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Evaluating the impacts of waterlogging stress on cowpea (Vigna unguiculata L.) growth traits and physiological performance

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Evaluating the impacts of waterlogging stress on cowpea (Vigna unguiculata L.) growth traits

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The progressive increase in the global population and the rapidly changing climate have put unprecedented pressure on crop production. Cowpea is one of the world's most important leguminous crops, contributing to food security and environmental sustainability. However, cowpea productivity is limited due to waterlogging stress. The main objective of this study was to explore physiological and biochemical mechanisms to understand how cowpea genotypes respond to waterlogging stress. Four studies were conducted in controlled and field conditions to achieve these objectives. Study 1 characterized the waterlogging tolerance of 30 cowpea genotypes in a controlled environment using 24 morphophysiological parameters with waterlogging tolerance coefficients and multivariate analysis methods. 10% of the genotypes exhibited high tolerance to waterlogging stress, and the genotypes UCR 369 and EpicSelect.4 were identified as the most and least waterlogging tolerant, respectively. Study 2 evaluated the key parameters influencing carbon fixation of UCR 369 and EpicSelect.4 at the reproductive stage. The less tolerant EpicSelect.4 experienced high downregulation of stomatal and non-stomatal limiting factors during waterlogging and recovery, resulting in decreased carbon assimilation rates. UCR 369 rapidly developed adventitious roots, maintained biomass, and

restored pigments and metabolites to sustain photosynthesis. A two-year field experiment was conducted in study 3 to quantify the effects of waterlogging on the yields, physiology, and biochemistry of cowpeas at different growth stages. The most apparent impact of waterlogging stress occurred at the reproductive stage, followed by the vegetative and maturity growth stages. Studies suggest that diverse cowpea genotypes have distinct physiological and biochemical mechanisms in response to waterlogging stress. In addition, the tolerant genotypes and traits identified herein can be used in genetic engineering and cowpea breeding programs that integrate increased yield with waterlogging stress tolerance

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CHAPTER I

GENERAL INTRODUCTION

Global food production will need to increase substantially by 2050 to meet the projected

demand from population growth and a more affluent population (Ray et al., 2019). In addition,

changing climate will sustainably increase the global food supply, the most critical issue facing

the world's population in the coming decades. As a result of climate change, abiotic stress events

such as drought, flood, and extreme temperatures have become more prevalent over the past few

decades, limiting agricultural productivity (Asseng et al., 2009). Among the various abiotic

stresses, waterlogging caused by excessive precipitation/irrigation and poor soil drainage is noted

to cause significant reductions in the yield of many crops (Hirabayashi et al., 2013). Recent

evidence suggests waterlogging affects over 16% of the world's cultivated land (Ploschuk et al.,

2018), and over 17 km^2 of the land surface is prone to flooding (Voesenek and Sasidharan,

2013). In the U.S., financial losses in crop production due to waterlogging stress were

approximately $3.75 billion in 2019 and $114 billion in total from 1995-2019 (Duffin, 2020).

Furthermore, with the rapid climate change, heavy precipitation events are projected to increase

by about 7% for every 1°C increase in global warming, leading to increased flood hazard

severity (high confidence) (IPCC, 2022). Hence, there is an increasing need to understand the

mechanisms of crop tolerance to waterlogging.

Cowpea, commonly referred to as black-eyed pea or southern pea, is a versatile crop

because it provides nutrition to humans, livestock, soil, and other crops (da Silva et al., 2018). A

large portion of high-quality cowpea is grown for dry bean, canning, and fresh markets in the

U.S. Cowpea seeds and pods contain approximately 25% of high-quality protein content in the

form of amino acids, carbohydrates, folic acid, chlorophyll, carotenoids, phenolics, and essential

minerals (Carvalho et al., 2019). It is widely adapted and grown in about sixty-five countries that

cut across six continents, particularly in the arid zone of tropical and sub-tropical countries

(Singh, 2014). The estimated global area for cowpea production is approximately 15.1 million

ha, with an annual output of 8.9 million tons and an average yield of 591 kg/ha (FAOSTAT,

2022). However, the global average yield in the farmer's field is below the optimum potential of

cowpea yield, estimated at 6000 kg ha^{-1} (Kamara et al., 2018). Even more than that, the FAO

reported a decline in cowpea by 19% between 2012 and 2017 despite cowpea's inherent

resilience to cope with the water deficit (FAOSTAT, 2019). Additionally, the demand for

cowpea is projected to increase by 2.68% annually instead of a 2.55% yield increase in the next

decade (Boukar et al., 2016). Unbalanced demand and yields for cowpea have been attributed to

harsh environmental conditions in many parts of the world (Omomowo and Babalola, 2021),

including seasonal flooding during winter and spring in the Lower Mississippi Alluvial Valley

(LMRAV).

The majority of cowpea produced in the southern U.S. are grown in the LMRAV, which

covers seven states, including Illinois, Missouri, Kentucky, Tennessee, Arkansas, Mississippi,

and Louisiana, before reaching the Gulf of Mexico (Kyaw, 2022). Yield loss due to waterlogging

is common in LMRAV due to heavy clay, poor surface drainage in areas with little slope, poor

soil structure, heavy rains, and cropping systems (Cornelious, 2004; Walne and Reddy, 2021).

Moreover, like many other legumes, cowpeas are considered sensitive to waterlogging; when the

soil water content is 2-3 cm above the soil level, the growth and development of cowpeas are

hampered (Minchin and Summerfield, 1976; Umaharan et al., 1997). Previous studies have found that depending on the growth stage, stress duration, soil type, and genotype, waterlogging stress can reduce grain yield by more than 50% in cowpeas (Hong et al., 1977; Minchin et al., 1978). For example, waterlogging in the vegetative growth period of cowpea can reduce the yield by 10% to 52%, and in the reproductive period, the yield can be reduced by 10% to 90% (Minchin et al., 1978; Timsina et al., 1994). The sensitivity of the reproductivity stage to waterlogging has been documented in other related crops, including soybeans (Scott et al., 1989), mung beans (Ahmed et al., 2002), and common beans (Costa et al., 2020). In these studies, yield losses due to excess water were due to reductions in root growth, nodulation, nitrogen fixation, photosynthesis, biomass accumulation, and stomatal conductance, as well as plant death due to stress severity and depth. Therefore, improving the tolerance of cowpea in waterlogging-prone environments to increase yield is crucial for future cowpea research.

Genetic improvement of cowpea genotypes adapted to waterlogging stress is an important strategy to address the challenge of yield reduction. However, compared with other legumes, the waterlogging stress tolerance of cowpea is understudied (Figure 1.1). It is also important to note that there has been little research on the effect of waterlogging stress on cowpea in the last 25 years compared to other abiotic stresses like drought and temperature stress (Figure 1.2). To improve waterlogging tolerance, it is first necessary to understand how waterlogging stress affects the growth and development of crops (Pan et al., 2021; Tian et al., 2021). Once the mechanism is understood, yields can be improved by modifying management practices, such as adjusting planting dates, cover cropping, surface drainage, raised beds, adaptive nutrient application, or hormonal/chemical treatments applied during sensitive growth stages. In addition, identifying desirable traits in cowpea to resist waterlogging can impact global

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productivity through selection, improvement, and the introduction of tolerant traits into elite lines.

Waterlogging Studies on Major Legumes (1971-2021)

	1971 - 1980	1981 - 1990	1991 - 2000	2001 - 2010	2011 -2021
Cowpea	3	5	4	1	3
Soybean	3	10	26	59	188
Common bean	7	14	9	15	42
Chickpea	0	3	9	6	10
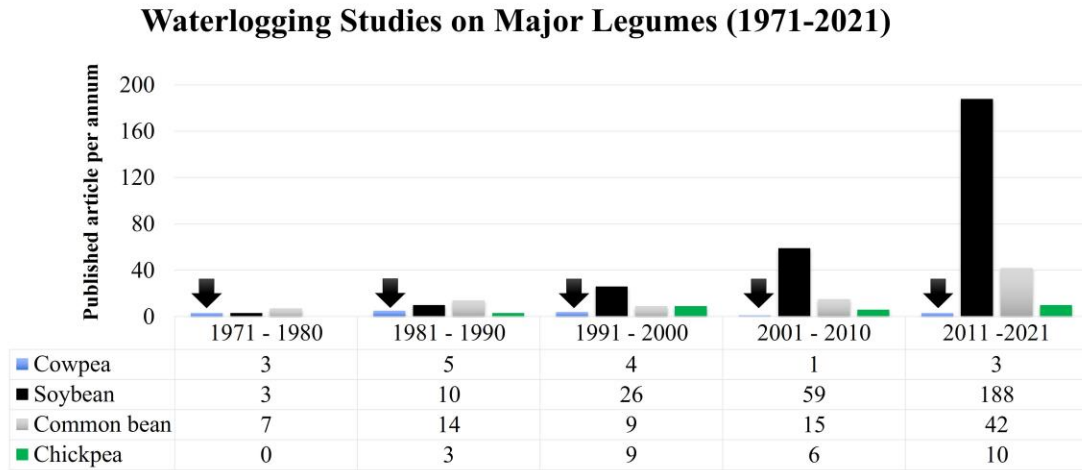
Figure 1.1   Annual publication rate for manuscripts dealing with waterlogging stress on important legumes (Data obtained from Scopus and Google Scholar)

Studies on Impacts of Abiotic Stress on Cowpeas 1971-2021

	1971 - 1980	1981 - 1990	1991 - 2000	2001 - 2010	2011 -2021
Waterlogging	3	5	4	1	3
Drought	7	39	52	86	202
Heat	10	18	25	32	62
Cold	2	7	11	14	19
Salinity	3	11	11	36	113
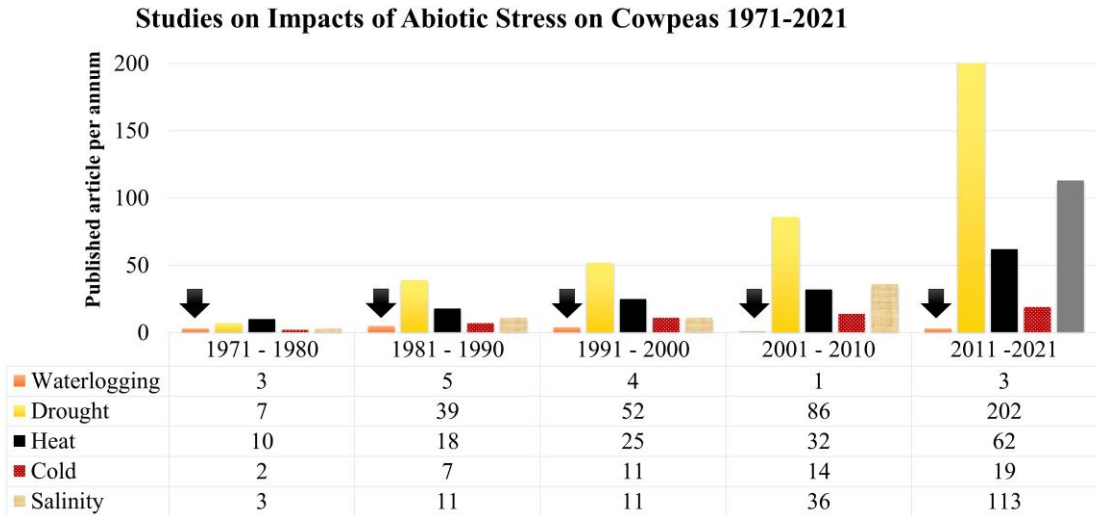
Figure 1.2   Annual publication rate for manuscripts dealing with common abiotic stress in cowpeas (Data obtained from Scopus and Google Scholar)

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1.1 Overall Goal and Specific Objectives

The overall goal of this study was to explore physiological and biochemical mechanisms to understand how cowpea genotypes respond to waterlogging stress. Specifically, this research was focused on the following.

- screen diverse cowpea genotypes for waterlogging tolerance at the vegetative stages using morphological and physiological traits,

- identify waterlogging-tolerant cowpea genotypes and early developmental traits associated with waterlogging tolerance,

- evaluate key parameters influencing carbon fixation of tolerant and sensitive cowpea genotypes under waterlogging and recovery at the reproductive growth stage,

- investigate the impact of waterlogging stress on cowpea genotypes at different growth stages through dynamic changes in growth, physiology, and metabolism,

- quantify differences in yield components and seed quality parameters between contrasting cowpea genotypes under waterlogging at different growth stages.

CHAPTER II

LITERATURE REVIEW

This chapter is written as a review of the current literature on the response of cowpeas to waterlogging stress. However, some parts of this review were collected from studies focusing on waterlogging stress of important legumes (such as soybeans (*Glycine max*), common beans (*Phaseolus vulgaris*), and chickpeas (*Cicer arietinum*), etc.) to provide information lacking in cowpea research. This chapter is divided into three parts that review the (1) Origin, classification, production, and importance of cowpeas, (2) Production constraints related to cowpeas with a specific focus on abiotic stress, and (3) Morphological, physiological, and biochemical response of cowpeas to waterlogging stress.

2.1   Origin, domestication, and diversity of cowpea

Cowpea (*Vigna unguiculata* (L.) Walp.), generally referred to as black-eyed peas or southern peas, is a vital leguminous crop with a noteworthy contribution to alleviating hunger, poverty, and health risks worldwide. Relative to other staple crops, cowpea is a versatile crop because it provides nutrients for humans, livestock, soil, and other crops (da Silva et al., 2018). In the U.S, a large portion of high-quality cowpea is grown for dry beans, canning, and fresh markets. Its seeds and pods contain approximately 25% of high-quality protein in amino acids, carbohydrates, folic acid, chlorophyll, carotenoids, phenolics, and essential minerals (Boukar et al., 2011; Carvalho et al., 2019). As a leguminous crop, cowpea fixes atmospheric nitrogen through Rhizobium symbiosis to improve soil fertility (Ehlers and Hall, 1996). For instance,

cowpea can fix 70-350 kg of nitrogen per hectare after harvest and contribute 40-80 kg/ha to the soil (Quin, 1995). These benefits make cowpea an excellent crop for studying the physiological, morphological, and biochemical basis of waterlogging stress tolerance in global food security and climate change.

Cowpea is perhaps the most antiquated human food source. It was first domesticated in prehistoric times (1700 to 1500 BCE) in tropical regions of Africa and was known in Sanskritic times (150 BCE) in Egypt, Arabia, and India (Rachie and Roberts, 1974). Many studies have speculated about the center of origin and domestication of cowpea. For instance, India and Egypt are independent centers of cowpea origin because these countries have diverse forms of cowpea types (Vavilov, 1951). Even so, recent evidence shows that there are no wild cowpeas in India that can be viewed as potential progenitors of cowpea (Singh, 2014). Simultaneously, many studies have noted different wild ancestral forms of cowpea, including some very primitive species, such as *Vigna unguiculata,* as far back as 2000 BCE, in the southern African region encompassing Namibia, Botswana, Zambia, Zimbabwe, Mozambique, Swaziland, and South Africa (Singh, 2014). This substantial evidence has made several authors conclude that the Southern African region is the primary center of cowpea origin. Around 2000 to 3500 years ago, cowpea moved to other African countries and Asia (Allen, 1983). In addition, West Africa was argued to be the epicenter of cultivated cowpea diversity (Ng and Marechal, 1985). This claim was supported by the discovery of the oldest archaeological evidence in the rock bunker at Kintampo in central Ghana using carbon dating of wild cowpeas (Agbicodo et al., 2009; Flight et al., 1976). The savannah region of Nigeria, southern Niger, parts of Burkina Faso, northern Benin, Togo, and the northwestern part of Cameroon are examples of West African countries. Another argument supporting this assertion could be based on the large area of land that is

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apportioned for cowpea production in these countries (Table 2.1). Therefore, given the current distribution of the different cowpeas worldwide, Western Africa is the secondary center of diversity (Fery, 1990). Later, around 300 BC, early travelers and explorers brought cowpeas from Africa to Asia (Steele and Mehra, 1980).

Interestingly, genetic variability and selection happened over time in the Indian subcontinent, making this region the third center of diversity. Even more than that, South Asia and Southeast Asia's climatic conditions were appropriate for cowpea growth and development (Singh, 2014). Moreover, in the 8th century BCE, cowpea was brought from Asia into Europe (particularly southern Europe) (Tosti and Negri, 2002).

Cowpea was brought to the U.S from Africa via Jamaica by slave traders around 1675 (Singh, 2014). The cowpeas carried to the U.S for food by Africans spread across the West Indies based on their rapid adaptability to the warm climate of the tropics and its high food source. From the West Indies, it was taken to Florida in 1700 and expanded in production in the Central and southern regions of the U.S, where it is utilized as fodder, cover crops, and food crops (Singh, 2014). Notably, most cowpea genotypes are more strongly associated with accessions from Asia or southern Europe than West Africa (Fang et al., 2007). In addition, the name "cowpea" is of American origin, first appearing in print in 1798 (Singh, 2014). Hence, cowpea got its name because it is an essential feed source for cows in the southeastern United States and other parts of the world (Timko et al., 2007).

2.2   Taxonomy and Botanical Classification of Cowpea

Cowpea is a dicotyledonous plant belonging to Fabales, Fabaceae family, Faboideae subfamily, Phaseoleae tribe, Phaseolinae subtribe, Catiang family, and *Vigna* genus (Verdcourt, 1970). *Vigna* is a pantropical genus with several species varying between 84 and 184 (Singh et

al., 1997). Cultivated cowpeas are classified as *Vigna* and are subdivided into four cultivar

groups, namely *Biflora* (or *cylindrical*), *textilis*, *sesquipedalis* (yard-long-bean), and *unguiculata*

(or *melanophthalmus*) (Singh, 2014). Importantly, each of these cultivar groups is

morphologically different from one another. For instance, the cultigroup *unguiculata* is the most

widely cultivated cowpea with the most germplasm and is characterized by a variety of plant and

leaf types; 10-30 cm long, thick pods, and seeds 5-12 mm long (Agbicodo et al., 2009; Boukar et

al., 2015). *Unguiculata* also includes the blackeye (e.g., California blackeye, Pinkeye Purple

Hull, Coronet, etc.), crowder (e.g., Knuckle Purple Hull, Mississippi Silver, Colossus, etc.), and

cream (e.g., White Acre and Texas Cream) seed-types that are commonly grown in the U.S

(Fery, 1990). *Biflora* are mainly grown in India and parts of Asia for fodder, vegetable pods, and

dried seed for pulse (Singh, 2014). They are distinguished by relatively small, smooth seeds in

shorter pods (7.5-13 cm long), which stand upright until maturity (Boukar et al., 2015; Singh,

2014). Cultivar group *textilis* is now scarce, and production in West Africa is declining rapidly.

They are traditionally grown along the banks of rivers Niger and Benue in Nigeria, where long

peduncles are made for fishing lines and as a source of fiber (Singh, 2014). Finally, the

sesquipedialis group (called the yardlong bean, long bean, asparagus bean, or snake bean) has

been widely developed in Asia to provide extremely long (40-100 cm) green pods for use as

mung beans (Agbicodo et al., 2009).

2.3   Cowpea Production

Cowpeas are widely adapted and grown throughout the globe. They are cultivated in

about 65 countries that cut across the six continents, particularly in the arid regions of tropical

and sub-tropical countries, covering a wide range of latitudes from 44°N to 35°S worldwide

(Gómez, 2004; Singh, 2020). Current data shows that the cultivated area of cowpeas is about

15.1 million hectares, and the world's cowpea production in 2020 was 8.9 million tonnes (FAOSTAT, 2022). The bulk of the world's production of cowpea is produced in Africa, especially West Africa, where the crop is often intercropped with maize, sorghum, cassava, or cotton but is sometimes grown as a single crop (Langyintuo et al., 2003; Timko et al., 2007). In 2020, the African continents contributed about 97% of world production (Figure 2.1). Of these, Nigeria produced the largest amount of cowpea grains in 2020, about 3.65 million tonnes, covering an area of 4.7 million hectares (Table 2.1). Other important producers were the Republic of Niger and Burkina Faso, with a production of 2.63 million and 666,023 tonnes, respectively (Table 2.1). Another important country for cowpea cultivation is Brazil (data not provided by FAO), which produces 700,000 tonnes per year on about 1.9 million hectares (Singh, 2014). Although cowpea production in Africa contributes the most substantial acreage to world production, the grain yields of cowpea in Africa are lower when compared to countries in other continents, such as Serbia, Yemen, Peru, and the U.S (Table 2.1). Low yields in Africa can be attributed to low-input cropping systems, inadequate technology, and the use of traditional varieties.

Table 2.1 The top 20 cowpea-producing countries in the world are based on FAO (2022) data.

Rank	Country	Production (tons)	Area (ha)	Yield (kg/ha)
1	Nigeria	3,647,115	4,695,849	777
2	Niger	2,637,486	5,723,820	461
3	Burkina Faso	666,023	1,376,717	484
4	Kenya	264,160	239,131	1,105
5	Senegal	253,897	289,895	876
6	Ghana	204,607	150,126	1,363
7	Mali	199,763	406,698	491
8	Cameroon	177,717	225,086	790
9	Sudan	148,205	853,088	174
10	Tanzania	139,555	126,987	1,099
11	Myanmar	111,345	119,673	930
12	Mozambique	92,439	349,589	264
13	Congo	70,983	159,681	445
14	Malawi	56,524	117,132	483
15	Yemen	55,940	23,105	2,421
16	Haiti	30,402	41,684	729
17	Madagascar	22,576	24,132	936
18	United States of America	17,980	7,650	2,350
19	Peru	16,514	11,956	1,381
20	Serbia	16,033	4,736	3,385
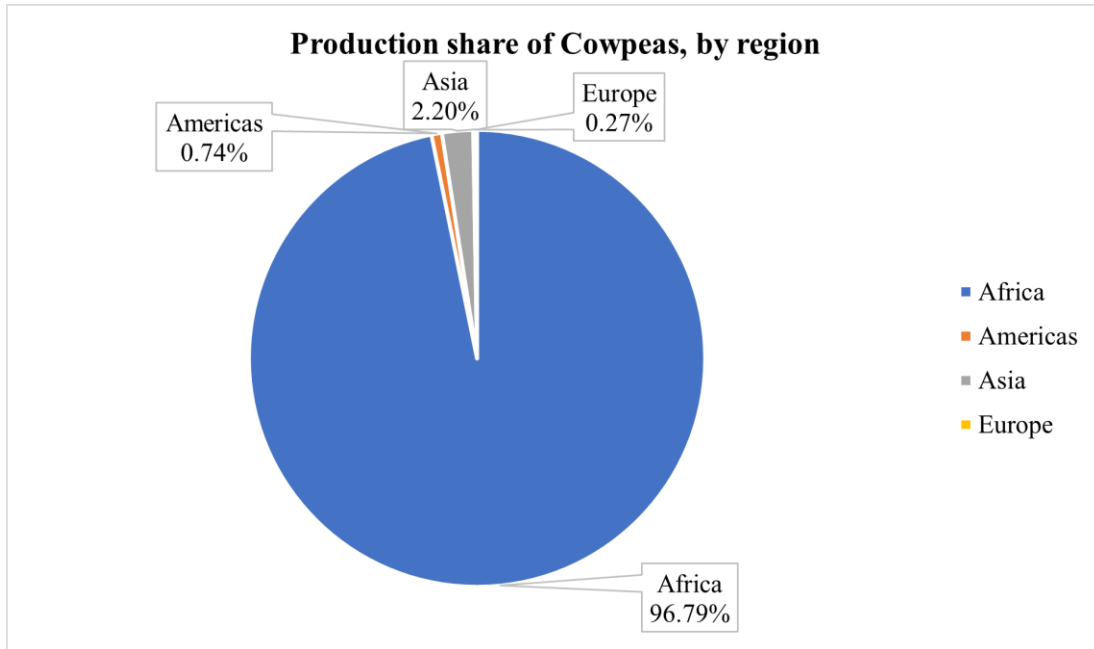
Figure 2.1   Production share of cowpeas by region in 2020 based on FAO (2022) data.

2.4   Importance of Cowpea

In this context, the global cultivation of cowpea is attributed to its capacity to deliver nutritional, agronomic, environmental, and economic benefits. Cowpeas are used for a variety of purposes, including food provisions for humans, feed for livestock, and nitrogen for soil fertility. Most parts of the cowpea plant are edible for humans: the young leaves and green pods are eaten as vegetables and green beans, while the seeds are eaten fresh, and when fully ripened and dried, they are eaten as pulses. Previous research has shown that cowpea plays a vital role in the lives of millions of people in Africa and other parts of the developing world (Sariah, 2010). Hence, cowpeas are a great source of dietary protein and can be used as a healthy supplement to low-protein grains and tubers (Agbicodo et al., 2009; Timko and Singh, 2008). In developed countries, cowpeas are also considered a healthy alternative to soybeans, as consumers want traditional foods that are low in fat, high in fiber, and have health benefits (Timko and Singh, 2008). Health benefits include components that maintain blood cholesterol levels, regulate blood

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sugar levels, aid in wealth loss planning, and help treat conditions associated with cardiovascular and pancreatic disease (Jayathilake et al., 2018).

Moreover, the early developmental qualities of some cowpea varieties provide a major harvest faster than most other crops during the production period. Early harvesting of cowpeas is a fundamental part of hunger-fighting techniques in Africa, where subsistence farmers experience food shortages a few months before the maturity of new crops (Timko et al., 2007). Based on its high protein content, cowpea grain is a significant component of children's food during the weaning period (Lambot, 2002).

Furthermore, cowpeas are regarded as a critical element in the crop-livestock systems because of the significant role their foliage plays as fodder in livestock's diet during the dry season. The mature pods are harvested, while the leaves and vines are cut into small bales. These bales are stored on the roof and used as a feed supplement during the dry season. On a dry weight basis, the harvest of cowpea hulls can provide more than 50% of the price of grains (Agbicodo et al., 2009). It is also a valuable and trustworthy commodity that can generate income for farmers and traders (Langyintuo et al., 2003; Singh, 2014). In the U.S., cowpeas are commonly grown in California and Texas and are used for wildlife forage. Deer selectively graze cowpea because of their high nutritional value, especially in the summer when other browse forms are not as readily available (Redmon and Rouquette Jr, 2000).

In addition, cowpea is an integral part of sustainable cropping systems in subtropical and arid regions of most parts of the world. It can be used as a rotational crop in many areas due to its ability to restore soil fertility (Carsky et al., 2002; Tarawali et al., 2002; Sanginga et al., 2003). Cowpea can improve soil fertility by converting atmospheric nitrogen (N_2) into usable nitrogen, which plays a vital role in biological nitrogen fixation (BNF) (da Silva et al., 2018). BNF occurs

in unique plant structures called nodules, formed by the mutualistic relationship between roots and *rhizobacteria*, providing legumes with the potential to satisfy their required plant's N (Udvardi and Poole, 2013). Cowpea can fix 70 to 350 kg of N per hectare and contributes 40 to 80 kg of N/ha to the soil after harvesting (Quin, 1995), particularly in poor soils with a pH as low as 4 to 5, organic matter below 0.2%, and sand content of over 85% (Kolawole et al., 2000; Sanginga et al., 2000). *Bradyrhizobiuim species* is the specific symbiotic nodular bacteria responsible for N formation (Gómez, 2004). In addition to their nitrogen-fixing ability, cowpea contributes to organic matter accumulation and carbon sequestration when used as a cover crop in crop rotation systems (da Silva et al., 2018). The cowpea used as a cover crop protects the soil from the impact of sunlight, rainfall, and wind, with the resultant benefits of controlling soil erosion and increasing water infiltration. Notably, the accumulation of organic matter in the soil contributes to the improvement of soil fertility and soil physical properties.

Cowpeas have high plasticity in various environments and can alleviate farmers' economic difficulties in severe drought and high-temperature conditions (Dadson et al., 2005). The crop can be grown under harsh conditions where other crops cannot grow. However, cowpeas are very sensitive to frost, resulting in limited growth in the far north, where temperatures are cooler (Gómez, 2004; Duke, 2012). Cowpea is more drought-resistant than common bean (Singh, 2014). Drought tolerance is one reason cowpea is an important crop in many tropical and subtropical regions. Mainly because cowpea has physiological and biochemical mechanisms to adapt to water-stressed conditions. These mechanisms include having a long taproot, turning the leaves upward to keep them from getting too hot, and the quick closure of the stomata when the plant is water-deficient (Gómez, 2004). Cowpeas also exhibit early flowering (at least 12 days earlier) to escape terminal drought (Fatokun et al., 2012).

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Moreover, cowpeas are shade-tolerant and can be intercropped with many staple crops, including cereals, root and tubers, cotton, sugarcane, and plantation tree crops (Henriet et al., 1997; Singh et al., 2003). In addition, several cowpea genotypes can tolerate poor soils with aluminum toxicity, acidity (pH 5.5 to 6.5), high alkalinity, and high boron concentrations (Gómez, 2004). Cowpeas are essential to sustainable livelihoods in tropical and marginal arid environments. Due to their rapid growth and surface cover, they are characterized by sandy soils with little rainfall and extremely low organic matter content (Carsky et al., 2001; Mortimore et al., 1997).

2.5   Production Constraints

Globally, cowpea remains one of the most important crops, mainly contributing to food security and environmental sustainability. However, cowpea yields are below 6000 kg/ha (da Silva et al., 2018), particularly in Africa, where the average yield is less than 1000 kg/ha (Table 2.1). This may be due to several biotic (insect pests, diseases, parasitic weeds, nematodes) and abiotic (drought, temperature extremes, waterlogging, low fertility) constraints, which adversely affect the growth and development of crops (Boukar et al., 2016; Chamarthi et al., 2019). The low yield of cowpea can also be attributed to their cultivation in low-input systems: poor soil, minimal fertilizer, no irrigation, etc. Importantly, low-input systems caused crop yields in the U.S to decrease by more than 90% Between 1937 and 1964 (Fery, 1990). Another limiting factor is the cultivation of conventional cultivars, which are predominant in Africa. They grow traditional cultivars with low yields due to their viny growth habits and late maturity (Henriet et al., 1997; Singh et al., 1997).

As mentioned in section 2.3, cowpeas are grown mainly in the southern U.S, especially in Texas and California, where they are prone to various biotic and abiotic stresses. These stresses could lead to a substantial decrease in cowpea production. It is worth noting that the production

of cowpeas in these areas is mainly constrained by biotic stress in the form of insect pests (e.g., lygus bud, cowpea curculio, and aphid) and diseases (e.g., black-eyed cowpea mosaic virus, bacterial blight, root node nematodes, and fusarium wilt). However, the critical stages of cowpea (seedling, flowering, and reproduction) are often prevented from reaching their genetic growth potential due to abiotic stresses such as drought, waterlogging, and extreme temperatures (heat and chilling). These abiotic stresses cause significant reductions in cowpea yield and total biomass (Timko and Singh, 2008). As a result, plant breeders have worked tirelessly to develop improved cowpea varieties to adapt to current and future climates while increasing yields. They are also developing cowpea genotypes resistant to a wide range of abiotic and biotic factors with enhanced agronomic features. The improved cowpea genotype can be applied to various agricultural ecological sites and can be used for agronomic, horticultural, economic, and nutritional purposes. Additionally, improved cowpea cultivars could help equilibrate the imbalance in global cowpea demand (2.68%) and supply (2.55%) (Fatokun et al., 2012; Boukar et al., 2016; Chamarthi et al., 2019).

2.6   Climate Change and Waterlogging Stress

The productivity of crops largely depends on the prevailing environmental conditions resulting from climate change and weather variability. Mainly because climatic parameters (including temperature, rainfall, and many other parameters) are essential factors affecting crop growth, development, and yield (Dawson et al., 2016); thus, any changes in global and regional climates would significantly impact the capacity of the world to produce food to feed the growing population. Many studies have shown higher concentrations of atmospheric carbon dioxide (CO_2), temperature extremes, and extreme weather events as evidence of climate change (Al Jaouni et al., 2018; IPCC, 2014). Global atmospheric CO_2 is rising (419.66 ppm in 2022) and

is projected by climate models to reach between 540 and 970 ppm by the end of this century because of human activities, declining carbon sinks, and natural global cycles (Prentice et al., 2001; Stocker et al., 2013). Recent climate models also predict that due to the increasing rate of CO_2 and other greenhouse gases in the atmosphere, the global temperature may rise by 1.5 °C to 4.5 ⁰C by 2100 (IPCC, 2022). While it is true that rising atmospheric CO_2 may increase crop productivity (especially C3 crops), it is also important to note that other stresses as a result of climate change (such as temperature extremes, heat waves, flooding, and increased incidence of droughts) may hamper the productivity of several crops. The reason for this significant impact is based on the fact that climatic parameters are critical in the photosynthesis, physiological, metabolic, and developmental processes that occur in many crops (Dong et al., 2020; Reddy et al., 1998). Hence, it is expected that the increase in atmospheric CO_2, extreme temperatures, long-term droughts, and floods will affect the growth and development of several crops, including cowpeas.

Climate change variables cause various abiotic stresses by exerting pressure on different plant species, leading to massive losses in global food production (Onyekachi et al., 2019). The primary abiotic environmental parameters that affect crop growth are fluctuations in rainfall distribution (drought and waterlogging), temperature extremes (low-cold and high-heat), elevated CO_2, light (intensity, quality, and duration), chemical factors (heavy metals and pH), soil nutrient content and availability (Taiz et al. 2015). The variation of these abiotic factors beyond their normal range usually has adverse biochemical and physiological effects on crop growth. It more often prevents crops from reaching their optimal genetic potential. Previous studies have also shown abiotic stress to cause a reduction in the productivity of many crops on a regional and global scale (Hall, 2011; Lobell and Gourdji, 2012; Ray et al., 2019). Mantri et al.

(2012) documented abiotic stress to be responsible for more than 70% of global yield loss of major crops, which agrees with the findings of Boyer (1982). Based on comprehensive estimates of climate change and crop yield models, it is predicted that the productivity of major crops such as rice, cowpea, corn, and cowpeas will further decline, which may have a severe impact on food security (Tigchelaar et al., 2018; Waqas et al., 2019). For example, the U.S. National Climate Assessment report (USGCRP, 2018) predicts the middle of this century (2036-2065) that due to climate change, global cereal yield will decrease by 5% to over 25%, below the inferred trend, while legumes in the southern U.S will fall by more than 25%. A corresponding study by Zhao et al. (2017) estimated that for every 1 ⁰C rise in the average growing season temperature, global cowpea production might drop by 6.0%, rice production will drop by 3.2%, corn production will drop by 7.4%, and soybean production will drop by 3.1%. Moreover, only 3.5% of the world's arable land is not constrained by climate change factors in the form of abiotic stress (FAO, 2011). The projected climate assessment shows that abiotic stress factors will affect more than 90% of the land in rural areas (Cramer et al., 2011).

Consequently, most crops cultivated do not usually reach their full genetic potential because their performance (genotypic and phenotypic capacity) is determined by the interaction with the environment in which the crop is grown. Imperatively, when a crop is grown in a particular environmental condition, it is often subjected to a combination of abiotic conditions that may include one or more stresses, such as heat and drought stress, because they depend on each other. The interaction of these factors facilitates a variety of responses in crops based on the crop developmental phase. When the crop is cultivated under ideal environmental conditions, the competition for resources between the different plant organs or developmental phases is minimal (Taiz et al., 2015). However, when the crop is subjected to a sup-optimal environmental

condition, most of the plant phenological stages (e.g., seedling, vegetative phase) would be terminated prematurely. The reproductive stage may also be compromised. Because the plant is yet to reach its full size, resulting in a reduction in crop performance and yield. Studies have also shown that crop performance can be improved by modifying the plant's genetic traits through plant breeding. This involves developing new crop varieties that can withstand abiotic stress conditions while producing yield at its optimum potential. The selection of tolerant cultivars and genetic traits in a population is crucial to developing new cultivars that can adapt to various environmental conditions. This can only be obtained by subjecting the crop of interest to different abiotic stress conditions and determining the responses of various growth and yield-related traits to other stressors, at the same time, evaluating the effect of these abiotic stresses on crop physiological, photosynthetic, and biochemical processes.

Waterlogging poses a greater risk to plant growth among various environmental stressors and has been selected for investigation in this study, primarily because water availability is a critical determinant constraining the photosynthesis, physiological, metabolic, and developmental processes of many crops in the world (Osakabe et al., 2014). Thus, the projected change in global temperature and precipitation, which has been linked to the increasing frequency and intensity of floods, will affect several cowpea growth and development. Also, the results of many climate change prediction scenarios show changes in precipitation distribution patterns. These changes in distribution may result in more precipitation in an area than in history (Walthall et al., 2013). These disruptions also mean that an area may experience extreme rainfall, and cowpeas may experience flood pressure during one of the critical stages of growth. In addition, water is essential for plants because it is the most abundant compound in the cytosol, in which significant biochemical and physiological reactions occur. Its hydraulic properties

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promote cell division and development (Sha Valli Khan et al., 2014). Soil moisture levels and their interactions with plant morpho-physiological factors, including mineral uptake cell division, photosynthesis, respiration, and protein synthesis, further demonstrated the importance of water stress on plant growth. Hence, it is pertinent to understand the mechanisms associated with cowpea's response to waterlogging stress in managing future food production.

2.6.1   Significance of waterlogging stress

Waterlogging stress is one of the most critical environmental stressors limiting crop production. It is ranked second to drought based on its devastating effects on crop yields as it reduces yields by 40-80% (Phukan et al., 2016; Shaw et al., 2013). Recent evidence suggests that over 16% of the world's cultivated land is affected by waterlogging stress (Ploschuk et al., 2018), and over 17 million km^2 of the land surface is prone to flooding (Voesenek and Sasidharan, 2013). In the U.S., Bailey-Serres et al. (2012) reported that financial losses in crop production due to waterlogging stress were estimated to exceed $24 billion in the last decade. According to the National Aeronautics and Space Administration (NASA) climate simulation model, heavy precipitation will increase sharply by 30% by 2030. The loss of crops is estimated to reach $3 billion yearly (Rosenzweig et al., 2002). Furthermore, with the rapid climate change, heavy precipitation events are projected to increase by about 7% for every 1℃ increase in global warming, leading to increased flood hazard severity (high confidence) (IPCC, 2022). Simultaneously, soil containing a high concentration of clay or soil that has been highly compacted due to repeated use of agricultural machinery can have poor drainage, leading to an increase in the occurrence of waterlogging events (Najeeb et al., 2015; Ploschuk et al., 2018). Hence, there is an increasing need to understand the mechanisms of plant tolerance to waterlogging.

Waterlogging is simply described as excessive soil moisture because of high rainfall/irrigation and poor soil drainage. In most academic literature, waterlogging can be depicted as soil flooding, submergence, soil saturation, anoxia, and hypoxia, usually based on the duration, depth, and severity of soil moisture on the field (Ahmed et al., 2012). The term "waterlogging or soil flooding" refers to a situation where only the plant roots are covered with excess soil moisture. Partial flooding is similar to soil flooding but differs because the soil is not fully saturated, emphasizing the depth and degree of the flooding assessed. Submergence, on the other hand, refers to a more severe situation in which both the below and above-ground plant parts are entirely immersed in water. Finally, when part of a plant's shoots and roots are inundated with excess water, it is called partial submergence. Past studies have evaluated plant height relating to the depth of waterlogged soil (Sasidharan et al., 2017).

2.6.2   Impact of waterlogging on soil properties

Waterlogging on agricultural fields induces a series of changes to the soil properties, including reduced oxygen diffusion rate, low light intensity, increased soil acidity, decreased soil nutrients, and increased vulnerability to pests and diseases. Consequently, crops subjected to such impaired environmental conditions tend to experience hypoxia (low oxygen level) or anoxia (zero oxygen level) (Nishiuchi et al., 2012). Under anoxic conditions, gas exchange within the soil pores is altered (10^4 lesser in drained soil), followed by reductions in important soil elements, including NO_3^-, SO_2^-, CO_2, Mn_4^+, and Fe_3^+ (Kaur et al., 2020). The rapid declines in oxygen concentrations and important soil elements are often reflected in the reductions in soil redox potential (Ponnamperuma, 1972). Moreover, in acidic soils, waterlogging increases soil pH due to proton depletion, or decreases soil pH in alkaline soils due to CO_2 accumulation, thereby neutralizing the alkalinity (Sahrawat, 2005). The capacity of the soil pH to change in

waterlogged soil depends on the soil type, microbial population, temperature, organic matter, depth, and duration of waterlogging (Kaur et al., 2020).

Waterlogging also affects nutrient cycling by reducing available NO_3^- in the soil via surface run-offs or erosion, nitrate leaching, and denitrification (Kaur et al., 2020; Zurweller et al., 2015). During nitrification, oxygen breaks down NH_4^+ (toxic to plants) into NO_3^- or NO_2^-, essential for plant growth and development. However, the absence of oxygen in waterlogged soil leads to denitrification, a reversal of nitrification. Under anaerobic conditions caused by waterlogging, denitrifying agents are activated, reducing available soil NO_3^- to molecular N, which can be lost to the atmosphere through soil layers. In addition, waterlogging leads to nitrate leaching, which is the movement of nitrate from the rooting zone of plants. The process of nitrate loss in the soil is more often dependent on other soil factors, including soil structure, temperature, and texture (Cannell et al., 1985). Soil N depletion due to waterlogging may lead to a decrease in available N for plant uptake and assimilation and the quality of surface water and groundwater. Therefore, it is vital to understand how plants respond to waterlogging to prevent its effects on crop growth and yield.

2.6.3   Impact of waterlogging on crops

Plants are obligate aerobes and require molecular oxygen to enable cellular respiration and other essential processes. However, due to waterlogging, plants often suffer from oxygen deprivation by shifting root metabolism from aerobic respiration to anaerobic fermentation, decreasing plant energy by about 37.5% (Gibbs and Greenway, 2003). Under oxygen-deficient conditions, plants experience a series of anatomical, morphological, and physiological changes, such as the development of adventitious roots (Yamauchi et al., 2018), limited gas exchange (Voesenek and Bailey-Serres, 2015), reduced hydraulic conductivity (Tournaire-Roux et al.,

2003), changes in gene expression (Xuewen et al., 2014), and increased oxidative damage (Shabala et al., 2014). Collectively, these changes redirect the energetic resources of the plant and prevent them from reaching their true genetic potential. In addition, anaerobic conditions can adversely affect leaf water potential (Schildwacht, 1989), enzymatic activity (Hasanuzzaman et al., 2017), nutrient absorption and assimilation (Arduini et al., 2019), plant growth and development (Pan et al., 2021), and ultimately lead to a decline in crop yields and mortality.

Previous research has shown that plants respond to waterlogging stress in three stages (Dennis et al., 2000; Wei et al., 2013). The first stage is characterized by reduced signal transduction, including carbohydrate assimilation and photosynthetic product utilization. Second, there is the activation of fermentative pathways. The survival of plants in flooded conditions is largely due to their ability to improve the gas exchange between plants and their surrounding environment and the transport of CO_2 from above ground to lower organs (Pan et al., 2021). In the third phase, there will be morphological and anatomical changes in the roots, stems, or other bud organs, such as the formation of aerenchyma (aerated tissue) and adventitious roots. It should be pointed out that many wetland plants, including rice, have constitutively developed aerated tissues, which are necessary for plants to improve gas transportation and distribution in submerged plant tissues (Mustroph, 2018). Waterlogging-tolerant crops can exhibit specific adaptations, such as the formation of aerenchyma and adventitious roots (Thomas et al., 2005; Yamauchi et al., 2018), but this does not maintain resilience in productivity. Therefore, unraveling the mechanistic responses that lead to waterlogging tolerance in crops is critical to helping current breeding programs to integrate waterlogging tolerance with improved yield.

2.6.4 Cowpea Response to Waterlogging Stress

Cowpea is an important grain legume with inherent resilience to cope with abiotic stress, such as drought and heat stress (Goufo et al., 2017; Hall, 2004; Olorunwa et al., 2021). However, cowpea growth and development are susceptible to waterlogging stress due to their lower ability to absorb nutrients than wetland crops (Hong et al., 1977; Minchin et al., 1978). Waterlogging stress threatens plant growth and development by decreasing soil oxygen levels resulting in hypoxia and anoxia in soil (Fukao and Bailey-Serres, 2004). A hypoxic condition is characterized by anaerobic respiration, which facilitates energy deficits up to 37.5% in plants because oxygen diffusion in waterlogged soil is 10,000 times lower than in well-drained soil (Gibbs and Greenway, 2003). Although studies exploring the response of cowpea to waterlogging are limited, research in related crops (e.g., soybeans, common beans, etc.) has shown that energy deficit significantly reduces the rate of all metabolic processes in plants, adversely affecting growth, development, and yield traits (Garcia et al., 2020; Pampana et al., 2016; Velasco et al., 2019). The extended exposure of cowpeas to the waterlogged condition could significantly reduce the water conductivity of the root system and leaf water potential (Ashraf, 2012). Thus, affecting the absorption of water and nutrients by the plant as well as causing a series of morphological, physiological, and biochemical changes.

2.6.4.1 Physiological response of cowpea to waterlogging stress

Understanding the concept and components of cowpeas' response to waterlogging stress is a key factor in improving the waterlogging resistance of cowpeas. Oxygen plays a crucial role in plant physiological processes; thus, hypoxia affects plant physiological functions and, in turn, various plant processes. Previous studies have shown that waterlogging stress at early vegetative

growth significantly impairs cowpea's morphological and physiological traits, damaging yields (Hong et al., 1977; Olorunwa et al., 2022). For instance, pigeon pea genotypes subjected to 6 days of waterlogged conditions experienced a 42% decrease in relative water content (RWC) (Sairam et al., 2009). RWC is an important plant trait that reflects plants' absolute water content and health status. It is also essential for maintaining the chlorophyll content of plants (Yang et al., 2021). RWC is closely associated with cell size and can demonstrate the equilibrium between the flow of solvents to the leaf and the rate of transpiration in plants. In addition, RWC is an essential indicator for monitoring the physiological response of plants under waterlogging stress because it can reflect the actual condition of crop growth. (Min and Bartholomew, 2005; Yang et al., 2021). It has been proposed that the water state of the plant, rather than the function of the plant, controls crop growth under waterlogging conditions (Kumutha et al., 2009). Hence, those cowpeas that can maintain higher RWC are waterlogging-resistant only because of their superior internal water status. The ability to reduce cell damage caused by excess water and maintain good leaf shape, structure, and function is also an attribute of tolerant crop varieties under waterlogging conditions (Sathi et al., 2022). In agreement, the RWC of waterlogging-sensitive genotypes of mung bean significantly decreased, while tolerant genotypes maintained RWC after nine days of waterlogging stress during the vegetative growth stage (Kumar et al., 2013). However, there are currently no studies investigating the effects of waterlogging stress on the RWC of cowpea genotypes. Therefore, a better understanding of how the RWC of cowpea genotypes responds under flooded conditions can help in breeding programs that integrate abiotic stress resistance with improved yield.

Decreased RWC due to waterlogging stress causes a reduction in cell turgor pressure, resulting in leaf chlorosis and photosynthesis apparatus damage (Sairam et al., 2009). Under

waterlogged conditions, leaf chlorophyll and soluble assimilates are degraded, and the photosynthetic system of plant leaves is severely damaged, affecting carbon assimilation (Garcia et al., 2020; Ren et al., 2016; Zeng et al., 2021). For instance, within the first day of waterlogging treatment, soybean plants' net photosynthesis (A) rate declined rapidly (Garcia et al., 2020). Thus, even in a short time, the significant reduction in A under waterlogging conditions could lead to a decline in plant energy reserves, showing the existence of a common metabolic pattern.

Imperatively, the factors affecting the A of plants are primarily divided into stomatal and non-stomatal limitations. Due to limited oxygen under waterlogging conditions, plants close their stomata to maintain plant water status, causing a decline in stomatal conductance (g_s) and inhibiting the exchange of CO_2 required by the plant's basic processes (Voesenek and Bailey-Serres, 2015). Consequently, a reduction in g_s eventually leads to a corresponding decrease in A and rate of transpiration (E) (Pedersen et al., 2013). These physiological changes have been thoroughly demonstrated in leguminous crops, except for cowpeas. After ten days of waterlogging, the chlorophyll content of soybean leaves decreased by 51%, resulting in a 97% decrease in A during the R3 growth stage (Lapaz et al., 2020). Similarly, the eight days of waterlogging decreased chlorophyll content and A by 34% and 81% in soybeans during the V4 growth stages (Pereira et al., 2020). These physiological changes were also observed in chickpeas (Palta et al., 2010), mung beans (Ahmed et al., 2002), common beans (Velasco et al., 2019), field peas (Ploschuk et al., 2018), and cowpeas (Takele and McDavid, 1994).

Another limitation of underwater photosynthesis is that light decreases exponentially with depth (Colmer et al., 2011). Light penetration in floodwaters is further reduced by dissolved organic matter and suspended particles (Voesenek and Bailey-Serres, 2015). Consequently, the A of submerged terrestrial leaves is significantly lower than that of aerial leaves.

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Furthermore, exposure of cowpeas to waterlogging stress could result in a decline in the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), chloroplast damage, and loss of leaf pigments (Ahmed et al., 2002; Bansal and Srivastava, 2015). Consequently, the physiological processes involved in crop growth and development decline significantly. Alterations of these processes could result in lodging and plant death, negatively impacting cowpea productivity (Minchin et al., 1978; Zhang et al., 2020). In addition, waterlogging stress can promote an imbalance between photochemical activity in the photosystem (PSII) and the photosynthetic electron transport chain, leading to a subsequent decline in A (Shao et al., 2013). This was evident in Velasco et al. (2019). They reported an over 50% decrease in A, g_s, E, chlorophyll, carotenoid, electron transport rate, and actual quantum yield when common beans were submerged for 14 days.

Waterlogging stress can change the dynamics of carbon and nitrogen metabolism in plants, causing soluble sugar and protein content variability. Moreover, there is an increased accumulation of leaf carbohydrates in crop genotypes susceptible to waterlogging stress, like cowpea, due to the anaerobic condition in the root system (Yan et al., 2018). Therefore, limiting sugar transport from the root to the shoot by decreasing sugar availability has detrimental effects on growth and development. Conversely, the loss of photosynthesis pigments may occur because of nitrogen loss in waterlogged soil and afterward result in decreased A and g_s with a declined photochemical capacity of PSII (Herzog et al., 2016; Yan et al., 2018). However, no study has been carried out on cowpea plants.

2.6.4.2 Morphological response of cowpea to waterlogging stress

The growth, development, and survival rate of cowpeas are the main effects of waterlogging stress worldwide. The adverse effects of excess soil water stress on RWC and A

would decrease the above and below-ground traits of the cowpeas. Waterlogging at all levels of cowpea's growth stages can cause plants to respond in various ways to maintain their growth and development using their morphological traits (Umaharan et al., 1997). Especially those related to the leaves, which are the site of photosynthesis. Under water stress, the leaf's area of cowpeas is reduced to maintain the balance between the water absorbed by the root and the water transpired by the leaf (to reduce the transpiration per unit leaf area) (Rivas et al., 2016). The leaf area is an important morphological parameter for evaluating the response of cowpeas to waterlogging stress in the early vegetative stage of growth (Olorunwa et al., 2022). Drought also leads to reduced lateral shoots, decreased number of branches, and reduced leaves per branch, resulting in decreased leaf area. Smaller leaf areas may lead to reduced water absorption and have a detrimental impact on E. Previous studies speculated that the reduction in the leaf area of cowpeas was due to damaged cell expansion in young leaves, leaf germination, reduced leaf size and growth rate, and accelerated leaf senescence (Hong et al., 1977; Olorunwa et al., 2022).

Cowpea damage caused by waterlogging includes chlorosis, necrosis, growth retardation, leaf loss, reduced nitrogen fixation, and plant death, resulting in yield loss (Ahmed et al., 2012; Minchin et al., 1978; Timsina et al., 1994). All these symptoms occur in several vegetative and reproductive stages of the plant. In the vegetative stages of growth, only two days of flooding significantly caused a yield loss of 18%, while in the reproductive stages of soybean propagation, this loss may exceed 26% (Minchin et al., 1978; Scott et al., 1989).

