Assessing the Efficacy of Iodine Seed Priming in Enhancing the Tolerance of Wheat (*Triticum aestivum* L) Genotypes to Water Stress Conditions

Nkosinathi Hlatshwayo

Submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy

in Discipline of Crop Science

School of Agricultural, Earth and Environmental Sciences
College of Agriculture, Engineering and Science
University of KwaZulu-Natal

Pietermaritzburg
South Africa

December 2024

DECLARATION

The research contained in this thesis was completed by the candidate while based in the Discipline of Crop Science, School of Agricultural, Earth and Environmental Sciences, in the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa. The research was financially supported by the South African Cultivar & Technology Agency (SACTA).

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

[redacted]

Signed: N. Hlatshwayo

Date: 27 November 2024

[redacted]

Signed: Prof. T. Mabhaudhi *(Supervisor)*

Date: 29 November 2024

Signed: Prof A.T. Modi *(Co-Supervisor)*

Date: 29 November 2024

PREFACE

This thesis is a compilation of manuscripts where individual chapter is an independent article introduced disjointedly. Hence, some repetition between individual chapters has been inevitable.

ACKNOWLEDGEMENTS

My special and most sincere gratitude goes to the following:

- The South African Cultivar & Technology Agency (SACTA) for initiating and funding my study.
- Prof. Albert Thembinkosi Modi, for his guidance, perseverance, belief and support throughout my studies.
- Prof. T. Mabhaudhi for agreeing to serve as my supervisor during challenging times and providing unwavering support throughout my studies
- The staff and management at Ukulinga Research Farm, Mr. Armstrong, Thandazani, Nokulunga, Mam Flo and Mr. Zuma, for assisting with field trials at both sites.
- My family for supporting me through thick and thin

.

DEDICATION

This work is dedicated to my grandmother “Ngane Kama Shanda” and mother ‘’Tholakele ‘’, both of whom have provided unwavering support and wisdom, profoundly shaping my journey.

GENERAL ABSTRACT

Worldwide, wheat (*Triticum aestivum* L.) is a vital cereal crop that provides over 20% of daily protein intake and calories. However, research has predicted that its production will continue to decrease due to climate change which will accelerate environmental stresses such as drought stress. Drought remains a significant environmental factor that negatively impacts wheat growth and development from germination to maturity stages, ultimately affecting yield and seed quality traits. Although iodine application has the potential to stimulate growth and development and enhance responses to tolerate abiotic stresses, there is a lack of knowledge on its effects on agronomic and physiological traits under drought stress conditions. Therefore, this study aimed to evaluate the potential of iodine fertilizer on seed quality, growth, physiological traits, and yield of wheat genotypes under drought-stress conditions. The study was conducted through a series of trials, including laboratory, tunnel, and field trials, which were carried out Controlled Environment Research Unit (CERU) and Ukulinga Research Farm of the University of KwaZulu Natal, Pietermaritzburg. The laboratory experiment focused on determining the optimal iodate dose and its effects on seed quality parameters. The pot trials aimed to investigate the impact of iodine priming on wheat seed quality, yield and physiological traits under drought stress conditions. Finally, the field trial evaluated the efficacy of iodine fertilization on wheat yield and yield parameters under rainfed and irrigated conditions.

In a laboratory experiment, a 3 x 5 factorial experiment was conducted in a randomized complete block design (RCBD) and replicated three times. The wheat genotypes (SST806, SST8135 and PAN3111) and five iodine concentration (0, 0.001, 0.01, 0.1 and 0.5 M IO_3^-) were used as treatment factors. The results revealed that wheat seeds primed with iodate doses higher than 0.01 M (IO_3^-) did not germinate, rendering concentrations of 0.1 and 0.5 M (IO_3^-) unsuitable for the experiment. However, the study found that iodate priming significantly ($p < 0.05$) influenced germination tests, such as germination rate index and meantime germination in wheat genotypes. The final germination percentage was not significantly ($p > 0.05$) affected by iodate concentration or wheat genotype. The vigour tests showed insignificant ($p > 0.05$) differences between the interaction genotypes and iodate concentrations, except for the cold test and seedling length. Despite these findings, genotype SST806 had higher seed quality than other genotypes.

In the first pot trial, the experiment was laid out using a factorial design arranged in a complete block design (CRD), where irrigation (well-watered (WW) and water deficit (WD)), wheat genotypes (PAN3111, SST806 and SST8135) and iodine concentrations (0, 0.001 and 0.01 M IO_3^-) were the treatment factors replicated three times. Tolerance growth indices, membrane stability index (MSI), relative water content (RWC) and photosynthetic pigments were determined in the study. The results demonstrated that iodine priming during early stages of growth significantly ($p < 0.05$) improved plant height stress index (PHSI), root length stress index (RLSI) and dry matter stress index (DMSI) and decreased carotenoids compared to control. Between iodate concentrations, genotypes primed with 0.001

M IO_3^- performed better than genotypes primed with 0.01 M IO_3^-. Overall, genotype SST806 had more resistance to drought than other wheat genotypes.

The second pot trial evaluated the agronomic and some physiological responses of wheat genotypes using iodine application (seed priming and foliar spraying) under drought stress conditions at different growth stages. The two season trails were conducted using a factorial design where wheat genotypes (SST806 and SST8135), iodine application [seed priming (0.001M) and foliar spraying (0.05% w/v)] of IO_3^- and water stress levels (water deficit (WD) at tillering, WD at flowering, well-watered (WW) at tillering and WW at flowering) were the treatment factors replicated three times. Data collected included plant height (PH), leaf gas exchange variables, relative water content (RWC) and yield and yield-related traits that were determined during the trial and at harvest. The findings showed that drought stress significantly ($p < 0.05$) affected all the studied traits, however, the application of iodine through seed priming and foliar applications significantly increased ((rate of photosynthesis (P_n), transpiration rate (Tr), number of tillers (NT), number of spikelets per spike (SPS), number of grains per spike (NGS), 1000 grain weight (TGW) and biological yield (BY)) under water stress and normal conditions in wheat genotypes. Nevertheless, some traits showed not only increases but also significant decreases ($p > 0.05$) compared to the control. Interestingly, genotype SST806 performed better than genotype SST8135.

The two-season field trial was conducted to evaluate the efficacy of iodine fertilization on wheat yield and yield parameters under rainfed and irrigated conditions. The experiment was arranged in a split-split plot design with three replications. Irrigation (well-watered and water deficit) was assigned to main plots, while wheat genotypes (PAN3111, SST806, and SST8135) were assigned to subplots. Iodine concentrations (0, 0.001 and 0.01 M IO_3^-) were assigned to sub-subplots. Wheat genotype transplanted seedlings were irrigated for one week after planting thereafter irrigation was withdrawn for rainfed plots. Plant height, days to maturity and yield and yield-related parameters were collected during the trial. The results of the study showed that iodate seed priming in wheat genotypes ameliorated the negative effects of drought stress experienced by the genotypes by improving yield traits such as spike length, number of kernels per spike, spikelet per spike, number of productive tillers, and grain yield. Based on the findings, it was concluded that iodine application has a potential to improve wheat growth, development and yield under drought stress conditions and the effective iodate seed priming concentration recommended is 0,001 M IO_3^-.

