Cyanidin-3-(6”-malonylglucoside) (C3-6’MG). The P_2 generation was omitted from the data presentation because it had a value of 0.00 for both grain and cob.

3.4.3. Discussion

The glycosylated anthocyanins observed in the chromatograms of the six generations of maize grain and cob align with the order of detection as noted by Lao and Guisti (2016). The differences in retention times are primarily attributed to adjustments in the methodology and conditions applied, which are influenced by the column length utilized in this research.

The ANOVA indicated significant differences in means for total phenolic content, total monomeric content, and cyanidin-3-glucoside in both grain and cob across all four crosses, suggesting a considerable level of genetic variability for all traits examined among the segregating populations derived from contrasting parental lines. This study observed a downward trend in the average values of all traits assessed in maize grain and cob across the four crosses, except the F_1 generation, where a rise was noted compared to the mid-parent value in both grain and cob. Oladipo and Abe (2022) found a 7 % increase in total phenolic content in the F_1 generation, while a 3 % decrease was noted in the F_2 generation compared to the mid-parent value in maize. Moreover, a separate study by Pfeiffer and Rooney (2016) in sorghum reported increases of 25 % and 15 % in total phenolic content for F_1 and F_2, respectively, compared to the mid-parent value. In contrast to our findings, Noubissié *et al.* (2012) observed a 5 % reduction in mean phenolic content in the F_1 generation relative to the mid-parent value. Previous studies assessed mean values from backcrosses between the F_1 and recurrent parents, which were lower than those of the F_1 and F_2 generation means. Regarding total monomeric anthocyanin content, both grain and cob showed an increase in the F_1 means. A decrease in the F_2 mean is aligned with the findings of Harakotr *et al.* (2016), who noted increases of 38 % and reductions of 30 % in mean total monomeric anthocyanins for F_1 and F_2, respectively, in maize cob, while in maize grain, reductions of 15 % and 80 % were recorded for F_1 and F_2 means, respectively. Our analysis revealed that mean cyanidin-3-glucoside levels in the F_1 generation exceeded the mid-parent value for both grain and cob, whereas a decline was seen in the F_2 generation. Harakotr *et al.* (2016) found decreases of 28 % and 25 % in cyanidin-3-glucoside concentrations in the grain for F_1 and F_2 generations, respectively. For maize cob, they reported an increase of 45 % and a decrease of 18 % in F_1 and F_2 generations, respectively. These findings highlight the differing relative influences of additive effects and dominance deviation on cyanidin-3-glucoside levels in purple maize grain and cob. Additionally, the variances observed in the parents and F_1 generation were minimal, suggesting a consistency in this anthocyanin derivative across these generations.

Epistasis refers to any non-allelic interaction (Philips, 1998; Gaoh *et al.,* 2020). In this context, a thorough understanding of gene actions and interactions can facilitate the selection of breeding strategies that effectively leverage genetic variance, which can also assist in interpreting the influence of breeding systems on crop evolution

(Singh *et al.,* 2024). The presence of notable epistasis can distort the estimation of variance components. For instance, polygenic inheritance models featuring a wide range of allelic interactions can lead to underestimations or overestimations of heritability, primarily of the narrow-sense type, which may further result in additional inaccuracies in predicted gains. Generation mean analysis incorporates several foundational generations from crosses between two inbred lines and provides estimates for epistatic effects. To evaluate these effects, we utilized the six-parameter model proposed by Hayman (1960) in our current study to analyze four crosses. In our results, we observed a higher magnitude of additive effect for monomeric anthocyanin content and cyanidin-3-glucoside in both grain and cob, compared to the dominance, indicating the gene correlation. In other words, we can say that one parent had genes with high performance, whereas the other one had genes with low performance. For the total phenolic content in grain and cob, dominant effects were greater than additive ones, indicating the use of hybrid development to harness heterosis. Significant additive and dominance genetic effects were consistently observed for all examined traits across all four crosses, suggesting that both additive and non-additive genetic effects substantially influence the inheritance of genes related to phenolic content, monomeric anthocyanin content, and cyanidin-3-glucoside in maize grain and cob. Our findings demonstrated that all three epistatic effects were significant for each trait analyzed in grain; however, for cob, the total phenolic content exhibited non-significant additive x additive and additive x dominance interactions, while the additive x additive interaction was non-significant for cyanidin-3-glucoside. Similar findings were documented by Pfeiffer and Rooney (2016), who reported that additive, additive × dominance, and dominance × dominance gene effects significantly contributed to the inheritance of total phenols in black sorghum, whereas, unlike our results, Oladipo and Abe (2022) indicated that all gene effects were non-significant for total phenolic content in maize. Harakotr *et al.* (2016) noted the existence of additive, dominance, and all three epistatic gene effects for cyanidin-3-glucoside in grain and for monomeric anthocyanin content in cob. They found a non-significant additive x additive effect for monomeric anthocyanin content in grain and a non-significant additive x additive and additive x dominance effect for cyanidin-3-glucoside in cob.