Generally, the number of leaves in cowpeas subjected to waterlogging stress is significantly reduced due to the reduced rate of node emergence and accelerated leaf senescence. The reduced number of leaves in waterlogged cowpeas may be due to insufficient energy that inhibits plant growth processes such as photosynthesis, cell size changes, and division. In this

way, the emergence of leaves is reduced and promotes leaf senescence and shedding under waterlogging, while those on the control plants remain green and continue to exist (Hong et al., 1977; Olorunwa et al., 2022).

In addition, reduced plant biomass followed a similar trend with leaf number under waterlogging, especially at the early vegetative stage (Olorunwa et al., 2022). For example, eight days of waterlogging decreased leaf biomass in cowpeas by 36%, compared to the control plants (Hong et al., 1977). Carbohydrate assimilation in plants and altered pod development may alter this abscission response, resulting in less nutrient availability for flowers and young pods (Dennis et al., 2000; Pan et al., 2021). Similarly, moderate waterlogging significantly improved the biomass accumulation and growth of tolerant cowpea genotypes, while severe waterlogging considerably reduced the leaf area and plant biomass of sensitive cowpea genotypes (Takele and McDavid, 1994). Later, four cowpea genotypes of cowpeas experienced a reduction in plant height, leaf area, leaf number, and shoot and root biomass when subjected to 4 days of waterlogging stress (Umaharan et al., 1997). The decrease in root biomass is caused by a decline in anaerobic respiration and root tissue death due to prolonged root submergence (Pampana et al., 2016). In addition, waterlogging promotes rapid senescence of the old leaves (Hong et al., 1977). This may result from either a reduced leaf chlorophyll content or the accumulation of reactive oxygen species (ROS) in the leaves (Ahmed et al., 2012; Pan et al., 2021).

The plant root system is comprised of different kinds of roots that change in morphology and function. The root architecture illustrates the root system's spatial arrangement in the soil, which is crucial for plants to obtain the water and nutrients required for growth and development (Santos et al., 2020). Previous research has shown that plant root systems suffer from waterlogging stress by reducing size due to inhibited growth and damaging the existing primary

root system (Herzog et al., 2016; Palta et al., 2010). The root biomass of two genotypes of chickpeas declines markedly by 67%, while new adventitious roots develop (Palta et al., 2010). The formation of adventitious roots contains aerenchyma with the internal oxygen movement to the apex enabling growth, but to a limited distance, into hypoxic conditions (Herzog et al., 2016). Also, waterlogging-sensitive crops like cowpea respond to submergence stress by decreasing their root hydraulic conductance to cope with the reduced gas diffusion, which ultimately causes a drastic reduction in the water uptake in the root system (Schildwacht, 1989). Decreasing root hydraulic conductance and reducing water uptake results from a disruption of aquaporin function (Tournaire-Roux et al., 2003). Studies have shown that biomass accumulation and the formation of the adventitious root are coping mechanisms that tolerant crops utilize to ameliorate the anoxic/hypoxic conditions to adapt to waterlogging stress (Barickman et al., 2019; Garcia et al., 2020). Hong et al. (1977) reported that adventitious roots and aerated tissues were observed on the cowpeas plant's stems, roots, and nodules after eight days of submergence. Similarly, waterlogged plants of soybean, mung bean, and faba bean produce adventitious roots after 4-8 days of flooding (Henshaw et al., 2007; Kumar et al., 2013; Pampana et al., 2016). Long-term waterlogging causes adventitious roots to form in the internodes of hypocotyls or at the base of plant stems, promoting gas exchange and water and nutrient absorption (Pan et al., 2021). Under waterlogging condition, ensuring oxygen enter the root is crucial to avoiding further cell damage. Soybean plants had thickened, presumably due to the growth of aerenchyma tissue caused by 14 days of waterlogging (Henshaw et al., 2007; Thomas et al., 2005). Many studies on legumes have shown that aeration tissue benefits nitrogen fixation under waterlogged conditions by providing a way for gas to diffuse to submerged nodules (Minchin et al., 1978; Hong et al., 1977; Shimamura et al., 2002).

The formation of adventitious roots can replace primary roots that died due to hypoxia to a certain extent, maintain metabolism, and promote normal growth and development (Eysholdt-Derzsó and Sauter, 2019). The degree to which various cowpeas generate such roots may explain their ability to survive alternating waterlogging and drying in a flooded environment (Thomas et al., 2005; Timsina et al., 1994). Previous research has revealed that the plant growth regulator, ethylene, may stimulate the formation of adventitious roots (Rasmussen et al., 2017; Hong et al., 1977). Kim et al. (2018) discovered that pre-waterlogging treatment with ethephon, a synthetic plant growth regulator that produces ethylene when metabolized, promoted the formation of adventitious roots.

The ability of plants to move their leaves up to reach the water and restore contact with the air is another important morphological response under waterlogged conditions. This is achieved via petiole epinasty growth, which means the change from the angle of the leaf into a more upright position, observed for both non-wetland and wetland plants (Mustroph, 2018). Submergence of the roots and a lack of oxygen in the rhizosphere can both promote the hyponastic growth of leguminous leaves (Cox et al., 2003; Striker and Colmer, 2017). It is imperative to note that the hyponastic growth of plants, when subjected to hypoxic conditions, can be associated with ethylene production (Jackson, 2008). Another important adaptive feature of cowpeas and related crops under waterlogging stress is the tolerance to radial oxygen loss (ROL) (Solaiman et al., 2007; Striker and Colmer, 2017; Pan et al., 2021). The root barrier structure also restricts the entry of toxic compounds or metals accumulated during long-term water storage. Other characteristics of leguminous species, such as lenticels at the base of the stem and pressure-driven rhizomes, contribute significantly to promoting internal ventilation under hypoxia (Pampana et al., 2016; Pan et al., 2021).

However, the evaluation of root structural characteristics is one of the main problems in root system research due to the difficulties associated with segregating the entire root system from the soil and the large amount of labor required for root system evaluation. In addition, analysis of the root architecture to understand the response of cowpea under waterlogging stress is characterized by the destructive harvesting of the root system, which can only be carried out at the end of the experiment. Consequently, it does not seem feasible to monitor the root system's development during the crop's growth stage to understand its response to waterlogging. Several encouraging assays have been performed in many studies to alleviate the problem, such as a root observation chamber, a soil-free medium, and an image-based phenotyping platform (Hund et al., 2009; Iyer-Pascuzzi et al., 2010). Many researchers have also utilized root imaging tools to understand the plant's root response to abiotic stress, including Root-Flow, RHIZO, and RootTrace (Armengaud et al., 2009; van der Weele et al., 2003; Wijewardana et al., 2019).

2.6.4.3 Biochemical response of cowpea to waterlogging stress

Under waterlogging, the gaseous components of plants (such as O_2, CO_2, and ethylene) change rapidly as their diffusion rates drop by more than 10,000 times relative to air (Bailey-Serres et al., 2012; Voesenek and Bailey-Serres, 2015). During the early stages of waterlogging, aerobic microorganisms alter the oxygen influx to the root (Bailey-Serres et al., 2012). On the other hand, waterlogging hinders the diffusive escape and oxidative decomposition of ethylene and CO_2, causing their accumulation to adversely affect root growth and function (Voesenek and Sasidharan, 2013). Previous studies have documented the damaging effects of accumulated ethylene and CO_2 in legumes due to waterlogging, thereby inhibiting root growth (Boru et al., 2003; Smith, 1987).

The degree of oxygen deprivation in plants depends on several factors, including oxygen replenishment through photosynthesis, inward diffusion from the water layer, and cell oxygen consumption through metabolic activities (Bailey-Serres et al., 2012). For example, prolonged waterlogging (anoxia) can lead to restricted aerobic metabolism, thereby inhibiting the production of the energy required for basic plant processes (Bailey-Serres and Voesenek, 2008; Zabalza et al., 2009). However, plants can cope with these energy deficiencies by producing more ATP through the catabolism of soluble sugars and starches during glycolysis (Bailey-Serres et al., 2012). In addition, the increased glycolytic influx is accompanied by the regeneration of NAD^+ from the mitochondrial electron transport chain to provide an alternative energy source (Nakamura and Noguchi, 2020). Although the glucose metabolized during anaerobic fermentation is 2 moles compared to aerobic metabolism, which produces 36 moles, the plant cell can still survive with the available glucose (Gibbs and Greenway, 2003). Moreover, the increased duration of waterlogging may lead to plant cell death when limited ATP expels protons to the apoplast to avoid membrane apoptosis while maintaining neutral cytosolic pH (Bailey-Serres and Voesenek, 2008; Felle, 2006).

Under waterlogged conditions, plants suffer from severe disorders caused by cell acidification, low energy supply, and reduced intracellular environment. Consequently, the production of ROS is increased due to the high reduction of the intracellular environment and the low energy supply (Gill and Tuteja, 2010). The increased accumulation of ROS is one of the first biochemical responses of plants to waterlogging stress (Garcia et al., 2020; Sairam et al., 2009). ROS oxidizes proteins, lipids, and nuclear acids at higher concentrations, leading to oxidative damage (Gill and Tuteja, 2010; Hasanuzzaman et al., 2017). The effects of ROS generation in

plants vary based on the duration, intensity, and frequency of waterlogging stress and the physio-chemical condition of the plant cell (Kumar et al., 2013; Scott et al., 1989).

The sensitivity of cowpeas and related crops under hypoxic and anoxic conditions is attributed to ROS accumulation, which causes oxidative damage in the plant cell (Borella et al., 2019; Posso et al., 2018; Sairam et al., 2009). As a cellular indicator of waterlogging stress, ROS has harmful effects on A and leads to the down-regulation of photosynthetic apparatus (Fukao and Bailey-Serres, 2004). A high level of ROS (e.g., H_2O_2) can also inhibit the various processes involved in the glycolysis pathway (Ashraf, 2012). For example, 4-6 days of waterlogging stress promotes the overproduction of H_2O_2 in pigeon peas due to NADPH-oxidase activation (Sairam et al., 2009). The concentration of H_2O_2 in common bean waterlogged leaves was higher than in non-stressed leaves (Costa et al., 2020). Further, seven days of waterlogging resulted in ROS accumulation in soybean genotypes, with adverse impacts on chlorophyll content and A (Garcia et al., 2020). Similar results were observed during recovery, which resulted in reduced g_s and pigment damage (Garcia et al., 2020). Under these conditions, light trapping can cause an overload of the electron transport chain in the chloroplast, leading to electron leakage and ROS accumulation (Gill and Tuteja, 2010). However, the pretreatment of soybean seeds with H_2O_2 improved the tolerance of soybeans to waterlogging stress by increasing photosynthetic pigments, A, shoot and root biomass accumulation (Andrade et al., 2018). The study also revealed that pretreatment of soybean seeds with H_2O_2 increased the activity of antioxidant enzymes with low H_2O_2 content, and cell membrane damage was correspondingly reduced. So far, studies on cowpeas' genotype using ROS production to evaluate waterlogging resistance are limited. Hence, biochemical analysis of cowpea genotypes under waterlogging stress is needed to

confirm and clarify the accumulation of H2O2 responsible for cowpea waterlogging tolerance signal transduction.

Recent studies have shown that oxidative damage caused by waterlogging does not usually appear immediately but relatively soon after the water level drops during the recovery period of complete submergence (Barik et al., 2019; Ploschuk et al., 2018). After draining the waterlogged soil, a second and more pronounced increase in ROS and loss of redox homeostasis in the plant occurs (Da-Silva and do Amarante, 2020; Garcia et al., 2020). The O_2 content increased after flooding during reoxygenation, resulting in redox imbalance and ROS accumulation in plant roots (Shikov et al., 2020). Furthermore, during the reoxygenation process, the inhibition of aquaporin exacerbated by waterlogging lowers root water absorption and causes water deficit, resulting in an increase in ROS on the leaf surface (Borella et al., 2019; Posso et al., 2018). Under these conditions, ROS needs to be removed to maintain normal plant growth.

The balance of excess ROS produced by soil flooding stress in plant cells should be maintained to maintain the normal metabolic process of plant cells (Hasanuzzaman et al., 2017). Counteracting oxidative stress under waterlogging involves the activity of plant antioxidant enzymes to scavenge excessive buildup of ROS in the plant cell (Irfan et al., 2010). Plant cells use enzymatic (e.g., superoxide dismutase, malondialdehyde, catalase, ascorbate peroxidase, and glutathione peroxidase) and non-enzymatic antioxidants (such as glutathione, ascorbic acid, tocopherols, carotenoids, flavonoids, phenolics, and ascorbic acid) to prevent oxidative damage caused by the excessive ROS production (Gill and Tuteja, 2010; Barik et al., 2019; Da-Silva and do Amarante, 2020). Higher antioxidant enzyme activity can improve waterlogging resistance by increasing the ability to protect against oxidative damage. Thus, these antioxidant mechanisms

can contribute to maintaining gas exchange parameters and morphological attributes of cowpeas when subjected to waterlogged conditions.

Although the research on cowpea under waterlogging stress is limited, the response of the antioxidant system under waterlogging has been studied in many related plant species. Seven days of waterlogging upregulated the activity of SOD and catalase in soybeans (Da-Silva and do Amarante, 2020; Garcia et al., 2020). Significant increases in SOD, catalase, and peroxidase activity were also observed in pigeon pea that was flooded for 2-8 days, and the activity changed according to the duration of the stress (R. K. Sairam et al., 2009). Subsequently, a corresponding increase in glutathione and SOD was observed in waterlogging-tolerant mung beans (Sairam et al., 2011). However, under flood conditions, sensitive mung beans' catalase and SOD activities decreased (Ahmed et al., 2002). Thus, these antioxidant mechanisms can contribute to maintaining gas exchange parameters and morphological attributes of cowpeas when subjected to waterlogged conditions.

Waterlogging and eventual anaerobic metabolism can lead to acute growth inhibition or even death of most crops. Mainly due to energy limitation, accumulation of toxic products such as lactic acid, and carbon loss (ethanol loss through roots) (Tamang et al., 2014). Some cowpea-related plants, such as soybeans, common beans, and peas, accumulate alanine, an amino acid produced by alanine aminotransferase under hypoxic conditions (Borella et al., 2017). The alanine synthesis significantly regulates glycolytic flux by preventing excessive pyruvate accumulation while maintaining carbon and nitrogen in the cell (Rocha et al., 2010; Zabalza et al., 2009). Conversely to lactic acid and ethanol production, alanine accumulation does not cause adverse effects on plant cells.

CHAPTER III

SCREENING OF COWPEA (*VIGNA UNGUICULATA* (L.) GENOTYPES FOR

WATERLOGGING TOLERANCE USING MORPHO-PHYSIOLOGICAL

TRAITS AT THE EARLY GROWTH STAGE.

Abstract

The majority of cowpea (*Vigna unguiculata* (L.) Walp.) produced in the U.S. is planted

shortly after the summer rains and subsequently depends on rain or artificial irrigation.

Therefore, excessive precipitation and poor soil drainage will cause cowpea plants to suffer

temporary waterlogging, reducing the submerged tissue's oxygen level. Although cowpea is

sensitive to waterlogging, excessive moisture can induce several morpho-physiological changes

with adverse impacts on yield in its early stages of development. The current study subjected 30

cowpea genotypes to 10-days of waterlogging at the seedling stage under a controlled

environment. The dynamic changes of 24 morpho-physiological parameters under waterlogging

and optimal water conditions were analyzed to understand cowpea's response to waterlogging.

Most of the measured parameters observed significant waterlogging treatment, cowpea

genotypes, and their interactions ($p < 0.001$). The results indicated the cowpea genotypes' plant

height, leaf area, and biomass significantly decreased under waterlogging compared to the

control treatments. Similar results were obtained for net photosynthesis (A), stomatal

conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (E). However, the

water use efficiency (WUE) and adventitious roots increased linearly under waterlogging

conditions. Waterlogging also declined chlorophyll fluorescence parameters except for non-photochemical quenching (qN), which increased with excess soil moisture. In addition, waterlogging tolerance coefficient (WTC) and multivariate analysis (MCA) methods were used to characterize cowpea genotypes for waterlogging tolerance. Accordingly, the cowpea genotype Dagupan Pangasinan, UCR 369, and Negro were classified as waterlogging tolerant, while EpicSelect.4 and ICARDA 140071, as the most waterlogging sensitive. The cowpea genotypes and morpho-physiological traits determined from this study may be helpful for genetic engineering and breeding programs that integrate cowpea waterlogging tolerance.

3.1   Introduction

Waterlogging stress is one of the most crucial environmental factors limiting crop production. It is ranked second to drought based on its devastating effects on crop yields and considerable economic losses. Recent evidence suggests that over 16 % of the global cultivated area is affected by waterlogging stress (Ploschuk et al., 2018), and over 17 km^2 of the land surface worldwide is exposed to flooding (Voesenek and Sasidharan, 2013). For example, waterlogging was directly linked to 65 % of financial damage in crops due to abiotic stresses (FAO, 2018), with an estimated global financial loss of $74 billion annually (Kaur et al., 2020). In the past two decades, the economic loss caused by flooding in the U.S. has been estimated to be approximately $114 billion (Duffin, 2020).

Moreover, the National Aeronautics and Space Administration (NASA) climate simulation model predicts an increase in heavy precipitation events by 30% in 2030. As a result, the loss of crops is estimated to reach $3 billion each year (Rosenzweig et al., 2002). Ouyang et al. (2020) also forecasted an increase in annual precipitation and average wet days in many regions of the Lower Mississippi River Alluvial Valley. Hence, waterlogging may threaten the

sustainability of cowpea yields because the majority of cowpea produced in the U.S. are grown shortly after the summer rains.

Cowpea (*Vigna unguiculata* L.) is an important grain legume with inherent resilience to cope with abiotic stress, such as drought and heat stress (Goufo et al., 2017; Hall, 2004; Olorunwa et al., 2021). However, cowpea growth and development are highly sensitive to waterlogging stress due to their lower ability to absorb nutrients than wetland crops (Hong et al., 1977; Minchin et al., 1978). Waterlogging stress threatens plant growth and development by decreasing soil oxygen levels resulting in hypoxia and anoxia in soil. A hypoxic condition is characterized by plant anaerobic respiration, which facilitates energy deficits up to 37.5% and reduced diffusion rates of gases that could cause cell and tissue death in waterlogging-sensitive crops, including cowpeas (Gibbs and Greenway, 2003; Limami et al., 2014). Due to energy deficits, plants experience a series of morphological, physiological, and metabolic changes, preventing them from reaching their true genetic potential (Barickman et al., 2019; Hasanuzzaman et al., 2017). Anaerobic conditions can also adversely affect enzymatic activity, carbon assimilation, source-sink relationship, plant growth, and development, ultimately leading to a decline in crop yields (Bailey-Serres et al., 2012; Velasco et al., 2019).

Depending on the growth stage, stress duration, soil type, and genotype, waterlogging stress has been documented to severely diminish grain yield by more than 50% in cowpeas (Hong et al., 1977; Minchin and Summerfield, 1976). Minchin et al. (1978) reported a 52% reduction in the grain yield of cowpeas when plants were waterlogged during the seedling stage. Hong et al. (1977) revealed cowpea to be most sensitive to waterlogging in the early stages of development, especially from the second leaf stage (V2) to the fourth leaf stage (V4), where the roots were the first to be damaged by waterlogging. After eight days of waterlogging treatment

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except for adventitious roots, most of the roots decayed, and the plants could not absorb the required nutrients, resulting in nitrogen deficiency due to leaching and denitrification (Hong et al., 1977; Minchin et al., 1978). Consequently, the root biomass of waterlogged cowpeas declined by 70% compared to the control treatments (Hong et al., 1977). Also, the nitrogen content of waterlogged cowpeas and the content of amino nitrogen compounds in the nodules were significantly decreased, resulting in slower growth and yield loss (Hong et al., 1977; Timsina et al., 1994). When waterlogging occurred 30 days after seeding, the yield of mung bean was reduced by 56%, peanut by 49%, and soybean by 37% (Timsina and Kathmandu, 1991). Previous studies have also established the sensitivity of seedling stages to waterlogging stress in different crops, including maize (Liu et al., 2010), cucumber (Barickman et al., 2019), canola (Zou et al., 2014), and field peas (Ploschuk et al., 2018). Moreover, plants with waterlogging tolerance in the seedling stage can show the same tolerance in the later stages of development (Kołton et al., 2020; Pan et al., 2019; Pang et al., 2004). Recent studies have also shown that evidence of plants' tolerance to waterlogging in the later stages of development may be linked to their performances during the recovery period of complete submergence (Arduini et al., 2019; Jayasundara et al., 1997). Hence, screening diverse cowpea genotypes for waterlogging tolerance during and after waterlogging period can be more effective at the seedling stage.

Developing cowpea genotypes adapted to waterlogging stress through genetic improvement is an effective and economic strategy to productively reduce the adverse effects of waterlogging on cowpea. However, compared with the inherent resilience traits of the progenitors of cultivated cowpeas, the modern breeding of cowpea with increased yields and the capacity to adapt to waterlogging conditions, has not been successful (Boukar et al., 2016; Omomowo and Babalola, 2021). Many studies have found that the wild ancestral forms of

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cowpea have demonstrated varying genetic abilities to acclimate to sub-optimal environmental conditions, particularly waterlogging (Agbicodo et al., 2009; Hong et al., 1977; Singh, 2014). Therefore, identifying waterlogging-tolerant germplasm from wild cowpea genotypes and combining the superior waterlogging-tolerant traits of wild cowpea into cultivated genotypes may help increase cowpea productivity.

Screening diverse crop genotypes are critical to identifying waterlogging-tolerant germplasm for breeding programs to develop waterlogging-tolerant and high-yielding crop cultivars (Liu et al., 2010; Zou et al., 2014). This approach involves the utilization of relevant and reliable traits for waterlogging tolerance, accurate phenotyping methods, and the appropriate developmental stage of cowpea that may be prone to waterlogging stress when identifying waterlogging-tolerant genotypes. Previous studies have reported a series of agronomic, morphological, and physiological traits for screening for waterlogging tolerance, including grain yield (Iseki et al., 2021; Minchin et al., 1978), biomass accumulation (Hong et al., 1977; Takele and McDavid, 1994), formation of adventitious root (Kumar et al., 2013; Pampana et al., 2016), leaf and shoot morphologies (Jayasundara et al., 1997; Umaharan et al., 1997), gas exchange parameters (Barickman et al., 2019; Garcia et al., 2020), chlorophyll fluorescence (Smethurst and Shabala, 2003; Velasco et al., 2019), and mineral nutrition (Arduini et al., 2019; Limami et al., 2014). All these variables have been utilized in screening for waterlogging tolerance in various plant species. Some of them have been employed as selection criteria in legumes to confer waterlogging resistance. For instance, leaf area (LA), plant height (PH), dry weight (DW), net photosynthesis (A), intercellular CO_2 concentration (C_i), transpiration rate (E), stomatal conductance (g_s), and quantum efficiency of PSII (F_v'/F_m') are considered as important parameters in waterlogging tolerance in Lucerne (*Medicago sativa*) (Smethurst and Shabala,

2003). The authors further demonstrated a significant correlation between these traits with waterlogging tolerance (Smethurst and Shabala, 2003). However, these traits have not been used in waterlogging-tolerant cowpea breeding programs, emphasizing the importance of screening cowpea germplasm for waterlogging tolerance.

Waterlogging tolerance is a polygenic feature in many crop species, particularly cowpeas. Hence, cowpeas' genetic variability and waterlogging tolerance will require simple, repeatable, and relevant methodologies. Experimental facilities such as the controlled environment that can simulate environmental conditions in farmer's fields are also needed to understand waterlogging tolerance in crops (Zou et al., 2014). In this context, a gas exchange portable system equipped with a fluorescence chamber, such as LI-6800 (LI-COR Biosciences, Lincoln, NE), can provide real-time measurement of photosynthesis and chlorophyll parameters, including A, E, g_s, C_i, and F_v'/F_m'. Assessing the stability of PSII through a chlorophyll fluorescence analysis under controlled environmental settings can also form a reliable, non-invasive, and effective technique in the study of flood tolerance of cowpeas (Mauchamp and Méthy, 2004; Ramazan et al., 2021).

Moreover, a selection index based on the relative ranking of groups using a single value index, cumulative index, percentile, or statistical separation based on means under single or multiple stress sources has been designed to assess the response of diverse crop genotypes to waterlogging stress. Many authors regard them as Waterlogging Tolerance Coefficient (WTC), representing the polygenic nature of waterlogging stress in crops (Jiménez et al., 2017; Liu et al., 2010; Zou et al., 2014). In addition, principal component analysis (PCA) and multivariate cluster analysis (MCA) have been proposed as valuable methods for classifying diverse crop genotypes based on their performance under waterlogging stress (Gedam et al., 2021; Panozzo et al., 2019; Zou et al., 2014). These statistical methods can also distinguish the genetic variation between the

cowpea genotypes studied. Therefore, combining the LI-6800 portable system, WTC, PCA, and MCA to screen diverse cowpeas genotypes under waterlogging stress could contribute to advancing breeding programs for waterlogging tolerance in cowpea.

Despite the adverse effects of waterlogging stress, little progress has been made in breeding cowpea varieties that are resistant to waterlogging. Besides, compared with other important legumes, there is currently no published data quantifying the genotypic differences in cowpea's waterlogging tolerance in the past 25 years. Also, there has been a lack of information on screening waterlogging tolerance on cowpea genotypes associated with early growth, development, and physiological performance. Thus, in this study, 30 cowpea genotypes from 21 countries were screened for waterlogging tolerance in their early developmental stages under a controlled condition. The main objectives of this study were (1) to evaluate the dynamic changes of morpho-physiological parameters of cowpea genotypes under waterlogging; (2) to identify waterlogging-tolerant cowpea genotypes based on their morpho-physiological response; and (3) to assess the suitability of various morpho-physiological parameters in screening for waterlogging tolerance of cowpea genotypes at the seedling stage. This study hypothesized that cowpea genotypes differ in tolerance to waterlogging stress. Moreover, their morphological and physiological traits can be used to discriminate the tolerance of cowpea plants to waterlogging stress at the seedling stage, with WTC, PCA, and MCA being beneficial for the indication of more tolerant and sensitive genotypes.

3.2   Materials and Methods

3.2.1   Plant materials and growth conditions

A total of 30 cowpea genotypes originating from 21 countries with contrasting climatic regions and soil types were selected for this study (Table 3.1). Among these 30 genotypes, 16 were obtained from the National Genetic Resources Program through the Germplasm Information Network (USDA, Griffin, GA) and selected based on geographic origin information. Additionally, 14 genotypes were acquired from the University of Arkansas. Seeds were increased at the North Mississippi Extension and Research Centre (NMREC) during the summer of 2020.

The experiment was conducted in the Vegetable Physiology Greenhouse at NMREC from 25 March to 28 April 2021. The greenhouse environment was set and recorded with a Seed 16 controller (Wadsworth, Arvada, CO). Photosynthetic photon flux density (PPFD) inside the greenhouse was measured with an LI-190R quantum sensor (LI-COR, Inc., Lincoln, NE) connected to a CR1000x data logger (Campbell Scientific, Logan, UT). The value of the PPFD during the experiment was up to 1686.5 ± 33.2 µmol m^{-2} s^{-1}. Plants were grown at a temperature of 30/20 °C (day/night) for a 16/8 h period, respectively. Also, the average relative humidity during the experiment was 69% and 64%, respectively, for March and April 2021.

The cowpea seeds were inoculated before sowing with *Bradyrhzobium japonicum* (Visjon Biologics, Wichita Falls, TX) at the rate of 141 g per 22.68 kg of seeds. Inoculated seeds were sown into 10-cm pots filled with Pro-Mix BX soilless medium (Premier Tech Horticulture, Quebec, Canada). Pots were arranged in a randomized complete block design of thirty cowpea genotypes subjected to two waterlogging treatments in four replications. Initially, four seeds previously selected by size and quality of each genotype were sown in each pot, and approximately 14 days after sowing (DAS), the plants were thinned to one plant per pot.

Table 3.1   Thirty cowpea accessions were selected for screening waterlogging tolerance at the seedling stage

No.	Line_ID	Plant Name	Country of origin	Seed Lot Origin
1	PI 610654	UCR 5274	Australia	USDA Griffin
2	PI 291140	Negro	Australia	UARK
3	PI 582824	UCR 834	Botswana	UARK
4	PI 527559	Inkore	Burundi	USDA Griffin
5	PI 582398	UCR 238	Tanzania	USDA Griffin
6	PI 632784	TVu 13776	Brazil	USDA Griffin
7	PI 583075	SALAK 01	Cameroon	USDA Griffin
8	PI 432345	Louvi	Cyprus	USDA Griffin
9	PI 610504	UCR 5360	Greece	USDA Griffin
10	PI 610652	UCR 5272	Ghana	USDA Griffin
11	PI 292898	Tvu 1890	Hungary	UARK
12	PI 186386	New Era	Uruguay	UARK
13	PI 152197	Tupi Pyta	Paraguay	UARK
14	PI 610519	UCR 5375	Italy	USDA Griffin
15	PI 582555	UCR 370	Kenya	USDA Griffin
16	PI 582554	UCR 369	Kenya	UARK
17	PI 190191	TVu 1557	Mexico	UARK
18	PI 255774	TVu 2428	Nigeria	UARK
19	PI 406290	IFH 27-8	Nigeria	UARK
20	PI 406292	IFH 113-1	Nigeria	UARK
21	PI 487486	Dagupan Pangasinan	Philippines	USDA Griffin
22	PI 339588	Tvu 1933	South Africa	UARK
23	ARBlackeye#1	ARBlackeye#1	United States	UARK
24	PI 653132	ICARDA 140071	Tajikistan	USDA Griffin
25	PI 339609	Tvu 1645	Tanzania	UARK
26	PI 663152	CR 22-2-21	United States	USDA Griffin
27	Grif 14490	Grif 14490	Sri Lanka	USDA Griffin
28	PI 663059	Texas Purple Hull 49	United States	UARK
29	PI 664529	US-311	United States	USDA Griffin
30	EpicSelect.4	EpicSelect.4	United States	USDA Griffin

Line ID (Plant Introductions (PIs), seed lot origin (U.S. Department of Agriculture (USDA) Germplasm Resources Information Network (GRIN), University of Arkansas (UARK).

3.2.2   Waterlogging treatments

After 14 DAS, uniformly emerged seedlings of cowpea genotypes at the V2 leaf stage were subjected to two experimental treatments: waterlogging and control treatments for ten days. Waterlogging treatments were imposed on cowpea plants by placing six pots of each cowpea genotype into four replicated 11 L containers (Rubbermaid Inc., Wooster, OH, USA). Each container was filled with enough water containing 5-15-29 water-soluble nutrient solutions until the water level reached 2 cm above the substrate surface to simulate waterlogging conditions. Pots containing cowpea plants under control (non-waterlogging) treatments were maintained at optimum field capacity (well-watered). After ten days of waterlogging (DOW), waterlogged and control plants were evaluated for various morpho-physiological traits.

3.2.3   Physiological performance: photosynthesis and chlorophyll fluorescence parameters

Photosynthesis and fluorescence parameters were measured on the second most fully expanded leaf trifoliate after ten days of waterlogging. The A, g_s, C_i, and E were measured in situ with chlorophyll fluorescence parameters at the North Mississippi Research and Extension Center (10:00 – 14:00 CST) using an LI-6800 portable photosynthesis system (LI-COR Biosciences, Lincoln, NE). Measured leaves were allowed to adjust to the measurement conditions before the values were recorded. Measurements were conducted on six representative plants of each cowpea genotype subjected to waterlogging stress and the control. The ratio of A/g_s was used to calculate the intrinsic water use efficiency (WUE) (Martin and Ruiz-Torres, 1992). The internal to external CO_2 ratio was calculated by the relationship C_i/C_a. The LI-6800 provided a PPFD of 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a CO_2 concentration of 410 $umol \cdot mol^{-1}$, and the

relative humidity was set to 50%. The measurement chamber temperature was kept at 28°C, corresponding to the daytime temperature.

With the aid of the LI-6800 portable photosynthesis system, chlorophyll fluorescence parameters were measured in situ in the survey measurements on the second, most fully developed trifoliate. The light-adapted chlorophyll fluorescence techniques, as described by Flexas et al. (1999), were utilized to measure the maximum fluorescence yield upon application of saturating flash of light, which corresponded to the light level of cowpea genotypes in each treatment at the time of measurement (10:00 – 14:00 CST). Steady-state fluorescence (F_s) was measured using modulation light settings recommended for light-adapted leaves, and maximum fluorescence intensity (F'_m) was estimated using the multi-phase flash protocol. When all reaction centers are closed during light-adapted fluorescence, the multiphase method is usually necessary for the actual measurement of the apparent F'_m because plant leaves produced in a controlled environment (especially under artificial light) usually do not reach the saturation of the photosystem when using a high-intensity rectangular flash (Earl and Ennahli, 2004; Pilon et al., 2018). The quantum efficiency by oxidized (open) PSII reaction center in light was estimated as $(F'_v/F'_m) = (F'_m-F'_o)/F'_m$, where F'_m = maximal fluorescence of light-adapted leaves, F'_o = minimal fluorescence of a light-adapted leaf. The light-adapted, effective quantum yield of PSII photochemistry (Φ_{PSII}) was also estimated according to (Maxwell and Johnson, 2000) as $\Phi_{PSII} = (F'_m-F_s)/F'_m$. The electron transport rate (ETR), photochemical quenching in the puddle (qP) and lake model (qL), and non-photochemical quenching (qN) were calculated according to Genty et al. (1989).

3.2.4   Morphological performance and plant harvest

Six representative cowpea plants from each genotype and treatment were destructively harvested to obtain phenotype and growth data on the impacts of waterlogging stress on cowpeas. Cowpea phenotypic data of plant height (PH), node number (NN), and leaf number (LN) were measured for each treatment combination. The LA was measured using the LI-3100 leaf-area meter (LI-Cor Bioscience, Lincoln, NE). The number of new adventitious roots that originated from the hypocotyl of the waterlogged plants was counted. Plant component fresh weight was measured from all plants by using a weighing scale. The samples of the plant's fresh weight were dried in a forced-air oven at 75°C for two days to obtain cowpea DW.

3.2.5   Total waterlogging tolerance coefficient

The thirty cowpea genotypes were classified for early vegetative waterlogging tolerance using a computed WTC described by Liu et al. (2010) with modifications. The individual waterlogging tolerance coefficient (IWTC) for each parameter measured was estimated as the value of the parameter under waterlogging treatment of a given genotype divided by the value of the same parameter under control treatment. Then, the total WTC (TWTC) for each cowpea genotype was calculated as the summation of all the 24 IWTC derived from morphological and physiological parameters.

3.2.6   Data analysis

The experiment was a randomized complete block design with two waterlogging treatments, thirty cowpea genotypes, four replications, and six plants in a factorial arrangement. In total, 1440 plants (4 replicates x 2 waterlogging treatments x 30 cowpea genotypes x 6 plants) were utilized in this study. SAS (version 9.4; SAS Institute, Cary, NC) was used to perform a

statistical analysis of the data. Replicated values of all morpho-physiological parameters

measured in this study were analyzed using a two-way analysis of variance of the general linear

model (PROC GLM) to determine the effects of waterlogging treatments, cowpea genotypes, and

their interactions. Fishers-protected least significant difference tests at $P \leq 0.05$ were employed

to test the differences among interactions of genotypes and treatments for measured parameters.

The standard errors of the mean were calculated using the pooled error term from the ANOVA

table and presented in the figures as error bars. Diagnostic tests, such as Shapiro–Wilk in SAS,

were conducted to ensure that treatment variances were statistically equal before pooling.

Pearson correlation analysis was utilized to study the relationship between the measured morpho-

physiological parameters.

Principal component analysis (PCA) was used to assign ranks to cowpea genotypes and

classify which are more sensitive or tolerant to waterlogging stress. The PCA analysis was

performed using Minitab statistical software (Minitab Inc., PA, USA), and the results were

reported in biplots, which are plots of the mean PC scores for waterlogging treatments for the

first two PCs. This study performed PCA on the correlation matrix of 30 cowpea genotypes and

24 measured parameters. Ward's cluster analysis (WCA) was also used to classify the cowpea

genotypes based on hierarchical cluster analysis.

3.3   Results

3.3.1   Morphological parameters of cowpea genotypes

The analysis of variance showed that, except for DW: FW ($P \geq 0.05$), there were

significant differences in all morphological attributes among cowpea genotypes, treatments, and

their interactions ($P \leq 0.001$) (Figure 3.1). The considerable variation of cowpea genotype and

genotype x treatment under waterlogging and control indicates sufficient genetic variation among the studied cowpea genotypes.
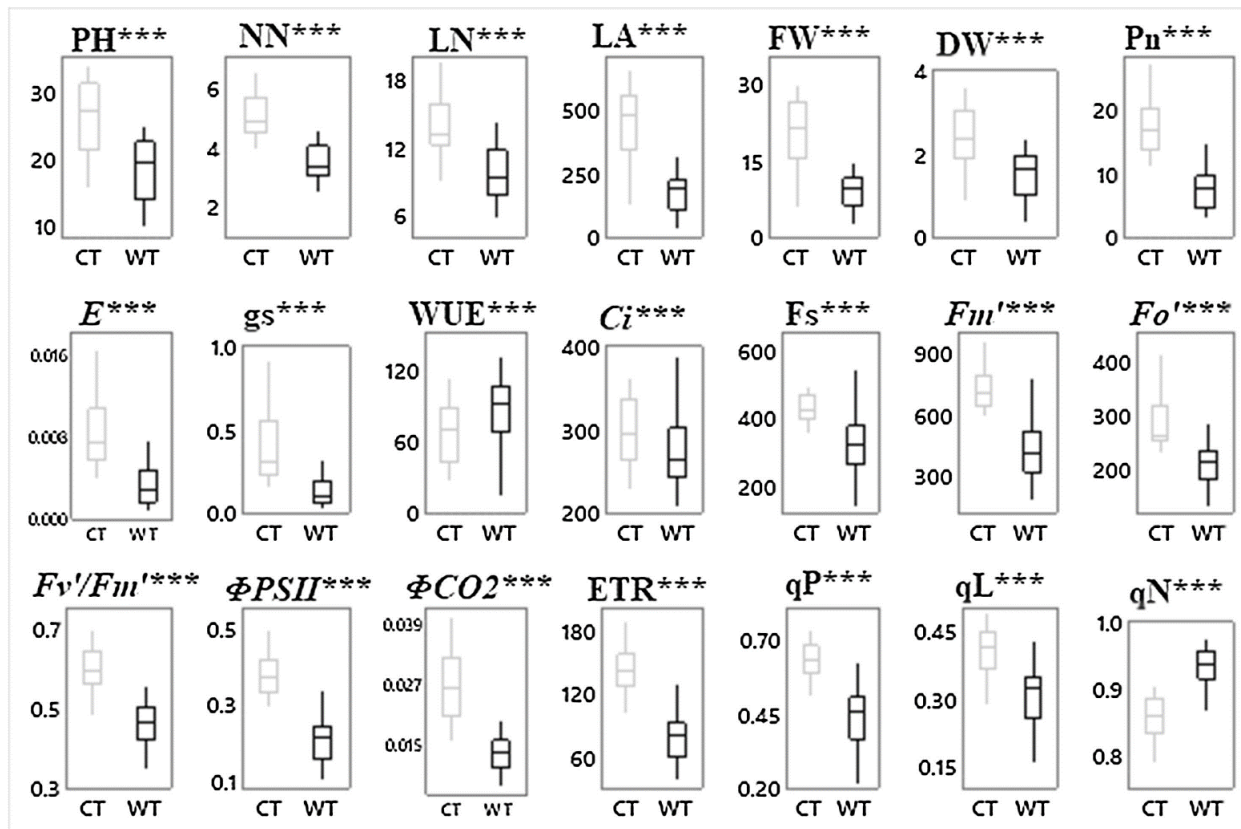
Figure 3.1   Performance of measured morphophysiological parameters under control (CT) and waterlogging treatments (WT). Asterisks (***) indicate that the parameter means significantly different ($P < 0.001$) between the waterlogging and control conditions.

Morphological parameters such as plant height, leaf number, node number, leaf area, and leaf senescence are important plant traits in understanding waterlogging tolerance. The current study observed that the leaf of cowpea genotypes subjected to waterlogging stress began to degenerate and fall off. At the same time, those on the control plants remain green and continue to exist. Waterlogging also delayed branch formation, decreasing cowpea genotypes' final node and leaf number (Figure 3.1). Cowpea genotype UCR 5272 had the highest node (4 and 6) and

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leaf numbers (14 and 19) under both waterlogging and control treatments, respectively (Figure

3.2). In contrast, cowpea genotype IFH 27-8 had the lowest node (2 and 4) and leaf number (6

and 9) under both waterlogging and control treatment, respectively (Figure 3.2). Furthermore, 16

cowpea genotypes (56%) studied under waterlogging treatments showed lower node and leaf

numbers with an overall average of 3 and 9, respectively. Thus, signifying the sensitivity of

cowpea genotypes to waterlogging.
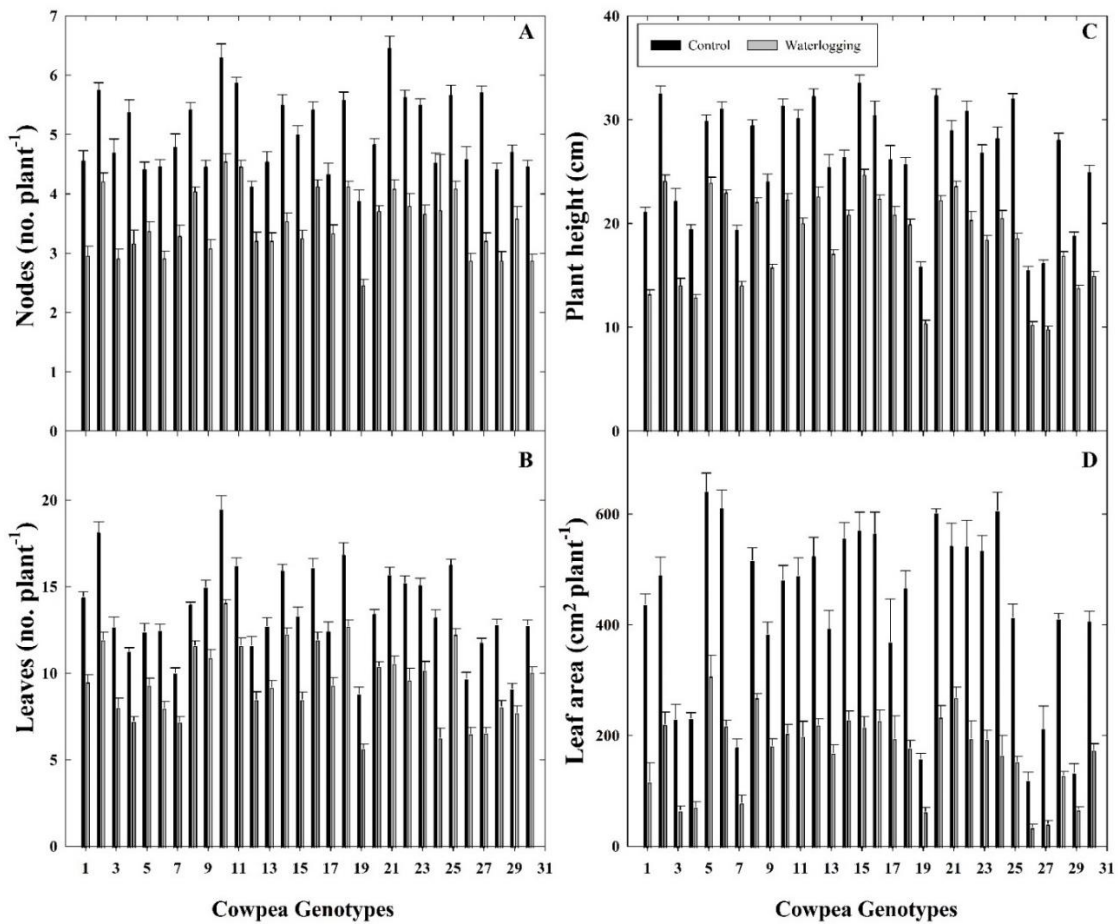
Figure 3.2   A. Mean node number, B. mean leaf number, C. mean plant height, and D. mean
leaf area of 30 cowpea genotypes subjected to 10-day waterlogging treatments.
The error bar on the vertical bar indicates the standard error of the mean ± 4
replications of each morphological trait.

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Cowpea genotypes that received the control treatment had higher plant height and significantly differed from the cowpea genotype grown under the waterlogging treatment (Figure 3.1). Under waterlogging treatment, the plant height of cowpea genotypes ranged from 24.7 cm (UCR 370) to 9.8 cm (Grif 14490) with a mean value of 18.3 cm, whereas at control treatment, plant height varied from 15.5 cm (CR 22-2-21) to 33.6 cm (UCR 370) with the average of 26.2 cm (Figure 3.2C). Interestingly, among the 30 cowpea genotypes studied, 17 cowpea genotypes (56%) showed higher PH than the overall mean value of 18.3 cm under waterlogging treatments.

The development of cowpea leaves, determined by cell division and expansion, tends to experience various morphological changes in waterlogging stress. The leaf area of cowpea genotypes decreased linearly under waterlogging treatment (Figure 3.1). The genotypic variability in the leaf area of the 30 cowpea genotypes ranged between 34.4 cm^2 and 307.7 $cm^{2,}$ with an average of 169.7 cm^2 under waterlogging stress. Under the control treatments, the leaf area ranged from 119.3 cm^2 to 641.2 cm^2. This range depicts maximum and minimum declines in leaf area of CR 22-2-21 (71%) and UCR 238 (52%), respectively, under waterlogging stress (Figure 3.2D).

During the early developmental stages of cowpeas, this study observed a significant decline in DW, which equates to plant biomass (Figure 3.3). On average, genotype UCR 238 maintained the highest dry weight of 3.6 g/plant and 2.4 g/plant under waterlogging and control treatments, respectively (Figure 3.3A). However, genotype CR 22-2-21 demonstrated the lowest dry weight of 0.8 g/plant and 0.3 g/plant under waterlogging and control treatments, respectively (Figure 3.3). Compared to the control treatment, 10-day waterlogging resulted in the loss of dry weight, which varied among cowpea genotypes, ranging from 72% in Grif 14490 to 7% in Louvi

(Figure 3.3). Among the 30 cowpea genotypes studied in this study, 16 cowpea genotypes had a higher dry weight than the average value of 1.5 g/plant.
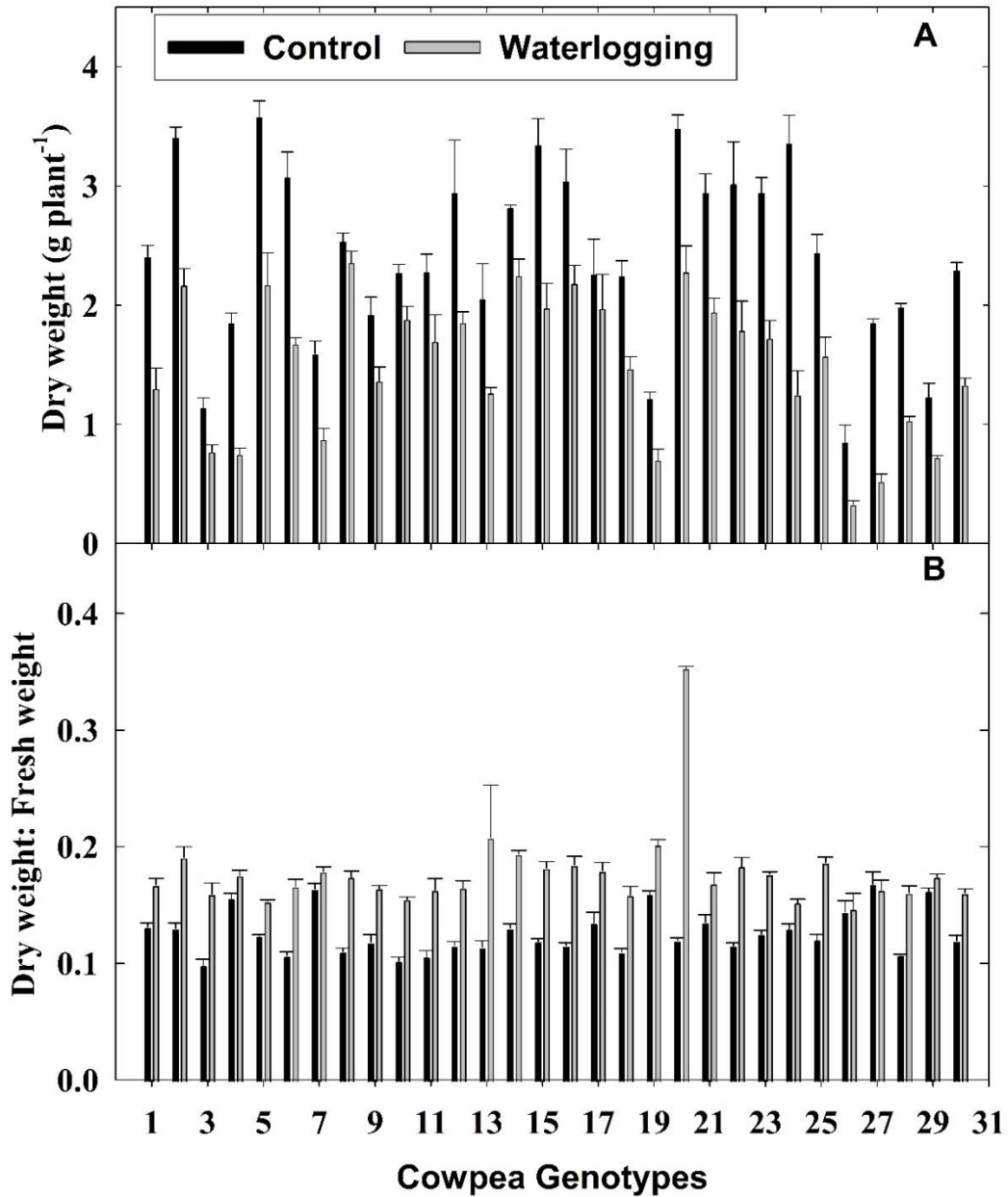
Figure 3.3   Waterlogging effects on A. Dry weight per plant and B. Dry weight: Fresh weight of the 30 cowpea genotypes. Measurements were recorded after 10-day of waterlogging. The error bar on the vertical bar indicates the standard error of the mean ± 4 replications of each morphological trait.