In conclusion, this study demonstrated that iodine fertilization has the potential to enhance the tolerance of wheat genotypes to water stress conditions. The findings provided valuable insights into the use of iodine as a means to improve crop productivity and mitigate the effects of water stress on crop growth and development.

TABLE OF CONTENTS

DECLARATION I

PREFACE II

ACKNOWLEDGEMENTS III

DEDICATION IV

GENERAL ABSTRACT V

TABLE OF CONTENTS VII

CHAPTER 1 GENERAL INTRODUCTION 1

CHAPTER 2 POTENTIAL OF IODINE PRIMING UNDER ABIOTIC STRESSES TO IMPROVE SEED QUALITY, AGRONOMIC AND PHYSIOLOGICAL TRAITS: A REVIEW 4

CHAPTER 3 EXPLORING THE EFFECTS OF IODATE PRIMING AND THE EFFECTIVE DOSE ON SEED GERMINATION, VIGOUR AND SEEDLING DEVELOPMENT IN WHEAT (*TRITICUM AESTIVUM*) GENOTYPES 20

CHAPTER 4 ENHANCING DROUGHT TOLERANCE IN WHEAT (*TRITICUM AESTIVUM* L.) THROUGH IODATE SEED PRIMING: EFFECTS ON GROWTH, PHYSIOLOGICAL ATTRIBUTES AND STRESS INDICES 36

CHAPTER 5 ASSESSING THE EFFICACY OF IODINE APPLICATION ON MORPHOPHYSIOLOGICAL TRAITS OF WHEAT GENOTYPES UNDER DROUGHT STRESS AND VARIED GROWTH STAGES OVER TWO WINTER SEASONS (2021 AND 2022) 54

CHAPTER 6 IODINE SEED PRIMING ENHANCES YIELD AND STRESS RESILIENCE IN WHEAT *(TRITICUM AESTIVUM L.)* UNDER RAINFED AND IRRIGATED CONDITIONS 75

CHAPTER 7 GENERAL DISCUSSION 97

CONCLUSION 98

RECOMMENDATIONS 100

REFERENCES 101

CHAPTER 1

GENERAL INTRODUCTION

1.1. BACKGROUND

Wheat (*Triticum aestivum* L.) is crucial staple crop worldwide and provides 20% of total calories. It is a rich source of carbohydrates (78.1%), protein (14.7%), minerals (2.1%), fat (2.1%) and considerable proportion of vitamins (Arzani & Ashraf, 2017). The global production of wheat is approximately 750 million tons on 220 million hectares, while in sub-Saharan Africa (SSA), it is estimated to be around 7.5 million tons on 2.9 million hectares (FAO, 2019). The germination and seedling establishments are crucial in the plant life cycle and can contribute to increasing total crop production. However, wheat productivity and yield were not satisfactory in the last decades to meet future demand. Climate change enhances the possible calamities such as drought, changes in rainfall, temperature, and floods, which adversely impact the crop productivity of crops such as wheat (Yadav et al., 2020).

Drought is one of the major causes of yield and quality losses of cereal and other crops worldwide, posing a significant problem to wheat productivity and food insecurity, given that it is consumed by two thirds of world's population especially in sub-Saharan Africa (SSA) (Farooq et al., 2009b; Khatiwada et al., 2020). Most agro-ecosystems and climate conditions in sub-Saharan Africa (SSA) are vulnerable to drought conditions, with subsistence farmers who depend on wheat for food security being most affected. They are unable to afford seeds developed through modern technologies such as conventional and modern breeding or biotechnology as they are expensive. Therefore, sustainable and cost-effective technologies for increasing wheat productivity are paramount, especially for SSA.

In this context, seed priming remains most feasible, sustainable, and cost-effective strategy that can be implemented to increase wheat crop production especially to the marginalized population. Seed priming can improve the seed quality by enhancing the germination and establishment in many crops such as wheat (*Triticum aestivum L.)* and maize (*Zea mays*) (Elias et al., 2006; Peñaloza et al., 2005; Ventura et al., 2012). It is an old age strategy for enhancing rapid and uniform emergence, leading to better stand establishment and yield (Ibrahim, 2019). Direct benefit due to seed priming include faster and uniform germination, increase nutrient uptake, relieve phytochrome induced photo and thermo-dormancy and increased temperature range for germination and improve water-use and synchronous maturity of crop (Hasanuzzaman & Fotopoulos, 2019).

In agriculture, iodine has recently gained relevance due to its ability to induce crop tolerance to abiotic stresses. Iodine is a non-essential nutrient for plant growth, nevertheless, studies have shown that fertilizing with iodine base products at lower doses can be beneficial to crop and it acts as a bio-stimulant which enhances the physiological and antioxidant properties in plants to tolerate stress (Medrano-Macías et al., 2016). This modified tolerance mechanism varies from specie to specie, dose and iodine type applied. Gupta et al. (2015) showed an increase in soybean (*Glycine max L.)* antioxidant defense systems when stressed with salinity and heavy metals. Presently, there are limited published studies on the impact of priming with iodine in crops including wheat under both normal and stressed conditions. However, other studies have shown an impact of iodine when applied with other elements such as selenium (Rady et al., 2020) and zinc (Candan et al., 2018). The review of Nascimento et al., (2022) reported that iodine can be used like other micronutrients such as potassium, silicon, iron and selenium, etc. Therefore, this prompted the study to be conducted, as it is crucial to simultaneously improve tolerance of crops to drought and increase wheat crop productivity. Applying iodine through seed priming can be a sustainable strategy as it is a simple, low-cost, and effective method to improve crop growth and productivity (Hasanuzzaman & Fotopoulos, 2019). Moreover, seed priming induces abiotic tolerance to plants through rapid and advanced germination; enhanced mechanisms of protections caused by oxidative stress and retained memory of previous stress (Chen & Arora, 2013).

Such information can be useful to plant breeders to establish genotypes that could be used to create improved varieties with higher drought tolerance, iodine (I) content, and yield. Moreover, the study will fill in the information gap in this area of knowledge. Considering the above matter, the study was conducted with the hypothesis that iodine priming will have positive effect on seed quality, grain, growth characteristics, physiological traits and the availability of micronutrients under drought stress conditions.

1.2. Aims and objectives.

The study was conducted with the aim of determining the effect of applied iodine fertilizers on seed quality, growth characteristics, physiological traits and yield of wheat genotypes under drought-stress conditions. To address the study aim, the following objectives were formulated:

i. to assess the effect of iodine priming and its effective dose in wheat seed germination and vigour.

ii. to investigate the effect of iodate priming and its effective dose on germination and seedling performance of wheat genotypes under water stress.

iii. to investigate the effect of iodate priming and foliar spraying in plant tissue at different growth stages and physiological parameters of wheat genotypes under water stress.

iv. to investigate the effect of iodate priming and its effective dose on yield and yield-related components in wheat genotypes under water stress.