It is crucial to recognize that the negative dominance value observed for total monomeric anthocyanin content and cyanidin-3-glucoside in both grain and cob signifies that this gene effect tends to reduce anthocyanin levels, depending upon the selection of the parental line designated as P_1. The sign of the dominance effect correlates with the mean value of the F_1 generation in relation to the mid-parent value, indicating that alleles from the parental line with high anthocyanin content (Morado San Luis Potosí) played a significant role. Similar findings were presented by Harakotr *et al.* (2016). The predominance of dominance [d] and dominance × dominance [dd] epistatic effects for the total phenolic content, total monomeric anthocyanin content, and cyanidin-3-glucoside in both purple maize grain and cob suggests that the expression of these traits is primarily governed by genes exhibiting dominance characteristics. Furthermore, the significance of dominance and its epistatic effects on these traits in maize grain and cob suggests that breeding and selection strategies for purple maize could be adapted to take advantage of this dynamic epistatic effect by postponing selection to subsequent generations, thereby stabilizing additive genes. This breeding strategy can preserve larger populations before selection, maximizing chances for favorable gene combinations to emerge (Khajoane, 2022). The dominance × dominance [dd] epistasis was found to contribute to the levels of anthocyanin and its derivatives, which aligns with expectations, given that the F_1 generation exhibited substantial heterosis. In contrast, additive × additive [aa] and additive × dominance [ad] epistatic effects showed no contribution to heterosis activity. This finding offers valuable insights for maize breeders seeking to enhance populations or hybrids with higher levels of anthocyanin and its derivatives. Duplicate gene interaction in the form of epistatic effects was evident for all traits in both maize grain and cob, displaying contrasting signs for the estimates of dominance [d] and dominance × dominance [dd] effects. The contrary signs between additive [a] and additive × additive epistatic effects for cyanidin-3-glucoside in the maize grain imply that there exists an oppositional nature in the interactions of these traits (Table 3). Epistatic effects and linkage can enhance dominance, leading to partial dominance that may appear as pseudo-overdominance (Liang *et al.,* 2015; Shang *et al.,* 2016). These findings are based on a single growing season and may be subject to some bias due to environmental factors and their interactions, necessitating further research. Nonetheless, prior studies have indicated that genotype represents the primary source of variation in anthocyanin levels in colored maize germplasm (Harakotr *et al.,* 2015).

3.5. Conclusion

In this research, the genetic factors influencing the inheritance of total phenolic content, anthocyanin, and its derivatives were examined in the six fundamental generations resulting from a cross between purple and white maize. To summarize, the majority of additive [a], dominance [d], and interaction effects were found to be significant for all traits analyzed in both the maize grain and cob. These findings highlight the crucial role of additive [a], dominance [d], and epistatic gene actions in governing the inheritance of anthocyanin levels in purple maize. Given the strong presence of nonadditive gene effects found in this study for all traits in the grain and cob, it was concluded that the selection process in maize could be altered to stabilize additive genes by postponing selection until subsequent generations. Furthermore, the most effective method to elevate anthocyanin levels to their maximum potential is to utilize inbred lines with purple grains and cobs as the maternal parent for creating purple-hued hybrid maize. In cases of duplicate epistasis identified in the inheritance of all observed traits, employing a combination of various breeding strategies that ensure the accumulation and stabilization of favorable alleles—such as recurrent selection and selection from early to advanced generations—would be essential for enhancing these traits.

GENERAL CONCLUSION

It has been observed that the induced drought stress during the reproductive stage caused significant damage to the photosystem II, imposing a negative impact on morphological and physiological attributes. The genotypes P-3011w, P-3092, and iku20 used in this study showed varying responses to the imposed drought stress. The iku20 performed better under stress conditions than P-3011w and P-3092. Consequently, we conclude that iku20 might tend to tolerate drought stress in the arid northeast of Mexico.

We observed that the seed thermopriming resulted in reduced physiological damage to the plants caused by drought stress because of increased antioxidant activity in all the genotypes evaluated. However, no significant variations were observed between thermopriming treatments of 40°C and 50°C. Therefore, we concluded that both treatments showed effectiveness in enhancing the tolerance to the drought stress imposed during the reproductive stage in maize.