The results also revealed that cowpea genotypes subjected to 10-day waterlogging developed new adventitious roots primordia on their hypocotyls, which significantly varied among genotypes (Figure 3.4). The adventitious roots among the genotypes ranged from 4 in IFH 27-8 to 29 in TVu 2428, with an overall average of 14.1 under waterlogging stress. Among the 30 cowpea genotypes, 15 genotypes (50%) showed a higher number of adventitious roots on their hypocotyls than the overall average number. However, no adventitious root was discovered on the hypocotyls of genotypes under control treatment.
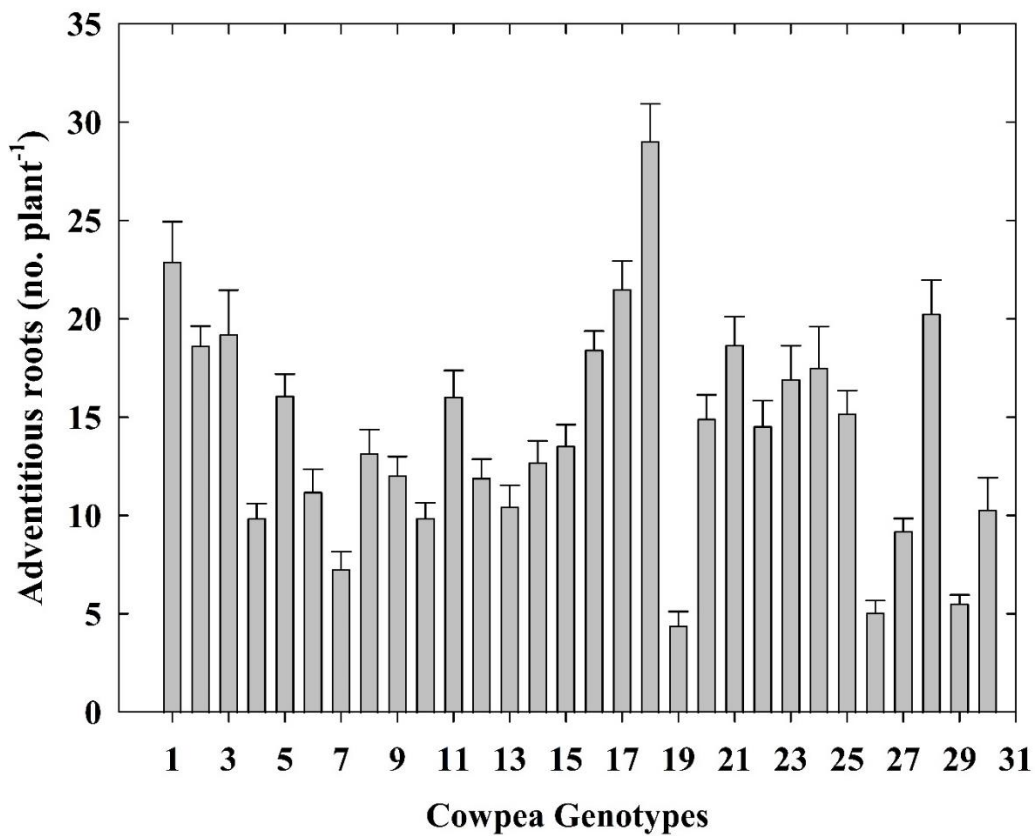
Figure 3.4   The number of adventitious roots of 30 cowpea genotypes under 10-day waterlogging treatment. The error bar on the vertical bar indicates the standard error of the mean ± 4 replications.

3.3.2 Photosynthetic parameters of cowpea genotypes

Significant differences ($P \leq 0.001$) were found between all the photosynthetic parameters measured among the 30 cowpea genotypes in this study. The A, g_s, C_i, and E were lower for all the studied cowpea genotypes subjected to 10-day waterlogging than the control treatment (Figure 3.1). Waterlogging significantly decreased the average A of cowpea genotypes by 57% compared to the non-waterlogging treatment. Cowpea genotypes Dagupan Pangasinan and Epic Select.4 showed the minimum and maximum decrease of A by 5% and 83%, respectively, when waterlogging was compared to the control treatment. The A among the 30 genotypes significantly varied with values ranging from 2.83 µmol m^{-2} s^{-1} in Epic Select.4 to 14.5 µmol m^{-2} s^{-1} in UCR 369 with an average of 7.4 µmol m^{-2} s^{-1} under waterlogging treatment, whereas UCR 5272 (26.9 µmol m^{-2}s^{-1}) and ICARDA 140071 (11.1 µmol m^{-2}s^{-1}), respectively had the maximum and minimum A under the control treatment. Notably, 19 cowpea genotypes had their A decreased by more than 50% under waterlogging (Table 3.2). In addition, the E of cowpeas significantly reduced ($P \leq 0.001$) under waterlogging stress and differed among genotypes (Figure 3.1). Compared to the control treatment, three cowpea genotypes (UCR 369, Dagupan Pangasinan, and Negro) significantly increased their E by 2%, 12%, and 37%, respectively, under waterlogging. In contrast, Epic Select.4, UCR 5275, UCR 5360, and IFH 27-8 showed a maximum E decline of 90%, 89%, 88%, and 88%, respectively, when waterlogged plants were compared to non-waterlogged plants (Table 3.2).

After 10-day waterlogging, UCR 369 exhibited the highest stomatal opening of 0.32 mol m^{-2} s^{-1}, closely followed by Negro (0.27 mol m^{-2} s^{-1}), UCR 5272 (0.26 mol m^{-2} s^{-1}), and TVu 2428 (0.24 mol m^{-2} s^{-1}) with the smallest g_s value recorded for Epic Select.4 (0.02 mol m^{-2} s^{-1}), and an overall mean of 0.13 mol m^{-2} s^{-1} (Table 3.2). Average values of g_s ranged from 0.15 mol

m^{-2} s^{-1} and 0.91 mol m^{-2} s^{-1} under control treatment (Table 3.2). Cowpea genotype Dagupan Pangasinan revealed the lowest stomatal opening of 0.15 mol m^{-2} s^{-1} at non-waterlogging treatment. However, this genotype showed an increment in g_s of 0.17 mol m^{-2} s^{-1} under waterlogging treatment. Contrasting trends of cowpea genotypes were observed in C_i under both waterlogging and non-waterlogging treatment (Figure 3.1). Waterlogging significantly decreased the C_i of 23 cowpea genotypes studied compared to the control treatment. In contrast, the C_i of 7 waterlogged cowpea genotypes increased by 3% in UCR 369 and 38% in Grif 14490 (Table 3.2).

Furthermore, the C_i/C_a and intrinsic WUE (A/g_s) of cowpea genotypes significantly increased under waterlogging stress (Table 3.2). When cowpea was subjected to non-waterlogging conditions, WUE ranged between 14.5 in Grif 14490 and 128.7 in Epic Select.4. Similarly, genotype Grif 14490 had the highest C_i/C_a values of 0.9. In contrast, Epic Select.4. had the minimum C_i/C_a value of 0.48 (Table 3.2). Interestingly, seven genotypes (23%) among the studied cowpea genotypes decreased linearly in the range of 8% in UCR 369 to 81% in Grif 14490. Contrastingly, 23 of the studied cowpea genotypes had higher linear increments of WUE, ranging between 6% in ICARDA 140071 and 252% in UCR 5375 under waterlogging compared to the control treatment.

Table 3.2   Mean responses of leaf transpiration rate (E), net photosynthesis (A), intercellular CO_2 concentration (C_i), intercellular/ambient CO_2 ratio (C_i/C_a), stomatal conductance (g_s), and intrinsic water use efficiency (WUE) of cowpea genotypes after 10 days of control (CT) and waterlogging treatment (WT).

Genotype	E [mol m^{-2}s^{-1}]		A (µmol m^{-2}s^{-1})		C_i [µmol mol^{-1}]		C_i/C_a		g_s [mol m^{-2} s^{-1}]		WUE	
	CT	WT	CT	WT	CT	WT	CT	WT	CT	WT	CT	WT
ARBlackeye#1	0.0067	0.0020	16.51	6.46	295.92	243.75	0.70	0.57	0.29	0.07	67.97	103.34
CR 22-2-21	0.0109	0.0046	23.68	7.20	332.75	360.55	0.79	0.84	0.58	0.19	43.50	29.67
Dagupan	0.0040	0.0044	11.27	10.69	262.06	280.79	0.62	0.66	0.15	0.18	89.12	77.34
EpicSelect.4	0.0070	0.0007	17.04	2.83	272.64	206.88	0.64	0.48	0.29	0.02	82.37	128.71
Grif 14490	0.0081	0.0015	20.76	3.87	279.92	386.72	0.66	0.90	0.40	0.06	77.40	14.53
ICARDA140071	0.0043	0.0014	11.05	4.22	265.34	260.21	0.63	0.61	0.17	0.05	88.07	93.40
IFH 113-1	0.0058	0.0043	15.75	9.33	260.66	286.50	0.62	0.67	0.24	0.16	89.78	75.32
IFH 27-8	0.0109	0.0013	24.72	4.49	326.07	257.64	0.78	0.60	0.56	0.05	47.00	96.20
Inkore	0.0093	0.0032	21.88	7.92	305.06	301.75	0.72	0.71	0.47	0.13	61.26	67.26
Louvi	0.0149	0.0048	22.23	7.24	353.53	251.39	0.84	0.59	0.81	0.20	30.07	99.01
Negro	0.0046	0.0062	13.50	11.54	227.96	290.40	0.54	0.68	0.18	0.27	111.61	72.33
New Era	0.0049	0.0019	13.66	6.39	263.64	241.16	0.63	0.57	0.20	0.07	87.21	104.28
SALAK 01	0.0082	0.0011	19.19	4.22	313.53	311.18	0.74	0.73	0.38	0.04	56.54	62.67
Texas Hull 49	0.0073	0.0025	15.40	6.16	301.35	257.44	0.71	0.60	0.29	0.09	64.41	96.15
Tupi Pyta	0.0058	0.0007	14.01	3.30	266.65	236.28	0.63	0.55	0.24	0.03	86.85	109.68
TVu 13776	0.0048	0.0023	13.27	7.76	244.49	233.06	0.58	0.55	0.20	0.09	99.39	109.88
TVu 1557	0.0102	0.0046	13.32	9.64	326.60	288.16	0.77	0.68	0.49	0.18	49.51	74.36
Tvu 1645	0.0076	0.0028	17.89	8.80	279.83	236.56	0.66	0.55	0.32	0.10	77.07	107.77
Tvu 1890	0.0069	0.0046	15.31	11.24	304.54	264.94	0.72	0.62	0.28	0.18	62.59	88.58
Tvu 1933	0.0068	0.0039	17.44	8.99	351.33	263.50	0.83	0.62	0.30	0.16	32.84	90.14
TVu 2428	0.0120	0.0059	16.25	8.58	358.05	321.60	0.85	0.75	0.60	0.24	29.01	53.66
UCR 238	0.0047	0.0026	11.44	8.31	270.68	238.99	0.64	0.56	0.20	0.10	84.11	104.93
UCR 369	0.0077	0.0076	16.10	14.46	294.28	303.02	0.70	0.72	0.31	0.32	68.63	63.22
UCR 370	0.0044	0.0045	13.64	9.54	242.47	287.34	0.57	0.68	0.17	0.19	100.71	75.06
UCR 5272	0.0163	0.0063	26.85	10.83	348.20	306.28	0.83	0.72	0.92	0.26	31.92	62.74
UCR 5274	0.0062	0.0026	19.52	7.90	259.60	245.18	0.61	0.57	0.25	0.10	89.74	102.67
UCR 5360	0.0075	0.0009	16.54	3.38	286.50	218.18	0.68	0.51	0.31	0.03	73.42	121.12
UCR 5375	0.0147	0.0016	22.55	4.69	353.32	242.49	0.84	0.57	0.75	0.06	29.88	105.16
UCR 834	0.0140	0.0048	16.69	7.22	362.67	344.90	0.86	0.81	0.72	0.21	26.02	39.66
US-311	0.0105	0.0016	19.74	4.26	342.39	278.96	0.81	0.65	0.55	0.06	38.42	82.69
Minimum	0.0040	0.0007	11.05	2.83	227.96	206.88	0.54	0.48	0.15	0.02	26.02	14.53
Maximum	0.0163	0.0076	26.85	14.46	362.67	386.72	0.86	0.90	0.92	0.32	111.61	128.71
Mean	0.0082	0.0032	17.24	7.38	298.40	274.86	0.71	0.64	0.39	0.13	65.88	83.72
Treatment (T)	***		***		***		***		***		***	
Genotype (G)	***		***		***		***		***		***	
G * T	***		***		***		***		***		***	

*** represent significance levels at $p \leq 0.001$.

3.3.3　Chlorophyll fluorescence of cowpea genotypes

The current study demonstrated that most of the chlorophyll fluorescence parameters were significantly affected by waterlogging (**Error! Reference source not found.**). The value of the non-photochemical quenching (qN) significantly increased while values of F'_m, F'_o, F_s, ETR, Φ_{PSII}, Φ_{CO2}, F'_v/F'_m, qP, and qL significantly declined after 10-day waterlogging (Figure 3.1). The normalized values revealed that cowpea genotypes subjected to waterlogging differed for chlorophyll fluorescence parameters.

When cowpea genotypes of waterlogged were compared to non-waterlogged, Φ_{CO2}, Φ_{PSII}, ETR, F'_m, and F'_v/F'_m were the most affected parameters. F'_v/F'_m values of cowpeas genotypes declined in the range of 6% in UCR 370 and 42% in IFH 27-8 under waterlogging compared to the control treatments. On the other hand, F'_v/F'_m values of Tvu 1890 and UCR 369 increased in the range of 1% and 4%, respectively. All the studied cowpea genotypes subjected to waterlogging decreased their Φ_{CO2} and Φ_{PSII}. Genotype UCR 369 ($\Phi_{PSII} = 0.34$; $\Phi_{CO2} = 0.02$) appears to be the most tolerant, while the most affected cowpea genotypes were UCR 5375 ($\Phi_{PSII} = 0.10$) and IFH27-8 ($\Phi_{CO2} = 0.01$). The same genotypes showed similar F'_o and F'_m trends under waterlogging and non-waterlogging stress. Interestingly, the ETR of Dagupan Pangasinan increased linearly by 4%, while UCR5375 had the highest decline of 75% under waterlogging compared to the control treatment. Moreover, photochemistry quenching parameters can be used to understand plants under stress. In the current study, UCR 369 had the maximum value of qP = 0.62 and qL =0.42, while UCR 5375 (qP = 0.21; qL =0.16) was revealed to be the most sensitive under waterlogging.

3.3.4 Classification of cowpea genotypes based on waterlogging tolerance coefficient

The WTC-based technique was utilized to identify genotype variability for waterlogging tolerance in cowpea genotypes. Cowpea genotypes showed significant differences in waterlogging tolerance, with the WTC varying from 0.67 in EpicSelect.4 to 0.92 in Dagupan, with a standard deviation of 0.07 (Table 3.3). Based on the standard deviation of their WTC, the cowpea genotypes were classified into four groups; three were classified as waterlogging tolerant, seven were moderately waterlogging tolerant, eleven were moderately waterlogging sensitive, and nine were waterlogging sensitive genotypes (Table 3.3). In addition, a positive and significant regression coefficient between the WTC of chlorophyll fluorescence and total WTC ($R^2 = 0.94$; $P \leq 0.001$) was observed (Figure 3.5). Corresponding results were obtained between the WTC of photosynthetic parameters and total WTC ($R^2 = 0.78$; $P \leq 0.001$). However, a poor regression coefficient ($R^2 = 0.20$; $P \leq 0.013$) was observed between WTC for morphological parameters and total WTC (Figure 3.5).
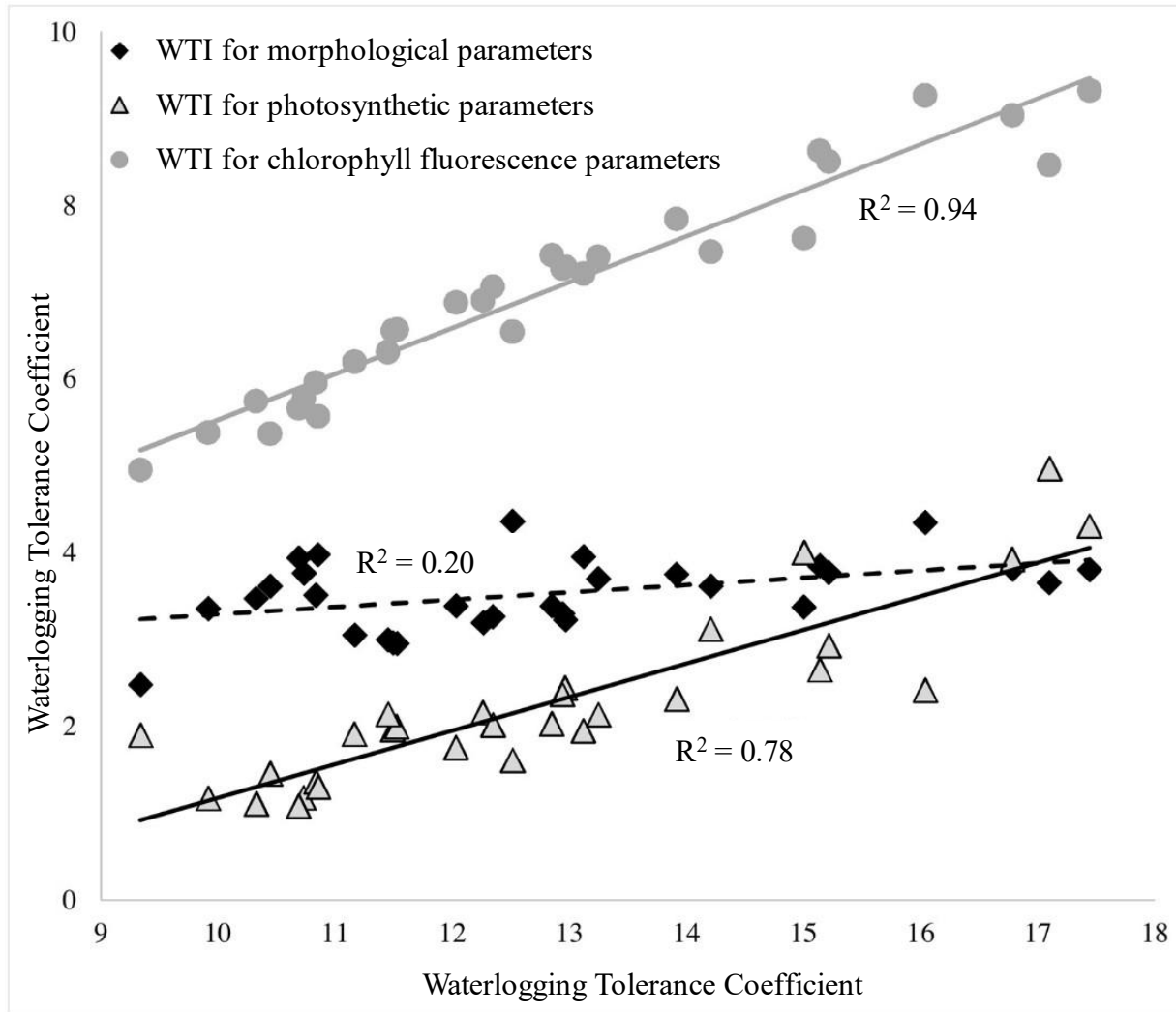
Figure 3.5   Correlations among waterlogging tolerance coefficient (WTC) of 30 cowpea genotypes, measured after 10-day waterlogging treatment.

Table 3.3   Classification of 30 cowpea genotypes into waterlogging tolerance groups based on total WTC (unitless) and standard deviation (SD).

Classification	Genotype	WTC value
Waterlogging-sensitive (WTC ≤ 0.74)	EpicSelect.4	0.67
	ICARDA 140071	0.70
	SALAK 01	0.71
	Tupi Pyta	0.71
	CR 22-2-21	0.71
	UCR 5360	0.72
	Texas Purple Hull 49	0.73
	Inkore	0.74
	ARBlackeye#1	0.74
Moderately waterlogging-sensitive (0.74 < WTC ≤ 0.81)	UCR 5274	0.75
	UCR 834	0.76
	New Era	0.76
	US-311	0.76
	UCR 5375	0.77
	Tvu 1645	0.77
	TVu 13776	0.78
	Grif 14490	0.79
	UCR 5272	0.81
	TVu 2428	0.81
	IFH 27-8	0.81
Moderately waterlogging-tolerant (0.81 < WTC ≤ 0.88)	UCR 370	0.83
	Tvu 1933	0.83
	UCR 238	0.84
	Louvi	0.84
	Tvu 1890	0.86
	IFH 113-1	0.87
	TVu 1557	0.88
Waterlogging-tolerant (0.88 < WTC ≤ 0.95)	Negro	0.91
	UCR 369	0.91
	Dagupan Pangasinan Collection No. 1.3	0.92

SD = 0.07. Waterlogging sensitive: WTC ≤ WTCmin + 1.0 SD; Moderately waterlogging-sensitive: WTCmin + 1.0 SD < WTC ≤ WTCmin + 2.0 SD; Moderately waterlogging-tolerant: WTCmin + 2.0 SD < WTC ≤ WTCmin + 3.0 SD; Waterlogging-tolerant: WTCmin + 3.0 SD < WTC ≤ WTCmin + 4.0 SD.

3.3.5 Principal component analysis and ward's cluster analysis of waterlogging tolerance

PCA analysis was performed to identify the principal components of morpho-physiological parameters of cowpea genotypes that best described the response to waterlogging and, hence, to identify tolerant and susceptible genotypes under waterlogging treatment. The orthogonal transformation was defined, so PC1 has the largest variance of 46.2%, while PC2 accounted for 23.3% of the total variation among the cowpea genotypes. Both PCs explain 69.6% of the total variance of analyzed parameters.

The biplot analysis demonstrated a positive correlation among the morpho-physiological parameters except for qN, WUE, C_i, and C_i/C_a (Figure 3.6). Furthermore, the PCA analysis revealed higher eigenvectors values for A, Φ_{CO2}, F'_m ETR, and Φ_{PSII}, and negative values for qN and WUE. Thus, cowpea genotypes with higher scores for PC1 tend to have higher values of A, Φ_{CO2}, F'_m, ETR, and Φ_{PSII}, while cowpea genotypes with low scores for PC1 tend to have low values and vice-versa. However, PC2 differed from PCI, by having higher eigenvectors values for C_i and C_i/C_a and low values for WUE, DW, LN, FW, and LA. Consequently, a biplot of PC1 vs. PC2 was used to classify cowpea tolerance to waterlogging stress (Figure 3.6B). Group 1 (highest PC1 and PC2) includes: Dagupan Pangasinan, Negro, UCR 369, IFH 113-1, Tvu 1890, and UCR 370 are classified as waterlogging-tolerant, while genotype UCR 5375, US-311, EpicSelect.4., UCR 5360, IFH 27-8, SALAK 01, and Tupi Pyta grouped as waterlogging-sensitive genotypes.
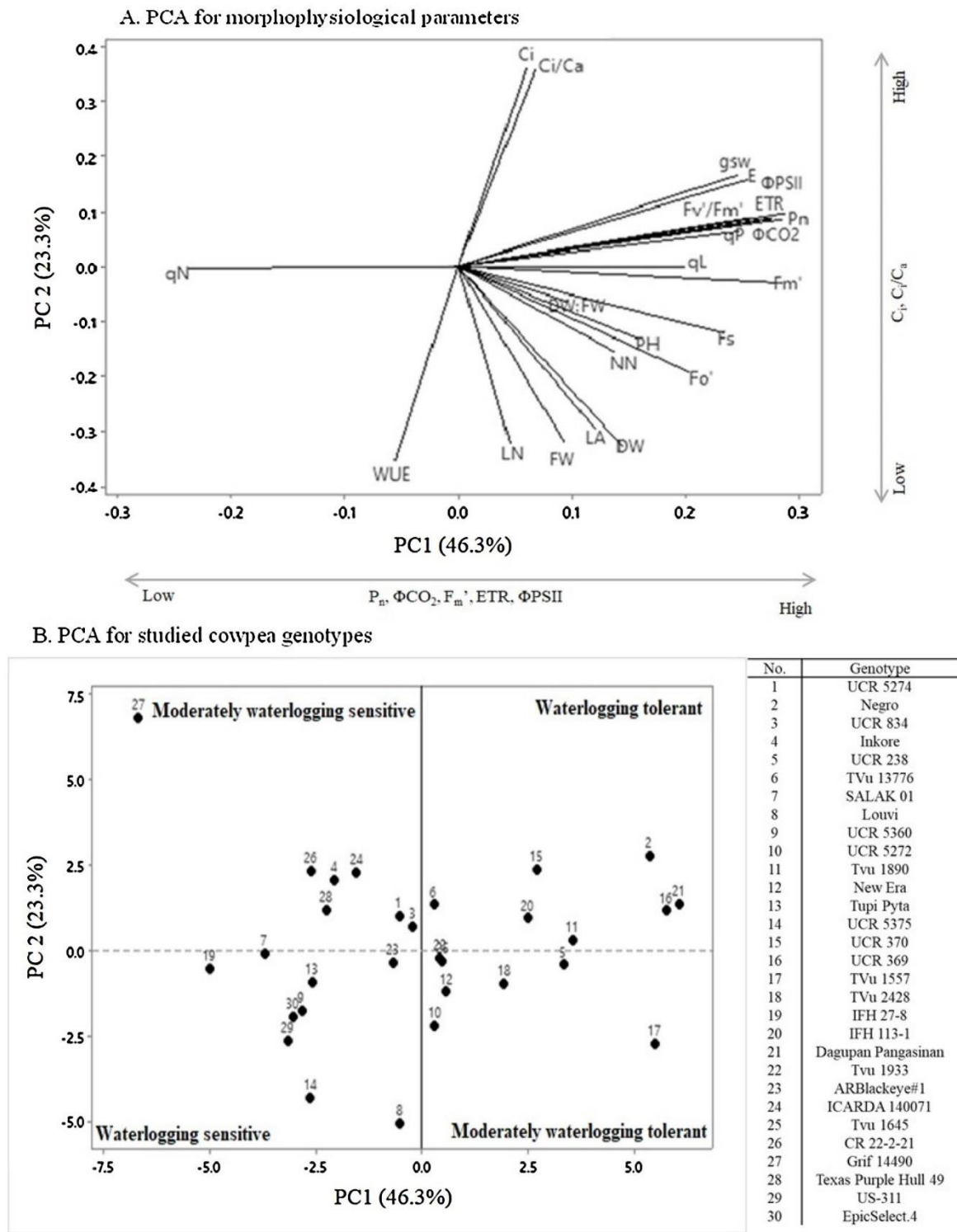
Figure 3.6   Principal component analysis (PCA) for (A). 24 morphophysiological parameters and (B). 30 cowpea genotypes based on WTC calculated for morphophysiological parameters.

The WCA was performed using the WTC of the 24 morpho-physiological parameters in this study (Figure 3.7). The cowpea genotypes were clustered and displayed in a dendrogram, with the distance between the clusters indicating the genotypes' similarity. The waterlogging-tolerant genotypes Negro, UCR 370, Dagupan Pangasinan, Tvu 1890, and UCR 369 were clustered together on the dendrogram in red (Figure 3.7). SALAK 01, US 311, IFH 27-8, Epic.select.4, and Tupi Pyta, which are waterlogging-sensitive (dendrogram colored in purple), showed a similar trend coupled with cowpea genotypes grouped in the moderately waterlogging-tolerant and moderately waterlogging-sensitive. It is interesting to note that a similar classification was recorded using WTC values and PCA.
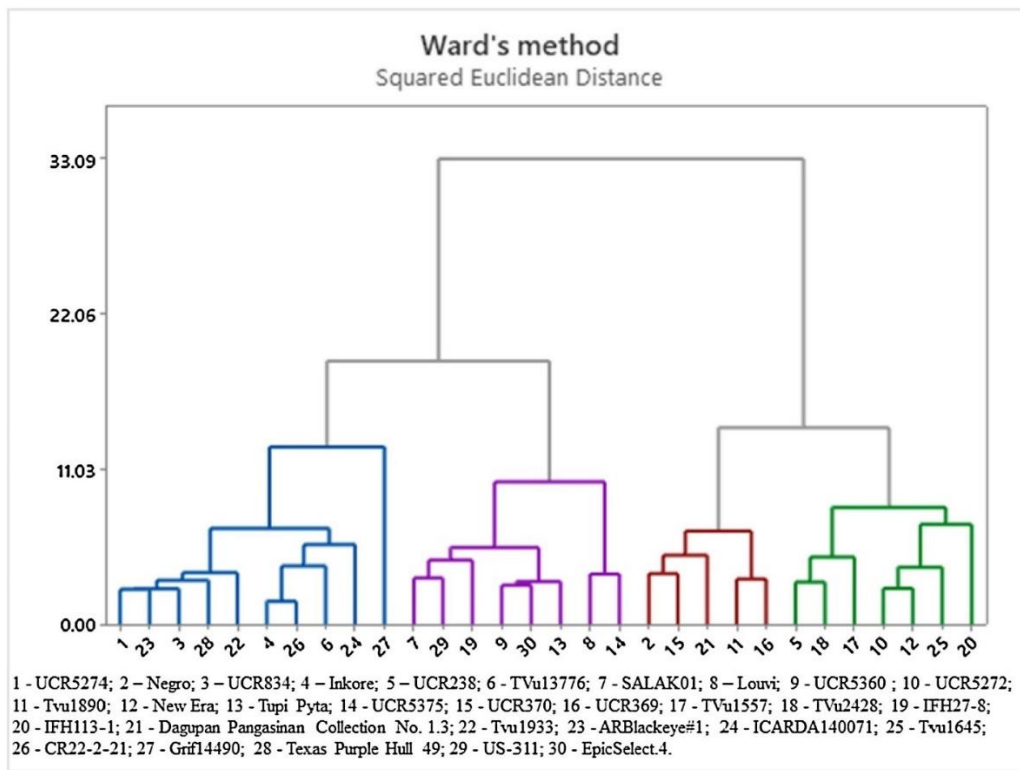
Figure 3.7   Dendrogram of 30 cowpea genotypes resulting from cluster analysis using WCA methods of WTC calculated for the 24 morphophysiological parameters. Dendrograms colored red, green, purple, and blue indicate waterlogging-tolerant, moderately waterlogging-tolerant, moderately waterlogging-sensitive, and waterlogging-sensitive groups, respectively.

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3.4   Discussion

Waterlogging stress can instigate a series of morphological and physiological changes in cowpeas, resulting in yield reduction (Hong et al., 1977; Timsina et al., 1994). Furthermore, recent studies on the integration of climate change and crop yield models have projected a decrease in cowpea productivity, which might exacerbate global food security (Hall, 2011; Ray et al., 2019). Thus, there is a critical need for a nuanced solution to this menace. Developing cowpea genotypes adapted to waterlogging stress through genetic improvement is an important strategy to address this challenge. However, to date, information on screening cowpea genotypes under waterlogging stress is limited. Therefore, evaluating the response of 30 cowpea genotypes to 10-day waterlogging stress in the early vegetative phase may help to understand the mechanism of waterlogging tolerance. In addition, it is understood that this is the first study to provide data on morpho-physiological parameters to evaluate the genetic variability and waterlogging tolerance of cowpea genotypes. Hence, understanding the performance of cowpeas under waterlogging conditions will be valuable for genetic engineering and breeding programs that integrate cowpea waterlogging tolerance.

3.4.1   Waterlogging induces morphological changes in cowpea genotypes

Exposure of cowpeas or related crops to waterlogging stress can cause significant changes in plant morphology, which are reflected in plant height, leaf area, biomass, and other growth functions (Ashraf, 2012; Takele and McDavid, 1994). Previous studies have ratified the decrease in shoot growth as an index for evaluating plants' response to waterlogging stress, which can be used for screening tolerant cultivars (Zou et al., 2014). In the current study, waterlogging of cowpea genotypes over ten days adversely affected the plant height and node numbers of all tested genotypes compared to the control treatments. Similar changes in plant

height were observed during the vegetative stage of soybeans (Dhungana et al., 2019), common beans (Ntukamazina et al., 2017), and mung beans (Kumar et al., 2013) under waterlogging stress. Previous findings in cowpeas also support that waterlogging stress impaired plant height, leading to stunted growth (Umaharan et al., 1997). According to these studies, the significant decrease in plant height under waterlogging conditions could be attributed to increased ethylene biosynthesis, mainly 1-aminocyclopropane-1-carboxylic acid (ACC), induced under hypoxic conditions (Pan et al., 2019). Increased ACC levels, although not measured in the current study, have been linked to the inhibition of abscisic acid, which limits shoot elongation, and the reduction of photosynthetic capacity via an apparent inhibition of ribulose 1,5-bisphosphate carboxylase activity, thereby inhibiting overall plant growth (Ntukamazina et al., 2017).

Furthermore, waterlogging stress at the vegetative stage can cause plants to respond in various ways to maintain their growth and development using their morphological traits, especially those related to the leaf, which is the site of photosynthesis. The significant reduction of leaf area and number and the occurrence of leaf senescence are common indicators of waterlogging stress, which worsens with the severity of flooding, especially in legumes (Kumar et al., 2013). It is important to note that in the current study, these adverse changes in the plant's leaves were found in all the cowpea genotypes tested under waterlogging compared to control treatments. The marked decrease in the leaf area of cowpea genotypes waterlogged for ten days can be attributed to the decline in A due to stomatal closure, as observed in Takele and McDavid (1994). Changes in the photosynthetic capacity can be attributed to differences in enzyme carboxylation, reduction in chlorophyll content, and reduction in leaf area, which are due to inhibition of leaf formation, expansion, damage, and shedding (Aldana et al., 2014).

In addition, in the present study, cowpea genotypes with higher leaf area and number were observed under waterlogging conditions to maintain the optimal photosynthetic capacity to ensure growth and development. In agreement, compared to waterlogging-sensitive genotypes, the identified tolerant pigeon pea genotypes have a higher leaf area under waterlogging (Kumutha et al., 2005). A larger leaf area was further used to illustrate the rapid and better recovery of waterlogging-tolerant genotypes of pigeon peas after 4 days of waterlogging (Kumutha et al., 2009). Thus, the identified genotypes with higher leaf area and number under waterlogged conditions may have an efficient protective mechanism (stomatal and non-stomatal gas exchange) that promotes increased leaf growth to ensure optimal A.

Previous studies have shown that waterlogging stress significantly reduces the biomass yield of cowpeas (Hong et al., 1977; Umaharan et al., 1997). Interestingly, we observed that the biomass of the cowpea genotype was significantly reduced under waterlogging stress. Previous studies reported that 16-day waterlogging duration in glasshouse conditions decreased shoot biomass of all cowpea genotypes by 60% compared to the control plants (Minchin and Summerfield, 1976). Correspondingly, a 6-day waterlogging duration significantly reduced the dry weight of all cowpea genotypes by 33% through accelerated leaf senescence, reduction in leaf area and plant height, and energy deficits, which limits A and E (Umaharan et al., 1997). However, almost doubling the waterlogging duration in the current study caused a 38% reduction in shoot biomass. The results of this study are consistent with the earlier reports on cowpeas and related crops (Hong et al., 1977; Kumar et al., 2013; Maekawa et al., 2011). Due to the genetic diversity among cowpea genotypes studied under waterlogging and control treatments, changes in biomass yield were also observed. Selective cowpea genotypes, namely Louvi, Tvu 1557, UCR 5272, and Dagupan Pangasinan, had relatively higher biomass under both treatments and

were thus categorized as either moderately or highly tolerant to waterlogging. In contrast, few cowpea genotypes, viz. Grif 14490, ICARDA 140071, Inkore, CR 22-2-21, which exhibited over 60% decrease in both biomass accumulation when waterlogging treatment was compared to control treatment, were designated as waterlogging sensitive. Several studies on other crops, such as Maize (Kaur et al., 2019; Liu et al., 2010), Lucerne (Smethurst and Shabala, 2003), Brassica (Zou et al., 2014), and Barley (Bertholdsson, 2013) have reported similar findings.

The morphological adaptation of cowpeas to waterlogging appears to involve the formation of adventitious roots, which are water loss avoidance mechanisms utilized by plants to prevent radial oxygen loss from the stem to the roots during submergence (Minchin et al., 1978; Solaiman et al., 2007). In the current study, 10-day waterlogging also resulted in the development of adventitious roots, which deteriorate the submerged root system and cause the main stem to become hypertrophic above the waterline. The formation of adventitious roots has been linked to increased expression of ACC levels, auxin, and regulation of reactive oxygen species in various legumes during waterlogging, including cowpea (Hong et al., 1977), mung bean (Ahmed et al., 2002), field pea (Pampana et al., 2016), and fava bean (Solaiman et al., 2007). These studies further attribute the development of adventitious roots of legumes to a key response to waterlogging stress, which provides oxygen, water, and nutrients for plant survival. Consequently, cowpea's newly formed aerated tissues improve plants' gas exchange, water, and nutrient absorption and greatly help plants adapt to hypoxic conditions (Steffens and Rasmussen, 2016).

Moreover, the current study demonstrated genotypic variation in the formation of adventitious roots in the tested genotypes. Compared to the control treatments, tolerant genotypes such as Negro and Dagupan Pangasinan that had more than the average number of 18

newly formed aerated tissues under waterlogging had higher g_s and less than a 10% decrease in

A. Contrarily, genotypes sensitive to waterlogging, such as IFH 27-8, US-311, SALAK 01, and

Grif 14490, have less than an average of 10 newly formed adventitious roots, and their gas

exchange parameters declined by more than 80% after 10-day waterlogging. Hence, increased

aerated tissues can be used directly to evaluate crop performance under waterlogging conditions.

3.4.2   Waterlogging induces physiological changes in cowpea genotypes

Waterlogging stress damages cowpea morphology, adversely affecting the shoots'

physiological response, particularly carbon fixation and stomatal conductance (Ploschuk et al.,

2018). Since oxygen diffusion in the submerged state is 10,000 times lower, the stomata and cell

walls cannot easily exchange the CO_2 required for the fundamental processes of the plant

(Voesenek and Bailey-Serres, 2015). In addition, the low availability of CO_2 in flooded leaves

may limit the production of heterotrophic energy in mitochondria, resulting in a corresponding

reduction in *A*, *E*, and g_s (Pedersen et al., 2013). In this context, we observed decreased *A*, *E*, g_s,

and C_i in all tested cowpea genotypes. A similar reduction in gas exchange parameters has been

reported in cowpeas, soybeans, and mung beans under submergence due to g_s (Ahmed et al.,

2002; Garcia et al., 2020). Consistent with the research by Velasco et al. (2019), decreased g_s

under waterlogging prevents excessive water loss by *E*, thereby reducing water absorption

capacity to maintain a positive water balance. Therefore, the WUE of cowpea genotypes was

enhanced under waterlogging treatment. More often, the decline in the photosynthetic capacity of

cowpeas due to waterlogging causes the inhibition of ETR, leading to photoinhibition in PSII.

Although we did not measure changes in leaf pigments in this study, an alternative mechanism

that may be contributing to the overall decline of *A* and g_s is the decline of chlorophyll content,

which was in form of leaf chlorosis exhibited by the waterlogged plants. General oxidative

stress, decreased light under submerged state, degradation of leaf chlorophyll and soluble content are non-stomatal limitations of A under waterlogging condition (Garcia et al., 2020).

The significant A reduction in the waterlogged genotype depends on stomatal and non-stomatal factors, such as impaired PSII activity (Ploschuk et al., 2018). In this study, the waterlogging reduced chlorophyll fluorescence parameters (such as F'_v/F'_m, F'_o, F'_m, qP, and Φ_{PSII}, which are signs of damaged PSII) excepting qN. Moreover, many studies have used changes in chlorophyll fluorescence parameters to understand plants' performance under waterlogging (Smethurst and Shabala, 2003; Velasco et al., 2019). The photosynthetic capacity of plants under non-waterlogging conditions is more functional when compared to waterlogged plants (Rao et al., 2021). Hence, waterlogging significantly declined F'_v/F'_m, F'_o, and qP of all tested cowpea genotypes compared to the control treatments. The reduction of F'_v/F'_m under waterlogging denotes that the light energy absorbed by the PSII was used to decrease the efficiency of the primary electron acceptor (Q_A) and the potential vigor change of PSII (Rao et al., 2021). Thus, these changes may reflect the tolerance of plants to various environmental stresses, including hypoxia and anoxia (Zhu et al., 2016). Besides, the reduced F'_v/F'_m, F'_o, and F'_m of cowpea genotypes under waterlogging in the early stages of vegetative growth indicates that cowpeas are sensitive to flooding stress. These findings corresponded to Ploschuk et al. (2018), who revealed the sensitivity of field peas to waterlogging due to damaged PSII.

Moreover, Φ_{PSII} is associated with significant reductions of F'_v/F'_m, which reflects the actual efficiency of photosynthesis (Colom and Vazzana, 2003; Genty et al., 1989). In the current study, the decline of Φ_{PSII} was observed to be the highest under waterlogging. However, the decrease of Φ_{PSII} of cowpea genotypes classified as waterlogging tolerant (e.g., UCR 369 and Dagupan Pangasinan) was smaller than that of the waterlogging-sensitive genotype (such as

SALAK O1, UCR 5375, and EpicSelect.4), suggesting that during waterlogging stress, tolerant cowpea genotypes can avoid photodamage better than the sensitive genotypes. Waterlogging tolerance can also be ascribed to the ability of the xanthophyll cycle to protect photosynthetic apparatus from photo-inhibitory damage under waterlogging (Zhu et al., 2016). In addition, the qP and qL represent the proportion of the open redox state of PSII's reaction centers, which can be used as an indicator to evaluate the occurrence of photoinhibition (Maxwell and Johnson, 2000; Shou-Ren, 1999). In this study, compared with the control treatment, the qP and qL of cowpea genotypes under waterlogging conditions were significantly reduced, primarily because of the decrease in the efficiency of excitation energy capture of open PSII reaction centers. Therefore, the waterlogging stress damages the PSII reaction center by keeping Q_A in oxidized or reduced form during steady-state light (Caudle and Maricle, 2012). Other studies on legumes consistently show that qP and qL under waterlogging are reduced, including waterlogging-sensitive genotypes, tending to poorly maintain water status (Smethurst et al., 2005; Velasco et al., 2019).

On the other hand, qN of cowpea genotypes shows the degree of heat dissipation or amount of energy not used in photochemical reactions increased under waterlogging stress. Previous studies (Velasco et al., 2019; Wu et al., 2015; Zhang et al., 2019) reported similar results. These studies surmised that increased qN under waterlogging stress prevented the potential damage of photosynthetic apparatus caused by excessive absorption of light energy by PSII. Hence, the current research shows that the decline in the photosynthetic capacity of cowpea genotypes can be attributed to stomatal and non-stomatal factors in the early developmental stage.

3.4.3   Classification of cowpea genotypes and their associated traits under waterlogging

Plant waterlogging tolerance is a multifaceted trait that integrates plants' morphological, physiological, biochemical, and molecular characteristics (Yamauchi et al., 2018; Zou et al., 2014). Since a single method is insufficient to reflect plants' waterlogging tolerance accurately, WTC, PCA, and WCA were used to evaluate the waterlogging tolerance of cowpea genotypes in this study. The WTC method quantifies the overall performance of each cowpea genotype under waterlogging stress, thereby providing selection criteria for screening waterlogging-tolerant cowpea genotypes. Based on WTC, the cowpea genotypes were classified as waterlogging-tolerant, moderately waterlogging-tolerant, moderately waterlogging-sensitive, and waterlogging-sensitive. The identified tolerant genotypes in this study exhibited lower waterlogging sensitivity with relatively higher values for photosynthetic and chlorophyll fluorescence parameters. The increased gas exchange activity of tolerant genotypes can provide greater plant survival rates and higher yields. Hence, compared with other genotypes, the cowpea genotype with higher WTC had lower biomass yield reduction under waterlogging stress. The present study results are consistent with those of the previous study, where 25 rapeseed varieties were screened based on WTC and tolerant genotypes with high WTC were identified (Zou et al., 2014). Moreover, the identified tolerant genotypes, such as Dagupan Pangasinan, UCR 369, and Negro, can be used along with other agronomic measures for mitigating waterlogging to improve crop yields in commercial cowpea production.

In addition, gas exchange parameters were significantly correlated with the total WTC, indicating that WTC variability can be better explained by chlorophyll fluorescence and photosynthetic parameters than measured morphological parameters during the early developmental growth stage. Thus, cowpea genotypes that maintained their growth prevented

photoinhibition by enhancing F'_v/F'_m, and regulated their photosynthetic apparatus under waterlogging had a better waterlogging tolerance mechanism than the other genotypes. Therefore, these results reveal the importance of gas exchange parameters and WTC in selecting tolerant genotypes for waterlogging stress. Highly significant genotypic differences were also observed for all the studied morpho-physiological parameters, indicating that the cowpea genotypes tested have inherent genetic variation among their studied traits. These results are consistent with those of other studies on Maize (Liu et al., 2010), beans (Velasco et al., 2019), and peanuts (Zeng et al., 2020) under waterlogging stress. Hence, the study's results revealed the substantial genetic diversity of the studied genotypes, which can be used in breeding programs that integrate cowpea waterlogging tolerance.

MCA showed the relationship between more than two measured morpho-physiological parameters simultaneously through WCA. The WCA provided a wide range of variability, which aided in the classification of waterlogging tolerance in cowpea genotypes. WCA was developed using the squared Euclidean distance of 30 cowpea genotypes under waterlogging treatment, dividing the genotypes into four cluster groups. The dendrogram colored in red contained cowpea genotypes that showed more waterlogging tolerance and minimal stress susceptibility with enhanced morpho-physiological characteristics such as higher A, g_s, E, Φ_{PSII}, ETR, F'_v/F'_m, and qP. These genotypes were classified as waterlogging tolerant. Again, the cluster colored in green contained the moderately tolerant genotypes with higher morphological and physiological performance regarding plant height, leaf area, and biomass under waterlogging conditions. Furthermore, cowpea genotypes with moderate sensitivity to waterlogging stress were placed in the purple-colored cluster. These genotypes had good morphological and physiological performance under the control treatments. However, they could not keep their potential

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performance under waterlogging treatment. Lastly, the cluster colored in blue, which had their morphological and physiological traits adversely impaired by waterlogging, were classified as waterlogging sensitive. A substantial decline in the photosynthetic performance of this group could be attributed to the limited number of adventitious roots and decreased g_s, which increased their sensitivity to waterlogging. Several studies have utilized these methods of classifying genotypes based on their morphological and physiological traits in many crops. For instance, Neashat et al. (2020) grouped 12 mung beans genotypes into four clusters, with tolerant and moderately genotypes placed in clusters III and IV, respectively, with relatively higher morphological performance, whereas sensitive and highly susceptible genotypes with poor morphological growth under waterlogging were identified in cluster I and II, respectively. Kołton et al. (2020) also classified 19 cucumber and 16 tomato accessions into two clusters based on their waterlogging tolerance and morpho-physiological performance under waterlogging at the early vegetative growth stage.

Overall, the results of the PCA showed a similar classification of cowpea genotypes with WCA and WTC methods. From the biplot analysis, cowpea genotypes and measured parameters far from the origin revealed a superior agronomic potential to the other genotypes (Gedam et al., 2021). In this study, cowpea genotypes Dagupan and UCR 369 were positioned far from the origin and thus classified as waterlogging tolerant, which can be utilized in cowpea breeding for waterlogging tolerance. It is also interesting to note that the genotypes of the waterlogging tolerant group on the PCA significantly correlate with important gas exchange parameters that reflect waterlogging tolerance in plants. Corresponding findings in maize were reported by Panozzo et al. (2019). Hence, the current results showed that the ability of genotypes to maintain higher gas exchange capacity under waterlogging stress could be utilized for developing

waterlogging tolerance in plants. However, the negative correlation of qN with other gas exchange parameters illustrates a conceivable trade-off technique used by cowpea to adapt to waterlogging stress by ensuring the equilibrium of energy lost during photochemical processes in PSII.

3.5 Conclusion

Evaluating the morpho-physiological performance of cowpea genotypes during their early developmental stage under waterlogging stress is valuable for breeding programs that integrate cowpea waterlogging tolerance. This study revealed that the cowpea genotype significantly interacted with morpho-physiological parameters, indicating that most traits are quantitatively inherited and differentially expressed under waterlogging. The positive correlation between the total WTC and the photosynthetic and chlorophyll fluorescence parameters WTC further indicates that screening cowpea genotypes based on gas exchange parameters can provide reliable analysis and classification of waterlogging tolerance. Moreover, the PCA identified A, Φ_{CO2}, F'_m, ETR, and Φ_{PSII} as the parameters to best describe waterlogging tolerance in cowpeas.

This study suggested that WTC, PCA, and WCA could be used as reliable methods for screening cowpea genotypes and classifying them into different groups based on the variation in morpho-physiological performance under waterlogging stress. Selected genotypes of waterlogging-tolerant cowpeas, such as Dagupan Pangasinan, UCR 369, and Negro, may help breeders develop new cowpea genotypes that can withstand flooding conditions. These unique tolerant genotypes can be crossed with high-yielding commercial cowpea varieties to introduce waterlogging tolerance genes without affecting their inherent performance under waterlogging stress. However, these findings can be further verified in a field condition to assess their growth and yield performance under flooded conditions to ensure sustainable cowpea production.

CHAPTER IV

WATERLOGGING DURING THE REPRODUCTIVE GROWTH STAGE CAUSES

PHYSIOLOGICAL AND BIOCHEMICAL MODIFICATIONS IN THE LEAVES

OF COWPEA GENOTYPES WITH CONTRASTING TOLERANCE

Abstract

Waterlogging causes various metabolic, physiological, and morphological changes in crops, resulting in the yield loss of most legumes in rainfed and irrigated agriculture. However, research on cowpea genotypes using physiological and biochemical traits to measure tolerance to waterlogging stress is limited. We evaluated the impacts of 7 days of waterlogging and 7 days of recovery on the physiology and biochemistry of two cowpea genotypes (UCR 369 and EpicSelect.4) with contrasting waterlogging tolerance. Cowpea genotypes were grown in a controlled environment until the R2 stage and subjected to 7 days of waterlogging (DOW). Later, the waterlogged plants were drained to allow an additional 7 days of recovery (DOR). Overall, cowpea genotypes had a contrasting response to waterlogging using different mechanisms. Compared to the control, the carbon assimilation rate (A) of both cowpea genotypes was impaired under 7 DOW and could not recover at 7 DOR, with a larger decline in EpicSelect.4. There was a highly specific downregulation of the stomatal (g_s) and mesophyll conductance (g_m), maximum rate of Rubisco (V_{cmax}), and photosynthetic electron transport rate (J_{max}) as non-stomatal limiting factors decreasing A in EpicSelect.4. In addition, 7 DOW caused significant loss in the chlorophyll and carotenoid content of both genotypes. However, only waterlogged

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UCR 369 was not photo-inhibited and could restore the levels of chlorophyll and carotenoids after 7 days of recovery. In addition, waterlogging induced intense stress in UCR 369 with increased zeaxanthin, sucrose, and flavonoid content. At the same time, these metabolites were decreased in EpicSelect.4. On the other hand, glucose, fructose, and phenolic content were increased in EpicSelect.4 but decreased in UCR 369 at 7 days of recovery. In summary, compared to EpicSelect.4, UCR 369 restored its photosynthetic pigments and metabolites to the control levels at recovery, indicating a likely tolerance to waterlogging stress.

4.1   Introduction

Cowpea (*Vigna unguiculata* L.) is a versatile crop that provides dietary and low-cost protein for humans and livestock. Cowpeas also play an important role in the sustainability of cropping systems due to their atmospheric nitrogen fixation potential (Timko and Singh, 2008). This species is cultivated on an area of approximately 15.1 million hectares worldwide, with an annual production of 8.9 million tonnes (FAO, 2022). However, cowpea is sensitive to waterlogging (Minchin et al., 1978; Olorunwa et al., 2022), which has become one of the major environmental factors limiting the growth, development, and productivity of rainfed and irrigated crops. More than 50% of U.S. cowpea production occurs in the South. In this region, cowpeas are grown shortly after summer rains and then rely on rainfall or irrigation for proper growth and development to ensure optimum yields (Hong et al., 1977). Climate models predict a 20-40% increase in spring precipitation in the southern U.S. by the end of the 21st century (Easterling et al., 2017).

Previous studies have shown that waterlogging can reduce cowpea yields by 10-90%, especially during the reproductive stage (Jayawardhane et al., 2022; Minchin et al., 1978; Timsina et al., 199). Consequently, excessive precipitation due to climate change and poor soil

drainage can cause temporary waterlogging of cowpea plants, resulting in reduced oxygen levels in the roots, which causes various metabolic, physiological, and morphological changes in plants (Setter and Waters, 2003). Therefore, exploring the mechanisms of cowpea's response to waterlogging and uncovering tolerance traits is crucial for developing new and improved cultivars.