1.3. Thesis Outline

The thesis is written in paper format and each study is presented as a distinct manuscript, with details on whether it has been submitted to a journal and is currently under review included on the title page. The following is an overview of the multiple manuscripts that make up this thesis:

Chapter 2: The literature review addresses the justification for promotion and inclusion of iodine seed priming, and mechanisms involved in tolerance to drought.

Chapter 3: Addresses the first objective through the laboratory experiments that were conducted at the University of KwaZulu-Natal (UKZN), Pietermaritzburg. It aimed to determine the recommended iodate concentration levels, uptake and accumulation and its effect on seed quality.

Chapter 4: This chapter was written as a sequel to chapter 3 objective. Wheat genotypes were planted in controlled environmental conditions at UKZN facility with the aim to determine the effect of iodate priming in seedling growth under drought stress conditions.

Chapter 5: This chapter aimed to determine the exogenous iodate application using seed priming and foliar spraying at different growth stages under drought stress and well-watered conditions. The study evaluated the physiological and morphological traits.

Chapter 6: Addresses the fourth objective through a field experiment that was conducted at Ukulinga Research Farm, Pietermaritzburg. The study aimed to assess the impact of iodate priming on growth, yield, and yield components in rainfed conditions.

Chapter 7: Concluding the research, the final chapter discusses the overall findings of the separate manuscripts and their relevance to the study's aim. Additionally, the chapter offers suggestions for possible future studies.

CHAPTER 2

POTENTIAL OF IODINE PRIMING UNDER ABIOTIC STRESSES TO IMPROVE SEED QUALITY, AGRONOMIC AND PHYSIOLOGICAL TRAITS: A REVIEW

ABSTRACT

Environmental factors such as drought, salinity, nutrient deficiency and temperature are the leading causes of agricultural production and seed quality losses around the world. They also cause adverse physiological and biochemical changes in plants. Techniques that can induce stress tolerances, enhance plant growth and crop quality are needed to counteract the negative effects of constrained production environments. One of the techniques that have been promising in modern crop production is nutrient seed priming, particularly iodine. Iodine as one of the nutrients has been associated with increasing concentration of osmolytes, antioxidants that stimulates tolerance to stress. This mini review highlights the possible potential of seed priming with iodine as an option to increase germination, plant growth, yield, and seed quality under abiotic stress of crops. The physiological, biochemical, and morphological changes induced by iodine application in crops are also discussed based on the available data. There is a lack of knowledge about iodine seed priming under abiotic stress, and more research is required.

Keywords: Iodine; seed priming; abiotic stress; seed quality; plant growth

2.1. INTRODUCTION

Globally, agriculture faces a significant challenge to ensure an adequate food supply while maintaining high productivity and quality standards due to population growth and environmental constraints affecting crop productivity (Masipa, 2017). Various environmental constraints such as drought, temperature, heavy metals, nutrient deficiency, and soil pH, pose severe effects on plants. They affect germination, emergence uniformity, seedling developments, plant growth, and yield (Hasanuzzaman et al., 2018; Sharma et al., 2015), which impact crop quality and quantity. Moreover, they also cause changes in physiological and biochemical processes, which lead to the development of reactive oxygen species (ROS), which in turn lead to membrane damage, cell leakage, and destruction of photosynthetic apparatus during plant growth (Kabiri et al., 2014).

In In order to meet these challenges, several approaches have been used to alleviate the constraint of abiotic stresses and improve seed quality. Reportedly, one of the approaches is the use of seed treatments such as seed priming. Seed priming is the process of soaking seeds in a hydration solution to allow pregermination metabolism but not actual germination (Jisha et al., 2013). It is a cost-effective method that enhances physiological and biochemical processes involved in seed germination, establishment, and improves plant growth at normal or under abiotic stress (Farooq et al., 2019; Singh et al., 2020). Various types of priming techniques are used including hydropriming, biopriming, nutrient priming, hormonal priming, redox priming, etc. These well-documented techniques have positively influenced germination, plant growth, and yield (Farooq et al., 2019; Jisha et al., 2013; Paparella et al., 2015). Studies have shown that apart from the physical and chemical factors (e.g., temperature, duration, osmotic, water potential, and aeration), the efficiency of priming is mainly determined by the priming technique used and the treated plant species (Varier et al., 2010). In this context, a better technique that has gained more attention recently is the use of nutrient seed priming (Farooq et al., 2019; Groot, 2020a; Mondal & Bose, 2019; Waqas et al., 2019). This technique involves soaking seed in a nutrient solution instead of water and aims to improve seed quality and seed nutrients for higher germination and seedling development which may increase yield.

Germination and early optimum establishment are the primary aspects associated with an increase in productivity (Farooq et al., 2009b). In seeds, nutrients in reserves play a crucial role in all phases of plant life and regulate physiological and biochemical functions of plant metabolism (Boelt, 2018; Imran, 2012). Several researchers have revealed the critical roles of micronutrients such as B, Cu, Fe, Mn, and Zn and these play an eminent role in seed development, seedling vigour, and yield by being involved in plant metabolism, nutrient regulation, reproductive growth, chlorophyll synthesis, carbohydrate production, fruits and seed development (Farooq et al., 2019; Mondal & Bose, 2019; Pawar & Laware, 2018). Nevertheless, little has been shown about iodine influencing plant growth and improving seed quality.

In the past two decades literature, a substantial part of iodine application in the past literature reports over the past two decades have focused mainly on (1) increasing iodine in higher plants through biofortification in human and animals' health (White and Broadley, 2009) (2) simultaneous application of iodine with other elements (e.g., selenium) (Golubkina et al., 2021) (3) types of application (soil, foliar and hydroponics) and chemical forms and their effective doses (Dávila-Rangel et al., 2019) (4) iodine effect in physiological traits (antioxidants) (Gonzali et al., 2017; Riyazuddin et al., 2023) (5) uptake, distribution and accumulation of iodine in plants (6) iodine relations with other elements within the crop plants (Augustine and Archives, 2020), and (7) biofortification of iodine through breeding and metabolic engineering in plants (Gonzali et al., 2017) (**Error! Reference source not found.**). While this review complements their finding with recent reports, it also focuses on the potential of seed priming with iodine under various environmental conditions to improve seed quality and productivity. This review also discusses iodine's potential for agronomic and some physiological traits (e.g., photosynthesis, chlorophyll pigments, some osmolytes) in improving productivity.