The study of generation means analysis of phenolic compounds in purple maize grain and cob revealed that all the genetic effects, including additive, dominance, and epistatic interactions, significantly controlled the inheritance of total phenols, total monomeric anthocyanin, and cyanidin-3-gucoside in grain and cob to a varying extent. In total phenolic content, the dominant effects were higher in magnitude than the additive ones in both grain and cob, thus indicating the use of hybrid development to utilize the heterosis, whereas in other variables, additive effects were higher than the dominant ones, indicating the possibility of making selection in the early generations, like recurrent selection. Regarding epistatic interactions, the dominant x dominant interaction was found to be significant in grain and cob in all studied traits, which indicates the use of breeding strategies that prioritize the exploitation of non-additive gene action, particularly through hybrid development to maximize the genetic gain.

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CHAPTER I

Anjum, S. A., Ashraf, U., Tanveer, M., Khan, I., Hussain, S., Shahzad, B., Zohaib, A., Abbas, F., Saleem, M. F., Ali, I. and Wang, L. C. (2017). Drought-induced changes in growth, osmolyte accumulation, and antioxidant metabolism of three maize hybrids. *Frontiers in plant science*, *8*, 69.

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CHAPTER II

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CHAPTER III

Abd El-Aty, M. S., El-Hity, M. A., Abo Sen, T. M., El-Rahaman, I. A. A., Ibrahim, O. M., Al-Farga, A. and El-Tahan, A. M. (2023). Generation Mean Analysis, Heterosis, and Genetic Diversity in Five Egyptian Faba Beans and Their Hybrids. *Sustainability*, *15*(16), 12313.

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ANNEX

Publications

Morphological and Physiological Response of Maize (*Zea mays* L.) to Drought Stress during Reproductive Stage

Saba Yasin, Francisco Zavala-García*, Guillermo Niño-Medina, Pablo Alan Rodríguez-Salinas, Adriana Gutiérrez-Diez, Sugey Ramona Sinagawa-García, Eleazar Lugo-Cruz.

Published: Agronomy (MDPI) (JCR Impact Factor: 3.4)

Physicochemical Effects of Seed Thermo-priming in Improving Drought Tolerance of Maize (*Zea mays* L.)

Saba Yasin, Francisco Zavala-García*, Guillermo Niño-Medina, Pablo Alan Rodríguez-Salinas, Adriana Gutiérrez-Diez, Sugey Ramona Sinagawa-García, Eleazar Lugo-Cruz.

Published: Agricultural Research & Technology: Open Access (JCR Impact Factor: 2.37)

Generation mean analysis of phenolic content in pigmented maize (*Zea mays* L.) grain and cob

Saba Yasin, Francisco Zavala-García*, Guillermo Niño-Medina, Pablo Alan Rodríguez-Salinas, Adriana Gutiérrez-Diez, Sugey Ramona Sinagawa-García, Eleazar Lugo-Cruz.

Published: Agricultural Research & Technology: Open Access (JCR Impact Factor: 2.37).

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Article

Morphological and Physiological Response of Maize (*Zea mays* L.) to Drought Stress during Reproductive Stage

Saba Yasin, Francisco Zavala-García *, Guillermo Niño-Medina, Pablo Alan Rodríguez-Salinas, Adriana Gutiérrez-Diez, Sugey Ramona Sinagawa-García and Eleazar Lugo-Cruz

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Citation: Yasin, S.; Zavala-García, F.; Niño-Medina, G.; Rodríguez-Salinas, P.A.; Gutiérrez-Díez, A.; Sinagawa-García, S.R.; Lugo-Cruz, E. Morphological and Physiological Response of Maize (*Zea mays* L.) to Drought Stress during Reproductive Stage. *Agronomy* **2024**, *14*, 1718. https://doi.org/10.3390/agronomy14081718

Academic Editor: Rong Zhou

Received: 9 July 2024
Revised: 27 July 2024
Accepted: 29 July 2024
Published: 5 August 2024