Waterlogging conditions result in hypoxia stress, adversely affecting the entire plant's growth and survival. Hypoxia is described by limited aerobic metabolism, which inhibits ATP regeneration produced by mitochondrial oxidative phosphorylation and NAD^+ generated by the mitochondrial electron transport chain (Nakamura and Noguchi, 2020). Under these waterlogged conditions, plants suffer from severe disorders caused by cell acidification, low energy supply, and reduced intracellular environment. Consequently, reactive oxygen species (ROS) that oxidize proteins, lipids, and nucleic acids are overproduced in response to waterlogging stress, resulting in oxidative damage to the basic structure of plants (Gill and Tuteja, 2010). For example, Sairam et al. (2009) reported that 4-6 days of waterlogging stress promotes ROS accumulation in the leaves of pigeon pea genotypes. In addition, the concentration of the ROS in waterlogged stressed common bean leaves was higher compared to non-stressed leaves (Costa et al., 2020). However, recent studies have shown that oxidative damage caused by waterlogging does not usually appear immediately but relatively soon after the water level drops during the recovery period of complete submergence (Barik et al., 2019; Sarkar et al., 2006). After draining the waterlogged soil, a second and more pronounced increase in ROS and loss of redox homeostasis in the plant occurs (Da-Silva and do Amarante, 2020; Garcia et al., 2020).

Furthermore, plants subjected to severe and prolonged waterlogging have significantly declined their carbon assimilation rate (*A*) due to ROS accumulation, resulting in reduced growth

and productivity (Ahmed et al., 2012; Pan et al., 2021). The factors affecting the A of plants are primarily divided into two distinct metabolisms: stomatal and non-stomatal limitations. Due to limited oxygen under waterlogging conditions, plants close their stomata to maintain plant water status, causing a decline in stomatal conductance (g_s) and inhibiting the exchange of CO_2 required by the plant's basic processes (Voesenek and Bailey-Serres, 2015). Consequently, the reduction in g_s eventually leads to a corresponding decrease in A (Kreuzwieser and Rennenberg, 2014). Another potential limitation of A in the submerged condition is the alteration in mesophyll conductance (g_m), which is the diffusion of CO_2 from intracellular space to the carboxylation site in the chloroplast stroma (Black et al., 2005). Non-stomatal limitation of A under waterlogging in legumes is associated with the maximum rate of Rubisco carboxylation (V_{cmax}), ribulose-1,5-bisphosphate (RuBP) regeneration capacity mediated by maximum electron transport rate (J_{max}), photosystem II (PSII) activity, Rubisco activity, and loss of pigments related to leaf senescence (Araki et al., 2012; Pompeiano et al., 2019; Zhou et al., 2014). The effects of waterlogging on stomatal and non-stomata factors limiting A varies with crop genotype, duration, and severity of waterlogging stress, ranging from a significant decline in sensitive genotypes to little or no inhibition in tolerant genotypes (Bansal and Srivastava, 2015; Ploschuk et al., 2018). However, comparing these factors between waterlogging-tolerant and -sensitive genotypes is scarce in cowpeas. Hence, evaluating the key factors limiting the photosynthetic performance of cowpea genotypes could reveal the underlying mechanisms of their responses to waterlogging stress.

Previous studies demonstrated that the decline in A under waterlogging resulted from decreased leaf chloroplast pigments (such as chlorophyll and carotenoids), regarded as a common index for oxidative stress (Garcia et al., 2020; Kumar et al., 2013). However, high chlorophyll content shows low photoinhibition of photosynthesis because it ameliorates the

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adverse effect of waterlogging stress on the plant's photoassimilates. Thus, evaluating leaf pigments can help understand cowpea's resilience to waterlogging stress. For example, a 9-day waterlogging caused a significant reduction in the growth and the A of mungbean (*Vigna radiata*) genotypes due to a 63% reduction in chlorophyll content (Kumar et al., 2013). Recent evidence suggests that waterlogging-sensitive genotypes could lose up to 100% of their leaf pigments when subjected to severe water stress (Ploschuk et al., 2022). Loss of chlorophyll concentration is described as a waterlogging feedback mechanism to curtail light absorption in the leaf (Singh and Reddy, 2011). Since nitrogen is a component of chlorophyll content in plants, the same method of estimating chlorophyll content indirectly measures the nitrogen content in plants. Hence, determining chlorophyll content in cowpeas under waterlogging stress can be closely linked to efficient nutrient management.

On the other hand, carotenoids are accessory light pigments that function as ROS scavengers and have protective properties (Esteban et al., 2015; Gill and Tuteja, 2010). Vosnjak et al. (2021) surmised that zeaxanthin (ZEA), a pigment of the xanthophyll cycle, plays a vital role in stress response. Carotenoids protect both photosystems I and II (PSII) under stress by quenching excited triplet chlorophyll to dissipate excess energy and counteracting ROS accumulation by binding singlet oxygen (Frank and Cogdell, 1996; Tracewell et al., 2001). The de-epoxidation cycle produces zeaxanthin and antheraxanthin from violaxanthin to thermally dissipate excess excitation energy that cannot be utilized for photosynthesis (Vosnjak et al., 2021).

Plants use an important mechanism to respond to waterlogging stress: the accumulation of compatible organic solutes in the cytoplasm and the flow of mineral solutes into the vacuole to facilitate osmotic adjustments (Akula and Ravishankar, 2011). Plants' intrinsic characteristics of

osmotic adjustment aid adaptation to hypoxia by maintaining water balance, turgor pressure, and other physiological processes using different osmolytes or compatible solutes (Anjum et al., 2017; Taiz et al., 2015). For example, accumulating soluble sugars such as sucrose, glucose, and fructose in stressed plants improves waterlogging tolerance by preventing oxidative stress and stabilizing plasma membranes and biomolecules (Barickman et al., 2019; Kuai et al., 2014; Sairam et al., 2009). Increased soluble sugars in waterlogging-resistant genotypes resulted in better growth and biomass accumulation than in sensitive genotypes (Sairam et al., 2009; Takahashi et al., 2018). Elkelish et al. (2020) also revealed an enhanced accumulation of phenolics and flavonoids among tolerant tomato seedlings under waterlogging conditions. So far, studies on the genotype of cowpeas using compatible solutes as a measure of resistance to evaluating waterlogging stress are limited. Hence, biochemical analysis of cowpea genotypes under waterlogging stress is required to confirm and elucidate the accumulation of osmolytes responsible for waterlogging tolerance in cowpeas.

In the context of climate change, a better understanding of waterlogging stress limitations of cowpeas is critical, particularly in regions where rainfed and irrigated agriculture is essential for sustainable crop production. Furthermore, there is limited research on the effect of waterlogging stress and post-waterlogging recovery period on the secondary metabolites of the cowpea genotype during the R2 growth stage. Hence, this study was conducted to determine the effects of waterlogging stress on contrasting cowpea genotypes based on key physiological and biochemical traits. Two cowpea genotypes with contrasting waterlogging tolerance, UCR 369 and EpicSelect.4, were subjected to 7 days of waterlogging and 7 days of recovery during the R2 growth stages. We aimed to provide the physiological and biochemical basis for waterlogging tolerance in the contrasting cowpea genotypes using photosynthesis, Rubisco activity,

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carotenoids, chlorophyll, phenolics, and soluble sugar content. This study hypothesized that the sensitive genotype would significantly decrease their photosynthetic capacity and pigments. In contrast, the tolerant genotype would accumulate higher phenolics, flavonoids, and sucrose under waterlogging stress.

4.2   Materials and Methods

4.2.1   Plant material and growth conditions

The experiment was carried out in the Vegetable Physiology Greenhouse at the North Mississippi Research and Extension Center (Verona, MS, USA) from 22 October to 21 December 2021. The Seed 16 controller was used to set and record environmental conditions in the greenhouse (Wadsworth, Arvada, CO, USA). The temperature and relative humidity in the greenhouse were monitored using the HygroVUE™5 sensor (Campbell Scientific Inc., Logan, UT, USA). Daily light integrals in the greenhouse were measured using a photosynthetically active radiation sensor (SQ-110-SS; Apogee Instruments, Logan, UT, USA) connected to a CR1000x data logger (Campbell Scientific, Logan, UT, USA). During the experiment, the average daily temperature, relative humidity, and daily light integral in the greenhouse were 25.1 $\pm$ 4.6 °C (mean $\pm$ SD), 66.2 $\pm$ 11.8 %, and 20.2 $\pm$ 11.7 mol m^{-2} d^{-1}, respectively.

Two cowpea genotypes, UCR 369 (waterlogging-tolerant) and EpicSelect.4 (waterlogging-sensitive), were used to evaluate tolerance during and after waterlogging during the reproductive growth stage (R2). Compared with EpicSelect.4, which showed severe signs of leaf senescence after ten days of waterlogging, the genotype, UCR 369, exhibited superior photosynthetic capacity and the rapid development of adventitious roots. The selected cowpea genotypes have similar growth and duration of the life cycle of 65 to 70 days. Previous studies

have characterized these genotypes in terms of morphological and physiological traits under waterlogging conditions (Olorunwa et al., 2022).

4.2.2 Experimental design and waterlogging treatments

Plants were grown in one-gallon plastic nursery pots, 15.9 cm deep and 16.6 cm in diameter. At the bottom end of each pot, five drainage holes retained the substrate but allowed the draining of excess water to avoid waterlogging. Each pot was filled with Pro-Mix BX soilless medium (Premier Tech Horticulture, Quebec, Canada). A total of 160 pots were used per genotype (320 pots in total). Cowpea seeds were treated directly with *Bradyrhizobium sp.* (vigna) (Exceed® superior legume inoculant; Visjon Biologics, Wichita, TX, USA) at a rate of 70.87 g per 22.68 kg of seeds before planting. Four seeds per pot were sown, and after two weeks, the seedlings were thinned to one plant per pot. The plants were fertigated twice a week with a 5-15-29 water-soluble nutrient solution at a rate of 100 ppm. The pots were arranged in a randomized complete block design, with two treatments, two cowpea genotypes, and five replications in a 5 x 16 factorial arrangement.

Forty-five days after sowing (DAS), cowpea plants with 50-100% of opened flowers at the R2 growth stage were subjected to two experimental treatments, including waterlogging and control treatments, for 7 days. Cowpea plants were waterlogged by placing 6 pots of each cowpea genotype into five replicated containers (Husky 15-gallon Latch and Stack Tote, Home Depot, Atlanta, GA), similar to that described by Barickman et al. (2019) and Olorunwa et al. (2022). In brief, the container was filled with tap water to a height of 2-3 cm above the substrate surface to simulate 7 days of waterlogging treatments. Under the control treatments, pots containing cowpea plants were kept at the optimal field capacity. After 7 days of waterlogging,

the pots were removed from the 15-gallon container filled with water, and the plants were allowed to recover for an additional 7 days.

4.2.3   Leaf gas exchange measurements

The net CO_2 assimilation rate (A), stomatal conductance (g_s), transpiration rate (E), and intercellular CO_2 concentration (C_i) were measured on the topmost recently fully expanded leaf of cowpea plants. Measurements were taken using a portable infrared gas analyzer (LI-6800, LI-COR, Lincoln, NE, USA) under a relative humidity of 65%, a light intensity of 1500 µmol m^{-2} s^{-1}, a temperature of 30 °C and a CO_2 concentration of 415 ppm. Measurements were performed on five representative plants of each cowpea genotype subjected to control and waterlogging treatments during 3 and 7 DOW and 3 and 7 days of recovery (DOR) in five replicates.

Additionally, the CO_2 response curve (A/C_i) measurements were evaluated using the auto program settings in the LI-6800 at 7 DOR and 7 DOR. To measure the steady-state response of A/C_i, the leaf chamber settings were fixed at 50% relative humidity, 1500 µmol m^{-2} s^{-1} light intensity, and the temperature set to maintain ambient greenhouse temperature (28-30°C). Using the built-in program on the LI-6800, measurements were taken at 50, 100, 200, 300, 400, 500, 600, 800, 1000, 1200, and 1500 ppm CO_2, with early matching enabled, and wait times of 60-90 seconds between measurements. A/C_i analyses were performed according to Sharkey et al. (2007), with few changes as portrayed in Olorunwa et al. (2021) using the excel fitting tool 10.0 available at http://landflux.org/Tools.php. Representative individual curves were fitted separately, and the extracted parameters were averaged across replicates for each treatment. According to Bernacchi et al. (2001), the estimated A/C_i response curve data was further utilized to calculate the maximum rate of Rubisco carboxylation (V_{cmax}), the maximum rate of photosynthetic electron transport (J_{max}), and mesophyll conductance (g_m).

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4.2.4 Chlorophyll fluorescence measurements

The LI-6800 using pulse-amplitude modulated (PAM) fluorometry with a Multi-phase Flash Fluorometer (6800-01A, LI-COR Biosciences, Lincoln, NE, USA) was used to measure the chlorophyll fluorescence at 3 and 7 DOW and 3 and 7 DOR. During predawn hours (3.00 – 5:00 CST), the minimal fluorescence of dark-adapted leaves was measured on the second-most fully expanded leaf using a measuring light (0.005 µmol m^{-2} s^{-1}). The maximal fluorescence was quantified using a 1-second saturating pulse at 8,000 µmol m^{-2} s^{-1} in dark-adapted leaves. The leaves were continuously illuminated for 20 minutes with an actinic light (1,400 µmol m^{-2} s^{-1}) to record the steady-state yield of fluorescence. Maximal light-adapted fluorescence yield was determined by 8,000 µmol m^{-2} s^{-1}. The actinic light was turned off, and minimal fluorescence yield in the light-adapted state was determined after 5 seconds of far-red illumination. The chlorophyll fluorescence parameters, including the quantum yield of PSII photochemistry (Φ_{PSII}), the effective quantum efficiency by open PSII reaction center (F'_v/F'_m), the maximum quantum efficiency of PSII reaction centers (F_v/F_m), and the non-photochemical quenching (NPQ) were calculated according to Maxwell and Johnson (2000) with few modifications reported in (Olorunwa et al., 2022).

4.2.5 Plant harvest and processing

Leaves of five representative cowpea plants from each treatment, replication, and genotype were harvested at 3 DOW, 7 DOW, 3 DOR, and 7 DOR. Subsamples of leaf tissue were flash-frozen in liquid nitrogen and stored in an ultra-low −80 °C freezer until processing. Leaf samples were lyophilized for 72 hours using a FreeZone 2.5L freeze dryer (Labconco Corp., Kansas City, MO, USA) for pigment analysis.

4.2.6   Carotenoid and chlorophyll analysis

The extraction of carotenoids and chlorophylls from leaf tissue was conducted as described by Kopsell et al. (2004), with modifications reported by Brazel et al. (2021). Briefly, 0.1g of lyophilized tissue subsamples were ground 4 times using 0.8 mL of high-performance liquid chromatography (HPLC) Grade H_2O, 0.8 µL of Internal Standard Concentration (Sigma Chemical Co., St. Louis, MO, USA), and 2.5 mL of Tetrahydrofuran. After extraction, the samples were placed into a nitrogen evaporator (N-EVAP 112; Organomation Associates, Inc., Berlin, MA, USA) to reduce the sample volume to 0.5 mL. Then, acetone (Thermo Fisher Scientific, Waltham, MA, USA) was added to the reduced supernatant to produce a 5 mL sample. Samples were that collected using a 5 mL syringe and passed through a 0.22 µm polytetrafluoroethylene (PTFE) filter (Basix, Thermo Fisher Scientific, Waltham, MA, USA) into 2.0 mL vials for analysis.

Pigments were analyzed on an Agilent 1260 HPLC according to Emenhiser et al. (1996) and Davies and Köst (1988). Briefly, the reverse-phase column was maintained at 60°C in an Agilent 1260 (Agilent Technologies, Santa Clara, CA, USA) thermostatic column compartment. An injection of 5 µL from each sample was separated using a mobile gradient phase for chromatographic separations utilizing methanol, triethylamine (Fisher Chemical, Thermo Fisher Scientific, Waltham, MA, USA), and tert-Butyl methyl ether (Alfa Aesar, Thermo Fisher Scientific, Waltham, MA, USA). Peak assignment of individual pigments was performed by comparing retention times and line spectra obtained from photodiode array detection using a reliable external standard (beta-carotene (β-car), Chlorophyll α, Chlorophyll β, Lutein (LUT), neoxanthin (NEO), violaxanthin (VIO), antheraxanthin (ANTH), zeaxanthin (ZEA) from ChromaDex Inc., Irvine, CA, USA). Total chlorophyll content (chlorophyll α + β) was calculated

as chlorophyll α + chlorophyll β. Total carotenoid content (CAR) was calculated as NEO + VIO + ANTH + LUT + ZEA + β-car, while the total xanthophyll cycle pigment content (VAZ) was calculated as VIO + ANTH + ZEA. The de-epoxidation state (DEPS), involving xanthophyll cycle components, was calculated as (ZEA + 0.5 ANTH)/VAZ.

4.2.7 Total phenolic content

TPC was determined following the Folin-Ciocalteau methodology as described by Singleton et al. (1999), with minor modifications described by Ordoñez et al. (2006). Briefly, 5 mL of 96+% ACS-grade ethanol was added to 0.1 g of lyophilized tissue and incubated for 7 days at room temperature using a Multi-Pulse Vortexer (Glas-Col, Terre Haute, IN, USA). The extracted samples were then diluted in a 1:10 ratio using 96+% ACS-grade ethanol as the diluent. Next, the samples were reacted with a 0.2 N Folin-Ciocalteau reagent for 5 min. Then, 0.4 ml of the prepared sodium carbonate (Na_2CO_3) was added to the mixture and incubated for 2 h at room temperature. Gallic acid was used as a standard calibration curve, and absorbance was read at 760 nm using the Synergy® H1 Microplate Reader (BioTek, Winooski, VT, USA). Phenolics results were expressed as gallic acid equivalents from a pure standard using a standard calibration curve (Alfa Aesar, Thermo Fisher Scientific, Waltham, MA, USA).

4.2.8 Total flavonoid content

Total flavonoid content was determined using the aluminum chloride colorimetric method of Woisky and Salatino (1998), with minor modifications. Briefly, 0.5 mL of sample extracted in the previous section (**Error! Reference source not found.**) was reacted with a 2% $AlCl_3$-ethanol solution and incubated for 1 hr at room temperature. Next, the samples were centrifuged (Eppendorf centrifuge 5417R, Hamburg, Germany) at 14,000 RPM for 10 min at 4

°C. The supernatant was then filtered through 0.22 µm PTFE, and the absorbance was read immediately at 420 nm using the Synergy® H1 Microplate Reader. Flavonoid results were expressed as quercetin dihydrate equivalents using a standard calibration curve.

4.2.9 Soluble sugar analysis

Soluble sugars were extracted and quantified with an Agilent 1260 Infinity HPLC using a protocol published by Barickman et al. (2019). Peak assignments were made to individual sugars by comparing retention times from a refractive index detector using external standards for sucrose, fructose, and glucose (Sigma-Aldrich, St. Louis, MO, USA).

4.2.10 Statistical analysis

SAS (version 9.4; SAS Institute, Cary, NC) was used to analyze the data statistically. A three-way analysis of variance (ANOVA) using the generalized linear mixed model (PROC GLIMMIX) was used to assess the effects of factors (treatments, genotypes, and duration), along with their interactions, on the replicated values of physiological and biochemical parameters during waterlogging and recovery. The experiment's fixed effects consist of treatment (control and waterlogging), cowpea genotypes (UCR 369 and EpicSelect.4), and durations (3 DOW, 7 DOW, and 3 DOR, 7 DOR), where the replication (5 levels) was treated as a random effect. The Fisher's protected least significant difference test ($P \leq 0.05$) was used as the post hoc to identify statistically significant differences in the mean values of the parameters measured. The standard errors were calculated using the pooled error term from the ANOVA table. Diagnostic tests, such as Shapiro–Wilk in SAS, were conducted to ensure that treatment variances were statistically equal before pooling and to evaluate the normal distribution of data. Graphs were plotted with Sigmaplot 14.5 (Systat Software Inc., San Jose, CA, USA).

4.3 Results

4.3.1 Gas exchange parameters of cowpea genotypes during waterlogging and recovery

Waterlogging significantly decreased the photosynthetic parameters of cowpea plants (Figure 4.1). After 3 DOW, there was no significant difference in the A of UCR 369, but it was lower after 7 DOW than the control. The A was significantly decreased for EpicSelect.4 when subjected to 3 DOW compared to the control, which was consistently lower after 7 DOW (Figure 4.1A). Waterlogged EpicSelect.4 plants showed a significantly reduced A at 3 DOR, while the A of UCR 369 during 3 DOR was comparable to the control. However, both cowpea genotypes were unable to recover to the control level at 7 DOR, with a more comparable decline in EpicSelect.4 (74%), compared to UCR 369 (33%) (Figure 4.1B).

3 DOW did not affect the g_s of cowpea genotypes, but 7 DOW resulted in stomatal in UCR 369 and EpicSelect.4 by 45% and 98%, respectively (Figure 4.1C). However, the g_s of EpicSelect.4 did not recover at 3 and 7 DOR, while waterlogged UCR 369 presented similar g_s to the control at 3 DOR (Figure 4.1D).

In addition, 3 and 7 DOW decreased the E of EpicSelect.4 by 38% and 98%, respectively, whereas only 7 DOW caused a significant decline in E of UCR 369 by 57% (Figure 4.1E). However, E of EpicSelect.4 did not recover at 3 and 7 DOR. At 3 DOR, the E of UCR 369 was not significantly different from the controls (Figure 4.1F).

Moreover, the C_i of EpicSelect.4 was reduced by 42% and 38% when subjected to 3 and 7 DOW, respectively, relative to the control (Figure 4.1G). At 7 DOR, the C_i of EpicSelect.4 was significantly increased by 34% compared to the control. However, there were no significant differences in C_i of UCR 369 under all treatments compared to control plants (Figure 4.1H).
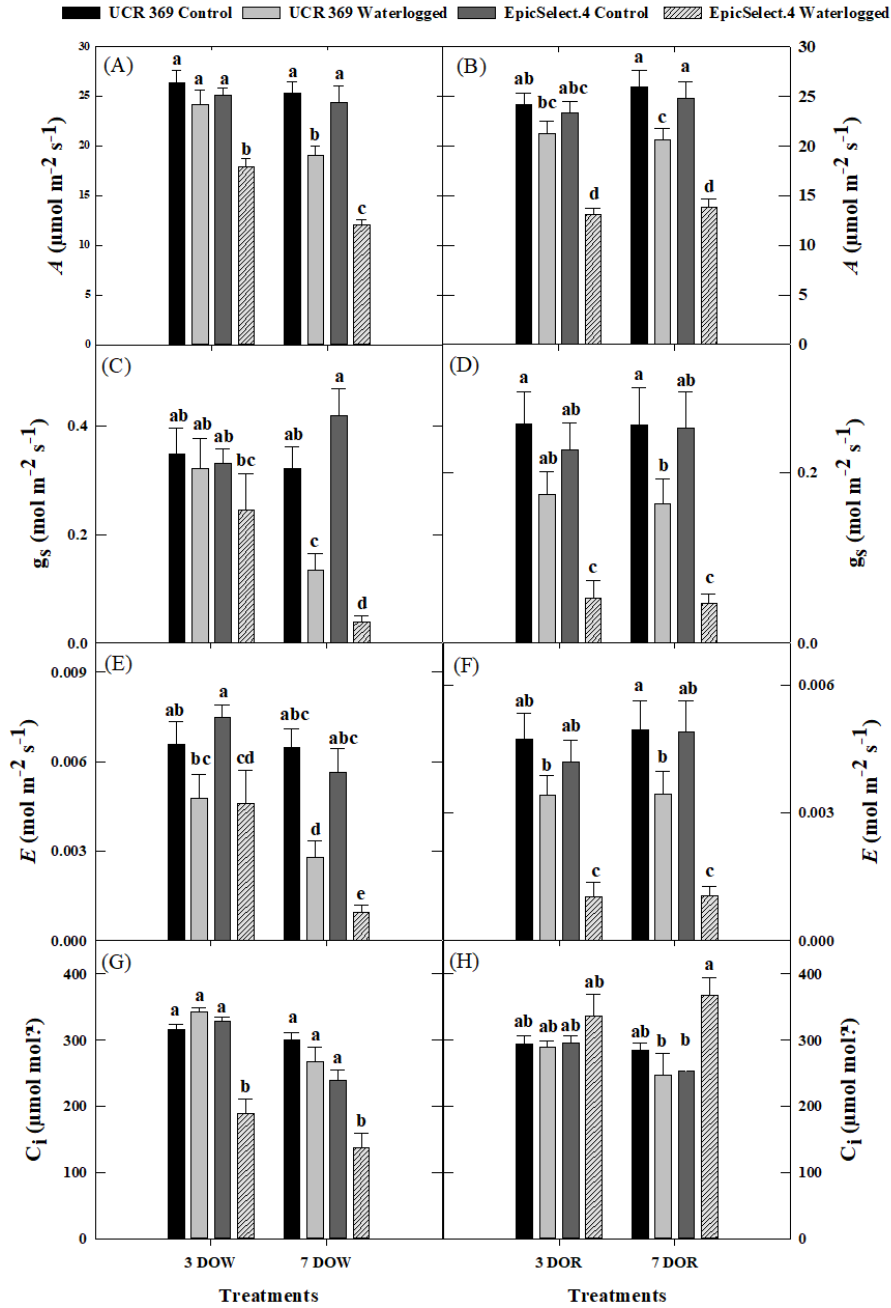
Figure 4.1   (A-B) CO_2 assimilation rate (A), (C-D) Stomatal conductance (g_s), (E-F) Leaf transpiration rate (E), and (G-H) Intercellular CO_2 concentration (C_i) of UCR 369 and EpicSelect.4 subjected to 3 and 7 DOW and DOR. DOW is days of waterlogging, and DOR is days of recovery. The error bar on the vertical bar indicates the standard error of the mean $\pm$ 5 replications of each leaf gas exchange trait. Different lowercase letters indicate significant differences between the genotype's means and treatments, respectively ($P < 0.05$), as determined by Fisher's LSD.

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4.3.2 The CO₂ response curve of cowpea genotypes during waterlogging and recovery

To investigate the biochemical limitation of A's response in cowpeas under waterlogging, the A/C_i curve was measured at 7 DOW (Figure 4.2A) and 7 DOR (Figure 4.2B). The shape of the A/C_i curve varied between cowpea genotypes and waterlogging treatments (Figure 4.2). The A of the genotypes subjected to waterlogging and control treatments increased with increasing C_i from 0 to 1500 µmol mol^{-1} (Figure 4.2). Conversely, the A was lower under waterlogging compared to the control conditions, with a substantial decline in EpicSelect.4 compared to UCR 369 at 7 DOW (Figure 4.2A). After 7 DORS, the A of waterlogged UCR 369 was comparable to the control plants, while the waterlogged EpicSelect.4 could not restore their A compared to the control plants (Figure 4.2B).
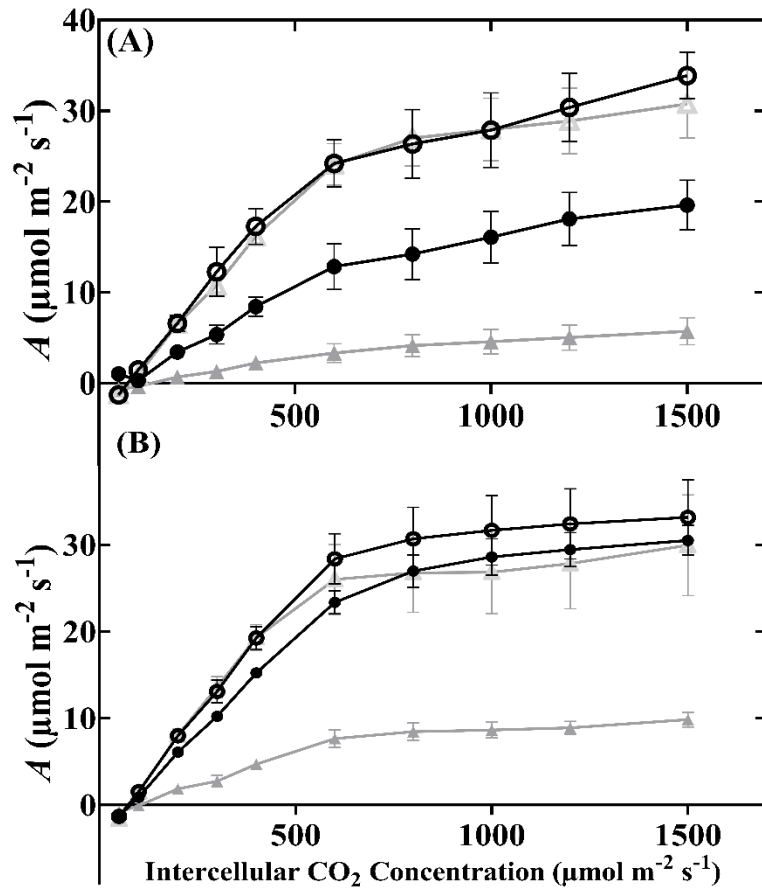
Figure 4.2   (A) A/C_i Curve in cowpea genotypes after 7 days of waterlogging treatments; (B) A/C_i Curve in cowpea genotypes after 7 days of recovery. The vertical bars represent the standard error of the mean (n = 5).

We used the A/C_i data of cowpea genotypes displayed in Figure 4.2 to estimate the g_m, V_{cmax}, and J_{max} at 7 DOW and 7 DOR. Waterlogging significantly affected the calculated V_{cmax} at 7 DOW. On average, UCR 369 had a significantly higher V_{cmax} of 90.22 $\mu mol\ m^{-2}\ s^{-1}$ than EpicSelect.4 (86.52 $\mu mol\ m^{-2}\ s^{-1}$) under control treatments (Figure 4.3A). In contrast to non-waterlogged plants, UCR 369 and EpicSelect.4 significantly reduced V_{cmax} by 31% and 48%,

respectively, at 7 DOW. However, genotype and waterlogging treatment independently and significantly affected g_m and J_{max}. Specifically, waterlogging significantly affected the g_m and J_{max} of UCR 369 by 55% and 34%, respectively, and by 85% and 73% in EpicSelect.4 (Figure 4.3B-C). In addition, the g_m, V_{cmax}, and J_{max} declined in cowpea genotypes at 7 DORs, especially in EpicSelect.4, where g_m, V_{cmax}, and J_{max} significantly decreased by 83%, 65%, and 70%, respectively, relative to the control plants. Interestingly, the values of g_m, V_{cmax}, and J_{max} of waterlogged UCR 369 after 7 DOR were statistically similar to the control plants (Figure 4.3).
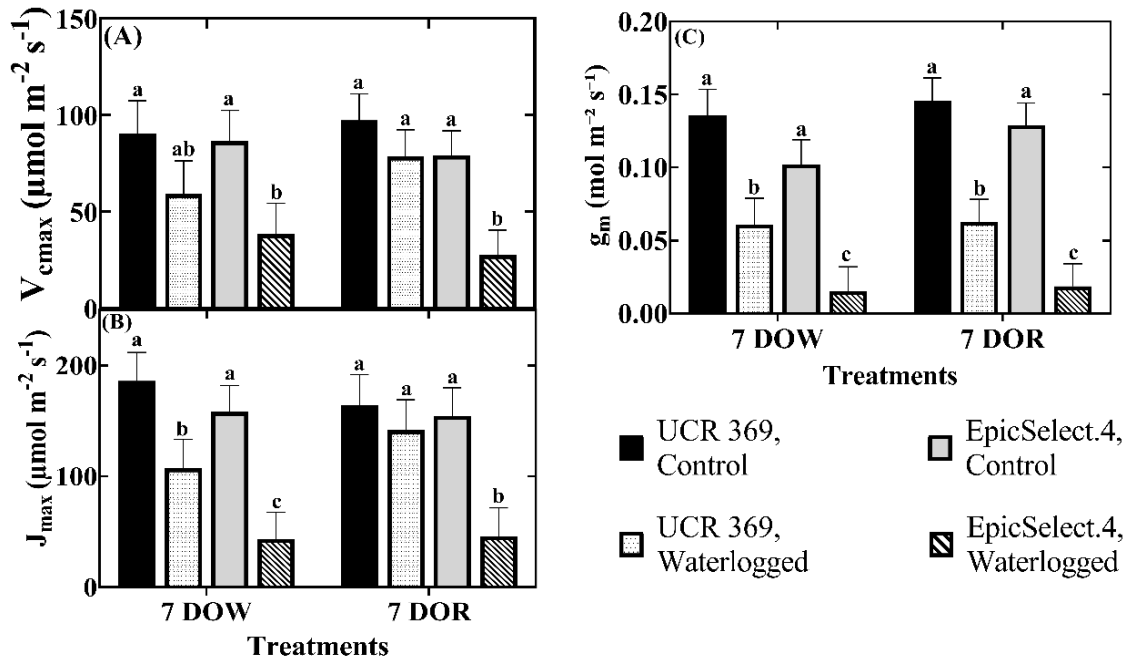
Figure 4.3   (A) Maximum rate of Rubisco carboxylation (V_{cmax}), (B) Maximum rate of photosynthetic electron transport (J_{max}), (C) Mesophyll conductance (g_m) of control and waterlogged UCR 369 and EpicSelect.4 after 7 DOW and 7 DOR. DOW is days of waterlogging, and DOR is days of recovery. Lowercase letters indicate significant differences between the genotype's means and treatments ($P < 0.05$), as determined by Fisher's LSD. The error bar on the vertical bar indicates the standard error of the mean ± 5 replications of each leaf gas exchange trait.

4.3.3 Chlorophyll fluorescence of cowpea genotypes during waterlogging and recovery

Throughout this experiment, the F_v/F_m of UCR 369 only decreased at 7 DOW, while at 3 and 7 DOW (Figure 4.4A). However, the F_v/F_m of EpicSelect.4 could not recover to the control level at both 3 and 7 DOR (Figure 4.4B). 3 and 7 DOW did not cause any change in the F'_v/F'_m of cowpea genotypes (Figure 4.4C). During recovery, the F'_v/F'_m in EpicSelect.4 genotype was only decreased at 3 and 7 DOR (Figure 4.4D).

In contrast, the NPQ of UCR 369 only increased at 7 DOR, whereas it significantly increased when subjected to 7 DOW and 7 DOR in EpicSelect.4 (Figure 4.4E-F).

The Φ_{PSII} in UCR 369 genotype was comparable to the control value at 3 DOW, but it decreased by 28% at 7 DOW (Figure 4.4G). Conversely, the Φ_{PSII} of EpicSelect.4 was reduced by 42% and 87% at 3 and 7 DOW (Figure 4.4G). At 3 DOR, only the Φ_{PSII} of EpicSelect.4 was significantly decreased, while 7 DOR resulted in a decline in both UCR 369 and EpicSelect.4 by 28% and 71%, respectively (Figure 4.4H).
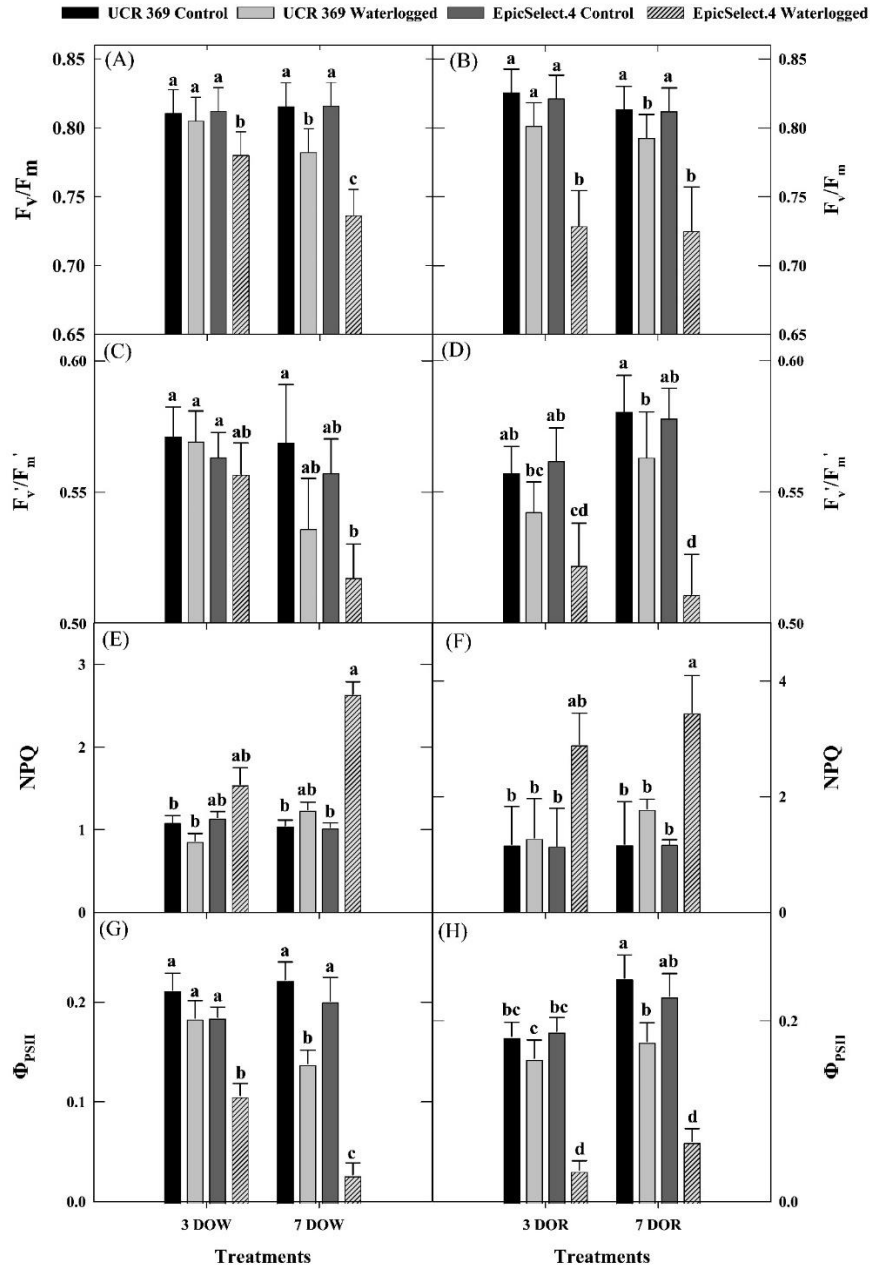
Figure 4.4   (A-B) Maximum quantum efficiency of PSII reaction centers (F_v/F_m), (C-D)
Effective quantum efficiency of PSII reaction centers (F'_v/F'_m), (E-F) Non-
photochemical quenching (NPQ), and (G-H) Quantum yield of PSII
photochemistry (Φ_{PSII}) of UCR 369 and EpicSelect.4 subjected to 3 and 7 DOW
and DOR. DOW is days of waterlogging, and DOR is days of recovery. The
error bar on the vertical bar indicates the standard error of the mean $\pm$ 5
replications of each leaf gas exchange trait. The lowercase letters indicate
significant differences between the genotype's means and treatments,
respectively ($P < 0.05$), as determined by Fisher's LSD.

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4.3.4 Chlorophylls and carotenoids of cowpea genotypes during waterlogging and recovery

Waterlogging resulted in a significant decrease in photosynthetic pigments in a genotype-dependent manner (Figure 4.5). At 3 DOW, chlorophyll α (Figure 4.5A) and β (Figure 4.5C) levels were significantly reduced in EpicSelect.4, while there was no difference in control and waterlogged UCR 369. 7 DOW resulted in reduction of chlorophyll α and β in UCR 369 and EpicSelect.4 by (45 & 48) % and (65 & 63) %, respectively. Interestingly, 3 and 7 DOR resulted in similar or even higher levels of chlorophyll α and β for UCR 369 (Figure 4.5 B, D). However, 3 and 7 DOR resulted in a significant decrease in chlorophyll α and β content in EpicSelect.4 and decreased by 61-77% relative to control (Figure 4.5B, D). The chlorophyll α + β of UCR 369 followed the same trend as chlorophyll α, while EpicSelect.4 lost 57-77% at 7 DOW and 7 DOR (Figure 4.5E-F). The ratio of chlorophyll α/β in UCR 369 and EpicSelect.4 was reduced at 3 and 7 DOW (Figure 4.5G). However, 7 DOR increased the chlorophyll α/β ratio in EpicSelect.4, but no significant change in waterlogged UCR 369 (Figure 4.5H).
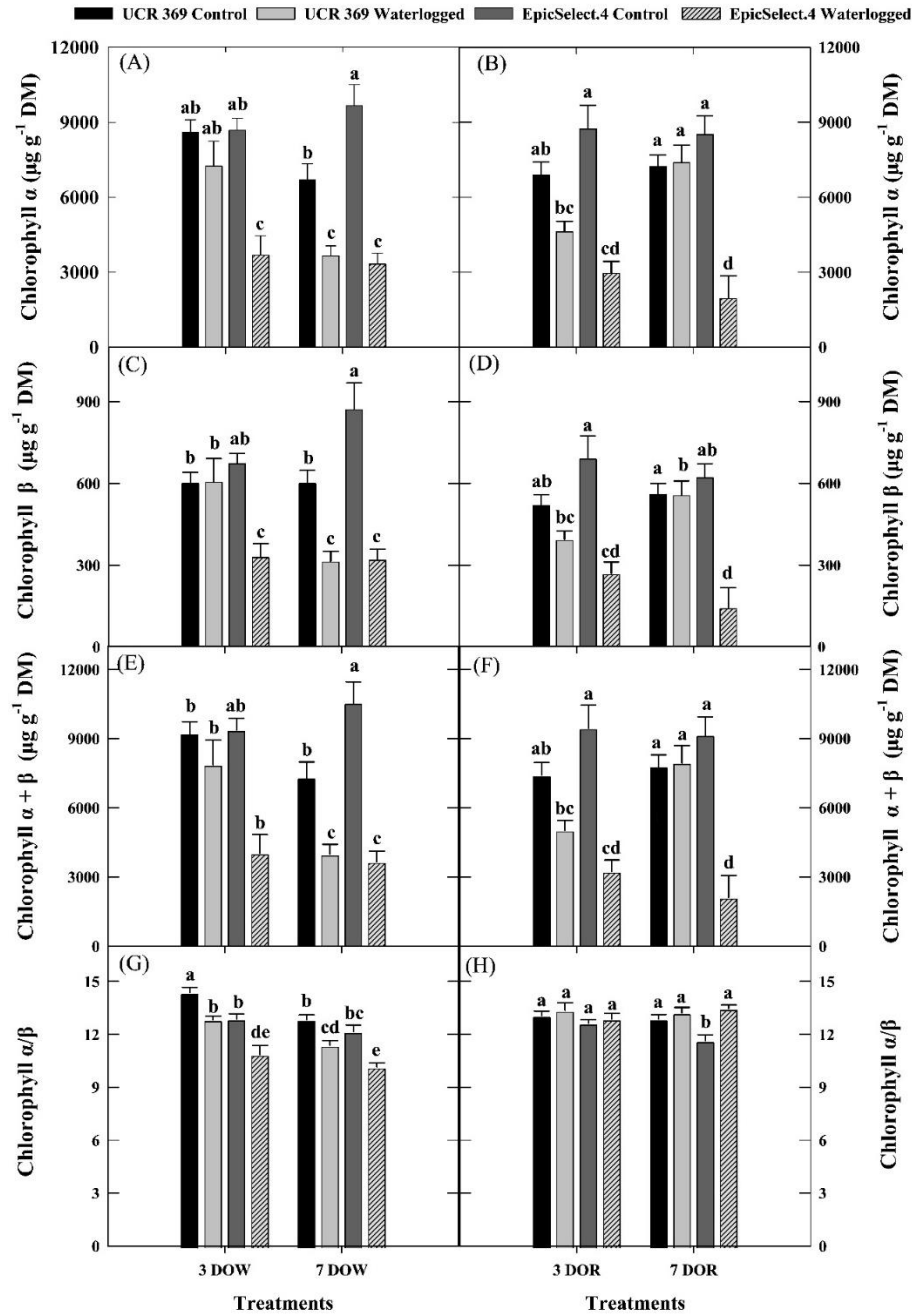
Figure 4.5   (A-B) Chlorophyll α, (C-D) Chlorophyll β, (E-F) Total chlorophyll content (chlorophyll α + β), and (G-H) chlorophyll α /β ratio of UCR 369 and EpicSelect.4 subjected to 3 and 7 DOW and DOR. DOW is days of waterlogging, and DOR is days of recovery. The error bar on the vertical bar indicates the standard error of the mean ± 5 replications of each leaf gas exchange trait. The lowercase letters indicate significant differences between the genotype's means and treatments ($P < 0.05$), as determined by Fisher's LSD.

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Correspondingly, the carotenoid parameters revealed a significant difference during and after waterlogging treatments (Figure 4.6). 3 DOW did not change in neoxanthin and violaxanthin content of UCR 369 but significantly decreased by 38% and 54%, respectively, in EpicSelect.4 (Figure 4.6A, C). Compared to the control, UCR 369 and EpicSelect.4 had more than a 50% reduction in neoxanthin and violaxanthin at 7 DOW, restored only in UCR 369 at 7 DOR (Figure 4.6 B, D).

Figure 4.6E revealed that 3 and 7 DOW significantly decreased the antheraxanthin levels in both UCR 369 and EpicSelect.4. However, the concentration of antheraxanthin in waterlogged UCR 369 was comparable to the control at 3 and 7 DOR. On the other hand, 3 and 7 DOR could not restore the concentration of antheraxanthin in waterlogged EpicSelect.4 (Figure 4.6F).

At 3 and 7 DOR, zeaxanthin concentrations decreased significantly in EpicSelect.4 but remained unchanged in UCR 369 compared to the control treatments. In addition, the concentration of zeaxanthin increased significantly in UCR 369 at 7 DOW but decreased in EpicSelect.4 (Figure 4.6G).
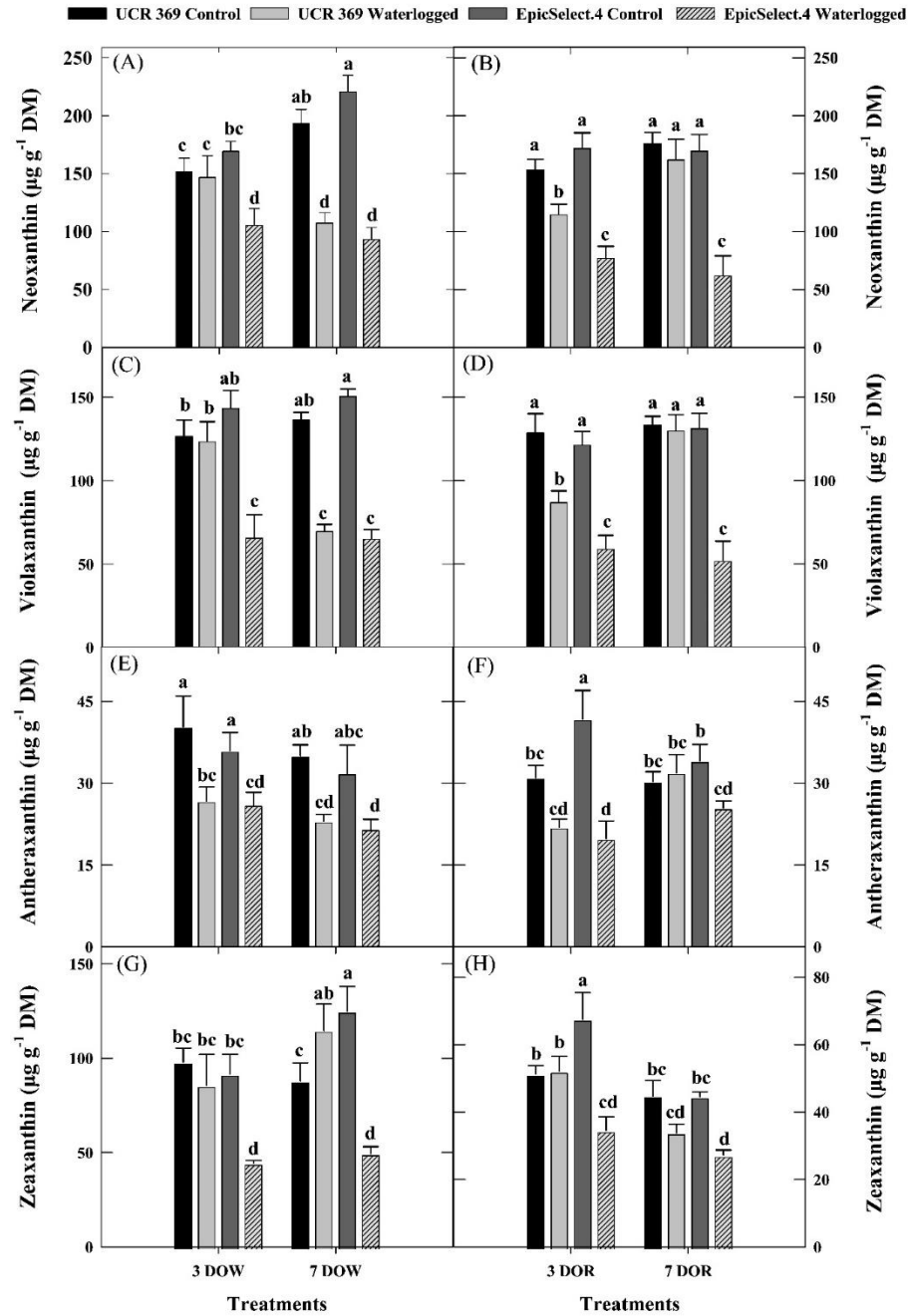
Figure 4.6   (A-B) Neoxanthin, (C-D) Violaxanthin, (E-F) Antheraxanthin, and (G-H) Zeaxanthin of UCR 369 and EpicSelect.4 subjected to 3 and 7 DOW and DOR. DOW is days of waterlogging, and DOR is days of recovery. The error bar on the vertical bar indicates the standard error of the mean ± 5 replications of each leaf gas exchange trait. The lowercase letters indicate significant differences between the genotype's means and treatments ($P < 0.05$), as determined by Fisher's LSD.

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At 3 DOW, there was no difference in the concentrations of lutein in UCR 369, which was reduced at 7 DOW (Figure 4.7A). Both plants of UCR 369 and EpicSelect.4 subjected to waterlogging also presented lower amounts of lutein on 3 DOR (Figure 4.7B). 7 DOR resulted in comparable concentrations of lutein in UCR 369 but decreased by 54% in EpicSelect.4 (Figure 4.7B). The levels of β-carotene in waterlogged UCR 369 were similar to the control plants during 3 and 7 DOW (Figure 4.7C). However, both waterlogging (Figure 4.7C) and recovery (Figure 4.7D) treatments caused a decline in β-carotene in EpicSelect.4.

It is interesting to note that the total carotenoids in UCR 369 only decreased at 7 DOW, while it was significantly reduced at all levels of treatments in EpicSelect.4 by 34-58% (Figure 4.7E). At 7 DOR, the total carotenoids in waterlogged UCR 369 were similar to the control plants, but 7 DOR was unable to restore in EpicSelect.4 (Figure 4.7F).