Table 2.1 Selected evidence of recent research on the use of iodine in biofortification

Focus	Title / Scope of reviews viewed	Reference
Human and crops	This article reviews aspects of soil science, plant physiology and genetics underpinning crop biofortification strategies, as well as agronomic and genetic approaches currently taken to biofortify food crops with the mineral elements most commonly lacking in human diets: iron (Fe), zinc (Zn), copper (Cu), calcium (Ca), magnesium (Mg), iodine (I) and selenium (Se).	White and Broadley (2009)
Crops	The possibilities for agronomic biofortification with individual micronutrients were discussed. The suitability of different mineral micronutrients for this approach is reviewed	Lyons and weh(2012)

Different crops and humans	The review aimed to provide an overview of the biofortification of iodine, presenting the progress in this important area of agricultural research. Information included was about the possible alternative use of iodine as an inductor of abiotic and biotic tolerance	Medrano-Macías et al. (2016a)
Crops	The review described the current knowledge regarding iodine physiology in higher plants and provides updates on recent agronomic and metabolic engineering strategies for biofortification	Gonzali et al. (2017)
Cereal crops and humans	The review focused on agronomic biofortification of cereals with selenium and iodine, and explore the proposal that simultaneous application of these micronutrients has the potential to reduce hypothyroidism and related iodine deficiency disorders (IDD) in areas with concurrent Se and I deficiencies	Lyons (2018)
Crops	The main focus was on the positive effects of beneficial elements on plant performance in restrictive environments and discussing some of the challenges of using these elements as biostimulants.	Gómez-Merino et al. (2018)
Different plants	It discussed the main issues related to the cultivation of plants enriched with iodine	Krzepilko et al, (2019)
Crops	This review showed the progress made in obtaining crop plants biofortified with iodine, showing the different techniques used and the results obtained.	Dávila-Rangel et al. (2019)
crops	The approach related to different micronutrients is reviewed. The efficacy studies of various elements like iron (Fe), iodine (I), cobalt (Co), Zinc (Zn) and Selenium (Se) and their concentration in different parts are also viewed	Augustine and Archives (2020)
Crop	This mini review presents the consequences of inadequate and excess iodine intake, the current status of iodine supplementation and the most recent alternative methods of the application of iodine in agriculture and its effect on the quality of used plant species.	Duborská et al (2021)
Crops	The present review discusses the outcome of joint plant Se–I biofortification, as well as factors affecting Se and I accumulation in plants, paying special attention to unsolved issues. A particular focus has been given to the prospects of herb sprout production enriched with Se and I, as well as the interactions between the latter microelements and arbuscular-mycorrhizal fungi (AMF).	Golubkina et al. (2021)
Crops	In this review, the role of iodine as a micro-nutrient and its interaction with phytohormones during the regulation of growth, development, and environmental stress tolerance in plants was discussed.	Riyazuddin et al. (2023)
Crops	This review aimed to elaborate on the role of iodine in plant: A commentary on benefit of this element	Nascimento et al. (2022)

2.2. Seed quality

Seed quality is the performance of a seed lot under a wide range of environmental conditions (Biemond, 2013). It is a crucial component impacting early performance and productivity of most crops, including wheat (Burris, 2015). Seed quality is characterized by the whole genetic quality (quality purity and longevity), physical purity (size and moisture content), physiological (germination capability, viability, and vigour), and seed health (pest and disease status) (Groot, 2020a; Perry, 1980). However, physiological indices such as germination capability (viability) and seed vigour are the most significant parameters (Domin et al., 2019; Odindo, 2007). Seed viability is the capacity of the embryo to germinate under favourable conditions, given that dormancy in the seeds is broken. It is regulated by predator and pathogen damage and environmental factors (Domin et al., 2019). It is regulated by predator and pathogen damage and environmental factors (Biemond, 2013). While seed vigour is defined as the indication of seed that gives the seed the potential for speedy and uniform emergence and development of normal seedlings across a wide range of field conditions. A number of studies have shown that high-quality seeds produce improved seedlings and high-yielding crops, as seeds are stimulated by faster and superior germination under both limited or normal environments (Elias et al., 2006; Peñaloza et al., 2005; Ventura et al., 2012). In contrast, poor quality seed tends to generate seedlings with low vigour, and low germinating rate ultimately leading to low yield (Elias et al., 2006; Peñaloza et al., 2005). Other researchers have shown that the properties that determine vigour in seeds include genetic background, storage, and environmental conditions during seed development (Peñaloza et al., 2005; Ventura et al., 2012). Environmental factors such as temperature, photoperiod, water deficits, and soil fertility are significant as they are the leading causes of major effects on seed quality (Ventura et al., 2012).

2.2.1. Environmental influence on seed quality

It is widely recognized that differences in environmental factors like water, nutritional status, temperature, and photoperiod can influence seed quality (Figure 2.1). These parameters are consistently related to changes in seed size, weight, germination capacity, seedling establishment, and yield (Madakadze & Kwaramba, 2004). It has been established that temperature impacts seed quality components such as dormancy, seed mass, and germination (Edwards et al., 2016; Peltonen-Sainio et al., 2011). Two scenarios commonly occur; for example, an increase in temperature leads to a decrease in seed mass, resulting in fewer dormant seeds and enhancing germination capacity. In the other species, it may limit the duration of seed filling, resulting in more dormant seeds, and could impair seed germination and vigour (Hampton et al., 2013). For example, Sershen et al. (2014) revealed that high temperature lowers germination but boosts seedling vigour in Natal mahogany *(Trichilia emetic)*. Grass and Burris (1995) observed that high temperature resulted in low values of both seed yield and physical attributes of seed quality in wheat *(Triticum durum*). Low temperature (typically at 4 °C) disrupts seed dormancy and enhances seed germination (Yan & Chen, 2020).

In plants, drought stress effect depends on its severity, time of stress (growth phases), and species (Blum, 2011; Farooq et al., 2009b; Khan et al., 2019a). Severe drought stress during seed development causes shrunken, light, and irregular form seeds, leading to impaired seed germination and vigour, which causes poor seed quality (Sehgal et al., 2018). It can also have little effect on seed quality. For example, Vieira et al. (1992) showed that drought did not influence seed quality in soybeans *(Glycine max)*, although yields were lowered. During seed filling, drought stress damages rice (*Oryza sativa)* seeds by promoting premature senescence and lowering the grain filling duration and seed production (Sehgal et al., 2018). Such situations arise due to diminished photosynthesis (Blum, 2011). A study by Bayu et al. (2006) demonstrated that drought stress affected the rate of germination, emergence, and early seedling growth of sorghum (*Sorghum bicolor)*. Similarly, Batool et al. (2014) observed that drought stress during germination led to reduced germination percent and rate, radicle length plumule, and crop stand in maize *(Zea mays)*.

Finding suitable areas for growing crops to yield good quality seeds is crucial, and the selection is dependent on soil attributes such as texture, pH, and fertility of the soil. In general, low soil fertility tends to yield low robust plants (Madakadze & Kwaramba, 2004). In maize (*Zea mays)*, Dlamini (2015) found that employing macronutrients (nitrogen, phosphorus, and potassium) in deficient soils resulted in reduced seed viability. Similarly, multiple studies have indicated that soil with micronutrient deficits (boron, zinc manganese) tends to induce distinctive damage, which can impair seed quality. For instance, Akgün et al. (2015) observed that zinc-deficient soil affects yield and lower quality of bread and durum wheat varieties. While Rashid and Ryan (2004) revealed that farming in boron-deficient soil leads to aberrant seedlings and causes a decrease in photosynthetic rate. The issue that may also affect low vigour in seed size or plants is the increase in competition for limited nutrients by weeds or crops owing to restricted spacing. Elmore and Lackobs (1984) revealed that these fluctuate with the amount of competition among plant species.