Abstract: Maize is among the most significant crops in the world regarding production and yield, but it is highly sensitive to drought, which reduces the growth, photosynthetic efficiency, grain quality, and yield production of a plant. Quantum yield efficiency of photosystem II is a critical photosynthetic component that is susceptible to drought stress. This study intended to investigate the effects of drought stress on growth and morpho-physiological parameters using three maize hybrids ('P-3011w', 'P-3092' and 'iku20') with contrasting soil moisture contents (100%, 40%) at the pre-flowering stage. The stress treatment (40%) was initiated at stage V7, for a period of 15 days; the experimental units were established in a completely randomized design with split-plot arrangement along with three repetitions in 42 L pots using a substrate of peat moss, black soil and poultry manure (1:2:1). The morphological, growth-related and physiological parameters were assessed, including chlorophyll fluorescence (Fv/Fm), which was measured using a LiCor-6400-40 fluorometer. The results showed that all morphological, growth-related and physiological variables decreased under drought stress during the reproductive stage, with the exception of leaf temperature and intercellular CO_2 concentration, which increased by 12% and 54%, respectively. Drought stress significantly reduced the photosynthetic chlorophyll fluorescence (43%), due to damage to photosystem II. The lowest percentage of damage to photosystem II (34%) was observed in the iku20 genotype. In contrast, P-3011w and P-3092 had the highest levels of significantly similar damage (49% and 46%, respectively). The correlation analysis showed a highly positive interaction of chlorophyll fluorescence (Fv/Fm) with net photosynthetic rate and stomatal conductance under drought conditions, and multiple regression analysis revealed that the maximum effect on net photosynthetic rate under drought was due to the damage it caused to photosystem II. Thus, iku20 might have a tendency to be able to withstand drought stress in the dry northeast region of Mexico. Overall, we concluded that the photosystem II was negatively impacted by drought stress thus causing a reduction in all physiological, morphological and growth-related variables.

Keywords: *Zea mays*; drought stress; photosystem II; chlorophyll florescence; photosynthesis; regression analysis; instantaneous water-use efficiency

1. Introduction

Maize is among the most extensively cultivated crops globally grown in tropical, subtropical and temperate regions [1]. After rice and wheat, it was the third-most significant crop in the world with a 1222.07 million metric ton production on a cultivated area of 201.98 million hectares [2]. Maize is mostly consumed by the people in Mexico in a form of variety of tejuino, pinole, tortillas, tamales, tostadas, atole and tortilla chips [3]. In 2022, the consumption of maize amounted to 1740 million bushels in Mexico [2]. In the northeastern

Agricultural Research & Technology
Open Access Journal
ISSN: 2471-6774

Juniper Publishers
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Research Article
Volume 29 Issue 3 - August 2025
DOI: 10.19080/ARTOAJ.2025.29.556449

Agri Res & Tech: Open Access J

Physicochemical Effects of Seed Thermo-priming in Improving Drought Tolerance of Maize (*Zea mays* L.)

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Submission: July 27, 2025; **Published**: August 13, 2025

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Abstract

Extreme temperatures and water deficits pose a significant threat to crop growth and food security in changing climates. Maize, a widely distributed crop, is susceptible to water deficiency. Seed priming, a low-cost and sustainable technology, can enhance drought tolerance, potentially improving crop productivity and food security. The current study aimed to investigate the impacts of seed thermopriming at physiological and biochemical levels under drought stress during the reproductive stage using three maize genotypes (Red, White, and P-3057w). The experiment was split into six treatments (control, drought, thermopriming at 40 °C, thermopriming at 40 °C + drought, thermopriming at 50 °C, and thermopriming at 50 °C + drought). Drought stress was induced at the pre-tasseling stage by withholding irrigation for 20 days in a split-plot under a completely randomized design. Our results showed that the drought stress significantly reduced photosynthetic rate (51%), stomatal conductance (65%), transpiration (69%), cell membrane injury (55%) chlorophyll a (85%), chlorophyll b (74%), total chlorophyll (81%) and carotenoids (66%) while increasing anthocyanins (7%), phenols (8%), DPPH (6%) and ABTS (11%) activity in non-primed seeds whereas thermopriming at 40oC and 50oC reduced significantly the negative effects of drought on photosynthesis (18%; 21%), stomatal conductance (16%), transpiration (15%; 16%) and cell membrane injury (36%; 37%) through increased anthocyanin content (25%; 24%), total phenols (30%; 29%), DPPH (10%; 11%) and ABTS activity (16%; 17%). However, there were no significant differences between thermopriming at 40 °C and 50 °C. Consequently, both thermopriming treatments were found to be effective for increasing the drought stress tolerance during the reproductive stage in maize.

Keywords: Anthocyanins; Cross stress tolerance; Photosynthesis; Reproductive stage

Introduction

The world's population is expected to increase significantly by 2050. As a result, it is believed that there will not be enough land available to cultivate cereal crops and meet the growing global demand for food. The loss of agricultural land as a result of climate change, the increase in biotic and abiotic stressors, and the acceleration of global climate change are some of the primary challenges facing agriculture and food production [1]. Drought, heat, salinity, and cold are examples of environmental stressors that can hinder crop quality and yield. Global climate change is predicted to increase the frequency and severity of severe weather events, such as extreme temperatures and decreased precipitation [2]. In the final decade of the 20th century, the average global temperature increased by 0.3 °C, and by 2100, it is expected to have increased by nearly 3 °C [3]. Two of the most prevalent abiotic stressors that can impact crop growth and productivity, and ultimately food security in a changing climate, are extreme heat and water scarcity [4]. Additionally, they have the potential to significantly alter the composition of the germplasm [5,6].