In contrast, DEPS increased significantly in EpicSelect.4 at 3 and 7 DOW (Figure 4.7G), while 7 DOR resulted in higher levels. Only 3 DOR caused the levels of DEPS in UCR 369 to increase when compared to the control (Figure 4.7H).
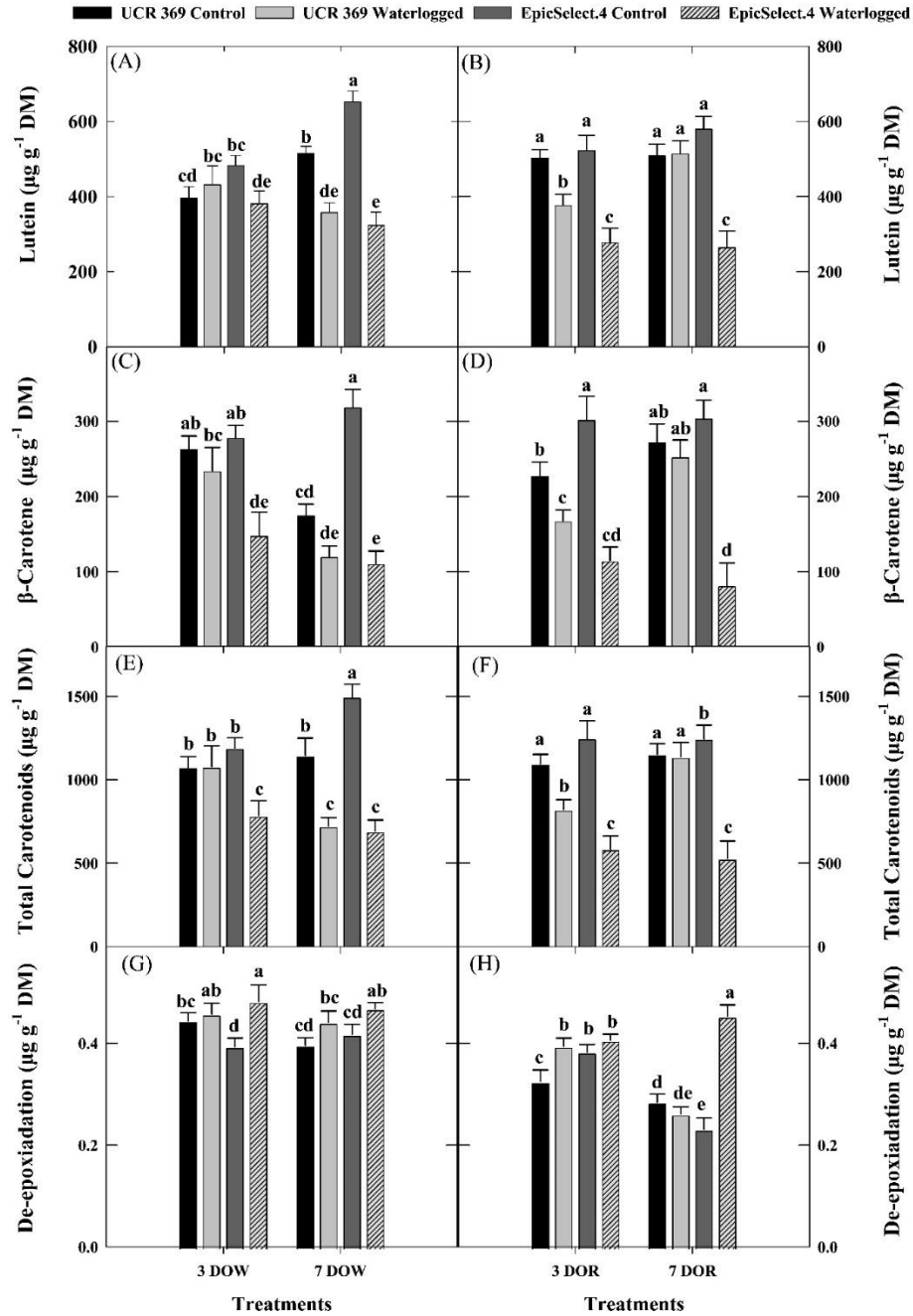
Figure 4.7   (A-B) Lutein, (C-D) β-Carotene, (E-F) Total carotenoids, and (G-H) De-
epoxidation state (DEPS) of UCR 369 and EpicSelect.4 subjected to 3 and 7
DOW and DOR. DOW is days of waterlogging, and DOR is days of recovery.
The error bar on the vertical bar indicates the standard error of the mean $\pm$ 5
replications of each leaf gas exchange trait. The lowercase letters indicate
significant differences between the genotype's means and treatments,
respectively ($P < 0.05$), as measured by Fisher's LSD.

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4.3.5   Total phenolic and flavonoid of cowpea genotypes during waterlogging and recovery

Compared to the control, the phenolic content increased in EpicSelect.4 at 3 DOW, with no difference in waterlogged UCR 369. 7 DOW increased the phenolics of UCR 369 but remained unchanged in EpicSelect.4 (Figure 4.8A). However, the total phenolic content in both genotypes was significantly higher during the recovery period (Figure 4.8B).

Waterlogged UCR 369 increased total flavonoid content compared to the control at 3 and 7 DOW (Figure 4.8C) but decreased at 7 DOR to a level comparable to the control (Figure 4.8D). However, in waterlogged EpicSelect.4, total flavonoid content increased only at 3 DOW and decreased from 7 DOW to 7 DOR compared to controls (Figure 4.8D).
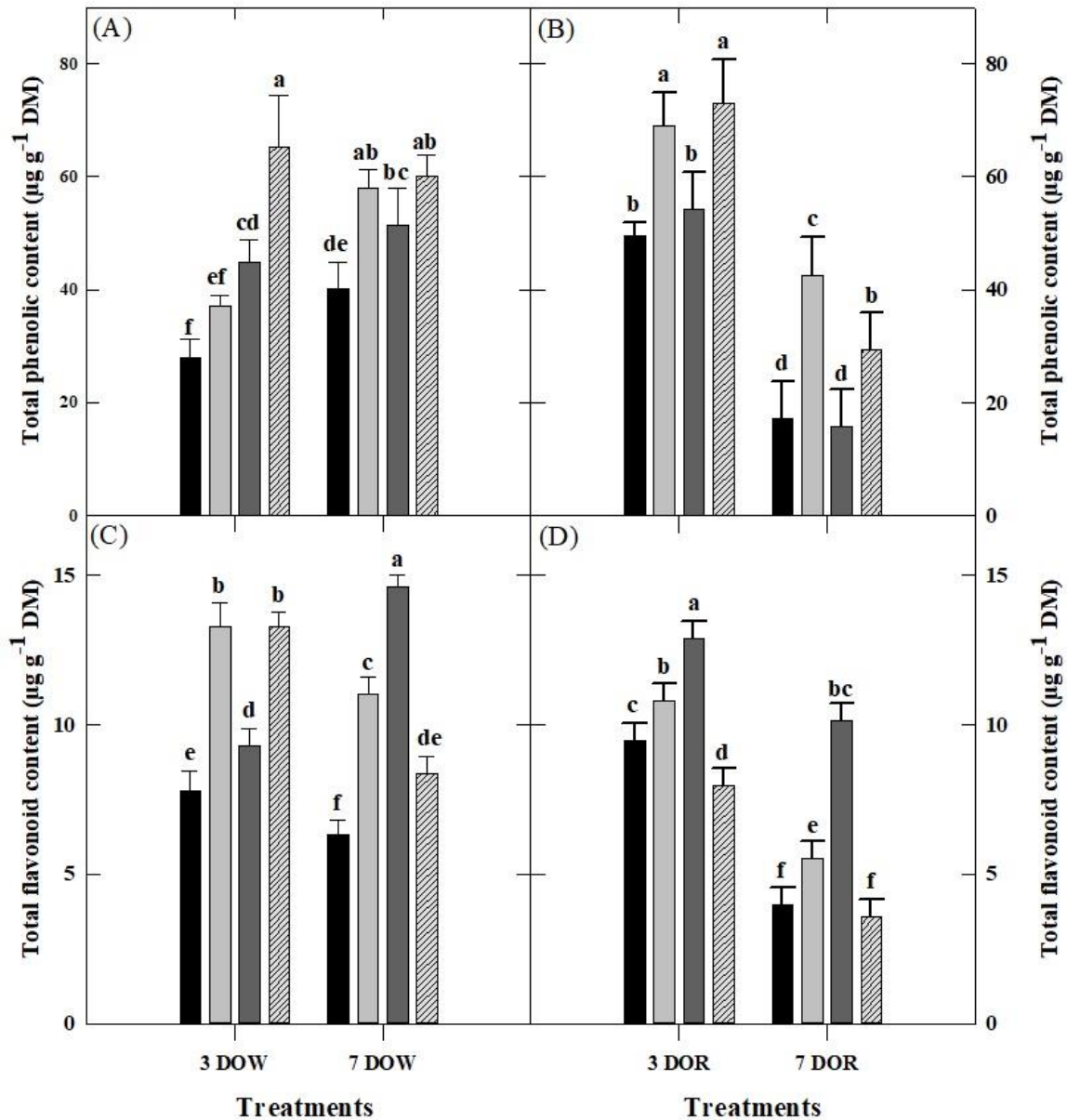
Figure 4.8   (A-B) Total phenolic content and (C-D) total flavonoid content of UCR 369 and EpicSelect.4 subjected to 3 and 7 DOW and DOR. DOW is days of waterlogging, and DOR is days of recovery. The error bar on the vertical bar indicates the standard error of the mean $\pm$ 5 replications of each leaf gas exchange trait. The lowercase letters indicate significant differences between the genotype's means and treatments ($P < 0.05$), as determined by Fisher's LSD.

4.3.6   Sucrose, glucose, and fructose of cowpea genotypes during waterlogging and recovery

The concentrations of fructose, glucose, and sucrose were quantified to determine the effect of waterlogging and recovery on cowpea's soluble sugars. No significant differences were detected in the glucose level in UCR 369 throughout the experiment. However, glucose levels increased significantly at 7 DOW (Figure 4.9A). Waterlogged EpicSelect.4 increased glucose levels compared to the control at 3 DOR and presented similar values at 7 DOR (Figure 4.9B). Interestingly, fructose followed the same pattern as glucose in both genotypes during waterlogging and recovery (Figure 4.9 C-D).

On the other hand, sucrose levels in UCR 369 significantly increased by 68% and 46% at 3 and 7 DOW, respectively (Figure 4.9E). At 3 DOW, sucrose levels decreased in EpicSelect.4 but were no different than in the control plant at 7 DOW (Figure 4.9E). At 7 DOR, the sucrose levels remained unchanged in EpicSelect.4 but increased in UCR 369 (Figure 4.9F).
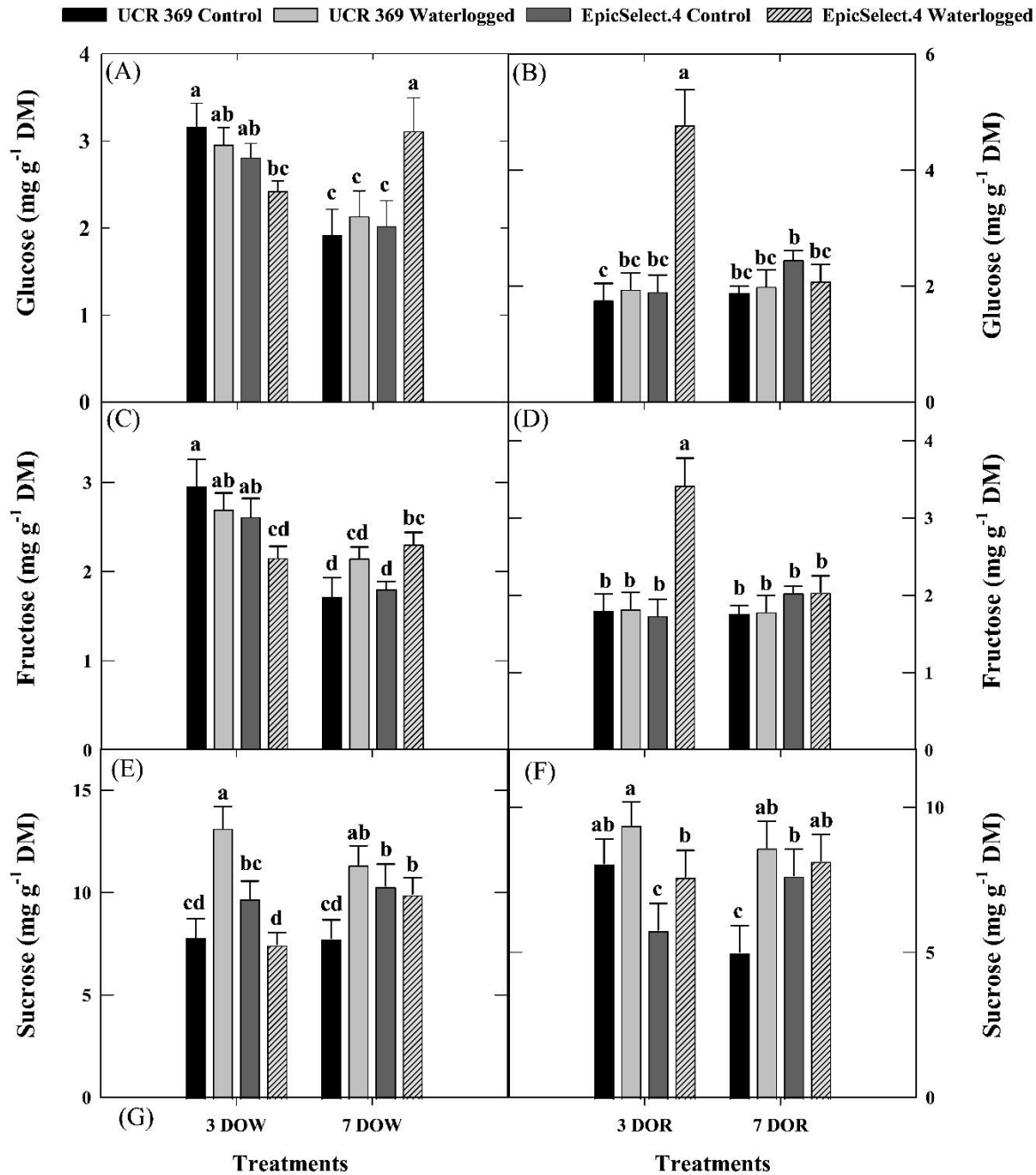
Figure 4.9   (A-B) Glucose, (C-D) Fructose, and (E-F) Sucrose concentrations of UCR 369 and EpicSelect.4 subjected to 3 and 7 DOW and DOR. DOW is days of waterlogging, and DOR is days of recovery. The error bar on the vertical bar indicates the standard error of the mean ± 5 replications of each leaf gas exchange trait. The lowercase letters indicate significant differences between the genotype's means and treatments (*P* < 0.05), as determined by Fisher's LSD.

4.4   Discussion

Waterlogging is important abiotic stress that affects plant growth and development, depending on the plant's sensitivity to the stress (Fukao et al., 2019). Like many other leguminous crops, cowpea is susceptible to soil waterlogging (Minchin et al., 1978; Umaharan et al., 1997), even though they are considered to be resilient crops under drought and heat stress. The sensitivity of cowpea's growth and development under hypoxic conditions is attributed to their inability to absorb nutrients and rapidly develop aerenchyma tissue in their roots (Hong et al., 1977; Olorunwa et al., 2022). Previous studies have investigated the impact of waterlogging during the vegetative stage on the growth and yield of cowpea (Hong et al., 1977; Olorunwa et al., 2022; Umaharan et al., 1997), but its effect during the R2 growth stage has been largely neglected. Therefore, in this study, the physiological and biochemical responses of two contrasting cowpea genotypes (more tolerant UCR 369 and less tolerant EpicSelect.4) identified in CHAPTER III were evaluated during and after waterlogging. After 7 DOW and 7 DOR, a considerable number of physiological and biochemical parameters of waterlogged cowpeas were differentially affected. Photosynthetic traits were significantly decreased and could not be restored after 7 DOR, whereas the secondary metabolites were upregulated and restored in UCR 369.

4.4.1   Impacts of waterlogging on gas exchange parameters

Waterlogging negatively affected the photosynthetic capacity of cowpea genotypes, and they could not recover during reoxygenation, but there was a more considerable decline in the sensitive EpicSelect.4 genotype. After 3 DOW, only EpicSelect.4 showed a reduction in A, indicating that this genotype is more sensitive to waterlogging than UCR 369, where A was significantly reduced at 7 days. Previous studies have shown that stomatal closure is a critical

factor in impairing A in legumes due to limited CO_2 supply to carboxylation sites under waterlogging stress (Garcia et al., 2020; Shao et al., 2013). Interestingly, this fact was corroborated in the current study. The decline of A in UCR 369 was associated with a significant reduction of g_s without any biochemical reduction of V_{cmax} and J_{max}. Thus, the photosynthetic downregulation in the tolerant genotype was primarily caused by stomatal-induced factors under waterlogging conditions. Ploschuk et al. (2018) reported similar findings in wheat and barley, demonstrating tolerance under two weeks of waterlogging.

Waterlogging-intolerant genotypes such as rapeseed, field peas, and peanuts (Ploschuk et al., 2018; Zeng et al., 2020) have demonstrated A decreases and C_i increases under waterlogging conditions. In contrast, the sensitive EpicSelect.4 genotype experienced a significant decline in A with decreased g_s, g_m, V_{cmax}, and J_{max} under waterlogging, indicating that both stomatal and non-stomatal limited photosynthesis according to the model of Farquhar et al. (1980). During the progressive waterlogging, an increase in Ci relative to the control treatment was also observed in the EpicSelect.4 genotype. Islam et al. (2008) also observed increased C_i in Vo1982A-G (sensitive mungbean genotypes) after 7 DOW and suggested that higher C_i limits Rubisco activity, resulting in the plant's inability to restore photosynthetic capacity during the recovery period. Therefore, these findings indicate that the reduction in A in the sensitive genotypes was primarily caused by photosynthetic apparatus damage rather than a lack of intercellular CO_2.

Many studies have evaluated the adverse effects of waterlogging on photosynthesis and how hypoxia and anoxia inhibit photosynthetic system activity by altering chlorophyll fluorescence parameters (Rao et al., 2021; Smethurst and Shabala, 2003), thereby reducing leaf carbon fixation. Under waterlogging, the inactivation of PSII in field peas (Ploschuk et al., 2018) and waxy corn (Zhu et al., 2016) results in a loss in photosynthetic capacity with detrimental

impacts on plant growth. Generally, healthy leaves' F_v/F_m values vary from 0.75 to 0.83, and a reduction in these values indicates damaged PSII (Krause and Weis, 1991). In the current study, the F_v/F_m of waterlogged UCR 369 ranged from 0.78 to 0.81 and was comparable to the control plants, ranging from 0.79 to 0.82. The absence of PSII photoinhibition further proved that no apparent A limitation in UCR 369 was due to non-stomatal factors. Therefore, it is suggested that UCR 369 may regulate photosynthesis through a better mechanism to adapt to waterlogging stress. On the other hand, there was significant photodamage of PSII activity indicated by a significant decrease in F_v/F_m and Φ_{PSII}, along with increased NPQ (excess energy dissipated as heat) during 7 DOW and 7 DOR. Previous studies have reported similar findings in sensitive crop genotypes (Ploschuk et al., 2018; Velasco et al., 2019). Thus, indicating the sensitivity of EpicSelect.4 to hypoxic conditions.

4.4.2 Impacts of post-waterlogging reoxygenation on gas exchange parameters

The adverse effects of waterlogging on photosynthesis observed in cowpea genotypes were exacerbated after soil drainage, particularly in EpicSelect.4 (Figure 4.1-Figure 4.4). After 7 DOR, it was observed that the EpicSelect.4 genotype could not reopen the stomata to restore A, J_{max}, and V_{cmax}, but continued to experience g_s and g_m reductions, resulting in higher C_i (Figure 4.1). Therefore, it is suggested that the total diffusion limitation (stomatal and mesophyll) continues to limit the photosynthetic performance of sensitive cowpea genotypes. However, the inability of A to fully recover at 7 DOR was only due to a decrease in g_s and g_m, while V_{cmax} and J_{max} fully recovered to control levels. Hence, it is plausible to suggest that only stomatal-induced factors limit the photosynthetic performance of UCR 369 during recovery. In addition, the Φ_{PSII} and NPQ values of UCR 369 were similar to control plants, which were in good agreement with F_v/F_m. The results indicate normal PSII functionality of the tolerant genotype during recovery.

In contrast, 7 DOR did not restore F_v/F_m to the control levels in EpicSelect.4, suggesting impaired photoinhibition of *A*. In addition to the decrease in F_v/F_m, an increase in NPQ was observed at 7 DOR, which confirms more substantial energy dissipation in sensitive cowpea genotypes. These results are consistent with the responses reported by Ploschuk et al. (2018) in waterlogging-tolerant wheat and -sensitive peas and Velasco et al. (2019) in common beans.

4.4.3 Impacts of waterlogging on photosynthetic pigments

Previous studies have shown that photosynthetic pigment reductions are more pronounced in relatively waterlogging-sensitive soybeans compared to the tolerant genotypes (Garcia et al., 2020). The decline in chlorophyll α, β, and total chlorophyll were other non-stomatal factors limiting *A* in cowpea genotypes, with the decrease in EpicSelect.4 being more pronounced and evident as leaf yellowing during waterlogging. Also, the substantial decline in *A* and total chlorophyll in EpicSelect.4 after 7 DOW during the R2 stage could be attributed to more accumulation of ROS, as observed by Zhang et al. (2016). This study showed that chlorophyll α was comparatively more sensitive than chlorophyll β in both genotypes under waterlogging. Moreover, chlorophyll α and β may be associated with nitrogen deficiency, manifested by the yellowing of waterlogged leaves. Minchin et al. (1978) found that the decrease in oxygen levels in the soil caused considerable suppression of nitrogen fixation in cowpea plants at 8 DOW compared to control plants. This suggests that the ability of cowpea genotypes to maintain their chlorophyll content may be linked to the efficient management of nitrogen under waterlogging conditions.

Waterlogging has been reported to reduce the concentrations of carotenoids (Barickman et al., 2019; Kumar et al., 2013), primarily due to the overproduction of ROS in the thylakoids, which damage the chloroplast, inhibiting photosynthesis (Cruz de Carvalho, 2008; Zhang et al.,

2021). In the current study, the changes in the levels of the major components of total carotenoids, including neoxanthin, violaxanthin, lutein, and β-carotene were similar to the responses of chlorophyll α and β, with the higher decline in EpicSelect.4 under 7 DOW. This result indicated that chloroplast ultrastructure and photosynthetic pigments of cowpea genotypes were sensitive to waterlogging. The effects of water stress on total carotenoid concentrations varied with the crop genotypes, severity, and duration of stress, as well as the individual pigments, studied by Šircelj et al. (2005). In the current study, the biosynthesis of zeaxanthin appeared to be the most sensitive to 7 DOW of all the total carotenoids examined in the cowpea leaves. The concentration of zeaxanthin was significantly higher in waterlogged UCR 369 than in control plants due to higher DEPS to dissipate excess light energy at 3 DOR. The upregulation of zeaxanthin indicates the adaptation of crops to oxidative stress (Demmig-Adams and Adams, 2018). Therefore, increasing the levels of zeaxanthin pigments enables the tolerant UCR 369 to quench excess light energy from the PSII reaction center.

Similarly, increased zeaxanthin as a result of hypoxia was reported in spinach (Wright et al., 2011). Carotenoids are photoprotective pigments that dissipate excess light energy as heat to prevent energy excitation and ROS formation in hypoxic conditions (Wright et al., 2011). As expected, the concentration of zeaxanthin in sensitive EpicSelect.4 decreased significantly during and after waterlogging, with a corresponding increase in NPQ. Wang et al. (2021) showed that degradation of the xanthophyll cycle resulted in low utilization of light energy received by leaves at the PSII reaction center. Hence, it was demonstrated that the photodamage induced by 7 DOW in EpicSelect.4 could not be ameliorated after 7 DOR due to the reduced concentration of photoprotective pigments.

4.4.4   Impacts of post-waterlogging reoxygenation on photosynthetic pigments

When 7 DOW was followed by 7 DOR, chlorophyll α and levels were restored in UCR 369, but EpicSelect.4 still showed a significant decrease compared to the control. The results of this study also revealed that chlorophyll β was comparatively more sensitive than chlorophyll α in EpicSelect.4 at 7 DOR, resulting in an increased chlorophyll α/β ratio. Thus, greater chlorophyll β damage was observed in the sensitive genotype during recovery, indicating a loss of light-harvesting antenna protein relative to reaction centers. This post-waterlogging response indicated that controlled downregulation of light harvesting occurred in the sensitive EpicSelect.4 genotype. However, there was no significant change in the ratio of chlorophyll α/β in waterlogged UCR 369 at 7 DOR, revealing that there was no programmed downregulation of light capture by the tolerant genotype rather UCR 369 upregulated its chlorophyll content when reoxygenated. Previous studies surmised that the differences in oxidase and chlorophyllase activities were responsible for higher chlorophyll in the relatively waterlogging-tolerant genotypes but not the waterlogging-sensitive genotypes (Ashraf and Mehmood, 1990; Muhammad et al., 2021).

On the other hand, UCR 369 contains a larger pool of total carotenoids compared to EpicSelect.4. at 7 DOR (Figure 4.7F). Carotenoids probably acted as an antioxidant defense protecting photosynthetic apparatus from ROS (Li et al., 2012) and may have been a strong antioxidant defense for UCR 369 during the recovery.

4.4.5   Impacts of waterlogging on compatible solutes

Waterlogging induces the accumulation of soluble leaf carbohydrates in crops, such as sucrose, glucose, fructose, maltose, trehalose, and raffinose (Kuai et al., 2014). Of these sugars,

elevated levels of sucrose are usually correlated to the tolerance of crop genotypes to waterlogging stress (Bertrand et al., 2003; Kumutha et al., 2008) because it can mitigate the response to oxidative stress by acting as a compatible solute to counteract the damaging effects of ROS. In the present study, sucrose levels in UCR 369 at 7 DOW were upregulated, while they remained unchanged in EpicSelect.4. Kumutha et al. (2008) reported a similar response among tolerant and sensitive pigeon pea after 6 DOW. They attributed the buildup of sucrose in tolerant genotypes under waterlogging to the activation of sucrose synthase involved in the biosynthetic pathway. Moreover, increased sucrose synthase activity could lead to the removal of substantial amounts of ROS, thereby reducing membrane damage under waterlogging, a quiescent adaptation strategy utilized by tolerant plants to adapt to waterlogging stress (Zhang et al., 2021). Sucrose is the primary carbohydrate translocated from the source to sink tissues. It has been shown that in the absence of oxidative phosphorylation, it can function as a source of energy in plants (Gibbs and Greenway, 2003). This suggests that sucrose accumulation in UCR 369 is responsible for reducing the effect of oxidative stress on photosynthetic performance. Thus, the decrease in *A* in EpicSelect.4 may be related to the lower sucrose content.

Under waterlogging, plants accumulate a variety of secondary metabolites, particularly phenolic and flavonoids, which provide antioxidant protection against ROS (Gill and Tuteja, 2010). Due to their remarkable ability to donate electrons and hydrogen atoms, phenolics and flavonoids protect against oxidative stress caused by ROS accumulation in stressed plants by neutralizing free radicals before damaging cells and inhibiting lipid peroxidation (Sharma et al., 2012; Vosnjak et al., 2021). Elkelish et al. (2020) reported an increased concentration of phenolics in tomato plants after 14 DOW. Previous studies have also shown that waterlogging-induced stress affects phenolics and flavonoids in mungbeans (Islam et al., 2022), potatoes

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(Orsák et al., 2020), and herbaceous peonies (Liu et al., 2021). Vogt (2010) ascribed the waterlogging-induced accumulation of phenolics and flavonoids to the overexpression of phenylalanine ammonia-lyase, which is critical in enhancing the plant's stress defense. The current study demonstrated that 7 DOW significantly increased phenolics and flavonoids in the leaves of UCR 369, signifying the development of a biochemical mechanism that scavenges ROS to adapt to hypoxia. However, flavonoid was downregulated in the sensitive EpicSelect.4 during and after waterlogging. This suggests that UCR 369 can potentially preserve tissue water content to avoid damage from waterlogging.

4.4.6   Impacts of post-waterlogging reoxygenation on compatible solutes

On the other hand, the concentration of fructose and glucose increased during reoxygenation in EpicSelect.4. Consistent with our results, Kumutha et al. (2008) reported increased glucose levels and fructose in waterlogging-sensitive pigeon peas after 5 DOR. However, the upregulation of these reducing sugars in EpicSelect.4 could not act as an energy source in the glycolytic pathway to combat the effects of 7 DOW on *A*. Primarily, because inhibited glycolysis leads to the accumulation of glucose, which triggers the repression of genes related to photosynthesis, thereby inhibiting *A* (Vosnjak et al., 2021). However, low glucose concentrations stimulate *A*, as demonstrated in UCR 369 in the current study. These results suggest that tolerant cowpea genotypes can modulate and alter carbohydrate metabolism upon exposure to hypoxia, thereby enhancing photoprotection and waterlogging tolerance.

Total phenolics and flavonoids in UCR 369 continued to increase during the 7 DOR period. However, the flavonoid was downregulated at 3 and 7 DOR in EpicSelect,4, which could inhibit the survival of plants during post-waterlogging events. Similar results were obtained in waterlogging-sensitive maize (Zhou et al., 2021). Taken together, these studies reveal that

flavonoids are more critical than phenolics in conferring waterlogging tolerance to cowpeas. Likewise, the antioxidant mechanisms of flavonoids can contribute to maintaining gas exchange parameters and morphological attributes of cowpeas when subjected to post-waterlogged conditions.

4.5   Conclusion

Waterlogging during the cowpea R2 growth stage affected both cowpea genotypes' physiological and biochemical parameters. Although the cowpea genotypes decreased their photosynthetic capacity due to waterlogging, the genotype, UCR 369, was more tolerant to waterlogging stress than the genotype, EpicSelect. 4. Seven days of waterlogging caused decreased the A of EpicSelect.4 due to rapid stomatal closure, decreased g_m, altered Rubisco activity, pigment degradation, photoinhibition of PSII, and reduced sucrose levels. However, only stomatal-induced factors were associated with the reduction in A in UCR 369 genotype. Additionally, the accumulation of compatible solutes, including zeaxanthin, sucrose, phenolics, and flavonoids, played essential roles in the waterlogging tolerance of the cowpea genotype, UCR 369.

After recovery, the cowpea genotype, UCR 369, overcame waterlogging damage by recovering most of the physiological and biochemical parameters to the control level. In contrast, EpicSelect.4 did not recover during reoxygenation and showed more damage to biochemical parameters from waterlogging. Overall, we found the potential of waterlogging-tolerant cowpea genotypes to maintain photosynthetic efficiency and adapt to short-term waterlogging may promote seed yield at the maturity study. Hence, the responses of contrasting genotypes at different growth stages should be further studied, especially at the field level, to elucidate cowpea waterlogging tolerance's physiological and metabolic mechanisms.

CHAPTER V

IMPACTS OF WATERLOGGING STRESS AT DIFFERENT GROWTH STAGES ON THE

GROWTH, YIELD, AND PHYSIOLOGICAL CHARACTERISTICS OF COWPEA

GENOTYPES UNDER FIELD CONDITIONS

Abstract

Excessive soil moisture in the rhizosphere is important abiotic stress that adversely affects normal plant growth and development by restricting oxygen supplies to the roots. Although cowpea is sensitive to waterlogging, recent evidence suggests that the impacts of waterlogging on grain yield depend on the growth stage and genotype of the crop. Here, a two-year field trial was conducted to evaluate the effects of waterlogging stress on the morphological attributes, photosynthetic characteristics, relative water content, biomass accumulation, chlorophyll content, soluble sugars, seed yield and components, and seed quality of cowpea genotypes at different growth stages. This experiment aimed to understand cowpea genotypes' growth, yield, and physiological responses to waterlogging and identify the most sensitive stage to waterlogging stress. Two cowpea genotypes (UCR 369 and EpicSelect.4) were subjected to 10-day waterlogging and control treatments at the vegetative (V4), reproductive (R2), and physiologically maturity (R7) growth stages during the 2021 and 2022 growing seasons. The results revealed that waterlogging in the R2 stage had the most significant effect on the leaf area, chlorophyll content, stomatal conductance, and actual photochemical efficiency of cowpea genotypes, followed by the V4 and R7 stages. Thus, these results show that waterlogging

significantly decreased the photosynthetic capacity of cowpeas, with adverse effects on biomass accumulation, pod dry weight, number of pods per plant, and seed weight. The decline was more significant in EpicSelect.4, which also experienced almost 100% mortality during waterlogging at the R2 stage than in UCR 369. UCR 369 exposed to waterlogging had the highest phenolic and flavonoid content at the R2 and V4 stages. Moreover, waterlogging increased the contents of flavonoids and sucrose in the leaves of UCR 369 at different growth stages, while the contents of fructose and glucose in EpicSelect.4 increased and decreased, respectively. The seed protein of UCR 369 did not change under waterlogging conditions at different growth stages but decreased in EpicSelect.4 seeds. Overall, the current findings revealed that UCR 369 was more tolerant to waterlogging stress than EpicSelect.4, with the most apparent effect of waterlogging on yields occurring at the R2 stage, followed by the V4 and R7 growth stages.

5.1 Introduction

Over the years, global climate change has increased the frequency, severity, and duration of flood events, adversely affecting the sustainability of crop production (Arias et al., 2021; Voesenek and Bailey-Serres, 2015). Recent climate models predict that climate change variables, including temperature and precipitation, will rise further over the next century (Masson-Delmotte et al., 2021). Annual daily precipitation events are projected to intensify by about 14% by 2050 and about 35% by the end of the 21st century (IPCC, 2021). This will lead to more frequent and extreme waterlogging events in many parts of the world, including the United States (USGCRP, 2017). In this context, waterlogging due to heavy precipitation, poor soil drainage, and over-irrigation has been documented to affect more than 1,700 million hectares of arable land globally each year (Voesenek and Sasidharan, 2013). Moreover, waterlogging is important for abiotic stress, limiting the average global crop yield by 32.9% (Tian et al., 2021). However, the impacts

of waterlogging on crop yield vary by species, growth stage, stress duration, soil type, and crop genotype (Langan et al., 2022). Previous studies have reported that waterlogging reduces the yield of wheat by 15-45% (Herzog et al., 2016), soybeans by 28-48% (Sathi et al., 2022), barley by 15-68% (Ploschuk et al., 2018), maize by 6-80% (Tian et al., 2019), and cowpeas by 10-91% (Timsina et al., 1994). Hence, maintaining yield in the face of increased climatic extremes is critical for future crop breeding programs.

Waterlogging causes major changes in the soil environment by inhibiting the diffusion of gases required for root growth and function (Arduini et al., 2019). Consequently, waterlogged soils become anaerobic after being saturated for a few hours because roots and soil microbes' oxygen demand exceeds the atmosphere's influx (Ploschuk et al., 2018). Previous studies have shown that a decrease in soil redox potential reflects oxygen deprivation in the soil and its rate of depletion depends primarily on soil depth and temperature (Cannell et al., 1985; Ponnamperuma, 1972). In addition, soil waterlogging inhibits the oxidative decomposition of gases such as ethylene and CO_2, leading to accumulation that impairs root growth and development (Herzog et al., 2016). Plants alleviate the effects of soil hypoxia through a series of anatomical, morpho-physiological, and metabolic responses. For example, waterlogged plants rapidly switch from aerobic metabolism to anaerobic fermentation, reducing ATP production from 36 moles of glucose metabolized to 2 moles (Gibbs and Greenway, 2003; Sousa and Sodek, 2002). Starch reserves are rapidly depleted during anaerobic fermentation as harmful byproducts such as alcohols, aldehydes, and reactive oxygen species (ROS) are produced (Langan et al., 2022; Sairam et al., 2011; Sauter, 2013).

Cowpea is one of the world's most critical leguminous crops, with widespread adaptation and cultivation. According to FAO (2022), the cultivated area of cowpea in 2022 was 15.1

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million hectares, with a global production of 8.9 million tonnes. Generally, like many other

legumes, cowpeas are considered sensitive to waterlogging; when the soil water content is 2-3

cm above the soil level, the growth and development of cowpeas are hampered (Minchin and

Summerfield, 1976; Umaharan et al., 1997). Previous studies have opined that significant

reductions in the water conductivity of cowpea's root system and leaf water potential are the

primary consequences of waterlogging (Ashraf, 2012; Takele and McDavid, 1994). When the

water potential of the leaves exceeds that of the soil, water uptake becomes difficult. In response,

plants close their stomata to avoid or delay extreme drops in leaf water potential (Ogbaga et al.,

2014), thus, limiting cowpea's photosynthetic capacity by causing a series of morphological

changes. For instance, increasing waterlogging duration gradually decreased the plant height,

leaf area, and biomass of four cowpea genotypes (Umaharan et al., 1997).

Waterlogging has multiple effects on cowpea growth and physiology, from disrupting

carbon metabolism to reducing biomass and yields. Moreover, waterlogging has a genotype-

dependent effect on cowpea's morphological characteristics and yields (Minchin et al., 1978).

For example, moderate waterlogging significantly improved the biomass accumulation and

growth of tolerant cowpea genotypes, while severe waterlogging considerably reduced the leaf

area, biomass, and seed yield of sensitive cowpea genotypes (Takele and McDavid, 1994).

Cowpea yield is a complex integration of different physiological processes. Therefore,

the negative impacts of waterlogging stress on the photosynthetic machinery would decrease

cowpea yield based on the severity and duration of the stress (Pampana et al., 2016; Tian et al.,

2021). Previous studies have shown that waterlogging at different growth stages reduces the

yields of leguminous crops, including soybean (Linkemer et al., 1998), cowpea (Umaharan et al.,

1997), mungbean (Ahmed et al., 2002), chickpea (Noori et al., 2017), and cool-season grain

legumes (Pampana et al., 2016). However, these studies concluded that the damage was more significant during the reproductive growth stage because this stage is associated with pollination, fertilization, gametogenesis, embryogenesis, and grain formation, all of which influence crop productivity (Wang et al., 2017). Minchin et al. (1978) revealed that waterlogging during flowering in cowpea resulted in more than 50% yield reduction due to flower abortion, grain abscission, poor pod formation, and seed set, possibly due to limited carbohydrate supply. Waterlogging has multiple effects on seed yield and associated traits, which may be related to three factors: crop genotype, crop duration, and crop growth stage (Tian et al., 2021). However, no comprehensive studies have assessed the effects of waterlogging on different growth stages of cowpea, even though their production is heavily dependent on rainfall and irrigation during the summer in the United States. Hence, a better understanding of the impact of waterlogging on the growth and physiology of cowpea genotypes at different growth stages is needed to improve management practices to optimize seed yield for economic gain.

In the current study, a two-year waterlogging experiment was conducted at the vegetative (V4), reproductive (R2), and physiological maturity (R7) stages of cowpea genotypes with different waterlogging tolerance. Growth attributes, leaf physiological parameters, seed yield-related traits, and quality of these genotypes were assessed after ten days of waterlogging to answer the following research questions: (1) What are the detrimental effects of waterlogging stress on morphological and physiological traits of cowpea at different growth stages?; (2) How does waterlogging affect cowpea seed yield and quality at different growth stages?; (3) When is the critical growth stage of waterlogging stress in cowpea genotypes?; and (4) Are there differences in the physiological and yield-related traits between waterlogged-tolerant and -sensitive cowpea genotypes? Therefore, this study will contribute to a better understanding of the

mechanisms of waterlogging tolerance in cowpea and provide data that may be beneficial in developing strategies to maintain cowpea yields in wetland environments.

5.2   Materials and Methods

5.2.1   Experimental Site

A two-year study was conducted in 2021 and 2022 in the experimental field of the Mississippi State University, located in the North Mississippi Research and Extension Center (NMREC; 34°09′54.2′ N, 88°43′14.3′ W, elevation 99 m). Summer at the NMREC is generally hot, with an average daily temperature of 26.7 °C in June, 27.2 °C in July, and 27.2 °C in August (Barickman et al., 2018). Figure 1 depicts the monthly average precipitation and temperature in 2021 and 2022. The site at the NMREC has an ora fine sandy loam with a pH of 6.8 and 1.2% organic matter. The soil analysis of the experimental site revealed 158.04 kg/ha phosphorus, 249.95 kg/ha potassium, 131.14 kg/ha magnesium, 3.14 kg/ha zinc, and 0.1 µmhos/cm of total soluble salts. The soil pH was 6.9, with an estimated CEC of 8.99 cmol$_c$/kg.

5.2.2   Plant material and field management

Two cowpea genotypes (EpicSelect.4 and UCR 369) with contrasting waterlogging tolerance, as determined by Olorunwa et al. (2022), were selected for this study. The selected cowpea genotypes have similar growth and duration of the life cycle. The cowpea seeds were inoculated a day before sowing with *Bradyrhzobium japonicum* (Visjon Biologics, Wichita Falls, TX) at 141 g per 22.68 kg of seeds. Twenty-five inoculated cowpea seeds of each genotype were directly sown at a distance of 7 cm, a depth of 1.5 cm, and a row spacing of 30 cm on May 9th and 25th in 2021 and 2022, respectively. Approximately 14 days after sowing (DAS), the plants were thinned to 12 per genotype on each stand.

General cultural practices, including fertilizer application, weed, and pest control, were carried out during the two-year experimental period. There was no irrigation, but the growth and development of plants were completely dependent on rainfall. Fertilizers were applied at NMREC based on soil test results from the Mississippi State University Extension Service Soil Testing Laboratory. Each year, 30 kg/ha N, 60 kg/ha P_2O_5, and 120 kg/ha K_2O were administered to the fields during field preparation. S-metolachlor (Dual II Magnum®, Syngenta, Greensboro, NC, USA) and Sethoxydim (Poast Plus, BASF Corporation, Research Triangle Park, NC, USA) were applied immediately after sowing to control weeds. Subsequently, weeds were manually controlled to prevent any competition with cowpea growth. Imidacloprid (Provoke™, Innvictis® Crop Care, Loveland, CO, USA) and spinosad (Entrust®, Dow AgroSciences, Indianapolis, IN, USA) were foliar sprayed to control aphids, leafhoppers, and whiteflies after 14 DAS. Subsequently, downy mildew and cutworms were controlled by applying zeta-cypermethrin (Mustang® Maxx, FMC Corporation, Philadelphia, PA, USA) and chlorothalonil (Praiz™, Winfield Solutions, St. Paul, MN, USA) at 21 DAS. During the reproductive stage, azoxystrobin (Quadris Top®, Syngenta, Greensboro, NC, USA) and zeta-cypermethrin were sprayed on the cowpea's foliage to control anthracnose and bean worms.

5.2.3   Experimental design and waterlogging treatment

The experimental design was a split plot with soil waterlogging treatments as the main plot and genotypes as the subplot. The experimental treatments consist of control and waterlogging treatments. The field was divided into three blocks as the different developmental stages of cowpea (vegetative, reproductive, and maturity). Each block was divided into two plots as control and waterlogging treatments, with a 2.5 m buffer zone between the two waterlogging treatments. The cowpea genotypes were planted in a randomized complete block design with

four replicates within each block. Before applying the treatment, levees targeting 5-10 cm in height were constructed to separate the control plot from the waterlogging plot to ensure the soil remained saturated during the treatment.

At approximately 30 DAS, when the plant began to branch, the cowpea plants were subjected to two experimental treatments consisting of waterlogging and control treatments to evaluate waterlogging stress during the V4 growth stage (first block). The waterlogged plots were irrigated at the same frequency as the control. Still, the soil profile was saturated entirely to the point of ponding (water level 2 cm above the soil surface) by pumping water from a nearby water source, usually every day, to create an oxygen-deficient environment. Morphological and physiological data were assessed on each plot after ten days of waterlogging treatment (DOW). Thereafter, cowpea plants were harvested to quantify seed yield-related traits and quality. Similar treatments were applied to the second and third blocks. The second block includes the application of waterlogging and control treatments on cowpea plants at their R2 growth stage (approximately 50 DAS; 75% flowering). The third block mirrored the second block at about 70 DAS to evaluate waterlogging stress on cowpea plants at the physiological maturity (R7) growth stage.

5.2.4   Leaf gas exchange parameters

After 10 days of waterlogging at the V4, R2, and R7 stages, the net photosynthetic rate (A), stomatal conductance (g_s), transpiration rate (E), and intercellular CO_2 concentration (C_i) were measured on the topmost fully expanded leaf of cowpea plants. Measurements were taken using a portable infrared gas analyzer (LI-6800, LI-COR, Lincoln, NE, USA) under a relative humidity of 55%, a light intensity of 1600 µmol m^2/s, an airflow rate of 600 µmol/s, a temperature of 28 °C and a CO_2 concentration of 410 ppm. Measurements were made between

10:00 and 14:00 CST on five representative plants of each cowpea genotype under control and waterlogging treatments. Chlorophyll fluorescence parameters, including the effective quantum yield of PSII (Φ_{PSII}) and the maximum quantum efficiency by open PSII reaction center (F'_v/F'_m), were measured in situ during the gas exchange using the light-adapted chlorophyll fluorescence method as portrayed in section 3.2.3. Electron transport rate (ETR) and photochemical quenching (qP) were calculated according to Genty et al. (1989).

5.2.5   Chlorophyll content index

The chlorophyll content index (CCI) of functional leaves was determined using a SPAD (Soil and Plant Analysis Development) analyzer (SPAD-502 Chlorophyll Meter, Konica Minolta, Tokyo, Japan). The SPAD value is the relative CCI of each leaf. CCI was obtained on five representative plants of each cowpea genotype subjected to 10 days of waterlogging at the V4, R2, and R7 growth stages.

5.2.6   Plant harvest and Processing

Five representative cowpea plants from each genotype and treatment were destructively harvested after 10 days of waterlogging at the V4, R2, and R7 growth stages to obtain biomass data on the genotypic variation of cowpea tolerance to waterlogging stress. Cowpea phenotypic data of plant height, leaf area, and leaf number were measured for each treatment combination. The leaf area was measured using the LI-3100 leaf-area meter (LI-COR Bioscience, Lincoln, NE, USA). The plant component's fresh weight was separated into stems and leaves and then measured using a weighing scale. The samples of the plant's fresh weight were then dried in a forced-air oven at 75 °C for two days to obtain cowpea dry weight.

Subsamples of leaf tissue were flash-frozen in liquid nitrogen and stored in an ultra-low −80 °C freezer until processing. Leaf samples were lyophilized for 72 hours using a FreeZone 2.5L freeze dryer (Labconco Corp., Kansas City, MO, USA) for pigment analysis.

5.2.7 Leaf relative water content (LRWC)

The cowpea's relative water content (RWC) was determined as per the method of Barrs and Weatherley (1962) with minor modifications. The RWC value is estimated as ((FW – DW/TW-DW) x 100). FW and DW are cowpea leaf fresh and dry weights, respectively. TM is the turgid mass, determined by soaking the FW of five replicated plants per treatment per genotype in distilled water and obtaining the weight after 24 hours.

5.2.8 Membrane stability index (MSI)

The membrane stability index (MSI) was determined according to the procedure described by Kumar et al. (2013). Briefly, 0.1 g cowpea leaf discs were placed in two sets of test tubes containing 10 ml of double distilled water. One set was heated in a water bath for 30 minutes at 40 °C. The initial electrical conductivity (C_1) was recorded after two hours using the digital EC meter (Fisherbrand™ Accumet AP85 portable waterproof pH/Conductivity meter, Thermo Fisher Scientific, Waltham, MA, USA). The second set was boiled for 10 minutes at 100 °C in a water bath, and its electrical conductivity was measured as C_2. MSI was computed using the following equation: MSI (%) = $[1 − (C1/C2)] \times 100$.

5.2.9 Carotenoids and Chlorophyll Analysis

The extraction and analysis of carotenoids and chlorophylls from leaf tissue were conducted as described in section 4.2.6.

5.2.10   Total Phenolic and Flavonoid Content

Total phenolic content was determined following the Folin-Ciocalteau methodology as described in section 4.2.7. Total flavonoid content was determined using the aluminum chloride colorimetric method portrayed in section 4.2.8.

5.2.11   Soluble Sugar Analysis

Soluble sugars were extracted and quantified with an Agilent 1260 Infinity HPLC using a protocol published by Barickman et al. (2019). Peak assignments were made to individual sugars by comparing retention times from a refractive index detector using external standards for sucrose, fructose, and glucose (Sigma-Aldrich, St. Louis, MO, USA).

5.2.12   Cowpea seed yield and yield-related traits

At the R7 stage, yield-related parameters, namely pod dry weight (PDW), number of pods per plant (NPP), number of seeds per pod (NSP), seed weight, and hundred-seed weight (HSW), were recorded for each genotype and treatment.

5.2.13   Seed quality measurements

Cowpea seed quality traits, including protein, starch, fiber, and moisture, were measured using the Perten DA7250 spectrometer (Perten Instruments, IL, USA) according to procedures described by Bheemanahalli et al. (2022). The phenolic seed content was determined following the Folin-Ciocalteau methodology, as described in section 4.2.7. The concentrations of sucrose in cowpea seeds were extracted and quantified with an Agilent 1260 Infinity HPLC using a protocol previously published by Barickman et al. (2019).

5.2.14   Statistical Analysis

Statistical analysis of the data was performed using SAS (version 9.4; SAS Institute, Cary, NC). The effects of waterlogging and cowpea genotypes, and their interactions, were analyzed using the PROC GLM analysis of variance at V4, R2, and R7 growth stages in 2021 and 2022. Then, Fisher's protected least significant difference test ($P \leq 0.05$) was conducted as the post hoc test. Also, variation in parameters measured due to cowpea growth stages was analyzed following the same procedure. The experiment's fixed effects consist of waterlogging treatment (2), growth stage (3), year (2), and cowpea genotypes (2), where the replication (4 levels) was treated as a random effect. The standard errors were calculated using the pooled error term from the ANOVA table. Diagnostic tests, such as Shapiro–Wilk in SAS, were conducted to ensure that treatment variances were statistically equal before pooling and to evaluate the normal distribution of data. Graphs of LSD means were plotted with Sigmaplot 14.5 (Systat Software Inc., San Jose, CA, USA).

5.3   Results

5.3.1   Morphological parameters

The adverse effects of waterlogging stress on the growth parameters of two cowpea genotypes at different growth stages are shown in Figure 5.1. Waterlogging had a differential impact on cowpea's plant height at different growth stages, and the alterations in the two growing seasons were similar (Figure 5.1 A-B). The effects of waterlogging on the plant height of the two cowpea genotypes were most significant in the R2 stage, followed by the V4 and R7 stages. Compared with the control, the plant height of UCR 369 and EpicSelect.4 decreased by 37% and 52%, respectively, after 10 days of waterlogging in the R2 growth stage.

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The adverse effects vary by growth stage, cowpea genotype, and growing seasons. After 10 days of waterlogging, the leaves of waterlogged cowpea genotypes senescence rapidly, decreasing the cowpea's leaf numbers, while those on the control plants remain green and continue to exist (Figure 5.1 C-D). EpicSelect.4 had the most significant decrease in leaf number after 10 days of waterlogging at R2, followed by V4 and R7 (Figure 5.1 C-D). A similar response was observed in UCR 369 in both growing seasons. Thus, the R2 growth stage is critical to waterlogging stress in cowpeas.

The most significant effect of waterlogging was the leaf area per plant reduction by more than 80% in EpicSelect.4 compared to the control treatments (Figure 5.1 E-F). The leaf area of UCR 369 in R2 was the most susceptible to waterlogging, followed by R7 and V4. However, damage to the leaf area in EpicSelect.4 was the greatest in R7 at 90%, followed by 84% in V4 and 82% in R2 (Figure 5.1 F).