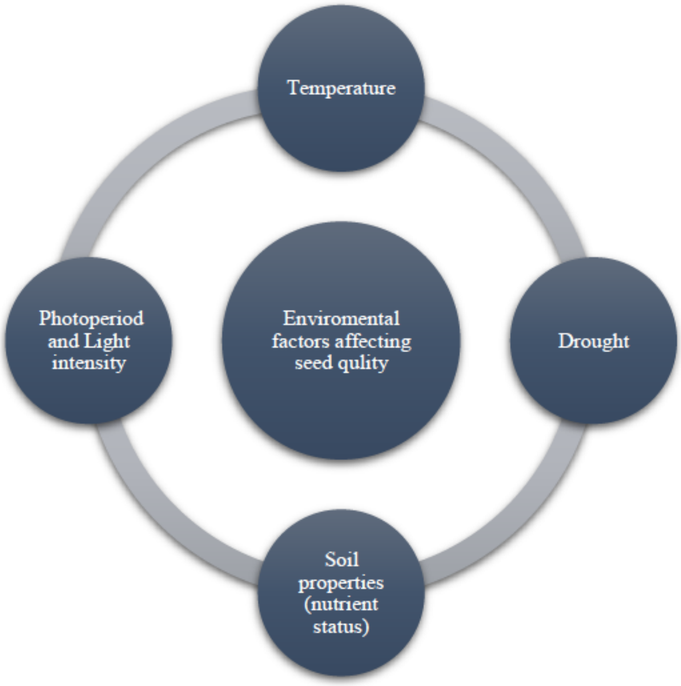


Figure 2.1. Major environmental factors affecting seed quality. Adapted from: (Madakadze & Kwaramba, 2004).

Seedling vigour, growth, and yield are also regulated by photoperiod and light intensity. Photoperiod is known to increase biomass accumulation, flower bud differentiation, and metabolite synthesis by modifying the timing of photosynthesis (Adams and Langton, 2005). While light intensity plays a key function in photosynthesis and photo-morphogenesis (Avercheva et al., 2009), it simultaneously serves as a developmental signal that triggers photomorphogenetic processes, directing plant structural adaptation through light-responsive mechanisms that regulate critical growth stages including germination and flowering. Longer photoperiod leads to more exposure to light, which in turn enhance photosynthesis, leading to higher seed output (Hendriks et al., 2003). A shorter photoperiod leads to reduced yield and yield components due to quicker flowering (Cho et al., 2017). For example, a longer day length promotes higher production, whereas a short-day length increases the weight and the number of seeds (Yunze & Shuangsheng, 2014). Contrary, low light intensity reduces growth, development, yield, and seed size due to limited photosynthetic capacity, thereby restricting assimilate partitioning. Conversely, excessive light intensity causes photoinhibition through diminished efficiency in both Photosystem II (PSII) and Photosystem I (PSI) (Yang et al., 2012). Such effects have been seen in numerous crops, including maize (*Zea mays)* (Kiniry & Ritchie, 1985), perennial ryegrass (*Lolium perenne) (Bean, 1980)*, clover (*Trifolium repens*) (Collins, 1978), and wheat *(Gascogne.)* (Khabiri & Jafari, 2012). Overall, these actions can induce an unfavourable change in physiological and biochemical processes during germination and seedling growth. Therefore, such environmental conditions make it valuable for researchers to create techniques to boost production.

2.3. Seed priming, mechanism of priming and its benefits

Seed priming is the process of soaking seeds in a hydration solution to allow pregermination metabolism without enabling actual germination (Jisha et al., 2013). Several studies have shown that seed priming increases early germination, promotes rapid and uniform emergence, breaks dormancy, stimulates early flowering and maturity, and improves tolerance to environmental factors such as drought, salinity, and fungal diseases such as *Sclerotium rolfsii L.* (Boelt, 2018; Farooq et al., 2019; Kasim et al., 2016; Pawar & Laware, 2018; Singh et al., 2015). Principally, the germination process is triphasic; Phase I) imbibition stage, Phase II) lag phase, and Phase III) growth (Sarkar et al., 2009; Toole et al., 1956). There is a rapid water uptake by dry seeds in phase I until the seed tissues are fully hydrated. In lag phase II, there is limited water uptake, and the activation of metabolic and physiological processes associated with germination processes such as mitochondria formation, protein synthesis, and the modification of soluble carbohydrates becomes stimulated (Varier et al., 2010). Phase III signifies the completion of germination, and the seedling starts with the resumption of radicle emergence and growth. However, seeds can undergo just phases I and II of the germination processes during priming. Phase II requires a lengthy time to accomplish metabolic activities and prevents phase III. After priming, drying of seed enables storage of seeds to prevent quality loss caused by rapid deterioration (Di Girolamo and Barbanti, 2012). Thereafter, seeds complete the germination process faster upon sowing whenever provided with water (

Figure 2.**2**). Gurusinghe and Bradford (2014) and Ibrahim (2019) point out that the period of storing primed seeds till time of sowing is determined by seed longevity and desiccation tolerance. For example, rapid dehydration may affect the soluble glucose levels, which reduces the resistance to dehydration and longevity.