Maize (*Zea mays* L.), the most widely distributed crop in the world, is grown in temperate, tropical, and subtropical regions [7]. It ranks as the third most significant crop globally after rice and wheat, with a production of 1241.56 million metric tons over 208.23 million hectares of cultivated land [8]. Maize is more susceptible to drought than crops like sorghum, resulting in a 66% decrease in maize yield and a 33% decrease in sorghum yield [9].

Drought stress is known to be the most harmful abiotic stressor to crops, affecting the growth, development, and production of a wide range of crops. It changes the physiology, biochemistry, and morphology of a plant [10]. Plant photosynthesis is severely

Agricultural Research & Technology
Open Access Journal
ISSN: 2471-6774

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Research Article
Volume 29 Issue 3 - October 2025
DOI: 10.19080/ARTOAJ.2025.29.556455

Agri Res & Tech: Open Access J

Generation Mean Analysis of Phenolic Content in Pigmented Maize (*Zea mays* L.) Grain and Cob

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Submission: September 20, 2025; **Published:** October 06, 2025

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Abstract

Pigmented maize is rich in phenolic compounds, carotenoids, and anthocyanins, which are linked to health-promoting and nutraceutical properties. Consequently, this type of grain is categorized as a functional food due to its high antioxidant activity and its potential preventive roles against cancer, diabetes, obesity, and neurodegenerative diseases. The numerous health advantages of anthocyanins in pigmented maize have recently drawn more attention from consumers. A better understanding of how genes influence anthocyanins is crucial for breeding initiatives aimed at increasing antioxidant levels. This study aimed to analyze the genetic influence on anthocyanin levels in the grain and cob of purple maize utilizing generation mean analysis, for which six generations (P_1, P_2, F_1, F_2, BCP_1, and BCP_2) were developed and planted in a randomized complete block design with three replications at the experimental station of the Facultad de Agronomía, Universidad Autónoma de Nuevo León in Marín, Mexico. A six-parameter model was used to estimate the genetic effects controlling the inheritance of anthocyanins, revealing the significance of all genetic effects for the studied traits in grain and cob, up to varying extents. Additive gene effects appeared to have a greater contribution in the inheritance of total monomeric anthocyanins and cyanidin-3-glucoside in grain and cob, indicating the possibility of making an early generation or recurrent selection, whereas dominant effects had a major contribution to total phenolic content in grain and cob. Moreover, among epistatic effects, the dominance x dominance gene interaction was important for all parameters in grain and cob, suggesting that the selection should focus on a strategy harnessing dominance, such as hybrid development.

Keywords: Additive and Dominance Effects; Epistatic Gene Effects; Cyanidin-3-glucoside; Monomeric Anthocyanins

Introduction

Mexico is the original center for the domestication and diversification of maize, maintaining a broad array of grain colors and traits, including growth speed, height of both plants and ears, physiological attributes, chemical makeup of grains, nutritional benefits, and various uses [1,2]. Consequently, the diversity of maize is primarily found among indigenous communities or ethnolinguistic groups [3]. There is a research bias concerning maize due to its commercial significance, with most investigations on maize grain composition concentrating on yellow and white varieties [4]; studies focusing on pigmented maize have only recently begun to receive attention over the last ten years [5].

Pigmented maize is rich in phenolic compounds, carotenoids, and anthocyanins, which are linked to health-promoting and nutraceutical properties. Consequently, this type of grain is categorized as a functional food due to its high antioxidant activity and its potential preventive roles against cancer, diabetes, obesity, and neurodegenerative diseases [6,7]. Although lutein, zeaxanthin, and α and β cryptoxanthin are the most frequently studied carotenoids in yellow maize [8], cyanidin-3-glucoside (C3G), cyanidin-3,5-diglucoside, pelargonidin, and peonidin-3-glucoside, along with their malonyl derivatives, are the main anthocyanins present in blue, red, and purple grains [9]. Anthocyanins, a kind of naturally occurring phenolic phytochemical found in many food sources, particularly fruits and vegetables, which have a well-established presence in the diet, are produced via the flavonoid pathway in plant tissues [10]. These compounds serve as pigments that display a spectrum of colors, including