The total dry weight of cowpea genotypes decreased with waterlogging at different growth stages (Figure 5.1 G-H). In response to 10 days of waterlogging, the reduction of cowpea biomass varied with genotypes, growth stages, and growing seasons. In UCR 369, 10-day waterlogging reduced leaf biomass by 49% and 42% in V4 and R2, respectively, but had no significant effect in the R7 stage of the 2021 growing season (Figure 5.1 G). In contrast, waterlogging reduced biomass in EpicSelect.4 by 79%, 60%, and 54% at R2, V4, and R7, respectively, in 2021. A similar response occurred during the 2022 growing season but with the greatest decline in the V4 stage of both genotypes (Figure 5.1 H).
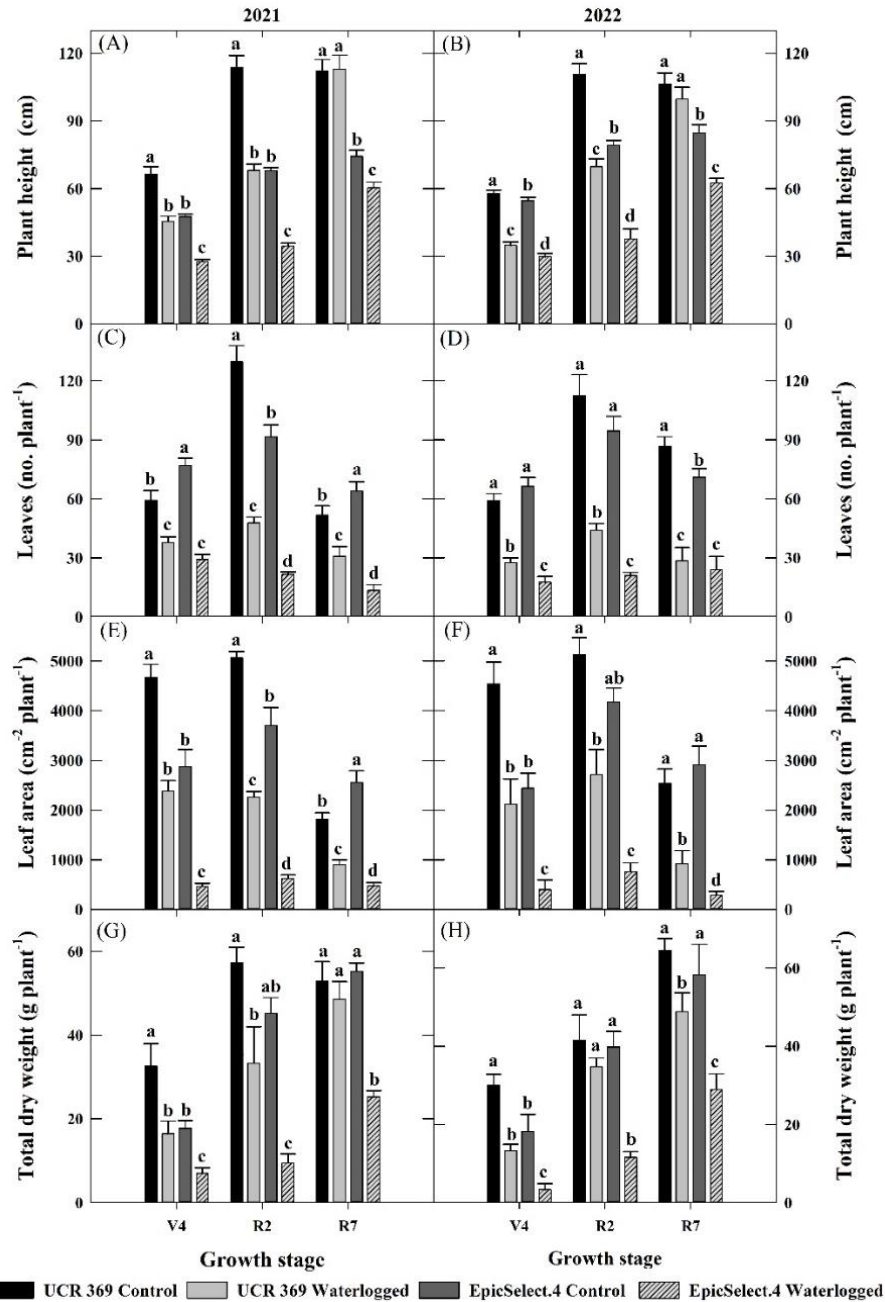
Figure 5.1   Mean plant height (A-B), mean leaf number (C-D), mean leaf area (E-F), and the mean total dry weight (G-H) of UCR 369 and EpicSelect.4 under 10 days of waterlogging at V4, R2, and R7 stages. V4, R2, and R7 illustrates vegetative, flowering, and physiological maturity growth stages during the 2021 and 2022 growing seasons. Different lowercase letters on the vertical bar indicate significant differences between the cultivar's means and treatments ($P < 0.05$), as determined by Fisher's LSD. The error bar on the vertical bar indicates the standard error of the mean $\pm$ 4 replications of each morphological trait. Cowpea morphological traits were measured 38, 54, and 76 d after sowing for V4, R2, and R7 growth stages.

5.3.2   RWC, MSI, and SPAD

After 10 days of waterlogging, the RWC of both cowpea genotypes decreased at different growth stages, and the changes were similar in the two-year study (Figure 5.2 A-B). The RWC of EpicSelect.4 decreased the most in the 10-day waterlogging treatment in the R2 stage, followed by R7 and V4. Waterlogged UCR 369 maintained a significantly higher RWC than EpicSelect.4 under waterlogging at different growth stages (Figure 5.2 A-B).

After 10 days of waterlogging, MSI significantly decreased by 29% and 32% in the V4 and R2 stages but remained unchanged in the R7 (Figure 5.2 C-D). However, there was no difference in the response of MSI to waterlogging at different growth stages of UCR 369 (Figure 5.2 C-D).

Compared with the control treatments, the SPAD value of both cowpea genotypes decreased at different growth stages in the 2021 and 2022 growing seasons (Figure 5.2 E-F). The largest reduction in SPAD occurred in the R7 stage of EpicSelect.4, followed by the R2 and V4 growth stages. However, in UCR 369, waterlogging at the V4 resulted in the greatest effect on the SPAD of the functional leaves, followed by R2 and R7. In addition, the impact of waterlogging on the SPAD of EpicSelect.4 was greater than that of UCR 369. The highest SPAD value was observed in UCR 369 at the V4 growth stage under the control treatment (Figure 5.2 E-F).
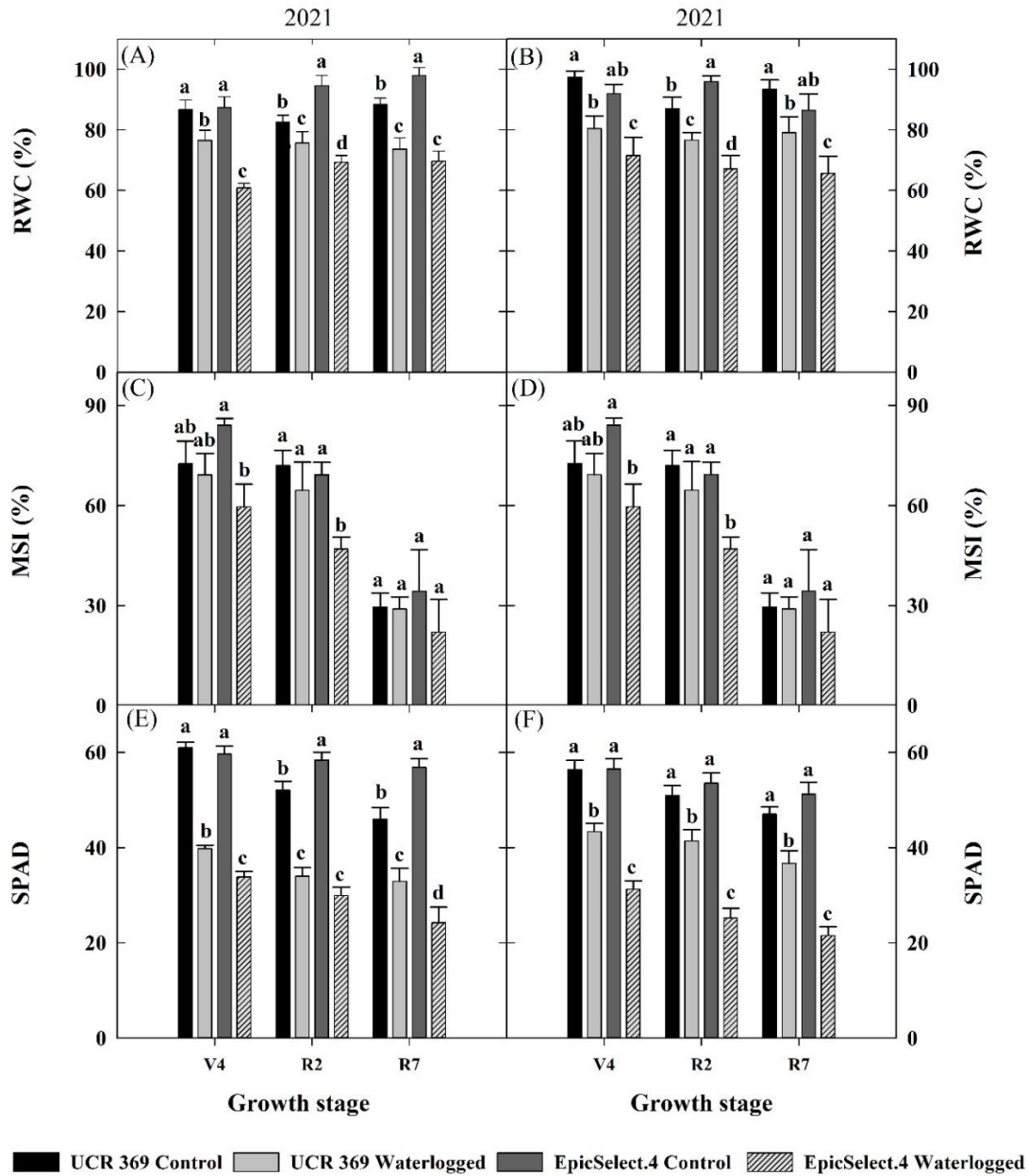
Figure 5.2   Relative water content (RWC) (A-B), membrane stability index (MSI) (C-D), and chlorophyll content index (SPAD) (E-F) of UCR 369 and EpicSelect.4 subjected to 10 days of control and waterlogging at V4, R2, and R7 growth stages. V4, R2, and R7 illustrates vegetative, flowering, and physiological maturity growth stages during the 2021 and 2022 growing seasons. Different lowercase letters on the vertical bar indicate significant differences between the cultivar's means and treatments ($P < 0.05$), as determined by Fisher's LSD. The error bar on the vertical bar indicates the standard error of the mean ± 4 replications of each morphological trait. RWC, MSI, and SPAD were measured 38, 54, and 76 d after sowing for V4, R2, and R7 growth stages.

5.3.3   Leaf gas exchange and chlorophyll fluorescence parameters

Waterlogging inhibited the leaf gas exchange parameters of the functional leaves of the two cowpea genotypes, which vary at different growth stages (Figure 5.3). The most significant effects of waterlogging on A, E, and g_s were observed at R2, followed by R7 and V4 stages. Changes in A, E, and g_s in UCR 369 were more detrimental in the 2021 growing season than in the two growing seasons in EpicSelect.4 (Figure 5.3 A-F). Moreover, the decline of A, E, and g_s was comparatively higher in EpicSelect.4 than in the UCR 369 genotype. On average, the A decreased by 67%, 63%, and 44% at the R2, R7, and V4 stages, respectively, relative to the control treatment. The corresponding A of EpicSelect.4 decreased by 92%, 86%, and 82% at R2, R7, and V4 stages, respectively, compared to the control treatments.

Decreased g_s of EpicSelect.4 in V4 and R2 increased C_i (Figure 5.3 G-H). However, there was no difference in C_i of UCR 369 at different growth stages (Figure 5.3 G-H). This implies that C_i did not play any role in the decreased A; instead, only g_s was responsible under waterlogging conditions.

The Φ_{PSII}, ETR, F_v'/F_m', and qP showed similar responses as the photosynthetic parameters. 10 days of waterlogging reduced Φ_{PSII} by 76% and 84% at R2, 70% and 77% at V4, and 68% and 80% at R7 in UCR 369 and EpicSelect.4, respectively (Figure 5.4 A-B). A corresponding response was measured for the ETR of both cowpea genotypes at different growth stages (Figure 5.4 C-D). The F_v'/F_m' and qP were significantly decreased after 10 days of waterlogging, with the most significant reductions in the R2 stage, which fell by 24% and 68% for UCR 369, respectively. EpicSelect.4 decreased by 39% and 79% compared to the control, respectively (Figure 5.4 E-H). Moreover, photoinhibition was comparatively higher in EpicSelect.4 than in UCR 369 cowpea genotypes.
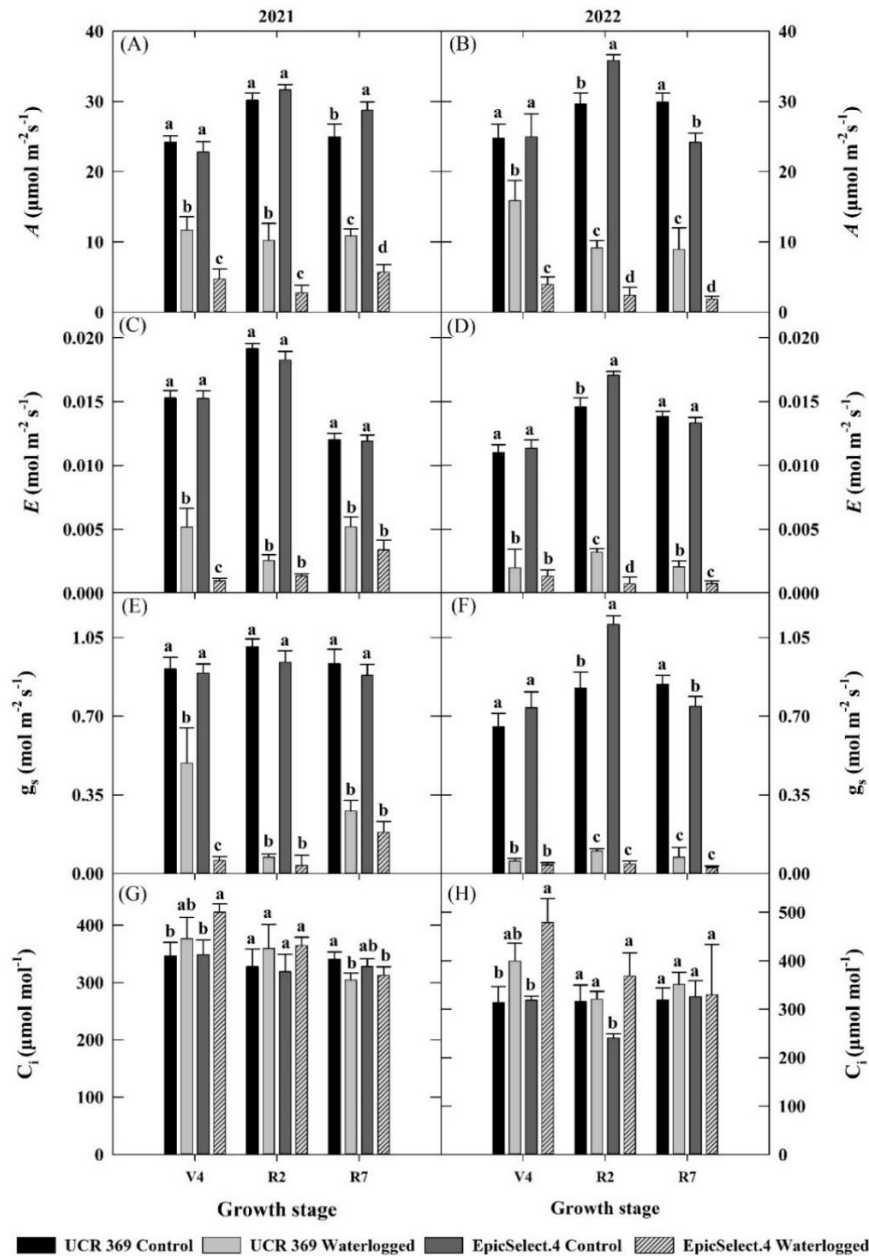
Figure 5.3   The CO$_2$ assimilation rate (*A*) (A-B), leaf transpiration rate (*E*) (C-D), stomatal conductance (g$_s$) (E-F), and intercellular CO$_2$ concentration (C$_i$) (G-H) of UCR 369 and EpicSelect.4 subjected to 10 days of control and waterlogging at V4, R2, and R7 growth stages. V4, R2, and R7 illustrates vegetative, flowering, and physiological maturity growth stages during the 2021 and 2022 growing seasons. Cowpea physiological parameters were measured at 37, 53, and 75 d after sowing for V4, R2, and R7 growth stages. Different lowercase letters on the vertical bar indicate significant differences between the cultivar's means and treatments (*P* < 0.05), as determined by Fisher's LSD. The error bar on the vertical bar indicates the standard error of the mean ± 4 replications of each physiological trait.

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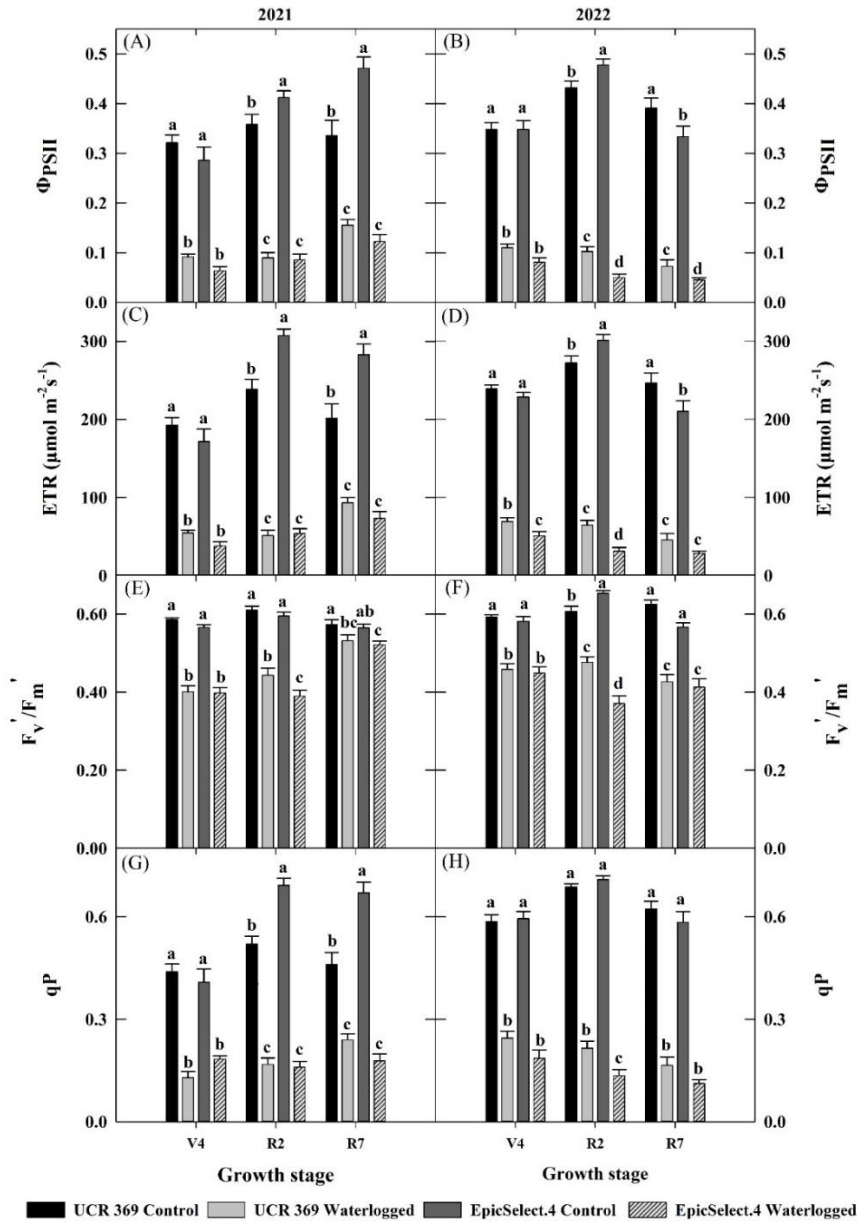
Figure 5.4   The effective quantum yield of PSII (Φ_{PSII}) (A-B), electron transport rate (ETR) (C-D), (and the maximum quantum efficiency of PSII (E-F), light-adapted state (F_v'/F_m'), and (G-H) Photochemical quenching (qP) (G-H) of UCR 369 and EpicSelect.4 subjected to 10 days of control and waterlogging at V4, R2, and R7 growth stages. V4, R2, and R7 illustrates vegetative, flowering, and physiological maturity growth stages during the 2021 and 2022 growing seasons. Cowpea physiological parameters were measured at 37, 53, and 75 d after sowing for V4, R2, and R7 growth stages. Different lowercase letters on the vertical bar indicate significant differences between the cultivar's means and treatments ($P < 0.05$), as determined by Fisher's LSD. The error bar on the vertical bar indicates the standard error of the mean ± 4 replications of each physiological trait.

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5.3.4 Plant pigments: chlorophyll and carotenoid

The photosynthetic pigments of the functional leaves of the two cowpea genotypes were significantly affected by 10 days of waterlogging during different growth stages compared with the control (Figure 5.5). The most significant effects of waterlogging on chlorophyll α (Figure 5.5 A-B) and β (Figure 5.5 C-D) levels occurred at R2, followed by V4 and R7. On average, chlorophyll α of UCR 369 and EpicSelect.4 were reduced by 43% and 75% after 10 days of waterlogging. Similarly, chlorophyll β decreased by 47% and 62% in UCR 369 and EpicSelect.4, respectively. It is interesting to note that waterlogging did not cause any change in chlorophyll α and β of UCR 369 at the R7 stage. These results showed that the waterlogging tolerance of UCR 369 was superior to that of EpicSelect.4 under the same waterlogging conditions.

The chlorophyll α/β ratio of UCR 369 and EpicSelect.4 decreased under waterlogging conditions at different growth stages but was not different in UCR 369 at the R7 stage (Figure 5.5 E-F). The most significant effect of waterlogging on chlorophyll α/β occurred at the R2 stage in EpicSelect.4 by 34%, while the lowest reduction was demonstrated in UCR 369 at R7 by 10%.

Waterlogging reduced total carotenoids by 40% and 54% at R2 and 35% and 57% at V4 in UCR 369 and EpicSelect.4 compared to the control treatment (Figure 5.5 G-H). However, at R7, there was no difference in total carotenoids in UCR 369, while total carotenoids in EpicSelect.4 were reduced by 34%.
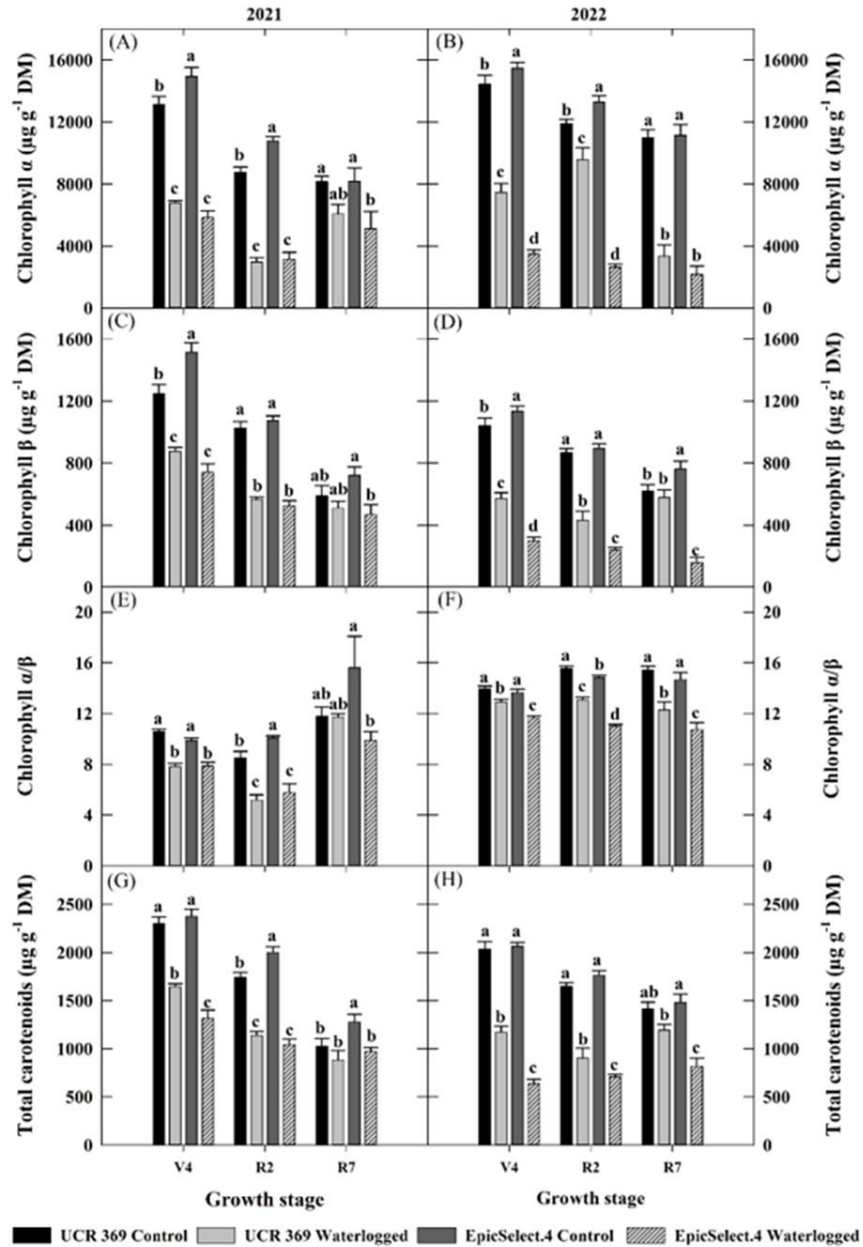
Figure 5.5   Chlorophyll α (A-B), chlorophyll β (C-D), chlorophyll α/β (E-F), and total carotenoids (G and H) of UCR 369 and EpicSelect.4 subjected to 10 days of control and waterlogging at V4, R2, and R7 growth stages. V4, R2, and R7 illustrates vegetative, flowering, and physiological maturity growth stages during the 2021 and 2022 growing seasons. Different lowercase letters on the vertical bar indicate significant differences between the cultivar's means and treatments ($P <$ 0.05), as determined by Fisher's LSD. The error bar on the vertical bar indicates the standard error of the mean $\pm$ 4 replications of each biochemical trait. Cowpea biochemical traits were measured at 38, 54, and 76 d after sowing for V4, R2, and R7 growth stages.

5.3.5   Total phenolic and flavonoid content

Total phenolic and flavonoid content are important compatible solutes in the cytoplasm of plants, and when plants are subjected to waterlogging, these osmolytes change to facilitate osmotic adjustments. Compared with the control plants, the total phenolic content of UCR 369 and EpicSelect.4 increased significantly by 44% and 23% at V4 and by 34% and 13% at R2 during the 2021 growing season (Figure 5.6 A), while no difference was found in the 2022 growing season (Figure 5.6 B). Also, UCR 369 showed no difference in total phenolic content at R7, whereas EpicSelect.4 showed a 15% decrease in total phenolic content during the 2021 growing season (Figure 5.6 A).

On the other hand, the total flavonoid content increased by 27% in UCR 369 at R2, while it decreased by 43% in EpicSelect.4 (Figure 5.6 C-D). At the R7 stage, the total flavonoid content of the two cowpea genotypes decreased by an average of 38% compared with the control.
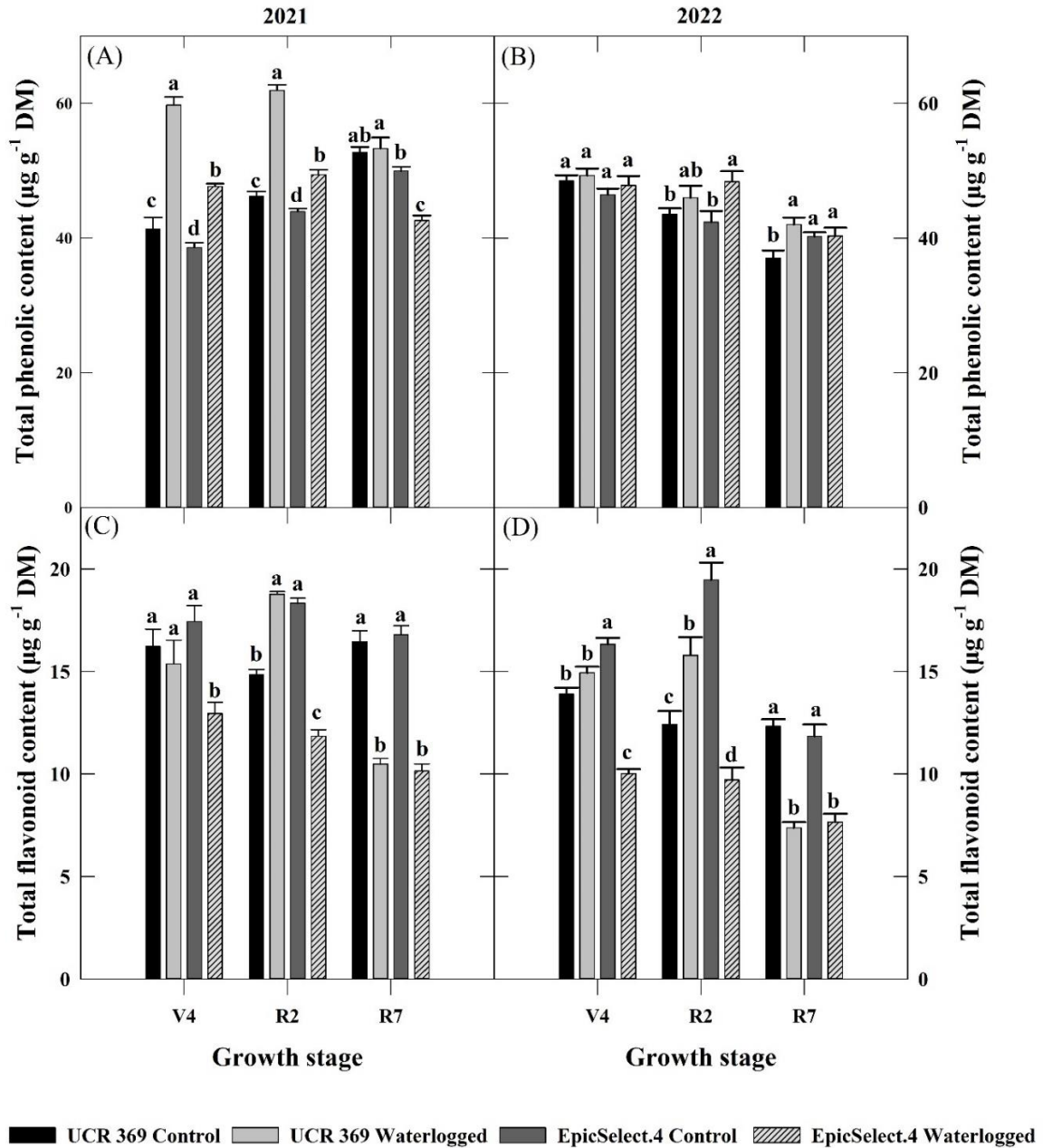
Figure 5.6   Total phenolic content (A-B), and total flavonoid content (C-D) of UCR 369 and
EpicSelect.4 subjected to 10 days of control and waterlogging at V4, R2, and R7
growth stages. V4, R2, and R7 illustrates vegetative, flowering, and physiological
maturity growth stages during the 2021 and 2022 growing seasons. Cowpea
biochemical traits were measured at 38, 54, and 76 d after sowing for V4, R2, and
R7 growth stages. Different lowercase letters on the vertical bar indicate
significant differences between the cultivar's means and treatments (P < 0.05), as
determined by Fisher's LSD. The error bar on the vertical bar indicates the
standard error of the mean ± 4 replications of each biochemical trait.

5.3.6   Soluble sugar accumulation

To determine the effect of waterlogging on cowpea's soluble sugars at different growth stages, the concentrations of fructose, glucose, and sucrose were quantified. The most significant effects of waterlogging on glucose and sucrose were observed at R2, followed by R7 and V4 stages in UCR 369, which showed no difference (Figure 5.7). Conversely, the V4 was observed with the highest increase in glucose and fructose in waterlogged EpicSelect.4 (Figure 5.7). Interestingly, the response of fructose and glucose in both genotypes at different growth stages followed a different pattern in both growing seasons, with higher concentrations in 2022.

After 10 days of waterlogging, the sucrose levels remained unchanged in UCR 369 at the V4 stage, while they significantly increased at the R2 and R7 stages during the 2021 growing season (Figure 5.7 E). However, waterlogging increased the sucrose levels in UCR 369 at different growth stages during the 2022 growing season (Figure 5.7 F). In EpicSelect.4, sucrose levels only increased at the V4 stage in 2022, while other treatments showed no difference with the control plants (Figure 5.7 E-F).
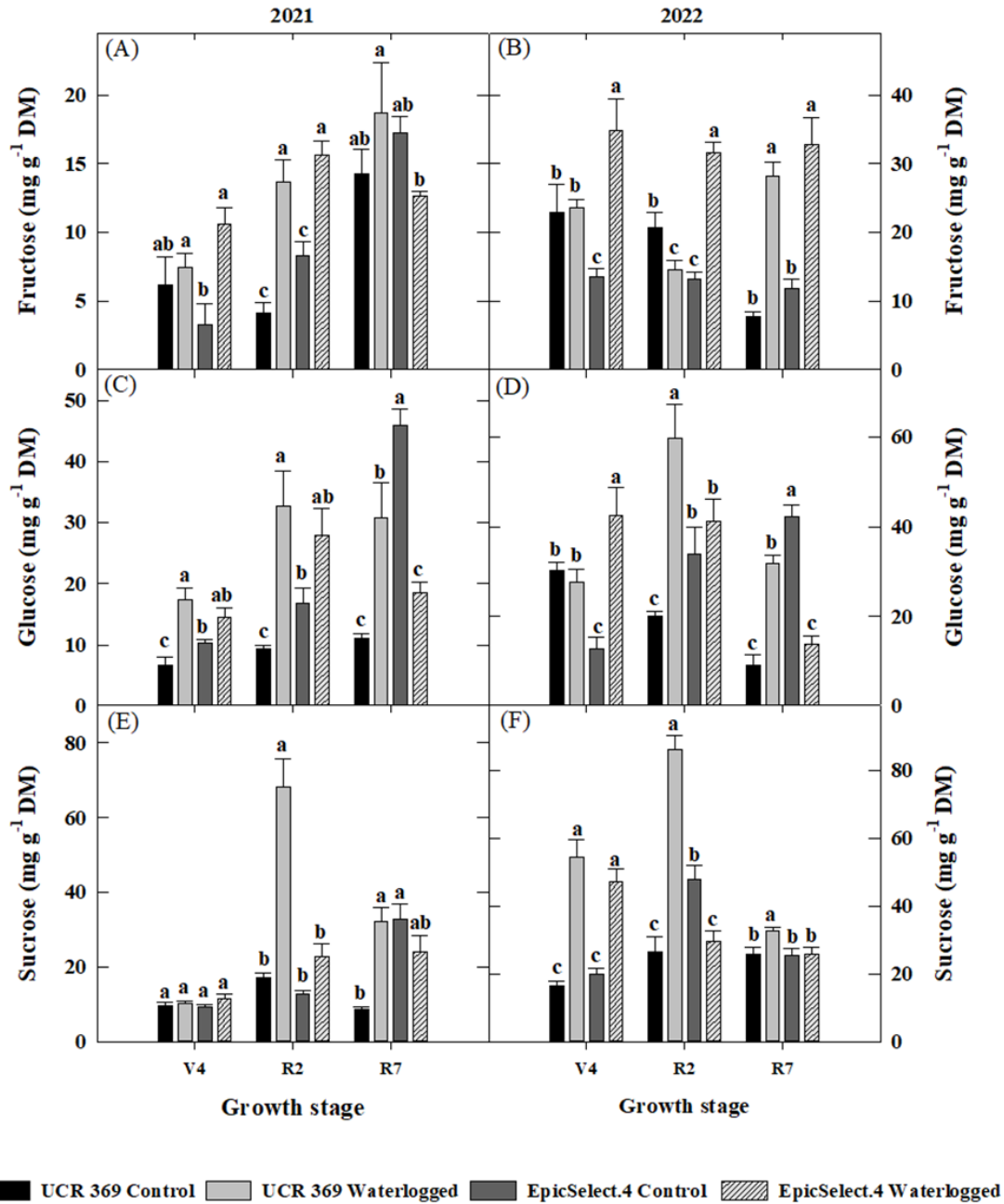
Figure 5.7   Fructose (A-B), glucose (C-D), and sucrose (E-F) of UCR 369 and EpicSelect.4 subjected to 10 days of control and waterlogging at V4, R2, and R7 growth stages. V4, R2, and R7 illustrates vegetative, flowering, and physiological maturity growth stages during the 2021 and 2022 growing seasons. Cowpea biochemical traits were measured at 38, 54, and 76 d after sowing for V4, R2, and R7 growth stages. The error bar on the vertical bar indicates the standard error of the mean $\pm$ 4 replications of each biochemical trait. Different lowercase letters on the vertical bar indicate significant differences between the cultivar's means and treatments (P < 0.05), as determined by Fisher's LSD.

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5.3.7   Seed yield and yield-related attributes

Waterlogging stress reduced cowpea seed yield and yield components at different growth stages, including PDW, NPP, NSP, and SW (Table 5.1). Cowpea SW per plant was most susceptible to waterlogging damage at R2, followed by V4 and R7, with the most significant reduction in EpicSelect.4. Compared to the control plants, waterlogging decreased SW by an average of 76% and 82% for UCR 369, respectively.

Waterlogging affected cowpea seed yield components with the most significant effect at the R2 stage, followed by V4 and R7. PDW, NPP, and NSP of UCR 369 were reduced after 10 days of waterlogging relative to the control, with the most significant decline of 73%, 67%, and 28% in R2, respectively (Table 5.1). EpicSelect.4 decreased by 81%, 79%, and 47% in PDW, NPP, and NSP at the R2 stage (Table 5.1).

There was no difference in the response of HSW in waterlogged UCR 369 at different growth stages, which was consistent in the two growing seasons (Table 5.1). However, only the HSW of EpicSelect.4 decreased by 37% and 19% in the 2022 and 2021 growing seasons, respectively.

Table 5.1   Effects of 10 days of waterlogging on cowpea genotypes pod dry weight (PDW), number of pods per plant (NPP), number of seeds per pod (NSP), seed weight (SW), and hundred-seed weight (HSW) of UCR 369 and EpicSelect.4 at vegetative (V4), flowering (R2), and maturity growth stage (R7).

Year	Genotype	Treatment	PDW	NPP	NSP	SW	HSW
			g plant^{-1}	no. plant^{-1}	no. plant^{-1}	g plant^{-1}	g
2021	UCR 369	Control	55.94 a	27.05 a	12.41 a	34.85 a	11.59 c
		V4	12.33 de	8.40 de	9.75 cd	8.25 c	11.84 c
		R2	6.13 de	5.60 ef	10.89 bc	6.37 c	11.58 c
		R7	41.42 b	20.7 b	11.38 ab	29.04 a	12.23 c
	EpicSelect.4	Control	25.66 c	18.35 bc	8.79 d	15.80 b	15.00 a
		V4	14.23 d	9.53 d	6.69 e	5.50 c	15.96 a
		R2	4.18 e	2.80 f	5.50 f	3.09 c	12.17 c
		R7	24.31 c	16.35 c	7.62 e	15.27 b	13.92 b
2022	UCR 369	Control	68.03 a	30.70 a	9.78 a	29.35 a	12.12 ab
		V4	22.30 cd	13.00 cd	7.88 ab	12.06 bc	11.02 bc
		R2	29.04 bc	14.05 cd	5.58 bc	8.62 bc	11.85 ab
		R7	46.01 ab	19.70 bc	9.75 a	15.69 b	13.22 ab
	EpicSelect.4	Control	54.54 ab	27.10 ab	5.18 bc	17.54 b	13.26 ab
		V4	16.22 de	12.40 d	2.93 bc	3.90 c	13.70 ab
		R2	11.30 e	7.00 d	2.21 c	2.90 c	8.34 c
		R7	42.57 ab	15.70 cd	4.75 bc	12.53 bc	15.08 a
	Treatment (Trt)		***	***	NS	***	NS
	Genotype (Gen)		*	NS	***	**	NS
	Trt * Gen		NS	NS	**	NS	NS

† NS represents non-significant $P > 0.05$. *, **, *** represent significance levels at $P \leq 0.05$, 0.01, and ≤ 0.001 respectively; within columns, values followed by the same letter are not significantly different.
†Cowpea seed yield and yield components subjected to waterlogging at R2 and R7 were measured 88 d after sowing. Cowpea seed yields subjected to waterlogging at V4 were collected 113 d after sowing.

5.3.8   Seed quality parameters

In UCR 369, 10 days of waterlogging did not cause any significant change in the total protein content of cowpea seed at different growth stages, which was consistent in the two growing seasons (Table 5.2). However, waterlogging decreased the seed protein of EpicSelect.4, with the greatest increase at the R2 stage. The interaction between waterlogging treatment and cowpea genotype significantly affected the starch content of cowpea seeds. (Table 5.2). Cowpea starch content increased the highest when UCR 369 was subjected to waterlogging at the R2 stage, while there is no difference in EpicSelect.4 (Table 5.2). Cowpea seed fiber of UCR 369 showed no difference but decreased in EpicSelect.4, with the greatest effect in the R2 stage (Table 5.2). The seed phenolics of UCR 369 were the lowest at the R2 stage, while they increased significantly in EpicSelect.4 (Table 5.2). Compared with the control, the sucrose of UCR seeds decreased at V4 and R7 stages but remained unchanged at R2. However, the seed sucrose in EpicSelect.4 only decreased at the R2 stage (Table 5.2).

Table 5.2   Effects of 10 days of waterlogging on cowpea genotypes seed quality traits of UCR 369 and EpicSelect.4 at vegetative (V4), flowering (R2), and maturity growth stage (R7)

Year	Genotype	Treatment	Protein	Starch	Fiber	Phenolics	Sucrose
			%	%		µg g^{-1}	mg g^{-1}
2021	UCR 369	Control	20.80 e	61.74 c	3.20 c	0.31 bc	28.74 a
		V4	20.47 e	65.62 a	2.68 d	0.38 a	17.09 b
		R2	19.87 e	50.85 g	4.07 b	0.20 e	32.17 a
		R7	20.53 e	62.53 b	2.67 d	0.27 d	13.01 bc
	EpicSelect.4	Control	28.37 a	52.70 f	4.43 a	0.18 e	16.95 b
		V4	27.10 cd	56.58 d	3.10 c	0.34 b	11.83 bc
		R2	26.74 d	54.41 e	3.83 b	0.27 cd	5.69 c
		R7	28.00 bc	54.37 e	3.78 b	0.19 e	16.10 ab
2022	UCR 369	Control	20.33 de	66.64 b	1.73 cd	0.33 bc	31.02 b
		V4	19.03 e	70.13 a	1.44 d	0.41 a	18.26 c
		R2	21.38 d	64.94 c	1.63 cd	0.26 cd	41.08 a
		R7	20.76 d	66.94 b	1.29 d	0.29 cd	13.93 cd
	EpicSelect.4	Control	27.58 a	59.42 e	2.70 b	0.20 e	19.11 c
		V4	26.16 bc	60.17 e	2.09 c	0.36 ab	12.58 cd
		R2	24.86 c	62.61 d	3.45 a	0.31 bc	6.55 d
		R7	26.30 ab	62.04 d	2.83 b	0.22 d	18.37 c
	Treatment (Trt)		***	**	**	***	*
	Genotype (Gen)		***	***	***	***	**
	Trt * Gen		NS	***	NS	***	NS

† NS represents non-significant $P > 0.05$. *, **, *** represent significance levels at $P \leq 0.05$, 0.01, and ≤ 0.001, respectively; within columns, values followed by the same letter are not significantly different.

†Cowpea seeds subjected to waterlogging at R2 and R7 were measured 88 d after sowing. Cowpea seed yields subjected to waterlogging at V4 were collected 113 d after sowing. The seeds were used for analysis.

5.4   Discussion

Waterlogging is one of the significant abiotic stresses, inhibiting plant growth, physiology, and metabolic processes, resulting in a severe decline in crop yields (Pan et al., 2021; Tian et al., 2021). The projected frequency of extreme events due to climate change will have profound effects on plants susceptible to waterlogging. Waterlogging reduces oxygen diffusion in the soil, leading to hypoxia, which limits plant root respiration, energy production, and nutrient uptake and damages root tissue through oxidative stress (Wang et al., 2017). These waterlogging effects on roots have devastating consequences on the shoot's physiology, hindering plant growth and development (Olorunwa et al., 2022). In this study, waterlogging also impaired cowpea's growth and physiological performance, adversely affecting seed yield and quality. These impacts varied between the two cowpea genotypes (more tolerant UCR 369 and less tolerant EpicSelect.4) and at different growth stages of cowpea. Regarding cowpea's growth stage, the impact of ten days of waterlogging on cowpea genotypes was ranked as R2 > V4 > R7. The R2 is the most critical growth stage for reproductive growth, and at this stage, ten days of waterlogging severely inhibited the morphological development, physiological processes, and yield formation of cowpea seeds. Previous studies have established the susceptibility of legumes to waterlogging during the R2 stage due to the high energy requirements for pollination, gametogenesis, fertilization, and pod filling (Noori et al., 2017; Pampana et al., 2016). In addition, the R2 stage occurred during the hottest period of the year, and high temperatures may have exacerbated the oxygen depletion caused by waterlogging, resulting in a significant loss of cowpea seed yield. In contrast, in the R7 stage, when cowpeas have completed vegetative and reproductive growth, ten days of waterlogging had minimal effects on morphological, physiological, and yield characteristics.

5.4.1 Effects of waterlogging on growth traits of cowpea genotypes at different growth stages

Waterlogging causes changes in plant biochemistry and physiology, leading to growth changes in many crops, including cowpeas (Olorunwa et al., 2022; Panozzo et al., 2019). In this study, the effects of 10-day waterlogging stress, genotype, and growth stage on cowpea's morphological parameters were significant. Earlier experiments in Chapter **Error! Reference source not found.** demonstrated that cowpea root growth was inhibited under 10-day waterlogging stress, resulting in restricted shoot growth. Previous studies have also revealed significant reductions in plant height, biomass, leaf number, and leaf area of cowpeas when subjected to waterlogging (Hong et al., 1977; Jayawardhane et al., 2022; Kumar et al., 2013). The results of this study showed that the plant height and leaf number of cowpea at different growth stages were reduced after ten days of waterlogging (Figure 5.1). Moreover, EpicSelect.4 had the most significant plant and leaf numbers reduction at the R2 stage, followed by the V4 and R7 stages. Waterlogging alters cowpea plant morphology due to reduced water and nutrient uptake and transportation caused by energy deficits during anaerobic respiration, resulting in limited cell expansion and leaf growth (Gibbs and Greenway, 2003). Therefore, due to the high energy demands of the various reproductive processes of cowpea, the most significant decline occurred in the R2 stage under waterlogging.

The effects of waterlogging stress on leaf area and biomass are dependent on plant growth stage, duration, and genotypes. Umaharan et al. (1997) reported greater reductions in leaf biomass in more tolerant cowpea cultivars compared to less tolerant cultivars with smaller leaf areas. In general, smaller leaves are better for reducing waterlogging stress than larger ones because their boundary layer conductivities are high, preventing heat build-up (Leigh et al.,

2017). Similarly, this study showed that the more tolerant genotype "UCR 369" with a larger leaf area experienced the most significant declines under waterlogging at R2, followed by R7 and V4 stages (Figure 5.1 C). Reducing leaf area to avoid water loss appears to be one of the morphological adaptation mechanisms of legumes (Ahmed et al., 2002; Bacanamwo and Purcell, 1999). In addition, the significant reduction in leaf area of cowpea genotypes at the R2 under waterlogging stress stage may be due to accelerated leaf senescence and shedding. Similar findings were reported in mungbean (Ahmed et al., 2002).

On the other hand, EpicSelect.4 showed the largest decrease in leaf biomass at R2, V4, and R7 stages. Previous studies on soybeans have reported higher reductions in biomass at the R2 growth stage (Ploschuk et al., 2022; Rhine et al., 2010). This decrease was associated with reductions in the chlorophyll content index (Figure 5.2 E-F) and the photosynthetic rate (Figure 5.3). Moreover, the decline of the total dry mass of cowpeas was lowest in UCR 369 at the R7 stage (Figure 5.1 G-H). This can be attributed to the fact that at the R7 stage, vegetative and reproductive growth is almost established, and changes in biomass under stress are generally smaller than at the R2 stage. Earlier research on cowpeas (Umaharan et al., 1997), mungbeans (Ahmed et al., 2002), and soybeans (Ploschuk et al., 2022) confirmed similar findings. Therefore, this study showed that the R2 and R7 growth stages were the most sensitive and tolerant to waterlogging in cowpea and related crops, respectively.

5.4.2   Effects of waterlogging on leaf physiological characteristics of cowpea genotypes at different growth stages

Waterlogging-induced anaerobiosis reduces root hydraulic conductivity, decreasing leaf turgor and stomatal conductance, and adversely affecting the photosynthetic process of plants (Shao et al., 2013). Previous studies utilized MSI and RWC as indicators of waterlogging

tolerance in legumes, where relatively high values were associated with waterlogging tolerance (Garcia et al., 2020; Kumar et al., 2013). In the current study, ten days of waterlogging significantly decreased the RWC and MSI of cowpea genotypes, particularly in EpicSelect.4 at the R2 stage (Figure 5.2). Analogous reductions in RWC and MSI in sensitive genotypes have been reported under waterlogging stress in mungbean (Kumar et al., 2013). The inability to maintain plant water status in EpicSelect.4 could be attributed to rapid stomata closure when subjected to waterlogging (Malik et al., 2001). Moreover, the significant decrease in RWC and MSI at the R2 stage suggests that flooded cowpea plants may suffer from impaired water transport, resulting in poor pod formation and reduced yield. Consistent with the current results, cowpeas and soybeans exposed to soil waterlogging stress during early flowering have been reported to exhibit impaired water relations and reduced hydraulic conductivity, resulting in a yield reduction of more than 50% (Hirasawa et al., 1994; Minchin et al., 1978).

The accumulation of dry matter and the formation of cowpea seeds depend on the process of photosynthesis, which is considered to be one of the most sensitive physiological processes to waterlogging (Shao et al., 2013; Tian et al., 2019). Previous studies have shown that waterlogging rapidly closes stomata, damages chlorophyll content, and alters the translocation of photosynthates, leading to a decline in A, with corresponding reductions in plant growth and seed yield (Ren et al., 2014; Shao et al., 2013). This study revealed that A was a sensitive parameter to waterlogging, which decreased in the two cowpea genotypes at different growth stages (Figure 5.3). The relative reduction in A was more pronounced in EpicSelect.4 than in UCR 369. In addition, the decrease in g_s and E was observed, along with the decline in A. Therefore, based on the experimental results, it can be concluded that the major impact of waterlogging on the gas exchange in cowpea leaves seems to be a decrease in g_s, which leads to a reduction in A. It can

also be found that after ten days of waterlogging, the A of UCR 369 decreased by 67%, 63%, and

44% in the R2, R7, and V4 stages, respectively, and by 92%, 86%, and 82% in EpicSelect.4. The

present results are in support of the previous findings in soybeans (Yamane and Iijima, 2016),

mungbean (Ahmed et al., 2002), and field beans (Pociecha et al., 2008). Hence, it indicated that

the photosynthesis of cowpea leaves decreased more obviously in the reproductive stage than in

the vegetative stage under waterlogging stress.