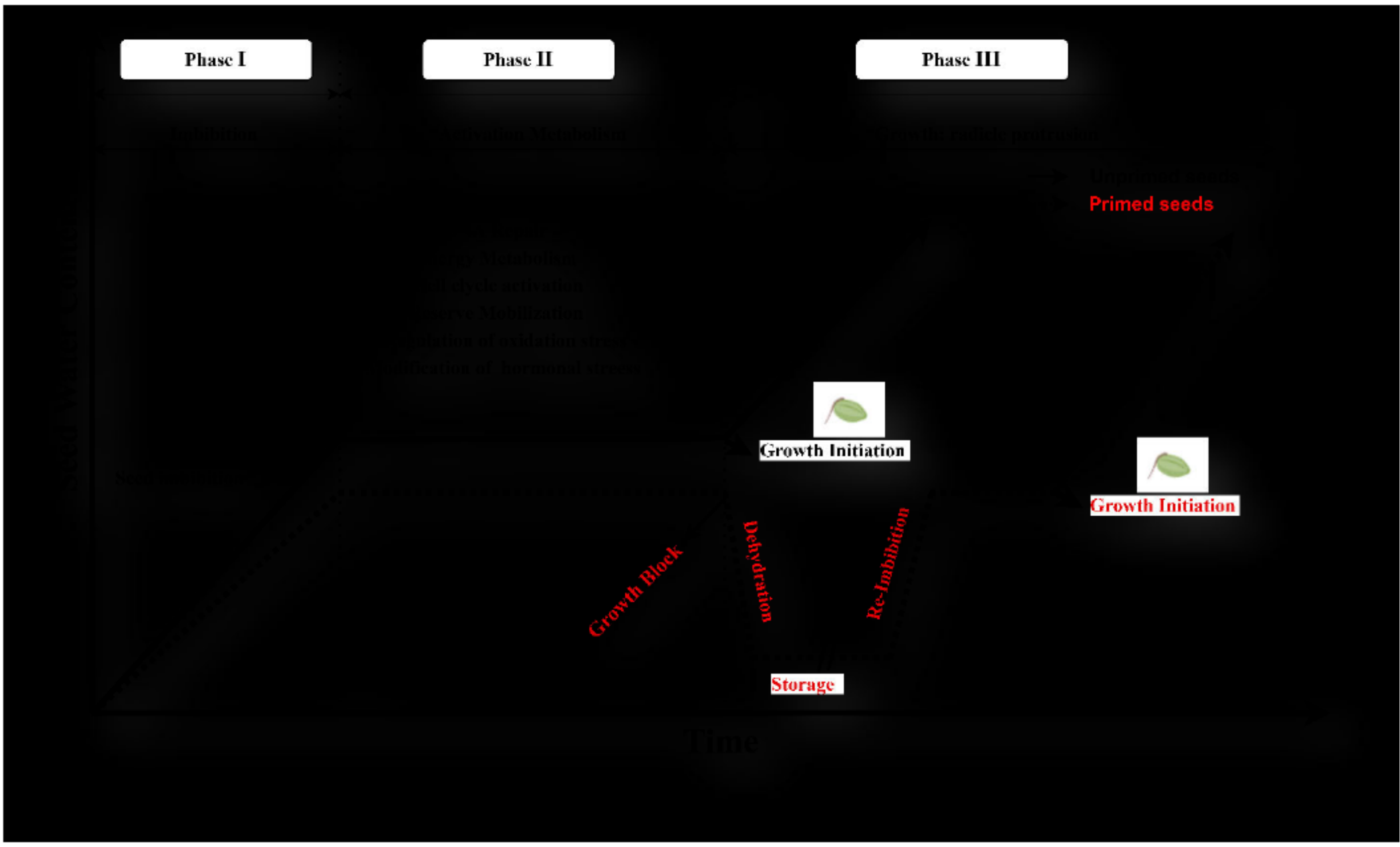


Figure 2.2. Diagram showing normal germination, seed priming process and time course of major events during germination and subsequent post-germination growth. Modified from: (Lutts et al., 2016; Marthandan et al., 2020).

According to Rakshit et al., (2020), priming seeds accelerate the signals for germination processes such as gene transcription factors, respiration, endosperm weakening, and early mobilizations of reserves to allow the modification of quiescent seeds into germinating state, which will translate into improving germination potential and increased yield. Moreover, physiological activities such as activation of the DNA repair system and antioxidant mechanisms occur early in seed imbibition to assert genomic integrity and ensure fast and uniform germination and seed development (Jisha et al., 2013; Paparella et al., 2015). Seed priming studies have been reported in various crop species such as wheat *(Triticum aestivum)* (Ali & Elozeiri, 2017; Pal et al., 2017), maize *(Zea mays)* (Dezfuli et al. 2008), chickpea *(Cicer arietinum)* (Kamithi et al., 2016), rice *(Oryza sativa)* (Waller et al., 2016), and sugar beets *(Beta vulgaris)* (Sadeghian & Yavari, 2004). In these investigations, early germination, uniform, and quick stand establishment were associated with enhanced yield. Moreover, the success depends also on the techniques used, which include hydropriming, biopriming, nutripriming (nutrient priming), osmo-priming, chemical priming, seed priming with plant growth regulators, priming with plant extracts, seed priming through nanoparticles, and priming through physical agents (Jisha et al., 2013; Paparella et al., 2015). However, the focus will be on nutrient priming, particularly with iodine.

2.3.1. Nutrient priming

Nutritional priming is the soaking of seeds in nutrient solutions instead of water (Paparella et al., 2015). It is one of the approaches that has gained attention to boost the availability of micronutrients in seeds (Abbas Khan et al., 2021; Majda et al., 2019; Reis et al., 2018). It combines the effect of seed priming with an increase in nutrient supply in the seeds. In crops, using micronutrients including iodine as a priming agent is very advantageous, as it does not only increase stand establishment, growth, water uptake efficiency, yield, and stress tolerance, but it also contributes to decreasing malnutrition as it enriches grains with nutrients (Mondal & Bose, 2019). Research on micronutrient priming has largely focused on boron, zinc, iron, and manganese under normal or poor environmental conditions (Carmona et al., 2020; Ramzan et al., 2020; Rehman & Farooq, 2016; Sirohi et al., 2015; Ullah et al., 2019; Zulfiqar et al., 2020). Few studies have used iodine as a priming agent, notably in cereals and in those studies; it is primarily used in combination with other elements.

2.4. Iodine

2.4.1. Iodine application

Different ways have been utilized to supply iodine in various crops; this includes foliar spraying, hydroponics, soil application, seed treatments, and irrigation water. The negative effect of using soil application is that higher doses are required due to leaching within the soil, such as iodine sorption, which is closely associated with fixation of both non-organic and organic fractions (copper, aluminium; humic, and fulvic acids), respectively (Fuge and Johnson, 2015). Contrary, hydroponics has been effective for leaf vegetables because of its rapid solubility (Sharma et al., 2019). Foliar application provides various advantages, including lower fertilizer use, no iodine fixation, and no negative nutritional influence on iodine absorption compared to soil treatment. However, it is also expensive and labour intensive, especially if one must apply fertilizer frequently (Medrano-Macías et al., 2016; Praharaj et al., 2021). Moreover, it is greatly dependent on the type of fertilizer used and the properties of the crop such as leaf qualities and genetic potential (Medrano-Macías et al., 2016). Seed treatments (i.e. seed priming and coating) are easier to apply, cost-effective, and less time-consuming. Nevertheless, there is still scant evidence about the effect of iodine in crops utilizing seed priming or coating on plant development and productivity.

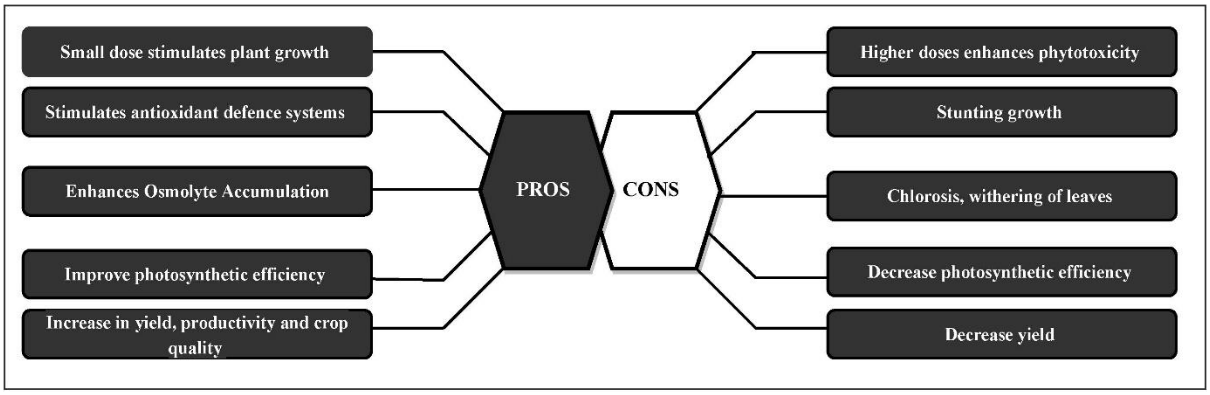


Figure 2.3. Diagram depicting possible morphological and physiological pros and cons of iodine application in crops (Modified from: (Gómez-Merino & Trejo-Téllez, 2018).