Previous studies in sensitive plants have shown that the increase in C_i due to the decrease

in g_s is a non-stomatal factor limiting A under waterlogging (Pompeiano et al., 2019; Yordanova

and Popova, 2007). In the current study, decreased g_s of EpicSelect.4 in V4 and R2 increased C_i

(Figure 5.3 G-H). Thus, the increase in C_i and the decrease in g_s under flooded conditions suggest

that stomatal and non-stomal limitations contribute significantly to A in the less tolerant

EpicSelect.4. However, there was no difference in the C_i of UCR 369 at different growth stages

(Figure 5.3 G-H). This implies that C_i did not play any role in the decreased A; instead, only g_s

was responsible under waterlogging conditions for tolerant UCR 369. Other non-stomatal factors

limiting A under waterlogged conditions include reduced SPAD (leaf greenness) and chlorophyll

concentrations. Plant leaf SPAD value and chlorophyll concentration are excellent indicators of

plant carbon fixation and nitrogen status under waterlogging stress (Mielke et al., 2010; Ploschuk

et al., 2022). In this study, waterlogging reduced SPAD by 22% to 35% in UCR 369 and 43% to

58% in EpicSelect.4 compared to controls (Figure 5.2 E-F). Interestingly, the concentration of

chlorophyll $\alpha + \beta$ declined more drastically than SPAD after waterlogging, showing reductions

of 22% to 66% in UCR 369 and 37% to 80% in EpicSelect.4 at different growth stages (Figure

5.5 A-D). Earlier studies in soybeans have reported similar findings and attributed significant

degradation of chlorophyll concentrations under waterlogging to inhibited oxygen supplies,

limiting oxidase and chlorophyllase activities (Lapaz et al., 2020; Pereira et al., 2020). Similar reductions in chlorophyll α and β concentrations in the leaves were observed in field peas at various growth stages by Pociecha et al. (2008). They reported the most significant decline in chlorophyll content occurred in leaves closer to the waterlogged roots. This explains the higher decrease in chlorophyll concentrations relative to the SPAD values. Moreover, because chlorophyll is integral to absorbing light energy during the photosynthetic process (Wang et al., 2017), decreased chlorophyll content under waterlogging may reduce the efficiency of cowpea's conversion of light energy into chemical energy. This will limit A and photosynthate and ultimately reduce biomass and seed yield.

Recent studies have established chlorophyll fluorescence as an effective tool to detect functional changes in the photosynthetic process of cowpea and related crops under waterlogging (Ahmed et al., 2002; Kim et al., 2018). This study demonstrated that ten days of waterlogging stress alters chlorophyll fluorescence parameters in the two cowpea genotypes as a non-stomatal factor limiting A. At different growth stages, declines in Φ_{PSII}, ETR, F_v'/F_m', and qP were observed in waterlogged cowpea genotypes compared to controls. These reductions in fluorescence indicate damage to the PSII, limiting light interception in cowpea's leaves, with a corresponding decrease in A. In addition, the results of this study showed that the adverse effects of waterlogging stress on stomatal factors were greater than those of non-stomatal factors. Therefore, changes in A are shown to be more strongly dependent on the reduction of g_s than on PSII photoinhibition. Overall, the findings indicated that waterlogging stress had the greatest impact on cowpea leaf physiology at the R2 stage, followed by the V4 and R7 stages. Consistent with the results of previous studies (Ahmed et al., 2002; Shao et al., 2013), these modifications in the leaf physiology consequently resulted in seed yield losses.

5.4.3 Effects of waterlogging on biochemical properties of cowpea genotypes at different growth stages

The lack of oxygen in waterlogged roots causes plants to switch from aerobic to anaerobic respiration as a plant response mechanism when stressed by waterlogging (Gibbs and Greenway, 2003). Under these conditions, plants suffer from oxidative damage mainly caused by ROS accumulation (Yang et al., 2011). Previous studies have attributed the susceptibility of cowpea and related crops to waterlogging stress to excess production of ROS, resulting in oxidative damage to photosynthetic cells (Borella et al., 2019; Posso et al., 2018; Sairam et al., 2009). In the current study, ten days of waterlogging damaged the photosynthetic apparatus, as evidenced by decreased A and g_s, low ETR, and reduced PSII states. In response to oxidative damage caused by ROS formation in waterlogged conditions, plants possess a suite of scavenging enzymatic or non-enzymatic antioxidants, such as phenolics, flavonoids, and carotenoids (Kang et al., 2021; Park and Lee, 2019). Flavonoids and phenolic compounds act as ROS scavengers by targeting and neutralizing free radicals before they damage the photosynthetic cells (Gill and Tuteja, 2010). In this study, UCR 369 and EpicSelect.4 showed no difference in phenolic content compared to individual controls under waterlogging conditions at different growth stages (Figure 5.6). This suggests that the two cowpea genotypes are not well equipped with an effective antioxidant system to protect them from oxidative damage caused by waterlogging. These results are consistent with previous studies on waterlogging stress responses in soybeans (Kang et al., 2021) and cowpeas (El-Enany et al., 2013).

Interestingly, reduced flavonoid contents in waterlogging-sensitive cowpea and soybean leaves have been reported previously (Jayawardhane et al., 2022; Kang et al., 2021), highlighting the importance of flavonoid biosynthesis during the R2 stage, which is the most critical stage of

the waterlogging effect. Furthermore, at the R2 stage, the flavonoid content in UCR 369 was higher than that in EpicSelect.4. Therefore, the increased content of UCR 369 flavonoids may be more inclined to detoxify ROS molecules formed during oxidative stress at the R2 stage. Conversely, decreased levels of phenolics and flavonoids in EpicSelect.4 and at the R7 stage indicated that the sensitive genotypes made less effort to counteract the adverse effects of hypoxia.

Another mechanism of plant response to waterlogging is through the glycolytic pathway rather than oxidative respiration to generate metabolic energy (Yang et al., 2011; Yin et al., 2009). Notably, glycolysis requires reducing sugars, such as glucose and fructose, as critical substrates (Gibbs and Greenway, 2003). Generally, declining sugar responses vary by genotype, growth stage, and duration of waterlogging (Sairam et al., 2009; Yang et al., 2011). In this study, waterlogging caused no difference in glucose levels at R2 in EpicSelect.4 but caused a decrease in glucose levels at the R7 stage. On the other hand, significant increases in glucose levels in response to waterlogging were found in UCR 369 at all growth stages (Figure 5.7 C-D). These findings further confirm that UCR 369 is more likely to be more tolerant to waterlogging than EpicSelect.4 because it has a greater supply of metabolic energy. Consistent with the current results, Sairam et al. (2009) revealed a significant decline in sensitive mungbean and an increase in tolerant genotypes. Corresponding results were demonstrated in pigeon peas (Kumutha et al., 2008).

There is substantial evidence that soluble sugars protect plants from oxidative damage caused by waterlogging by stabilizing membranes (Bertrand et al., 2003; Kumutha et al., 2008). In UCR 369 and EpicSelect.4, sucrose and fructose were the main soluble sugars whose concentrations increased during waterlogging, respectively (Figure 5.7). After ten days of

waterlogging, the sucrose concentration of UCR 369 increased at the R2 and R7 stages, while the sucrose concentration of EpicSelect.4 decreased under the same conditions (Figure 5.7). This result confirms the conclusions of previous studies (Bertrand et al., 2003; Kuai et al., 2014), which reported that energy and carbon are critical determinants of plant survival under waterlogging, especially at the R2 stage. Moreover, the accumulation of sucrose found in UCR 369 at the R2 stages is consistent with previous findings (Kuai et al., 2014; Zeng et al., 2021). Therefore, suggesting that the greater tolerance of UCR 369 may be related to slower glycolytic machinery, a strategy that allows the tolerant genotype to maintain oxidizable substrates for rapid recovery after waterlogging stress (Bertrand et al., 2003). In contrast, since EpicSelect.4 favors glycolysis under waterlogging stress at the R2 and R7 stages, it is not surprising to observe an upregulation of fructose concentration, as this process requires large amounts of fructose-6-P. Overall, these results suggest that sucrose is used as a direct substrate for the production of the energy needed for various reproductive processes and that sucrose metabolism is enhanced under waterlogging conditions at the R2 stage of cowpea. In addition, the responses of soluble and reducing sugars at the R2 stage suggest a cytoprotective mechanism that mitigates damage under waterlogged conditions, but enzymatic activity drops dramatically at the R7 stage.

5.4.4 Effect of waterlogging on seed yield, yield-related attributes, and seed quality of cowpea genotypes at different growth stages

In different growth stages, the effect of waterlogging on the seed yield of the two cowpea genotypes showed a downward trend, and the seed weight per plant decreased the most in the R2 stage, followed by the V4 and R7 stages (Table 5.1). Previous studies have shown that waterlogging can lead to poor pod setting, high flower abortion rate, and lower seed formation rate, thereby increasing the yield loss of cowpea seeds (Hong et al., 1977; Minchin et al., 1978;

Timsina et al., 1994), which is similar to the current study. Minchin et al. (1978) found that waterlogging during the reproductive stage significantly impacted cowpea yield and its components. The yield reduction was mainly affected by the number of pods per plant. Similar results were reported in the reproductive stage of mungbean relative to the vegetative stage (Ahmed et al., 2002). In the present study, the number of pods per plant decreased most during the R2 stage of the cowpea genotype, with a significant decrease in EpicSelect.4. The higher susceptibility to waterlogging in the R2 stage can be ascribed to a restricted supply of carbohydrates from the source to the sink, resulting in lower biomass and flower abortion (Minchin et al., 1978; Umaharan et al., 1997), which leads to a decrease in the number of seeds per plant. The current study showed that limited dry matter accumulation due to leaf senescence and impaired A in R2 under waterlogging conditions resulted in a significant decrease in pod dry weight per plant for both cowpea genotypes. EpicSelect.4 (81%) showed a more significant reduction than UCR 369 (73%), although this indicated the sensitivity of both genotypes to waterlogging. Given that pod-related parameters decreased the most under waterlogging, it is suggested that the development of fewer pods in cowpea genotypes could be a tolerance mechanism used by cowpea to adapt to waterlogging and maintain yield.

Waterlogging impairs cowpea seed yield and its composition and alters grain quality in a genotype-dependent manner. Previous analyses have determined that waterlogging reduces nitrogen accumulation in cowpea seeds, resulting in a significant decrease in seed protein content relative to the environment (Ravelombola et al., 2016; Weng et al., 2019). In this experiment, ten days of waterlogging at different growth stages did not cause any change in the seed protein content of UCR 369. However, waterlogging at the R2 stage resulted in lower seed protein content in EpicSelect.4, suggesting that waterlogging of the sensitive cowpea genotypes at the

reproductive stage is detrimental to seed protein content. In addition, the current study shows that the UCR 369 (tan) has a seed protein content of between 19% and 21%, while the EpicSelect.4 (pinkeye) is between 25% and 28%. This result supports the results of Weng et al. (2019), who reported that cowpea seed protein content ranged from 23% to 31% in pinkeye and from 19% to 20% in tan-colored genotypes. Moreover, waterlogging decreased the protein of cowpea seeds, increased the starch content, and reduced the sucrose and phenolic contents in the waterlogged cowpea seeds at the R2 stage. Previous studies attributed starch accumulation in seeds to increased ABA concentrations under waterlogging conditions, which increased the activity of key enzymes that convert soluble sugars to starch (Araki et al., 2012; Huang et al., 2022; Ren et al., 2018).

5.5   Conclusion

Based on experiments in 2021 and 2022, the results of this study show that waterlogging imposed at different growth stages of cowpea reduces parameters related to plant growth, physiology, biochemistry, and seed yield. Under waterlogging, there were significant differences in plant growth traits, physiological parameters, seed yield, and quality between the two cowpea genotypes. EpicSelect.4 was found to have a more substantial decline in yield and biomass accumulation due to leaf senescence, chlorophyll degradation, and damaged photosynthetic processes than UCR 369, most likely due to its elongated stem in the form of vines and genetics. Cowpea genotypes were most sensitive to waterlogging in the R2 stage, followed by the V4 and R7 stages. The R2 stage is particularly vulnerable to waterlogging due to the high energy demands of the cowpea reproductive process. Therefore, it becomes critical for producers to avoid waterlogging at the reproductive stage of the cowpea planting management process to sustain yield.

CHAPTER VI

GENERAL SUMMARY AND CONCLUSION

Waterlogging is critical abiotic stress intrinsically linked to global climate change, causing severe yield declines in many crops yearly. Understanding the direct and indirect effects of waterlogging on crops can play a pivotal role in achieving sustainable crop production. For instance, exploring waterlogging tolerance mechanisms to understand how plants respond to waterlogging would undoubtedly aid in improving crops. Improving and intensifying crop production becomes even more crucial as the frequency of extreme precipitation events increases and food demand rises due to population growth. The main objective of this study was to explore physiological and biochemical mechanisms to understand how cowpea genotypes respond to waterlogging stress. This research addresses cowpea, an agronomically and economically important legume, using greenhouse and field environmental conditions to waterlogging conditions. Four studies were conducted at various growth stages.

In the first study presented in Chapter **Error! Reference source not found.**, 30 cowpea genotypes from 21 countries were screened for waterlogging tolerance in their early developmental stages under controlled conditions. The dynamic changes of 24 morpho-physiological parameters under ten days of waterlogging and optimal water conditions were analyzed to understand cowpea's response to waterlogging. The results of this study revealed that waterlogging treatment and cowpea genotype interacted to affect 96% of the measured parameters, indicating that most traits were quantitatively inherited and differentially expressed

under waterlogging conditions. In addition, the waterlogging tolerance coefficient (WTC) and multivariate analysis were used to characterize the waterlogging tolerance of cowpea genotypes. Accordingly, 10% of the genotypes exhibited high tolerance to waterlogging stress. The genotypes UCR 369 and EpicSelect.4 were identified to be the most and least tolerant to waterlogging, respectively. The tolerant cowpea genotypes were observed to adapt to waterlogging by increasing adventitious root formation, whereas the photosynthetic parameters of the sensitive cowpea genotypes were adversely affected. The total WTC was highly positively correlated with the WTC of photosynthesis and chlorophyll fluorescence parameters, further indicating that screening cowpea genotypes based on gas exchange parameters can provide reliable analysis and classification of waterlogging tolerance. Moreover, the principal component analysis identified adventitious roots, stomatal conductance, carbon assimilation rate, electron transport rate, and effective quantum yield of PSII photochemistry as parameters that best describe the waterlogging tolerance of cowpea genotypes. The identified morpho-physiological traits determined from this study may be helpful for genetic engineering and breeding programs that integrate cowpea waterlogging tolerance.

Study 2 evaluated the key physiological and biochemical parameters influencing carbon fixation of UCR 369 and EpicSelect.4 during waterlogging and recovery periods. Compared with the control treatment, the carbon assimilation rates of both cowpea genotypes were adversely affected after seven days of waterlogging and could not be recovered in an additional seven days of reoxygenation, with a more significant decline in EpicSelect.4. There was a highly specific downregulation of stomatal and mesophyll conductance, maximum rate of Rubisco, electron transport rate, and quantum yield of PSII photochemistry as stomatal and non-stomatal factors limiting photosynthesis in EpicSelect.4, resulting in decreased shoot biomass. On the other hand,

the downregulation of photosynthesis in UCR 369 was mainly due to reduced stomatal and mesophyll conductance, while non-stomatal limiting factors were maintained under short-term waterlogging. In addition, seven days of waterlogging caused a significant loss in both genotypes' chlorophyll and carotenoid content. However, only waterlogged UCR 369 was not photo-inhibited and could restore the levels of chlorophyll and carotenoids after seven days of recovery. Waterlogging also induced intense stress in UCR 369 with increased zeaxanthin, sucrose, and flavonoid content. At the same time, these metabolites were decreased in EpicSelect.4. Conversely, glucose, fructose, and phenolic content were increased in EpicSelect.4 but fell in UCR 369 at seven days of recovery. The findings indicated that the tolerant UCR 369 genotype maintained higher photosynthesis under waterlogging stress, which was attributed to higher photochemical efficiency, Rubisco activity, chlorophyll content, and less stomatal restriction. After recovery, the incomplete restoration of photosynthesis can be attributed to the reduced stomatal conductance caused by severe waterlogging in both genotypes. Thus, promoting the rapid recovery of stomata from waterlogging stress may be crucial for the complete restoration of carbon fixation in cowpeas during the reproductive stage.

Finally, a two-year field experiment was conducted in **Error! Reference source not found.** to quantify the impacts of waterlogging on growth traits, physiological characteristics, seed yield and its components, and seed quality of cowpea genotypes at different growth stages. Cowpea genotypes UCR 369 and EpicSelect.4 were treated with waterlogging and non-waterlogging for ten days under field conditions in the 2021 and 2022 growing seasons, respectively. The results showed that waterlogging in the R2 stage significantly affected cowpea growth, physiology, seed yield, and quality, followed by V4 and R7 stages. Leaf area, relative water content, chlorophyll content, stomatal conductance, and electron transport rate decreased

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under waterlogging, resulting in a reduction in photosynthetic rate and total biomass, which ultimately led to a decline in seed yield and its attributes. The reduction in net photosynthesis under waterlogging was mainly due to the rapid closure of stomata, while the decrease in pod dry weight and number of pods per plant were the main drivers of seed yield loss. The sensitivity of the R2 stage to waterlogging was attributed to the high energy demands of various reproductive processes in cowpeas, which could not be met due to the energy deficit caused by hypoxia. Compared with the control, phenolics, flavonoids, and sucrose content in the leaves increased at the R2 but decreased at the R7 stage. As a result, seed weight, pod dry weight, number of seeds per pod, and number of pods per plant fell, and seed quality also suffered. Seed protein, phenolics, and sucrose decreased, but starch content in cowpea seed increased.

Moreover, the waterlogging effect was more significant in EpicSelect.4, which also experienced almost 100% mortality in the R2 stage compared to UCR 369. Overall, the findings suggest that R2 is the most sensitive to waterlogging in cowpea. Hence, maintaining growth and physiological performance after flowering may be a reasonable strategy to increase cowpea seed yield in flood-prone environments.

Furthermore, future studies can focus on more synergistic studies due to the current study's limitations. For instance, the evaluations of cowpea genotypes at different growth stages under waterlogging can be further investigated using a combination of phenomic, genomic, proteomic, and metabolomic approaches.

REFERENCES

Agbicodo, E.M., Fatokun, C.A., Muranaka, S., Visser, R.G.F., Linden van der, C.G., 2009. Breeding drought tolerant cowpea: constraints, accomplishments, and future prospects. Euphytica 167, 353–370. https://doi.org/10.1007/s10681-009-9893-8

Ahmed, F., Rafii, M.Y., Ismail, M.R., Juraimi, A.S., Rahim, H.A., Asfaliza, R., Latif, M.A., 2012. Waterlogging tolerance of crops: breeding, mechanism of tolerance, molecular approaches, and future prospects. BioMed Research International 2013, 963525. https://doi.org/10.1155/2013/963525

Ahmed, S., Nawata, E., Sakuratani, T., 2002. Effects of waterlogging at vegetative and reproductive growth stages on photosynthesis, leaf water potential, and yield in mungbean. Plant Production Science 5, 117–123. https://doi.org/10.1626/pps.5.117

Akula, R., Ravishankar, G.A., 2011. Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal Behavior 6, 1720–1731. https://doi.org/10.4161/psb.6.11.17613

Al Jaouni, S., Saleh, A.M., Wadaan, M.A.M., Hozzein, W.N., Selim, S., AbdElgawad, H., 2018. Elevated CO_2 induces a global metabolic change in basil (*Ocimum basilicum* L.) and peppermint (*Mentha piperita* L.) and improves their biological activity. Journal of Plant Physiology 224–225, 121–131. https://doi.org/10.1016/j.jplph.2018.03.016

Aldana, F., García, P.N., Fischer, G., 2014. Effect of waterlogging stress on the growth, development, and symptomatology of cape gooseberry (*Physalis peruviana* L.) plants. Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales 38, 393–400. https://doi.org/10.18257/raccefyn.114

Allen, D.J., 1983. The pathology of tropical food legumes: disease resistance in crop improvement. John Wiley and Sons, New York. 150-187.

Andrade, C.A., Souza, K.R.D. de, Santos, M. de O., Silva, D.M. da, Alves, J.D., 2018. Hydrogen peroxide promotes the tolerance of soybeans to waterlogging. Scientia Horticulturae 232, 40–45. https://doi.org/10.1016/j.scienta.2017.12.048

Anjum, S.A., Ashraf, U., Tanveer, M., Khan, I., Hussain, S., Shahzad, B., Zohaib, A., Abbas, F., Saleem, M.F., Ali, I., Wang, L.C., 2017. Drought-induced changes in growth, osmolyte accumulation, and antioxidant metabolism of three maize hybrids. Frontier in Plant Science 8, 69–69. https://doi.org/10.3389/fpls.2017.00069

Araki, H., Hamada, A., Hossain, M.A., Takahashi, T., 2012. Waterlogging at jointing and/or after anthesis in wheat induces early leaf senescence and impairs grain filling. Field Crops Research 137, 27–36. https://doi.org/10.1016/j.fcr.2012.09.006

Arduini, I., Baldanzi, M., Pampana, S., 2019. Reduced Growth and Nitrogen Uptake During Waterlogging at Tillering Permanently Affect Yield Components in Late Sown Oats. Frontiers in Plant Science 10, 1087–1087. https://doi.org/10.3389/fpls.2019.01087

Arias, P., Bellouin, N., Coppola, E., Jones, R., Krinner, G., Marotzke, J., Naik, V., Palmer, M., Plattner, G.-K., Rogelj, J., 2021. Climate Change 2021: The Physical Science Basis. Contribution of Working Group14 I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change; Technical Summary.

Armengaud, P., Zambaux, K., Hills, A., Sulpice, R., Pattison, R.J., Blatt, M.R., Amtmann, A., 2009. EZ-Rhizo: integrated software for the fast and accurate measurement of root system architecture. The Plant Journal 57, 945–956. https://doi.org/10.1111/j.1365-313X.2008.03739.x

Ashraf, M., Mehmood, S., 1990. Response of four Brassica species to drought stress. Environmental and Experimental Botany 30, 93–100. https://doi.org/10.1016/0098-8472(90)90013-T

Ashraf, M.A., 2012. Waterlogging stress in plants: A review. African Journal of Agricultural Research 7, 1976–1981. https://doi.org/10.5897/AJAR2016.12050

Asseng, S., Cao, W., Zhang, W., Ludwig, F., 2009. Crop physiology, modelling and climate change: impact and adaptation strategies, in Crop Physiology: Applications for Genetic Improvement and Agronomy, eds V. O. Sadras and D. F. Calderini (London: Elsevier), 511–543. https://doi.org/0.1016/b978-0-12-374431-9.00020-73

Bacanamwo, M., Purcell, L.C., 1999. Soybean dry matter and N accumulation responses to flooding stress, N sources, and hypoxia. Journal of Experimental Botany 50, 689–696. https://doi.org/10.1093/jxb/50.334.689

Bailey-Serres, J., Fukao, T., Gibbs, D.J., Holdsworth, M.J., Lee, S.C., Licausi, F., Perata, P., Voesenek, L.A.C.J., van Dongen, J.T., 2012a. Making sense of low oxygen sensing. Trends in Plant Science 17, 129–138. https://doi.org/10.1016/j.tplants.2011.12.004

Bailey-Serres, J., Lee, S.C., Brinton, E., 2012b. Waterproofing Crops: Effective Flooding Survival Strategies. Plant Physiology 160, 1698. https://doi.org/10.1104/pp.112.208173

Bailey-Serres, J., Voesenek, L.A.C.J., 2008. Flooding Stress: Acclimations and Genetic Diversity. Annu. Review of Plant Biology 59, 313–339. https://doi.org/10.1146/annurev.arplant.59.032607.092752

Bansal, R., Srivastava, J.P., 2015. Effect of waterlogging on photosynthetic and biochemical parameters in pigeonpea. Russian Journal of Plant Physiology 62, 322–327. https://doi.org/10.1134/S1021443715030036

Barickman, T.C., Simpson, C.R., Sams, C.E., 2019. Waterlogging causes early modification in the physiological performance, carotenoids, chlorophylls, proline, and soluble sugars of cucumber plants. Plants 8, 160. https://doi.org/10.3390/plants8060160

Barickman, T.C., Sublett, W.L., Miles, C., Crow, D., Scheenstra, E., 2018. Lettuce biomass accumulation and phytonutrient concentrations are influenced by genotype, N application rate and location. Horticulturae 4. https://doi.org/10.3390/horticulturae4030012

Barik, J., Panda, D., Mohanty, S.K., Lenka, S.K., 2019. Leaf photosynthesis and antioxidant response in selected traditional rice landraces of Jeypore tract of Odisha, India to submergence. Physiology and Molecular Biology of Plants 25, 847–863. https://doi.org/10.1007/s12298-019-00671-7

Barrs, H., Weatherley, P., 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Australian Journal of Biological Sciences 15, 413–428. https://doi.org/10.1071/BI9620413

Bernacchi, C.J., Singsaas, E.L., Pimentel, C., Portis Jr, A.R., Long, S.P., 2001. Improved temperature response functions for models of Rubisco-limited photosynthesis. Plant, Cell & Environment 24, 253–259. https://doi.org/10.1111/j.1365-3040.2001.00668.x

Bertholdsson, N.-O., 2013. Screening for Barley Waterlogging tolerance in nordic barley cultivars (*Hordeum vulgare* L.) using chlorophyll fluorescence on hydroponically-grown plants. Agronomy 3, 376–390. https://doi.org/10.3390/agronomy3020376

Bertrand, A., Castonguay, Y., Nadeau, P., Laberge, S., Michaud, R., Bélanger, G., Rochette, P., 2003. Oxygen deficiency affects carbohydrate reserves in overwintering forage crops. Journal of Experimental Botany 54, 1721–1730. https://doi.org/10.1093/jxb/erg182

Bheemanahalli, R., Poudel, S., Alsajri, F.A., Reddy, K.R., 2022. Phenotyping of Southern United States Soybean Cultivars for Potential Seed Weight and Seed Quality Compositions. Agronomy 12. https://doi.org/10.3390/agronomy12040839

Black, K., Davis, P., Mc Grath, J., Doherty, P., Osborne, B., 2005. Interactive effects of irradiance and water availability on the photosynthetic performance of Picea sitchensis seedlings: implications for seedling establishment under different management practices. Annals of Forest Science 62, 413–422. https://doi.org/10.1051/forest:2005037

Borella, J., Becker, R., Lima, M.C., Oliveira, D. dos S.C. de, Braga, E.J.B., Oliveira, A.C.B. de, Amarante, L. do, 2019. Nitrogen source influences the antioxidative system of soybean plants under hypoxia and re-oxygenation. *Scientia Agricola* (Piracicaba, Braz.) 76, 51–62. https://doi.org/10.1590/1678-992X-2017-0195

Borella, J., Oliveira, H.C., de Oliveira, D. dos S.C., Braga, E.J.B., de Oliveira, A.C.B., Sodek, L., do Amarante, L., 2017. Hypoxia-driven changes in glycolytic and tricarboxylic acid cycle metabolites of two nodulated soybean genotypes. Environmental and Experimental Botany 133, 118–127. https://doi.org/10.1016/j.envexpbot.2016.10.007

Boru, G., van Ginkel, M., Trethowan, R.M., Boersma, L., Kronstad, W.E., 2003. Oxygen use from solution by wheat genotypes differing in tolerance to waterlogging. Euphytica 132, 151–158. https://doi.org/10.1023/A:1024622405505

Boukar, O., Fatokun, C.A., Huynh, B.-L., Roberts, P.A., Close, T.J., 2016. Genomic Tools in Cowpea Breeding Programs: Status and Perspectives. Frontiers in Plant Science 7. https://doi.org/10.3389/fpls.2016.00757

Boukar, O., Fatokun, C.A., Roberts, P.A., Abberton, M., Huynh, B.L., Close, T.J., Kyei-Boahen, S., Higgins, T.J.V., Ehlers, J.D., 2015. Cowpea, in De Ron, A.M. (Ed.), Grain Legumes. Springer New York, New York, NY, pp. 219–250. https://doi.org/10.1007/978-1-4939-2797-5_7

Boukar, O., Massawe, F., Muranaka, S., Franco, J., Maziya-Dixon, B., Singh, B., Fatokun, C., 2011. Evaluation of cowpea germplasm lines for protein and mineral concentrations in grains. Plant Genetic Resources 9, 515–522. https://doi.org/10.1017/S1479262111000815

Boyer, J.S., 1982. Plant Productivity and Environment. Science 218, 443. https://doi.org/10.1126/science.218.4571.443

Brazel, S.R., Barickman, T.C., Sams, C.E., 2021. Short-term waterlogging of kale (*Brassica oleracea* L. var. acephala) plants causes a decrease in carotenoids and chlorophylls while increasing nutritionally important glucosinolates, in Acta Horticulturae. International Society for Horticultural Science (ISHS), Leuven, Belgium, pp. 175–180. https://doi.org/10.17660/ActaHortic.2021.1329.21

Cannell, R.Q., Belford, R.K., Blackwell, P.S., Govi, G., Thomson, R.J., 1985. Effects of waterlogging on soil aeration and on root and shoot growth and yield of winter oats (*Avena sativa* L.). Plant and Soil 85, 361–373. https://doi.org/10.1007/BF02220191

Carsky, R.J., Singh, B.B., Oyewole, B., 2001. Contribution of early season cowpea to late season maize in the savanna zone of West Africa. Biological agriculture & horticulture 18, 303–315. https://doi.org/10.1080/01448765.2001.9754894

Carsky, R.J., Vanlauwe, B., Lyasse, O., 2002. Cowpea rotation as a resource management technology for savannah cereal-based systems in the savannas of West Africa. In: Fatokun CA, Tarawali SA, Singh BB, Kormawa PM, Tamò M, editors. Challenges and Opportunities for Enhancing Sustainable Cowpea Production. Proceedings of the 3rd World Cowpea Conference. International Institute of Tropical Agriculture, Ibadan, Nigeria, pp. 252–266.

Carvalho, M., Matos, M., Castro, I., Monteiro, E., Rosa, E., Lino-Neto, T., Carnide, V., 2019. Screening of worldwide cowpea collection to drought tolerant at a germination stage. Scientia Horticulturae 247, 107–115. https://doi.org/10.1016/j.scienta.2018.11.082

Caudle, K.L., Maricle, B.R., 2012. Effects of flooding on photosynthesis, chlorophyll fluorescence, and oxygen stress in plants of varying flooding tolerance. Transactions of the Kansas Academy of Science (1903-) 115, 5–18. https://doi.org/10.1660/062.115.0102

Chamarthi, S.K., Belko, N., Togola, A., Fatokun, C.A., Boukar, O., 2019. Genomics-assisted breeding for drought tolerance in cowpea. In: Rajpal, V., Sehgal, D., Kumar, A., Raina, S. (eds) Genomics assisted breeding of crops for abiotic stress tolerance, Vol. II. Sustainable Development and Biodiversity, vol 21. Springer, Cham. https://doi.org/10.1007/978-3-319-99573-1_10.

Colmer, T.D., Winkel, A., Pedersen, O., 2011. A perspective on underwater photosynthesis in submerged terrestrial wetland plants. AoB PLANTS 2011. https://doi.org/10.1093/aobpla/plr030

Colom, M.R., Vazzana, C., 2003. Photosynthesis and PSII functionality of drought-resistant and drought-sensitive weeping lovegrass plants. Environmental and Experimental Botany 49, 135–144. https://doi.org/10.1016/S0098-8472(02)00065-5

Cornelious, B.K., 2004. Phenotypic evaluation and molecular basis for waterlogging tolerance in southern soybean populations. Ph.D. Dissertation, University of Arkansas, Fayetteville, Arkansas, USA.

Costa, B.G.P., Amâncio, B.C.S., Boas, L.V.V., Bastos, L. da S., Domiciano, D., Marchiori, P.E.R., 2020. Waterlogging effects upon the phenological phases of common bean cultivar BRSMG-Uai. Ciênc. agrotec. 44. https://doi.org/10.1590/1413-7054202044009520

Cox, M.C.H., Millenaar, F.F., van Berkel, Y.E.M. de J., Peeters, A.J.M., Voesenek, L.A.C.J., 2003. Plant movement. submergence-induced petiole elongation in *Rumex palustris* depends on hyponastic growth. Plant Physiology 132, 282–291. https://doi.org/10.1104/pp.102.014548

Cramer, G.R., Urano, K., Delrot, S., Pezzotti, M., Shinozaki, K., 2011. Effects of abiotic stress on plants: a systems biology perspective. BMC Plant Biology 11, 163. https://doi.org/10.1186/1471-2229-11-163

Cruz de Carvalho, M.H., 2008. Drought stress and reactive oxygen species: production, scavenging, and signaling. Plant Signaling & Behavior 3, 156–165. https://doi.org/10.4161/psb.3.3.5536

Da Silva, A.C., Da Costa Santos, D., Junior, D.L.T., Da Silva, P.B., Dos Santos, R.C., Siviero, A., 2018. Cowpea: a strategic legume species for food security and health, in legume seed nutraceutical research. , eds. J. C. Jimenez-Lopez and A. Clemente (London: IntechOpen). https://doi.org/10.5772/intechopen.79006

Dadson, R.B., Hashem, F.M., Javaid, I., Joshi, J., Allen, A.L., Devine, T.E., 2005. Effect of water stress on the yield of cowpea (*Vigna unguiculata* L. Walp.) genotypes in the Delmarva region of the united states. Journal of Agronomy and Crop Science 191, 210–217. https://doi.org/10.1111/j.1439-037X.2005.00155.x

Da-Silva, C.J., do Amarante, L., 2020. Time-course biochemical analyses of soybean plants during waterlogging and reoxygenation. Environmental and Experimental Botany 180, 104242. https://doi.org/10.1016/j.envexpbot.2020.104242

Davies, B.H., Köst, H.P., 1988. Chromatographic methods for the separation of carotenoids. In CRC Handbook of Chromatography, Plant Pigments, Vol. 1: Fat Soluble Pigments, H.P. Kö St, G. Zweig, and J. Sherma, Eds. CRC Press (Taylor and Francis Group), Boca Raton, FL, USA, pp. 1–185.

Dawson, T.P., Perryman, A.H., Osborne, T.M., 2016. Modelling impacts of climate change on global food security. Climatic Change 134, 429–440. https://doi.org/10.1007/s10584-014-1277-y

Demmig-Adams, B., Adams, W.W., 2018. An integrative approach to photoinhibition and photoprotection of photosynthesis. Environmental and Experimental Botany 154, 1–3. https://doi.org/10.1016/j.envexpbot.2018.05.006

Dennis, E.S., Dolferus, R., Ellis, M., Rahman, M., Wu, Y., Hoeren, F.U., Grover, A., Ismond, K.P., Good, A.G., Peacock, W.J., 2000. Molecular strategies for improving waterlogging tolerance in plants. Journal of Experimental Botany 51, 89–97. https://doi.org/10.1093/jexbot/51.342.89

Dhungana, S.K., Kim, H.-S., Kang, B.-K., Seo, J.-H., Kim, H.-T., Shin, S.-O., Park, C.-H., Kwak, D.-Y., 2019. Evaluation of flooding tolerance of soybean (*Glycine max* L. Merr.) in greenhouse under upland and paddy soil conditions. Journal of Crop Science and Biotechnology. 22, 283–290. https://doi.org/10.1007/s12892-019-0106-0

Dong, J., Gruda, N., Li, X., Tang, Y., Zhang, P., Duan, Z., 2020. Sustainable vegetable production under changing climate: The impact of elevated CO2 on yield of vegetables and the interactions with environments- A review. Journal of Cleaner Production 253, 119920. https://doi.org/10.1016/j.jclepro.2019.119920

Duffin, E., 2020. Economic damage caused by floods in the U.S. 2019 [WWW Document]. Statista. URL https://www.statista.com/statistics/237420/economic-damage-caused-by-floods-and-flash-floods-in-the-us/ (accessed 10.7.21).

Duke, J., 2012. Handbook of legumes of world economic importance. Springer Science & Business Media. New York: Plenum Press. pp. 52–57.

Earl, H.J., Ennahli, S., 2004. Estimating photosynthetic electron transport via chlorophyll fluorometry without Photosystem II light saturation. Photosynthesis Research 82, 177–186. https://doi.org/10.1007/s11120-004-1454-3

Easterling, D.R., Kunkel, K.E., Arnold, J.R., Knutson, T., LeGrande, A.N., Leung, L.R., Vose, R.S., Waliser, D.E., Wehner, M.F., 2017. Precipitation change in the United States, in Climate Science Special Report: Fourth National Climate Assessment, Volume I [Wuebbles, D.J., D.W. Fahey, K.A. Hibbard, D.J. Dokken, B.C. Stewart, and T.K. Maycock (Eds.)]. U.S. Global Change Research Program, Washington, DC, USA, pp. 207–230.

Ehlers, J.D., Hall, A.E., 1996. Genotypic classification of cowpea based on responses to heat and photoperiod. Crop Science 36, 673–679. https://doi.org/10.2135/cropsci1996.0011183X003600030026x

El-Enany, A. wahab, Alanazi, A., Dief, N., Al-Taisan, W., 2013. Role of antioxidant enzymes in amelioration of water deficit and waterlogging stresses on *Vigna sinensis* plants. Journal of Biology and Earth Sciences 3, B1 44-B1 53. Available online at: www.journals.tmkarpinski.com/index.php/jbes/article/download/59/54

Elkelish, A.A., Alhaithloul, H.A.S., Qari, S.H., Soliman, M.H., Hasanuzzaman, M., 2020. Pretreatment with *Trichoderma harzianum* alleviates waterlogging-induced growth alterations in tomato seedlings by modulating physiological, biochemical, and molecular mechanisms. Environmental and Experimental Botany 171, 103946. https://doi.org/10.1016/j.envexpbot.2019.103946

Emenhiser, C., Simunovic, N., Sander, L.C., Schwartz, S.J., 1996. Separation of Geometrical Carotenoid Isomers in Biological Extracts Using a Polymeric C30 Column in Reversed-Phase Liquid Chromatography. Journal of Agricultural and Food Chemistry. 44, 3887–3893. https://doi.org/10.1021/jf960104m

Esteban, R., Barrutia, O., Artetxe, U., Fernández-Marín, B., Hernández, A., García-Plazaola, J.I., 2015. Internal and external factors affecting photosynthetic pigment composition in plants: a meta-analytical approach. New Phytologist 206, 268–280. https://doi.org/10.1111/nph.13186

Eysholdt-Derzsó, E., Sauter, M., 2019. Hypoxia and the group VII ethylene response transcription factor HRE2 promote adventitious root elongation in Arabidopsis. Plant Biology 21, 103–108. https://doi.org/10.1111/plb.12873

Fang, J., Chao, C.-C.T., Roberts, P.A., Ehlers, J.D., 2007. Genetic diversity of cowpea [Vigna unguiculata (L.) Walp.] in four West African and USA breeding programs as determined by AFLP analysis. Genetic Resources and Crop Evolution 54, 1197–1209. https://doi.org/10.1007/s10722-006-9101-9

FAO, 2022. FAOSTAT database [WWW Document]. FAOSTAT. URL https://www.fao.org/faostat/en/#data/QCL (accessed 5.31.22).

FAO, 2018. The impact of disasters and crises on agriculture and food security 2017. Food and Agriculture Organization of the United Nations, Rome.

FAO, 2011. Save and Grow: A Policymaker's Guide to the Sustainable Intensification of Smallholder Crop Production. Food and Agriculture Organization of the United Nations, Rome, Italy.

Farquhar, G.D., von Caemmerer, S., Berry, J.A., 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. Planta 149, 78–90. https://doi.org/10.1007/BF00386231

Fatokun, C.A., Boukar, O., Kamara, A., Coulibaly, O., Alene, A., Boahen, S., Young, N.D., Myers, G.O., 2012. Enhancing cowpea productivity and production in drought-prone areas of Sub-Saharan Africa. in Four seasons of learning and engaging smallholder farmers: Progress of phase 1, ed T. Abate (Nairobi: International Crops Research Institute for the Semi-Arid Tropics), 81–112.

Felle, H.H., 2006. Apoplastic pH During Low-oxygen Stress in Barley. Annals of Botany 98, 1085–1093. https://doi.org/10.1093/aob/mcl193

Fery, R.L., 1990. The cowpea: production, utilization, and research in the United States. Horticultural Reviews 12, 197–222. https://doi.org/10.1002/9781118060858.ch4

Flexas, J., Escalona, J.M., Medrano, H., 1999. Water stress induces different levels of photosynthesis and electron transport rate regulation in grapevines. Plant, Cell & Environment 22, 39–48. https://doi.org/10.1046/j.1365-3040.1999.00371.x

Flight, C., Harlan, J.R., Dewet, J.M.J., Stemler, A.B.L., 1976. The Kintampo culture and its place in the economic prehistory of West Africa. In Harlan, J. R., DeWet, J. M. J., and Stemler, A. B. L. (eds.), Origins of African Plant Domestication, Mouton, The Hague, pp. 211–221.

Frank, H.A., Cogdell, R.J., 1996. Carotenoids in photosynthesis. Photochemistry and photobiology 63, 257–264. https://doi.org/10.1111/j.1751-1097.1996.tb03022.x

Fukao, T., Bailey-Serres, J., 2004. Plant responses to hypoxia – is survival a balancing act? Trends in Plant Science 9, 449–456. https://doi.org/10.1016/j.tplants.2004.07.005

Fukao, T., Barrera-Figueroa, B.E., Juntawong, P., Peña-Castro, J.M., 2019. Submergence and waterlogging stress in plants: A review highlighting research opportunities and understudied aspects. Frontiers in Plant Science 10. https://doi.org/10.3389/fpls.2019.00340

Garcia, N., da-Silva, C.J., Cocco, K.L.T., Pomagualli, D., de Oliveira, F.K., da Silva, J.V.L., de Oliveira, A.C.B., Amarante, L. do, 2020. Waterlogging tolerance of five soybean genotypes through different physiological and biochemical mechanisms. Environmental and Experimental Botany 172, 103975. https://doi.org/10.1016/j.envexpbot.2020.103975

Gedam, P.A., Thangasamy, A., Shirsat, D.V., Ghosh, S., Bhagat, K.P., Sogam, O.A., Gupta, A.J., Mahajan, V., Soumia, P.S., Salunkhe, V.N., Khade, Y.P., Gawande, S.J., Hanjagi, P.S., Ramakrishnan, R.S., Singh, M., 2021. Screening of onion (*Allium cepa* L.) genotypes for drought tolerance using physiological and yield based indices through multivariate analysis. Frontiers in Plant Science 12. https://doi.org/10.3389/fpls.2021.600371

Genty, B., Briantais, J.-M., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta (BBA) - General Subjects 990, 87–92. https://doi.org/10.1016/S0304-4165(89)80016-9

Gibbs, J., Greenway, H., 2003. Review: Mechanisms of anoxia tolerance in plants. I. Growth, survival and anaerobic catabolism. Functional Plant Biology 30, 1–47. https://doi.org/10.1071/PP98095

Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant physiology and biochemistry 48, 909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Gómez, C., 2004. Cowpea: post-harvest operations. in PHO—Post-Harvest Compendium, D. Mejía, Ed., Food and Agriculture Organization of the United Nations, Rome, Italy.

Goufo, P., Moutinho-Pereira, J.M., Jorge, T.F., Correia, C.M., Oliveira, M.R., Rosa, E.A.S., António, C., Trindade, H., 2017. Cowpea (*Vigna unguiculata* L. Walp.) metabolomics: osmoprotection as a physiological strategy for drought stress resistance and improved yield. Frontiers in Plant Science 8. https://doi.org/10.3389/fpls.2017.00586

Hall, A.E., 2004. Breeding for adaptation to drought and heat in cowpea. European Journal of Agronomy 21, 447–454. https://doi.org/10.1016/j.eja.2004.07.005

Hall, A. E. (2011). Breeding cowpea for future climates. In crop adaptation to climate change, eds S. S. Yadav, R. Redden, J. L. Hatfield, H. Lotze-Campen, and A. J. W. Hall (Hoboken: John Wiley & Sons).

Hasanuzzaman, M., Al Mahmud, J., Nahar, K., Anee, T.I., Inafuku, M., Oku, H., Fujita, M., 2017. Responses, adaptation, and ROS metabolism in plants exposed to waterlogging stress, in Khan, M.I.R., Khan, N.A. (Eds.), reactive oxygen species and antioxidant systems in plants: role and regulation under abiotic stress. Springer, Singapore, pp. 257–281. https://doi.org/10.1007/978-981-10-5254-5_10

Henriet, J., Van Ek, G.A., Blade, S., Singh, B., 1997. Quantitative assessment of traditional cropping systems in the Sudan savanna of northern Nigeria: I. Rapid survey of prevalent cropping systems. Samaru Journal of Agricultural Research, 14, 37-45. https://hdl.handle.net/20.500.12478/6447

Henshaw, T.L., Gilbert, R.A., Scholberg, J.M.S., Sinclair, T.R., 2007. Soya bean (*Glycine max* L. Merr.) genotype response to early-season flooding: I. Root and nodule development. Journal of Agronomy and Crop Science 193, 177–188. https://doi.org/10.1111/j.1439-037X.2007.00257.x

Herzog, M., Striker, G.G., Colmer, T.D., Pedersen, O., 2016. Mechanisms of waterlogging tolerance in wheat – a review of root and shoot physiology. Plant, Cell & Environment 39, 1068–1086. https://doi.org/10.1111/pce.12676

Hirabayashi, Y., Mahendran, R., Koirala, S., Konoshima, L., Yamazaki, D., Watanabe, S., Kim, H., Kanae, S., 2013. Global flood risk under climate change. Nature Climate Change 3, 816–821. https://doi.org/10.1038/nclimate1911

Hirasawa, T., Tanaka, K., Miyamoto, D., Takei, M., Ishihara, K., 1994. Effects of pre-flowering soil moisture deficits on dry matter production and ecophysiological characteristics in soybean plants under drought conditions during grain filling. Japanese Journal of Crop Science 63, 721–730. https://doi.org/10.1626/jcs.63.721

Hong, T.D., Minchin, F.R., Summerfield, R.J., 1977. Recovery of nodulated cowpea plants (*Vigna Unguiculata* (L.) Walp.) from waterlogging during vegetative growth. Plant and Soil 48, 661–672. https://doi.org/10.1007/BF00145776

Huang, C., Zhang, W., Wang, H., Gao, Y., Ma, S., Qin, A., Liu, Zugui, Zhao, B., Ning, D., Zheng, H., Liu, Zhandong, 2022. Effects of waterlogging at different stages on growth and ear quality of waxy maize. Agricultural Water Management 266, 107603. https://doi.org/10.1016/j.agwat.2022.107603

Hund, A., Trachsel, S., Stamp, P., 2009. Growth of axile and lateral roots of maize: I development of a phenotyping platform. Plant and Soil 325, 335–349. https://doi.org/10.1007/s11104-009-9984-2

IPCC, 2022. Summary for Policymakers [H.-O. Pörtner, D.C. Roberts, E.S. Poloczanska, K. Mintenbeck, M. Tignor, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem (eds.)]. In: Climate Change 2022: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (eds.)]. Intergovernmental Panel on Climate Change (IPCC), Cambridge University Press.

IPCC, 2021. Summary for Policymakers. In: Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Intergovernmental Panel on Climate Change (IPCC), Cambridge, UK and New York, New York, USA.

IPCC, 2014. Climate change 2014: impacts, adaptation, and vulnerability. Part A: Global and sectoral aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. C.B. Field, V.R. Barros, D.J. Dokken, K.J. Mach, M.D. Mastrandrea, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken, P.R. Mastrandrea & L.L. White, eds. (No. 1107058163). Cambridge University Press. 1132 pp., Cambridge, UK, and New York, USA.

Irfan, M., Hayat, S., Hayat, Q., Afroz, S., Ahmad, A., 2010. Physiological and biochemical changes in plants under waterlogging. Protoplasma 241, 3–17. https://doi.org/10.1007/s00709-009-0098-8

Iseki, K., Ikazaki, K., Batieno, J.B., 2021. Cowpea yield variation in three dominant soil types in the Sudan Savanna of West Africa. Field Crops Research 261, 108012. https://doi.org/10.1016/j.fcr.2020.108012

Islam, M.R., Hamid, A., Karim, M.A., Haque, M.M., Khaliq, Q.A., Ahmed, J.U., 2008. Gas exchanges and yield responses of mungbean (*Vigna radiata* L. Wilczek) genotypes differing in flooding tolerance. Acta Physiologiae Plantarum 30, 697–707. https://doi.org/10.1007/s11738-008-0168-0

Islam, M.R., Rahman, M.M., Mohi-Ud-Din, M., Akter, M., Zaman, E., Keya, S.S., Hasan, M., Hasanuzzaman, M., 2022. Cytokinin and gibberellic acid-mediated waterlogging tolerance of mungbean (*Vigna radiata* L. Wilczek). PeerJ 10, e12862. https://doi.org/10.7717/peerj.12862

Iyer-Pascuzzi, A.S., Symonova, O., Mileyko, Y., Hao, Y., Belcher, H., Harer, J., Weitz, J.S., Benfey, P.N., 2010. Imaging and analysis platform for automatic phenotyping and trait ranking of plant root systems. Plant Physiology 152, 1148–1157. https://doi.org/10.1104/pp.109.150748

Jackson, M.B., 2008. Ethylene-promoted elongation: an adaptation to submergence stress. Annals of Botany 101, 229–248. https://doi.org/10.1093/aob/mcm237

Jayasundara, H.P.S., Thomson, B.D., Tang, C., 1997. Responses of cool season grain legumes to soil abiotic stresses, in Sparks, D.L. (Ed.), Advances in Agronomy. Academic Press, pp. 77–151. https://doi.org/10.1016/S0065-2113(08)60242-4

Jayathilake, C., Visvanathan, R., Deen, A., Bangamuwage, R., Jayawardana, B.C., Nammi, S., Liyanage, R., 2018. Cowpea: an overview on its nutritional facts and health benefits. Journal of the Science of Food and Agriculture 98, 4793–4806. https://doi.org/10.1002/jsfa.9074

Jayawardhane, J., Goyali, J.C., Zafari, S., Igamberdiev, A.U., 2022. The response of cowpea (*Vigna unguiculata*) plants to three abiotic stresses applied with increasing intensity: hypoxia, salinity, and water deficit. Metabolites 12. https://doi.org/10.3390/metabo12010038

Jiménez, J. de la C., Cardoso, J.A., Leiva, L.F., Gil, J., Forero, M.G., Worthington, M.L., Miles, J.W., Rao, I.M., 2017. Non-destructive phenotyping to identify brachiaria hybrids tolerant to waterlogging stress under field conditions. Frontiers in Plant Science 8. https://doi.org/10.3389/fpls.2017.00167

Kamara, A.Y., Omoigui, L.O., Kamai, N., Ewansiha, S.U., Ajeigbe, H.A., 2018. Improving cultivation of cowpea in West Africa. in Achieving Sustainable Cultivation of Grain Legumes. Burleigh Dodds Science Publishing Limited, 2, 235–252.