2.4.2. Effect of iodine on crop growth and yield

It has been proven that the early phase of crop growth and establishment is critical to produce optimum yield. Iodine has been reported to alter plant growth and yield (Finch-Savage & Bassel, 2016). For instance, Golob et al. (2020a) evaluated the effect of iodine in pumpkins (*Cucurbita pepo*) through the soaking technique. The results demonstrated that iodine soaking decreased seed germination, seedling mass, and yield. Similarly, Germ et al. (2019) also tested soaking influence on growth in buckwheat (*Fagopyrum esculentum*) and results showed that iodine considerably affected seed germination and yield compared to untreated seeds. Jerše et al. (2017a) observed a decrease in plant height and sprout mass after soaking peas *(Pisum sativum)* seeds in 1000 mg L^{-1} iodate and selenium solution.

Other research has been largely focused mainly on foliar, soil application, and hydroponics, and a great variation occurs among the methods. For example, hydroponics research has demonstrated that iodine in the nutrient solution enhances biomass in crops such as spinach (*Spinacia oleracea*), lettuce (*Lactuca sativa)*, chinese cabbage *(Brassica oleracea*), soybean *(Glycine max)*, and rapeseed (*Brassica napus*) (Blasco et al., 2013a; Borst Pauwels, 1961; Dai et al., 2004; Mao et al., 2014; Weng et al., 2003; Zhu et al., 2003). In *Arabidopsis* plants in a nutrients solution in a floating system revealed an improvement in biomass and seed production, combined with a very clear hastening of flowering when applying plants with iodate or iodide (0.2 or 10 mM) (Kiferle et al., 2021). Moreover, Li et al. (2017) noticed an increase in biomass and quality in strawberries (*Fragaria ananassa*) when iodate doses of less than 0.10 and 0.25 mg L^{-1} iodine were utilized. Dobosy et al. (2020) pointed out that applying iodate at concentrations of 0.1 and 0.25 mg/L boosted growth in both bean (*Phaseolus vulgaris)* and lettuce *(Lactuca sativa)*, although higher concentration, higher than 0.50 mg/L, resulted in smaller leaf surface and same biomass when compared with the control. However, there have been studies that observed a decrease in biomass, for example, in carrots *(Daucus carota)* (Smoleń et al., 2014), lettuce *(Lactuca sativa*) (Smoleń et al., 2011), tomatoes

(*Solanum lycopersicum),* and potatoes (*solanum tuberosum*) (Caffagni et al., 2011). Studies conducted in the soil have also shown mixed effects, such as an increase and decrease in biomass (Dai et al., 2004).

There have also been results demonstrating no influence on plant growth or yield. These results have been recorded in diverse crops such as potato *(solanum tuberosum)* (Ledwożyw-Smoleń et al., 2020), Kohlrabi (*Brassica oleracea)* (Golob, Novak, et al., 2020), tomato (*Solanum lycopersicum),* and cabbage *(Brassica oleracea* var. *capitata)* (Dobosy, Endrédi, et al., 2020). Overall, based on the data, it can be stated that low application of iodine stimulates plant growth, and high application promotes impairment over a specific threshold that becomes harmful depending on the plant species based on the data by Lawson et al., (2015). In addition, the application of iodine on plant biomass is directly dependent on the amount applied the type of iodine fertilizer utilized and applied methods. Lastly, there is a need to perform more studies on iodine using seed treatments such as seed priming as both soil and foliar spray are expensive for small-scale farmers.

2.4.3. Physiological effect of iodine application

In addition to morphological effects, iodine also influences the physiology of plants. Physiological processes that are impacted by iodine in plants include photosynthesis, chlorophyll pigments, accumulation of osmotically active solutes, nutrient relations, and accumulation of both enzymatic and non-enzymatic antioxidants.

2.4.3.1. *Photosynthesis*

Photosynthesis contributes greatly to plant growth and is the foundation of all agricultural yields. It is controlled by complex networks of proteins such as photosystem I and photosystem (II) regulated by ATP synthase complex and cytochrome b6/f complex (Yamori et al., 2011). These proteins are extensively impacted under abiotic stress conditions (Yamori et al., 2011). They influence photosynthetic pigment, soluble proteins, electron transport chain, protein in the thylakoid membrane, and carbon dioxide fixation, which impedes plant growth (Chaves et al., 2002). Studies have shown that micronutrients influence photosynthesis through diverse ways of action. For instance, manganese has been proven to be involved in energy transmission, photosynthesis activities, and chlorophyll production (Lidon et al., 2004). At the same time, boron plays a critical function in carbohydrate metabolism, membrane integrity, calcium uptake, and blooming (Zewide and Sherefu, 2021). In addition, zinc is involved in photosynthesis, enzyme activation systems, protein systems, and carbohydrate metabolism (Ilyas et al., 2015; Roach & Krieger-Liszkay, 2014). On the other hand, although few reports have revealed that iodine modulates photosynthesis processes, little information has been shown on different crops. Venturi (2011) and Medrano-Macías et al. (2016) have suggested that iodine was one of the first inorganic antioxidants used by photosynthetic organisms that marine algae utilize during periods of oxidative stress and a similar phenomenon may occur in terrestrial plants. A study conducted by Blasco et al. (2011) demonstrated that applying iodine in low concentration increases net assimilation of photosynthesis by increasing stomatal conductance and transpiration rate in lettuce (*Lactuca sativa*) plants. Moreover, a recent study assessed the roles of iodine in *Arabidopsis* plants demonstrated that iodine is essential in plants metabolism, i.e., it stimulates numerous iodinated proteins which are constituents of photosystem II (PSII), photosystem I (PSI), cytochrome b6f (Cyt b6/f) and light-harvesting complex II (LHCII) and plastocyanin, which are involved in photosynthesis processes (Kiferle et al., 2021). A recent study by Lima et al. (2023) evaluated the effect of soybean (*Glycine max)* on different concentrations of potassium iodide (KI) under water deficit and the results showed that KI promoted photosynthetic improvements under adverse conditions and concluded that iodine may directly affect plant tolerance to water deficit.