Kang, S.-M., Adhikari, A., Khan, M.A., Kwon, E.-H., Park, Y.-S., Lee, I.-J., 2021. Influence of the rhizobacterium rhodobacter sphaeroides KE149 and biochar on waterlogging stress tolerance in *Glycine max* L. Environments 8, 94. https://doi.org/10.3390/environments8090094

Kaur, G., Singh, G., Motavalli, P.P., Nelson, K.A., Orlowski, J.M., Golden, B.R., 2020. Impacts and management strategies for crop production in waterlogged or flooded soils: A review. Agronomy Journal 112, 1475–1501. https://doi.org/10.1002/agj2.20093

Kaur, G., Zurweller, B., Motavalli, P.P., Nelson, K.A., 2019. Screening corn hybrids for soil waterlogging tolerance at an early growth stage. Agriculture 9, 33. https://doi.org/10.3390/agriculture9020033

Kim, Y., Seo, C.-W., Khan, A.L., Mun, B.-G., Shahzad, R., Ko, J.-W., Yun, B.-W., Park, S.-K., Lee, I.-J., 2018. Exo-ethylene application mitigates waterlogging stress in soybean (*Glycine max* L.). BMC Plant Biology 18, 254. https://doi.org/10.1186/s12870-018-1457-4

Kolawole, G.O., Tian, G., Singh, B.B., 2000. Differential response of cowpea lines to aluminum and phosphorus application. Journal of Plant Nutrition 23, 731–740. https://doi.org/10.1080/01904160009382055

Kołton, A., Kęska, K., Czernicka, M., 2020. Selection of tomato and cucumber accessions for waterlogging sensitivity through morpho-physiological assessment at an early vegetative stage. Agronomy 10, 1490. https://doi.org/10.3390/agronomy10101490

Kopsell, D.A., Kopsell, D.E., Lefsrud, M.G., Curran-Celentano, J., Dukach, L.E., 2004. Variation in lutein, β-carotene, and chlorophyll concentrations among *Brassica oleracea* cultigens and seasons. HortScience 39, 361–364. https://doi.org/10.21273/HORTSCI.39.2.361

Krause, G.H., Weis, E., 1991. Chlorophyll fluorescence and photosynthesis: the basics. Annual Review of Plant Physiology and Plant Molecular Biology 42, 313–349. https://doi.org/10.1146/annurev.pp.42.060191.001525

Kreuzwieser, J., Rennenberg, H., 2014. Molecular and physiological responses of trees to waterlogging stress. Plant, Cell & Environment 37, 2245–2259. https://doi.org/10.1111/pce.12310

Kuai, J., Liu, Z., Wang, Y., Meng, Y., Chen, B., Zhao, W., Zhou, Z., Oosterhuis, D.M., 2014. Waterlogging during flowering and boll forming stages affects sucrose metabolism in the leaves subtending the cotton boll and its relationship with boll weight. Plant Science 223, 79–98. https://doi.org/10.1016/j.plantsci.2014.03.010

Kumar, P., Pal, M., Joshi, R., Sairam, R.K., 2013. Yield, growth and physiological responses of mung bean [Vigna radiata (L.) Wilczek] genotypes to waterlogging at the vegetative stage. Physiol Mol Biol Plants 19, 209–220. https://doi.org/10.1007/s12298-012-0153-3

Kumutha, D., Ezhilmathi, K., Sairam, R.K., Srivastava, G.C., Deshmukh, P.S., Meena, R.C., 2009. Waterlogging induced oxidative stress and antioxidant activity in pigeonpea genotypes. Biologia plant. 53, 75–84. https://doi.org/10.1007/s10535-009-0011-5

Kumutha, D., Sairam, R.K., Ezhilmathi, K., Chinnusamy, V., Meena, R.C., 2008. Effect of waterlogging on carbohydrate metabolism in pigeon pea (*Cajanus cajan* L.): Upregulation of sucrose synthase and alcohol dehydrogenase. Plant Science 175, 706–716. https://doi.org/10.1016/j.plantsci.2008.07.013

Kyaw, T.Y., 2022. Assessing the flood tolerance, physiological mechanism, and nutrient mitigation potential of short rotation woody crops planted on seasonally flooded marginal land of the Lower Mississippi Alluvial Valley.

Lambot, C., 2002. Industrial potential of cowpea. In Challenges and opportunities for enhancing sustainable cowpea production, (eds) Fatokun CA Tarawali SA Singh BB Kormawa PM Tamò M. Proceedings of the World Cowpea Conference III held at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, 367–375.

Langan, P., Bernád, V., Walsh, J., Henchy, J., Khodaeiaminjan, M., Mangina, E., Negrão, S., 2022. Phenotyping for waterlogging tolerance in crops: current trends and future prospects. Journal of Experimental Botany erac243. https://doi.org/10.1093/jxb/erac243

Langyintuo, A.S., Lowenberg-DeBoer, J., Faye, M., Lambert, D., Ibro, G., Moussa, B., Kergna, A., Kushwaha, S., Musa, S., Ntoukam, G., 2003. Cowpea supply and demand in West and Central Africa. Field crops research 82, 215–231. https://doi.org/10.1016/S0378-4290(03)00039-X

Lapaz, A. de M., de Camargos, L.S., Yoshida, C.H.P., Firmino, A.C., de Figueiredo, P.A.M., Aguilar, J.V., Nicolai, A.B., Silva de Paiva, W. da, Cruz, V.H., Tomaz, R.S., 2020. Response of soybean to soil waterlogging associated with iron excess in the reproductive stage. Physiology and Molecular Biology of Plants 26, 1635–1648. https://doi.org/10.1007/s12298-020-00845-8

Leigh, A., Sevanto, S., Close, J.D., Nicotra, A.B., 2017. The influence of leaf size and shape on leaf thermal dynamics: does theory hold up under natural conditions? Plant, cell & environment 40, 237–248. https://doi.org/10.1111/pce.12857

Limami, A.M., Diab, H., Lothier, J., 2014. Nitrogen metabolism in plants under low oxygen stress. Planta 239, 531–541. https://doi.org/10.1007/s00425-013-2015-9

Linkemer, G., Board, J.E., Musgrave, M.E., 1998. Waterlogging effects on growth and yield components in late-planted soybean. Crop Science, 38: 1576-1584. https://doi.org/10.2135/cropsci1998.0011183X003800060028x

Liu, M., Zhang, Q., Xu, J., Bao, M., Zhang, D., Xie, A., Sun, X., 2021. Effects of waterlogging stress on the physiological characteristics and secondary metabolites of herbaceous peony (*Paeonia lactiflora* Pall.). American Journal of Plant Sciences, 12, 536-557. https://doi.org/10.4236/ajps.2021.124035.

Liu, Y., TANG, B., ZHENG, Y., MA, K., XU, S., QIU, F., 2010. Screening Methods for Waterlogging Tolerance at Maize (Zea mays L.) Seedling Stage. Agricultural Sciences in China 9, 362–369. https://doi.org/10.1016/S1671-2927(09)60105-X

Liu, Y., Tang, B., Zheng, Y., Ma, K., Xu, S., Qiu, F., 2010. Screening methods for waterlogging tolerance at maize (*Zea mays* L.) seedling stage. Agricultural Sciences in China 9, 362–369. https://doi.org/10.1016/S1671-2927(09)60105-X

Lobell, D.B., Gourdji, S.M., 2012. The influence of climate change on global crop productivity. Plant Physiology 160, 1686–1697. https://doi.org/10.1104/pp.112.208298

Maekawa, T., Shimamura, S., Shimada, S., 2011. Effects of short-term waterlogging on soybean nodule nitrogen fixation at different soil reductions and temperatures. Plant Production Science 14, 349–358. https://doi.org/10.1626/pps.14.349

Malik, A.I., Colmer, T.D., Lambers, H., Schortemeyer, M., 2001. Changes in physiological and morphological traits of roots and shoots of wheat in response to different depths of waterlogging. Functional Plant Biology. 28, 1121–1131. https://doi.org/10.1071/pp01089

Mantri, N., Patade, V., Penna, S., Ford, R., Pang, E., 2012. Abiotic stress responses in plants: present and future, in: Ahmad, P., Prasad, M.N.V. (Eds.), Abiotic stress responses in plants: metabolism, productivity and sustainability. Springer New York, 1–19. https://doi.org/10.1007/978-1-4614-0634-1_1

Martin, B., Ruiz-Torres, N.A., 1992. Effects of water-deficit stress on photosynthesis, its components and component limitations, and on water use efficiency in wheat (*Triticum aestivum* L.). Plant Physiology 100: 733–739. https://doi.org/10.1104/pp.100.2.733

Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S.L., Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L., Gomis, M.I., 2021. Climate change 2021: the physical science basis. Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change 2.

Mauchamp, A., Méthy, M., 2004. Submergence-induced damage of photosynthetic apparatus in Phragmites australis. Environmental and Experimental Botany 51, 227–235. https://doi.org/10.1016/j.envexpbot.2003.11.002

Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence—a practical guide. Journal of Experimental Botany 51, 659–668. https://doi.org/10.1093/jexbot/51.345.659

Mielke, M.S., Schaffer, B., Li, C., 2010. Use of a SPAD meter to estimate chlorophyll content in Eugenia uniflora L. leaves as affected by contrasting light environments and soil flooding. Photosynthetica 48, 332–338. https://doi.org/10.1007/s11099-010-0043-2

Min, X.-J., Bartholomew, D.P., 2005. Effects of flooding and drought on ethylene metabolism, titratable acidity and fruiting of pineapple, in Acta Horticulturae. International Society for Horticultural Science (ISHS), Leuven, Belgium, pp. 135–148. https://doi.org/10.17660/ActaHortic.2005.666.13

Minchin, F.R., Summerfield, R.J., 1976. Symbiotic nitrogen fixation and vegetative growth of cowpea (*Vigna unguiculata* (L.) Walp.) in waterlogged conditions. Plant Soil 45, 113–127. https://doi.org/10.1007/BF00011133

Minchin, F.R., Summerfield, R.J., Eaglesham, A.R.J., Stewart, K.A., 1978. Effects of short-term waterlogging on growth and yield of cowpea (*Vigna unguiculata*). The Journal of Agricultural Science 90, 355–366. https://doi.org/10.1017/S0021859600055465

Mortimore, M.J., Singh, B.B., Harris, F., Blade, S.F., 1997. Cowpea in traditional cropping systems. in Advances in Cowpea Research. Co-publication of the International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). Eds. Singh, B. B., Mohan Raj, D. R., Dashiell, K. E., Jackai, L. E. N. (Ibadan, Nigeria: IITA), pp. 99–113.

Muhammad, I., Shalmani, A., Ali, M., Yang, Q.-H., Ahmad, H., Li, F.B., 2021. Mechanisms regulating the dynamics of photosynthesis under abiotic stresses. Frontiers in Plant Science 11. https://doi.org/10.3389/fpls.2020.615942

Mustroph, A., 2018. Improving flooding tolerance of crop plants. Agronomy 8. https://doi.org/10.3390/agronomy8090160

Najeeb, U., Bange, M.P., Tan, D.K.Y., Atwell, B.J., 2015. Consequences of waterlogging in cotton and opportunities for mitigation of yield losses. AoB PLANTS 7. https://doi.org/10.1093/aobpla/plv080

Nakamura, M., Noguchi, K., 2020. Tolerant mechanisms to O_2 deficiency under submergence conditions in plants. Journal of plant research 133, 343–371. https://doi.org/10.1007/s10265-020-01176-1

Neashat, N., Islam, M., Islam, Md.M., Hasan, M., 2020. Assessment of waterlogging tolerance in mungbean genotypes utilizing morphological traits and SSR markers. Annals of Agrarian Science 18, 258–268.

Ng, N.Q., Marechal, R., 1985. Cowpea taxonomy, origin and germplasm. in Cowpea Research, Production and Utilization, eds S. R. Singh and K. O. Rachie (Chichester: John Wiley and Sons), 13–21.

Nishiuchi, S., Yamauchi, T., Takahashi, H., Kotula, L., Nakazono, M., 2012. Mechanisms for coping with submergence and waterlogging in rice. Rice 5, 2. https://doi.org/10.1186/1939-8433-5-2

Noori, K., Ghobadi, M.E., Ghamarnia, H., 2017. The effect of waterlogging on yield and yield components of chickpea under dry farming. Journal of Crop Production 10, 51–64. https://dx.doi.org/10.22069/ejcp.2018.10993.1861

Ntukamazina, N., Onwonga, R.N., Sommer, R., Mukankusi, C.M., Mburu, J., Rubyogo, J.C., 2017. Effect of excessive and minimal soil moisture stress on agronomic performance of bush and climbing bean (*Phaseolus vulgaris* L.). Cogent Food & Agriculture 3, 1373414. https://doi.org/10.1080/23311932.2017.1373414

Ogbaga, C.C., Stepien, P., Johnson, G.N., 2014. Sorghum (*Sorghum bicolor*) varieties adopt strongly contrasting strategies in response to drought. Physiologia Plantarum 152, 389–401. https://doi.org/10.1111/ppl.12196

Olorunwa, O.J., Adhikari, B., Brazel, S., Popescu, S.C., Popescu, G.V., Barickman, T.C., 2022. Short waterlogging events differently affect morphology and photosynthesis of two cucumber (*Cucumis sativus* L.) cultivars. Frontiers in Plant Science 13. https://doi.org/10.3389/fpls.2022.896244

Olorunwa, O.J., Adhikari, B., Shi, A., Barickman, T.C., 2022. Screening of cowpea (*Vigna unguiculata* (L.) Walp.) genotypes for waterlogging tolerance using morpho-physiological traits at the early growth stage. Plant Science 315, 111136. https://doi.org/10.1016/j.plantsci.2021.111136

Olorunwa, O.J., Shi, A., Barickman, T.C., 2021. Varying drought stress induces morpho-physiological changes in cowpea (*Vigna unguiculata* (L.) genotypes inoculated with Bradyrhizobium japonicum. Plant Stress 2, 100033. https://doi.org/10.1016/j.stress.2021.100033

Omomowo, O.I., Babalola, O.O., 2021. Constraints and prospects of improving cowpea productivity to ensure food, nutritional security and environmental sustainability. Frontiers in Plant Science 12, 2392. https://doi.org/10.3389/fpls.2021.751731

Onyekachi, O.G., Boniface, O.O., Gemlack, N.F., Nicholas, N., 2019. The effect of climate change on abiotic plant stress: A review, in Abiotic and biotic stress in plants. IntechOpen: London, UK. https://doi.org/10.5772/intechopen.82681

Ordoñez, A.A.L., Gomez, J.D., Vattuone, M.A., lsla, M.I., 2006. Antioxidant activities of Sechium edule (Jacq.) Swartz extracts. Food Chemistry 97, 452–458. https://doi.org/10.1016/j.foodchem.2005.05.024

Orsák, M., Kotíková, Z., Hnilička, F., Lachman, J., Stanovič, R., 2020. Effect of drought and waterlogging on hydrophilic antioxidants and their activity in potato tubers. Plant, Soil and Environment 66, 128–134. https://doi.org/10.17221/520/2019-PSE

Osakabe, Y., Osakabe, K., Shinozaki, K., Tran, L.-S.P., 2014. Response of plants to water stress. Frontiers in plant science 5, 86. https://doi.org/10.3389/fpls.2014.00086

Ouyang, Y., Zhang, J., Feng, G., Wan, Y., Leininger, T.D., 2020. A century of precipitation trends in forest lands of the Lower Mississippi River Alluvial Valley. Scientific Reports 10, 12802. https://doi.org/10.1038/s41598-020-69508-8

Palta, J.A., Ganjeali, A., Turner, N.C., Siddique, K.H.M., 2010. Effects of transient subsurface waterlogging on root growth, plant biomass and yield of chickpea. Agricultural Water Management 97, 1469–1476. https://doi.org/10.1016/j.agwat.2010.05.001

Pampana, S., Masoni, A., Arduini, I., 2016. Response of cool-season grain legumes to waterlogging at flowering. Canadian Journal of Plant Science 96, 597–603. https://doi.org/10.1139/cjps-2015-0268

Pan, J., Sharif, R., Xu, X., Chen, X., 2021. Mechanisms of waterlogging tolerance in plants: research progress and prospects. Frontiers in Plant Science 11, 2319. https://doi.org/10.3389/fpls.2020.627331

Pan, R., Jiang, W., Wang, Q., Xu, L., Shabala, S., Zhang, W.Y., 2019. Differential response of growth and photosynthesis in diverse cotton genotypes under hypoxia stress. Photosynthetica 57, 772–779. https://doi.org/10.32615/ps.2019.087

Pang, J., Zhou, M., Mendham, N., Shabala, S., Pang, J., Zhou, M., Mendham, N., Shabala, S., 2004. Growth and physiological responses of six barley genotypes to waterlogging and subsequent recovery. Australian Journal of Agricultural Research 55, 895–906. https://doi.org/10.1071/AR03097

Panozzo, A., Dal Cortivo, C., Ferrari, M., Vicelli, B., Varotto, S., Vamerali, T., 2019. Morphological changes and expressions of AOX1A, CYP81D8, and putative PFP genes in a large set of commercial maize hybrids under extreme waterlogging. Frontiers in Plant Science 10. https://doi.org/10.3389/fpls.2019.00062

Park, J.S., Lee, E.J., 2019. Waterlogging induced oxidative stress and the mortality of the Antarctic plant, Deschampsia Antarctica. Journal of Ecology and Environment 43, 29. https://doi.org/10.1186/s41610-019-0127-2

Pedersen, O., Colmer, T.D., Sand-Jensen, K., 2013. Underwater photosynthesis of submerged plants – recent advances and methods. Frontier in Plant Science 4. https://doi.org/10.3389/fpls.2013.00140

Pereira, Y.C., Silva, F.R. da, Silva, B.R.S. da, Cruz, F.J.R., Marques, D.J., Lobato, A.K. da S., 2020. 24-epibrassinolide induces protection against waterlogging and alleviates impacts on the root structures, photosynthetic machinery and biomass in soybean, Plant Signaling & Behavior 15, 11. https://doi.org/10.1080/15592324.2020.1805885

Phukan, U.J., Mishra, S., Shukla, R.K., 2016. Waterlogging and submergence stress: affects and acclimation. Critical Reviews in Biotechnology 36, 956–966. https://doi.org/10.3109/07388551.2015.1064856

Pilon, C., Snider, J.L., Sobolev, V., Chastain, D.R., Sorensen, R.B., Meeks, C.D., Massa, A.N., Walk, T., Singh, B., Earl, H.J., 2018. Assessing stomatal and non-stomatal limitations to carbon assimilation under progressive drought in peanut (*Arachis hypogaea* L.). Journal of Plant Physiology 231, 124–134. https://doi.org/10.1016/j.jplph.2018.09.007

Ploschuk, R.A., Miralles, D.J., Colmer, T.D., Ploschuk, E.L., Striker, G.G., 2018. Waterlogging of winter crops at early and late stages: impacts on leaf physiology, growth and yield. Frontiers in Plant Science 9, 1863. https://doi.org/10.3389/fpls.2018.01863

Ploschuk, R.A., Miralles, D.J., Striker, G.G., 2022. A quantitative review of soybean responses to waterlogging: agronomical, morpho-physiological and anatomical traits of tolerance. Plant and Soil 475, 237–252. https://doi.org/10.1007/s11104-022-05364-x

Pociecha, E., Kościelniak, J., Filek, W., 2008. Effects of root flooding and stage of development on the growth and photosynthesis of field bean (*Vicia faba* L. minor). Acta Physiologiae Plantarum 30, 529. https://doi.org/10.1007/s11738-008-0151-9

Pompeiano, A., Huarancca Reyes, T., Moles, T.M., Guglielminetti, L., Scartazza, A., 2019. Photosynthetic and growth responses of *Arundo donax* L. plantlets under different oxygen deficiency stresses and reoxygenation. Frontiers in Plant Science 10. https://doi.org/10.3389/fpls.2019.00408

Ponnamperuma, F.N., 1972. The chemistry of submerged soils. In Advances in Agronomy; Elsevier: New York, USA; 24, 29–96. ISBN 0065-2113. https://doi.org/10.1016/S0065-2113(08)60633-1

Posso, D.A., Borella, J., Reissig, G.N., Bacarin, M.A., 2018. Root flooding-induced changes in the dynamic dissipation of the photosynthetic energy of common bean plants. Acta Physiologiae Plantarum 40, 212. https://doi.org/10.1007/s11738-018-2790-9

Prentice, I.C., Farquhar, G.D., Fasham, M.J.R., Goulden, M.L., Heimann, M., Jaramillo, V.J., Kheshgi, H.S., LeQuéré, C., Scholes, R.J., Wallace, D.W., 2001. The carbon cycle and atmospheric carbon dioxide. n Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change, eds J. Houghton, Y. Ding, and D. Griggs (New York, NY: Cambridge University Press), 185–237.

Quin, F.M., 1995. Introduction, in B. B. Singh, D.R.M.-R. (Ed.), Advances in cowpea research. International Institute of Tropical Agriculture (IITA), Japan International Research Center for Agricultural Sciences (JIRCAS)), Ibadan, Nigeria, pp. ix–xv.

Rachie, K.O., Roberts, L.M., 1974. Grain legumes of the lowland tropics, in Brady, N.C. (Ed.), Advances in agronomy. Academic Press, pp. 1–132. https://doi.org/10.1016/S0065-2113(08)60869-X

Ramazan, S., Bhat, H.A., Zargar, M.A., Ahmad, P., John, R., 2021. Combined gas exchange characteristics, chlorophyll fluorescence and response curves as selection traits for temperature tolerance in maize genotypes. Photosynthesis Research 150, 213–225. https://doi.org/10.1007/s11120-021-00829-z

Rao, L., Li, S., Cui, X., 2021. Leaf morphology and chlorophyll fluorescence characteristics of mulberry seedlings under waterlogging stress. Scientific Reports 11, 13379. https://doi.org/10.1038/s41598-021-92782-z

Rasmussen, A., Hu, Y., Depaepe, T., Vandenbussche, F., Boyer, F.-D., Van Der Straeten, D., Geelen, D., 2017. Ethylene controls adventitious root initiation sites in arabidopsis hypocotyls independently of strigolactones. Journal of Plant Growth Regulation 36, 897–911. https://doi.org/10.1007/s00344-017-9692-8

Ravelombola, W.S., Shi, A., Weng, Y., Motes, D., Chen, P., Srivastava, V., Wingfield, C., 2016. Evaluation of total seed protein content in eleven Arkansas cowpea (*Vigna unguiculata* (L.) Walp.) lines. American Journal of Plant Sciences 7, 2288–2296. https://doi.org/10.4236/ajps.2016.715201

Ray, D.K., West, P.C., Clark, M., Gerber, J.S., Prishchepov, A.V., Chatterjee, S., 2019a. Climate change has likely already affected global food production. PLOS ONE 14, e0217148. https://doi.org/10.1371/journal.pone.0217148

Reddy, A.R., Reddy, K.R., Hodges, H.F., 1998. Interactive effects of elevated carbon dioxide and growth temperature on photosynthesis in cotton leaves. Plant Growth Regulation 26, 33–40. https://doi.org/10.1023/A:1006035517185

Redmon, L.A., Rouquette Jr, M., 2000. Wildlife forage areas and food plots for deer (SCS 2000–24). College Station, TX: Texas A&M University.

Ren, B., Zhang, J., Dong, S., Liu, P., Zhao, B., 2018. Responses of carbon metabolism and antioxidant system of summer maize to waterlogging at different stages. Journal of Agronomy and Crop Science 204, 505–514. https://doi.org/10.1111/jac.12275

Ren, B., Zhang, J., Dong, S., Liu, P., Zhao, B., 2016. Effects of waterlogging on leaf mesophyll cell ultrastructure and photosynthetic characteristics of summer maize. PLoS One 11, e0161424–e0161424. https://doi.org/10.1371/journal.pone.0161424

Ren, B., Zhang, J., Li, X., Fan, X., Dong, S., Liu, P., Zhao, B., 2014. Effects of waterlogging on the yield and growth of summer maize under field conditions. Canadian Journal of plant science 94, 23–31. https://doi.org/10.4141/cjps2013-175

Rhine, M.D., Stevens, G., Shannon, G., Wrather, A., Sleper, D., 2010. Yield and nutritional responses to waterlogging of soybean cultivars. Irrigation Science 28, 135–142. https://doi.org/10.1007/s00271-009-0168-x

Rivas, R., Falcão, H.M., Ribeiro, R.V., Machado, E.C., Pimentel, C., Santos, M.G., 2016. Drought tolerance in cowpea species is driven by less sensitivity of leaf gas exchange to water deficit and rapid recovery of photosynthesis after rehydration. South African Journal of Botany 103, 101–107. https://doi.org/10.1016/j.sajb.2015.08.008

Rocha, M., Licausi, F., Araújo, W.L., Nunes-Nesi, A., Sodek, L., Fernie, A.R., van Dongen, J.T., 2010. Glycolysis and the tricarboxylic acid cycle are linked by alanine aminotransferase during hypoxia induced by waterlogging of *Lotus japonicus*. Plant Physiology 152, 1501–1513. https://doi.org/10.1104/pp.109.150045

Rosenzweig, C., Tubiello, F.N., Goldberg, R., Mills, E., Bloomfield, J., 2002. Increased crop damage in the US from excess precipitation under climate change. Global Environmental Change 12, 197–202. https://doi.org/10.1016/S0959-3780(02)00008-0

Sahrawat, K.L., 2005. Fertility and organic matter in submerged rice soils. Current Science 88, 735–739. http://www.jstor.org/stable/24111259

Sairam, Raj K., Dharmar, K., Chinnusamy, V., Meena, R.C., 2009. Waterlogging-induced increase in sugar mobilization, fermentation, and related gene expression in the roots of mung bean (*Vigna radiata*). Journal of Plant Physiology 166, 602–616. https://doi.org/10.1016/j.jplph.2008.09.005

Sairam, R.K., Dharmar, K., Lekshmy, S., Chinnusamy, V., 2011. Expression of antioxidant defense genes in mung bean (*Vigna radiata* L.) roots under water-logging is associated with hypoxia tolerance. Acta Physiologiae Plantarum 33, 735–744. https://doi.org/10.1007/s11738-010-0598-3

Sairam, R. K., Kumutha, D., Ezhilmathi, K., Chinnusamy, V., Meena, R.C., 2009. Waterlogging induced oxidative stress and antioxidant enzyme activities in pigeon pea. Biologia Plantarum 53, 493–504. https://doi.org/10.1007/s10535-009-0090-3

Sanginga, N., Dashiell, K.E., Diels, J., Vanlauwe, B., Lyasse, O., Carsky, R.J., Tarawali, S., Asafo-Adjei, B., Menkir, A., Schulz, S., 2003. Sustainable resource management coupled to resilient germplasm to provide new intensive cereal–grain–legume–livestock systems in the dry savanna. Agriculture, Ecosystems & Environment 100, 305–314. https://doi.org/10.1016/S0167-8809(03)00188-9

Sanginga, N., Lyasse, O., Singh, B.B., 2000. Phosphorus use efficiency and nitrogen balance of cowpea breeding lines in a low P soil of the derived savanna zone in West Africa. Plant and Soil 220, 119. https://doi.org/10.1023/A:1004785720047

Santos, R., Carvalho, M., Rosa, E., Carnide, V., Castro, I., 2020. Root and agro-morphological traits performance in cowpea under drought stress. Agronomy 10, 1604. https://doi.org/10.3390/agronomy10101604

Sariah, J.E., 2010. Enhancing cowpea (*Vigna unguiculata* L.) production through insect pest resistant line in East Africa. University of Copenhagen, Faculty of Life Sciences.

Sarkar, R.K., Reddy, J.N., Sharma, S.G., Ismail, A.M., 2006. Physiological basis of submergence tolerance in rice and implications for crop improvement. Current Science 91, 899–906. http://www.jstor.org/stable/24094287

Sasidharan, R., Bailey-Serres, J., Ashikari, M., Atwell, B.J., Colmer, T.D., Fagerstedt, K., Fukao, T., Geigenberger, P., Hebelstrup, K.H., Hill, R.D., Holdsworth, M.J., Ismail, A.M., Licausi, F., Mustroph, A., Nakazono, M., Pedersen, O., Perata, P., Sauter, M., Shih, M.-C., Sorrell, B.K., Striker, G.G., van Dongen, J.T., Whelan, J., Xiao, S., Visser, E.J.W., Voesenek, L.A.C.J., 2017. Community recommendations on terminology and procedures used in flooding and low oxygen stress research. New Phytologist 214, 1403–1407. https://doi.org/10.1111/nph.14519

Sathi, K.S., Masud, A.A.C., Falguni, M.R., Ahmed, N., Rahman, K., Hasanuzzaman, M., 2022. Screening of Soybean Genotypes for Waterlogging stress tolerance and understanding the physiological mechanisms. Advances in Agriculture 2022, 1–14. https://doi.org/10.1155/2022/5544665

Sauter, M., 2013. Root responses to flooding. Current Opinion in Plant Biology, Physiology and metabolism 16, 282–286. https://doi.org/10.1016/j.pbi.2013.03.013

Schildwacht, P.M., 1989. Is a decreased water potential after withholding oxygen to roots the cause of the decline of leaf-elongation rates in *Zea mays* L. and *Phaseolus vulgaris* L.? Planta 177, 178–184. https://doi.org/10.1007/BF00392806

Scott, H.D., DeAngulo, J., Daniels, M.B., Wood, L.S., 1989. Flood duration effects on soybean growth and yield. Agronomy Journal 81, 631–636. https://doi.org/10.2134/agronj1989.00021962008100040016x

Setter, T.L., Waters, I., 2003. Review of prospects for germplasm improvement for waterlogging tolerance in wheat, barley and oats. Plant and Soil 253, 1–34. https://doi.org/10.1023/A:1024573305997

Sha Valli Khan, P.S., Nagamallaiah, G.V., Dhanunjay Rao, M., Sergeant, K., Hausman, J.F., 2014. Chapter 2 - Abiotic Stress Tolerance in Plants: Insights from Proteomics, in Ahmad, P., Rasool, S. (Eds.), Emerging Technologies and Management of Crop Stress Tolerance. Academic Press, San Diego, pp. 23–68. https://doi.org/10.1016/B978-0-12-800875-1.00002-8

Shabala, S., Shabala, L., Barcelo, J., Poschenrieder, C., 2014. Membrane transporters mediating root signalling and adaptive responses to oxygen deprivation and soil flooding. Plant, Cell & Environment 37, 2216–2233. https://doi.org/10.1111/pce.12339

Shao, G.C., Lan, J.J., Yu, S.E., Liu, N., Guo, R.Q., She, D.L., 2013. Photosynthesis and growth of winter wheat in response to waterlogging at different growth stages. Photosynthetica 51, 429–437. https://doi.org/10.1007/s11099-013-0039-9

Sharkey, T.D., Bernacchi, C.J., Farquhar, G.D., Singsaas, E.L., 2007. Fitting photosynthetic carbon dioxide response curves for C3 leaves. Plant, Cell & Environment 30, 1035–1040. https://doi.org/10.1111/j.1365-3040.2007.01710.x

Sharma, P., Jha, A.B., Dubey, R.S., Pessarakli, M., 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of botany 2012. https://doi.org/10.1155/2012/217037

Shaw, R.E., Meyer, W.S., McNeill, A., Tyerman, S.D., 2013. Waterlogging in Australian agricultural landscapes: a review of plant responses and crop models. Crop and Pasture Science 64(6) 549-562. https://doi.org/10.1071/CP13080

Shikov, A.E., Chirkova, T.V., Yemelyanov, V.V., 2020. Post-anoxia in plants: reasons, consequences, and possible mechanisms. Russian journal of plant physiology 67, 45–59. https://doi.org/10.1134/S1021443720010203

Shimamura, S., Mochizuki, T., Nada, Y., Fukuyama, M., 2002. Secondary aerenchyma formation and its relation to nitrogen fixation in root nodules of soybean plants (*Glycine max*) grown under flooded conditions. Plant Production Science 5, 294–300. https://doi.org/10.1626/pps.5.294

Shou-Ren, Z., 1999. A discussion on chlorophyll fluorescence kinetics parameters and their significance. Chinese Bulletin of Botany 16, 444. https://www.chinbullbotany.com/EN/Y1999/V16/I04/444

Singh, B., Mohan-Raj, D., Dashiell, K.E., Jackai, L., 1997. Advances in cowpea research.

Singh, B.B., 2014. Cowpea: The food legume of the 21st century. Crop Science Society of America, Inc., Madison, WI.

Singh, B.B., Ajeigbe, H.A., Tarawali, S.A., Fernandez-Rivera, S., Abubakar, M., 2003. Improving the production and utilization of cowpea as food and fodder. Field Crops Research 84, 169–177. https://doi.org/10.1016/S0378-4290(03)00148-5

Singh, S.K., Reddy, K.R., 2011. Regulation of photosynthesis, fluorescence, stomatal conductance and water-use efficiency of cowpea (Vigna unguiculata [L.] Walp.) under drought. Journal of Photochemistry and Photobiology B: Biology 105, 40–50. https://doi.org/10.1016/j.jphotobiol.2011.07.001

Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., 1999. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, in Methods in Enzymology. Academic Press, pp. 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1

Šircelj, H., Tausz, M., Grill, D., Batič, F., 2005. Biochemical responses in leaves of two apple tree cultivars subjected to progressing drought. Journal of Plant Physiology 162, 1308–1318. https://doi.org/10.1016/j.jplph.2005.01.018

Smethurst, C.F., Garnett, T., Shabala, S., 2005. Nutritional and chlorophyll fluorescence responses of lucerne (*Medicago sativa*) to waterlogging and subsequent recovery. Plant Soil 270, 31–45. https://doi.org/10.1007/s11104-004-1082-x

Smethurst, C.F., Shabala, S., 2003. Screening methods for waterlogging tolerance in lucerne: comparative analysis of waterlogging effects on chlorophyll fluorescence, photosynthesis, biomass and chlorophyll content. Functional Plant Biology 30, 335. https://doi.org/10.1071/FP02192

Smith, K.A., 1987. The effects of waterlogging on root growth and on symbiotic nitrogen fixation by legumes. In: Proceedings of the consultants' workshop on adoption of chickpea and pigeonpea to abiotic stress, 10–21 December 1984. ICRISAT, Patancheru, India, 77–89

Solaiman, Z., Colmer, T.D., Loss, S.P., Thomson, B.D., Siddique, K.H.M., Solaiman, Z., Colmer, T.D., Loss, S.P., Thomson, B.D., Siddique, K.H.M., 2007. Growth responses of cool-season grain legumes to transient waterlogging. Australian Journal of Agricultural Research 58, 406–412. https://doi.org/10.1071/AR06330

Sousa, C.A.F. de, Sodek, L., 2002. The metabolic response of plants to oxygen deficiency. Brazilian Journal of Plant Physiology 14, 83–94. https://doi.org/10.1590/S1677-04202002000200002

Steele, W.M., Mehra, K.L., 1980. Structure, evolution, and adaptation to farming systems and environments in Vigna. Structure, evolution, and adaptation to farming systems and environments in Vigna. Advances in legume science, Summerfield, R.J.; Bunting, A.H. (Eds). Royal Botanical Gardens 393–404.

Steffens, B., Rasmussen, A., 2016. The physiology of adventitious roots. Plant Physiology 170, 603–617. https://doi.org/10.1104/pp.15.01360

Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M., 2013. Climate change 2013: The physical science basis. Intergovernmental Panel on Climate Change, Working Group I Contribution to the IPCC Fifth Assessment Report (AR5)(Cambridge Univ Press, New York) 25.

Striker, G.G., Colmer, T.D., 2017. Flooding tolerance of forage legumes. Journal of Experimental Botany 68, 1851–1872. https://doi.org/10.1093/jxb/erw239

Taiz, L., Zeiger, E., Møller, I.M., Murphy, A., 2015. Plant Physiology and Development, 6th ed. Sinauer Associates, Sunderland, Massachusetts.

Takahashi, H., Xiaohua, Q., Shimamura, S., Yanagawa, A., Hiraga, S., Nakazono, M., 2018. Sucrose supply from leaves is required for aerenchymatous phellem formation in hypocotyl of soybean under waterlogged conditions. Annals of Botany 121, 723–732. https://doi.org/10.1093/aob/mcx205

Takele, A., McDavid, C.R., 1994. Effects of short-term waterlogging on cultivars of cowpea (*Vigna uniguiculata* (L.) Walp.). Tropical agriculture 71, 275–280.

Tamang, B.G., Magliozzi, J.O., Maroof, M. a. S., Fukao, T., 2014. Physiological and transcriptomic characterization of submergence and reoxygenation responses in soybean seedlings. Plant, Cell & Environment 37, 2350–2365. https://doi.org/10.1111/pce.12277

Tarawali, S.A., Singh, B.B., Gupta, S.C., Tabo, R., Harris, F., Nokoe, S., Fernández-Rivera, S., Bationo, A., Manyong, V.M., Makinde, K., Odion, E.C., 2002. Cowpea as a key factor for a new approach to integrated crop–livestock systems research in the dry savannas of West Africa. In: Fatokun, C.A., Tarawali, S., Singh, B.B., Kormawa, P.M., Tamo, M. (Eds.), Proceedings of the World Cowpea Conference III on Challenges and Opportunities for Enhancing Sustainable Cowpea Production. International Institute of Tropical Agriculture, Ibadan, Nigeria, pp. 237–255.

Thomas, A.L., Guerreiro, S.M.C., Sodek, L., 2005. Aerenchyma formation and recovery from hypoxia of the flooded root system of nodulated soybean. Annals of Botany 96, 1191–1198. https://doi.org/10.1093/aob/mci272

Tian, L., Li, J., Bi, W., Zuo, S., Li, L., Li, W., Sun, L., 2019. Effects of waterlogging stress at different growth stages on the photosynthetic characteristics and grain yield of spring maize (*Zea mays* L.) under field conditions. Agricultural Water Management 218, 250–258. https://doi.org/10.1016/j.agwat.2019.03.054

Tian, L., Zhang, Y., Chen, P., Zhang, F., Li, J., Yan, F., Dong, Y., Feng, B., 2021. How Does the Waterlogging Regime Affect Crop Yield? A Global Meta-Analysis. Frontier in Plant Science 12, 634898. https://doi.org/10.3389/fpls.2021.634898

Tigchelaar, M., Battisti, D.S., Naylor, R.L., Ray, D.K., 2018. Future warming increases probability of globally synchronized maize production shocks. Proceedings of the National Academy of Sciences 115, 6644–6649. https://doi.org/10.1073/pnas.1718031115

Timko, M.P., Ehlers, J.D., Roberts, P.A., 2007. Cowpea, in: Kole, C. (Ed.), Pulses, Sugar and Tuber Crops. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 49–67. https://doi.org/10.1007/978-3-540-34516-9_3

Timko, M.P., Singh, B.B., 2008. Cowpea, a Multifunctional Legume, in Moore, P.H., Ming, R. (Eds.), Genomics of Tropical Crop Plants. Springer New York, New York, NY, pp. 227–258.

Timsina, J., Garrity, D.P., Pandey, R.K., 1994a. Plant water relations and growth of cowpea cultivars subjected to varying degrees of waterlogging. Field Crops Research 39, 49–57. https://doi.org/10.1016/0378-4290(94)90070-1

Timsina, J., Garrity, D.P., Pandey, R.K., 1994b. The interaction of cowpea maturity with degree of waterlogging in the post-rice environment. Field Crops Research 39, 39–48. https://doi.org/10.1016/0378-4290(94)90069-8

Timsina, J., Kathmandu, N., 1991. Drought and waterlogging of food legumes: a review of effects, tolerances and implications for simulation. Simulation and systems analysis for rice production (SARP) 261.

Tosti, N., Negri, V., 2002. Efficiency of three PCR-based markers in assessing genetic variation among cowpea (*Vigna unguiculata* subsp. unguiculata) landraces. Genome 45, 268–275. https://doi.org/10.1139/g01-146

Tournaire-Roux, C., Sutka, M., Javot, H., Gout, E., Gerbeau, P., Luu, D.-T., Bligny, R., Maurel, C., 2003. Cytosolic pH regulates root water transport during anoxic stress through the gating of aquaporins. Nature 425, 393–397. https://doi.org/10.1038/nature01853

Tracewell, C.A., Vrettos, J.S., Bautista, J.A., Frank, H.A., Brudvig, G.W., 2001. Carotenoid photooxidation in photosystem II. Archives of Biochemistry and Biophysics 385, 61–69. https://doi.org/10.1006/abbi.2000.2150

Udvardi, M., Poole, P.S., 2013. Transport and metabolism in legume-rhizobia symbioses. Annual review of plant biology 64, 781–805. https://doi.org/10.1146/annurev-arplant-050312-120235

Umaharan, P., Ariyanayagam, R.P., Haque, S.Q., 1997. Effect of short-term waterlogging applied at various growth phases on growth, development and yield in *Vigna unguiculata*. The Journal of Agricultural Science 128, 189–198. https://doi.org/10.1017/S0021859696004121

USGCRP, 2018. Impacts, risks, and adaptation in the United States: Fourth National Climate Assessment, Volume II. U.S. Global Change Research Program, Washington, DC, USA.

USGCRP, 2017. Climate Science Special Report: Fourth National Climate Assessment, Volume I [Wuebbles, D.J., D.W. Fahey, K.A. Hibbard, D.J. Dokken, B.C. Stewart, and T.K. Maycock (eds.)]. U.S. Global Change Research Program, Washington D.C, USA. https://doi.org/10.7930/J0J964J6

van der Weele, C.M., Jiang, H.S., Palaniappan, K.K., Ivanov, V.B., Palaniappan, K., Baskin, T.I., 2003. A new algorithm for computational image analysis of deformable motion at high spatial and temporal resolution applied to root growth. Roughly uniform elongation in the meristem and also, after an abrupt acceleration, in the elongation zone. Plant Physiology 132, 1138–1148. https://doi.org/10.1104/pp.103.021345

Vartapetian, B.B., Jackson, M.B., 1997. Plant adaptations to anaerobic stress. Annals of Botany 79, 3–20. https://doi.org/10.1093/oxfordjournals.aob.a010303

Vavilov, N.I., 1951. The origin, variation, immunity and breeding of cultivated plants: selected writings.

Velasco, N.F., Ligarreto, G.A., Díaz, H.R., Fonseca, L.P.M., 2019. Photosynthetic responses and tolerance to root-zone hypoxia stress of five bean cultivars (*Phaseolus vulgaris* L.). South African Journal of Botany 123, 200–207. https://doi.org/10.1016/j.sajb.2019.02.010

Verdcourt, B., 1970. Studies in the Leguminosae-Papilionoïdeae for the'Flora of Tropical East Africa': III. Kew Bulletin 379–447.

Voesenek, L. a. C.J., Sasidharan, R., 2013. Ethylene – and oxygen signalling – drive plant survival during flooding. Plant Biology 15, 426–435. https://doi.org/10.1111/plb.12014

Voesenek, L.A.C.J., Bailey-Serres, J., 2015. Flood adaptive traits and processes: an overview. New Phytologist 206, 57–73. https://doi.org/10.1111/nph.13209

Vogt, T., 2010. Phenylpropanoid Biosynthesis. Molecular Plant 3, 2–20. https://doi.org/10.1093/mp/ssp106

Vosnjak, M., Sircelj, H., Hudina, M., Usenik, V., 2021. Response of chloroplast pigments, sugars and phenolics of sweet cherry leaves to chilling. Scientific Reports 11, 7210. https://doi.org/10.1038/s41598-021-86732-y

Walne, C.H., Reddy, K.R., 2021. Developing Functional Relationships between Soil Waterlogging and Corn Shoot and Root Growth and Development. Plants 10, 2095. https://doi.org/10.3390/plants10102095

Walthall, C.L., Anderson, C.J., Baumgard, L.H., Takle, E., Wright-Morton, L., 2013. Climate change and agriculture in the United States: Effects and adaptation.

Wang, S., Zheng, J., Wang, Y., Yang, Q., Chen, T., Chen, Y., Chi, D., Xia, G., Siddique, K.H.M., Wang, T., 2021. Photosynthesis, chlorophyll fluorescence, and yield of peanut in response to biochar application. Frontiers in Plant Science 12. https://doi.org/10.3389/fpls.2021.650432

Wang, X., Deng, Z., Zhang, W., Meng, Z., Chang, X., Lv, M., 2017. Effect of waterlogging duration at different growth stages on the growth, yield and quality of cotton. PLOS ONE 12, e0169029. https://doi.org/10.1371/journal.pone.0169029

Waqas, M.A., Kaya, C., Riaz, A., Farooq, M., Nawaz, I., Wilkes, A., Li, Y., 2019. Potential mechanisms of abiotic stress tolerance in crop plants induced by thiourea. Frontiers in Plant Science 10, 1336. https://doi.org/10.3389/fpls.2019.01336

Wei, W., Li, D., Wang, L., Ding, X., Zhang, Y., Gao, Y., Zhang, X., 2013. Morpho-anatomical and physiological responses to waterlogging of sesame (*Sesamum indicum* L.). Plant Science 208, 102–111. https://doi.org/10.1016/j.plantsci.2013.03.014

Weng, Y., Qin, J., Eaton, S., Yang, Y., Ravelombola, W.S., Shi, A., 2019. Evaluation of seed protein content in USDA cowpea germplasm. HortScience 54, 814–817. https://doi.org/10.21273/HORTSCI13929-19

Wijewardana, C., Alsajri, F.A., Irby, J.T., Krutz, L.J., Golden, B.R., Henry, W.B., Reddy, K.R., 2019. Water Deficit Effects on Soybean Root Morphology and Early-Season Vigor. Agronomy 9, 836. https://doi.org/10.3390/agronomy9120836

Woisky, R.G., Salatino, A., 1998. Analysis of propolis: some parameters and procedures for chemical quality control. Journal of apicultural research 37, 99–105. https://doi.org/10.1080/00218839.1998.11100961

Wright, A.H., DeLong, J.M., Gunawardena, A.H.L.A.N., Prange, R.K., 2011. The interrelationship between the lower oxygen limit, chlorophyll fluorescence and the xanthophyll cycle in plants. Photosynthesis Research 107, 223–235. https://doi.org/10.1007/s11120-011-9621-9

Wu, X., Tang, Y., Li, C., Wu, C., Huang, G., 2015. Chlorophyll fluorescence and yield responses of winter wheat to waterlogging at different growth stages. Plant Production Science 18, 284–294. https://doi.org/10.1626/pps.18.284

Xuewen, X., Huihui, W., Xiaohua, Q., Qiang, X., Xuehao, C., 2014. Waterlogging-induced increase in fermentation and related gene expression in the root of cucumber (Cucumis sativus L.). Scientia Horticulturae 179, 388–395. https://doi.org/10.1016/j.scienta.2014.10.001

Yamane, K., Iijima, M., 2016. Nodulation control of crack fertilization technique reduced the growth inhibition of soybean caused by short-term waterlogging at early vegetative stage. Plant Production Science 19, 438–448. https://doi.org/10.1080/1343943X.2016.1164573

Yamauchi, T., Colmer, T.D., Pedersen, O., Nakazono, M., 2018. Regulation of root traits for internal aeration and tolerance to soil waterlogging-flooding stress. Plant Physiology 176, 1118–1130. https://doi.org/10.1104/pp.17.01157

Yan, K., Zhao, S., Cui, M., Han, G., Wen, P., 2018. Vulnerability of photosynthesis and photosystem I in Jerusalem artichoke (*Helianthus tuberosus* L.) exposed to waterlogging. Plant Physiology and Biochemistry 125, 239–246. https://doi.org/10.1016/j.plaphy.2018.02.017

Yang, F., Liu, T., Wang, Q., Du, M., Yang, T., Liu, D., Li, S., Liu, S., 2021. Rapid determination of leaf water content for monitoring waterlogging in winter wheat based on hyperspectral parameters. Journal of Integrative Agriculture 20, 2613–2626. https://doi.org/10.1016/S2095-3119(20)63306-8

Yang, F., Wang, Y., Wang, J., Deng, W., Liao, L., Li, M., 2011. Different eco-physiological responses between male and female Populus deltoides clones to waterlogging stress. Forest Ecology and Management 262, 1963–1971. https://doi.org/10.1016/j.foreco.2011.08.039

Yin, D., Chen, S., Chen, F., Guan, Z., Fang, W., 2009. Morphological and physiological responses of two chrysanthemum cultivars differing in their tolerance to waterlogging. Environmental and Experimental Botany 67, 87–93. https://doi.org/10.1016/j.envexpbot.2009.06.006

Yordanova, R.Y., Popova, L.P., 2007. Flooding-induced changes in photosynthesis and oxidative status in maize plants. Acta Physiologiae Plantarum 29, 535–541. https://doi.org/10.1007/s11738-007-0064-z

Zabalza, A., van Dongen, J.T., Froehlich, A., Oliver, S.N., Faix, B., Gupta, K.J., Schmälzlin, E., Igal, M., Orcaray, L., Royuela, M., Geigenberger, P., 2009. Regulation of respiration and fermentation to control the plant internal oxygen concentration. Plant Physiology 149, 1087–1098. https://doi.org/10.1104/pp.108.129288

Zeng, R., Chen, L., Wang, X., Cao, J., Li, X., Xu, X., Xia, Q., Chen, T., Zhang, L., 2020. Effect of waterlogging stress on dry matter accumulation, photosynthesis characteristics, yield, and yield components in three different ecotypes of peanut (*Arachis hypogaea* L.). Agronomy 10, 1244. https://doi.org/10.3390/agronomy10091244

Zeng, R., Chen, T., Wang, X., Cao, J., Li, X., Xu, X., Chen, L., Xia, Q., Dong, Y., Huang, L., Wang, L., Zhang, J., Zhang, L., 2021. Physiological and expressional regulation on photosynthesis, starch and sucrose metabolism response to waterlogging stress in peanut. Front Plant Sci 12, 601771–601771. https://doi.org/10.3389/fpls.2021.601771

Zhang, F., Zhu, K., Wang, Y.Q., Zhang, Z.P., Lu, F., Yu, H.Q., Zou, J.Q., 2019. Changes in photosynthetic and chlorophyll fluorescence characteristics of sorghum under drought and waterlogging stress. Photosynthetica 57, 1156–1164. https://doi.org/10.32615/ps.2019.136

Zhang, Y., Chen, Y., Lu, H., Kong, X., Dai, J., Li, Z., Dong, H., 2016. Growth, lint yield and changes in physiological attributes of cotton under temporal waterlogging. Field Crops Research 194, 83–93. https://doi.org/10.1016/j.fcr.2016.05.006

Zhang, Y., Liu, G., Dong, H., Li, C., 2021. Waterlogging stress in cotton: Damage, adaptability, alleviation strategies, and mechanisms. The Crop Journal 9, 257–270. https://doi.org/10.1016/j.cj.2020.08.005

Zhao, C., Liu, B., Piao, S., Wang, X., Lobell, D.B., Huang, Y., Huang, M., Yao, Y., Bassu, S., Ciais, P., Durand, J.-L., Elliott, J., Ewert, F., Janssens, I.A., Li, T., Lin, E., Liu, Q., Martre, P., Müller, C., Peng, S., Peñuelas, J., Ruane, A.C., Wallach, D., Wang, T., Wu, D., Liu, Z., Zhu, Y., Zhu, Z., Asseng, S., 2017. Temperature increase reduces global yields of major crops in four independent estimates. Proc Natl Acad Sci USA 114, 9326. https://doi.org/10.1073/pnas.1701762114

Zhou, J., Tian, L., Wang, S., Li, H., Zhao, Y., Zhang, M., Wang, X., An, P., Li, C., 2021. Ovary abortion induced by combined waterlogging and shading stress at the flowering stage involves amino acids and flavonoid metabolism in maize. Frontiers in Plant Science 12. https://doi.org/10.3389/fpls.2021.778717

Zhou, S., Medlyn, B., Sabaté, S., Sperlich, D., Prentice, I.C., Whitehead, D., 2014. Short-term water stress impacts on stomatal, mesophyll and biochemical limitations to photosynthesis differ consistently among tree species from contrasting climates. Tree Physiology 34, 1035–1046. https://doi.org/10.1093/treephys/tpu072

Zhu, M., Li, F.H., Shi, Z.S., 2016. Morphological and photosynthetic response of waxy corn inbred line to waterlogging. Photosynthetica 54, 636–640. https://doi.org/10.1007/s11099-016-0203-0

Zou, X., Hu, C., Zeng, L., Cheng, Y., Xu, M., Zhang, X., 2014. A comparison of screening methods to identify waterlogging tolerance in the field in *Brassica napus* L. during plant ontogeny. PLOS ONE 9, e89731. https://doi.org/10.1371/journal.pone.0089731

Zurweller, B.A., Motavalli, P.P., Nelson, K.A., Dudenhoeffer, C.J., 2015. Short-term soil nitrous oxide emissions as affected by enhanced efficiency nitrogen fertilizers and temporarily waterlogged conditions. Journal of Agricultural Science 7, 1-14. http://dx.doi.org/10.5539/jas.v7n12p1