2.4.3.2. *Chlorophyll fluorescence*

Chlorophyll Chlorophyll is a green pigment found in chloroplast cells that is necessary for regulating photosynthesis, which aid the plants in absorbing energy from the sun. Chlorophyll fluorescence (CF) is an indirect tool for the physiological status of chlorophyll and used to determine photosynthesis performance (Areington, 2016; Murchie & Lawson, 2013). Moreover, CF measures the light that is reemitted from photosystem II. Upon measuring CF, various parameters are considered, such as the use of F_o (minimum value for chlorophyll fluorescence when PSII has been exposed to light), F_m (maximum value for chlorophyll fluorescence when PSII has not been exposed to light), F_v difference between (variable= F_m-F_o), which can be used as an indicator of stress in plants (Murchie & Lawson, 2013). For example, F_v/F_m ratio is the generally used calculation, and it is used to measure maximum quantum yield of PSII under dark adaption conditions. Higher $F_v/F_m >$ ~ 0.8 shows that leaves are healthy whole lower, indicating that plants have been undergoing stress (Murchie & Lawson, 2013). Dobosy et al. (2020) researched iodine build-up and employed diverse soil (sand, sandy silt, and silt) irrigation water in potatoes *(solanum tuberosum)*. The study revealed that photosynthetic efficiency and chlorophyll of potato plants grown in silt soil improve greatly when compared with other soil. In tomato (*Solanum lycopersicum)* and cabbage *(Brassica oleracea* var. *capitata)* plants, Dobosy et al. (2020) also evaluated absorption and translocation when iodine and selenium were given using different soil in lettuce *(Lactuca sativa)* and green bean (*Phaseolus vulgaris)*; the results revealed photosynthetic efficiency and chlorophyll content had no significant effect. Germ et al. (2019) noticed a small alteration (0.78 to 0.80) in F_v/F_m ratios, and chlorophyll-a was moderately stimulated when Buckwheat (*Fagopyrum esculentum*) microgreens were soaked in water containing iodine (1000 mg/L I^- or IO_3^-). In literature, the effect of iodine treatment on photosynthetic efficiency in various crops is poorly researched.

2.4.3.3. *Accumulation of osmolytes*

Proline and sugars

Biosynthesis and accumulation of various osmolytes are known as the early response of plants for withstanding osmotic and oxidative stress generated by different stimuli (Jogawat, 2019). These osmolytes include glutamate, proline, glycine betaine, and sugars; among these, proline and sugars are mainly researched, especially under stress conditions. Studies have indicated that osmolytes are important in maintaining cell osmotic balance, stabilizing membranes to prevent electrolyte leakage, and functioning as an antioxidant to regulate the level of reactive oxygen species (ROS) (Ahanger et al., 2018). In higher plants, proline biosynthesis occurs from the cytoplasm in glutamate and ornithine pathways. Through glutamate pathway, 1-pyrroline-5-carboxylic acid (P5C) is converted by two enzymes, P5C synthetase (PC5S) and P5C reductase (PC5R), to proline. While in ornithine, P5C is catalyzed by the ornithine aminotransferase (OAT) enzyme (Ahanger et al., 2018).

Accumulation of sugars also plays a critical role in osmotic adjustment, carbon storage, and free radical scavengers (Ahanger et al., 2018; Ashraf & Foolad, 2007). Many studies have revealed that the build-up of sugars is a crucial protective response to abiotic stress. During stress, carbohydrates stored by higher plants operate as osmolytes to maintain the cell turgor and protect membrane and protein structure (Ahanger et al., 2018; Ashraf & Foolad, 2007). In pea *(Pisum sativum)* seedlings, Jerše et al. (2017a) found that soaking seeds in iodine solution did not have an impact when compared with control in photochemical efficiency of PS II and photosynthetic pigments such as chlorophyll-a, b, lutein, neoxanthin, violaxanthin, antheraxanthin, and carotene. Wojciechowska et al. (2021) found out that iodide salt affects the efficiency of photosystem II in basil *(Ocimum basilicum*) leaves but does not influence the quantity of photosynthetic pigments. Studies indicating no substantial influence on total soluble sugars have also been shown in radishes (*Raphanus sativus*) (Strzelecki et al., 2010), carrots *(Daucus carota)* (Smoleń et al., 2014), and lettuce *(Lactuca sativa)* (Blasco, Rios, et al., 2011). On the other hand, Smolen et al. (2016) reported a build-up of sugars in lettuce *(Lactuca sativa)* when iodized. However, it should be mentioned that iodine was applied with the addition of selenium and salicylic acid in the solution (Smoleń, Kowalska, et al., 2016). Recently, Krzepiłko et al. (2021) . examined potassium iodide (KI) application at doses of 0.15, 0.30, 0.75, and 1.5 $mg·g^{-1}$ on two radish cultivars (Warta and Zlata). The results demonstrated differential cultivar responses: soluble sugar content remained unaffected in Warta compared to untreated controls, while Zlata exhibited significant reductions in soluble sugars across tested KI concentrations. In this study, it was concluded that the iodine effect depends on the dose of iodide and radish cultivars.

2.4.3.4. *Antioxidants and tolerance to stress*

In Table 2.2 we summarize the environmental factors also require the development of reactive oxygen species (ROS) in the cells of plants, which regulates metabolic processes activated in response to oxidative stress, causing damage or disturbance to plant development, photosynthesis, and biochemical processes. Kabiri et al. (2014) found that ROS build-up damages to protein, membrane lipids, and other biological components. These consequences ultimately lead to cell death. Examples of these ROS components are singlet oxygen (O_2), superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH) (Kabiri et al., 2014; Munné-Bosch & Peñuelas, 2003). To respond to such consequences, plants developed antioxidant defense mechanisms as an adaptative strategy (Gonzali et al., 2017). These include accumulating enzymatic and non-enzymatic antioxidants (Blokhina et al., 2003; Wang et al., 2000). Enzymatic antioxidant systems include superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POX), glutathione reductase (GR), etc.

Non-enzymatic antioxidant systems include ascorbate acids (AA), glutathione (GSH), α-tocopherol, carotenoids, and phenolic chemicals (Blokhina et al., 2003). These antioxidants protect plants by detoxifying ROS or from oxidative stress by direct scavenging. Several researchers have demonstrated that I biofortification has a favourable effect on antioxidant components; For instance, Blasco et al. (2008) reported an increase in the amount of AA, phenolics, GSH, SOD, and ascorbates peroxide when iodide and iodate were used in lettuce (*Lactuca sativa)*. Similarly, Incrocci et al. (2019) discovered higher total phenols and total antioxidant capacity (DPPH assay) in sweeter basil (*Ocimum basilicum*). The study of Medrano-Macías et al. (2016) carried out in greenhouse tomato (*Solanum lycopersicum),* seedlings indicated that foliar treatment of KI boosted ascorbate and glutathione levels while lowering superoxide dismutase activity (SOD). However, CAT, APX, and GPX's enzymatic activity were not altered. Smoleń et al. (2015) noted that applying 7.88 M IO_3^- to tomato (*Solanum lycopersicum),* plants boosted the level of ascorbic acid and total phenolic compounds. In water spinach (*Spinacia oleracea*), the application of iodide (I^-) increased ascorbic acid levels; however, iodate (IO_3^-) and iodoacetic acid (CH_2ICO_2) had significant detrimental effects (Weng, Yan, et al., 2008).

Research showing the influence of iodine under stress conditions have also been published, although are very limited (Table 2.2). Blasco et al. (2013) revealed that utilizing KIO_3 in doses of 20 and 40 µM improved the antioxidant response and raised the content of total phenolic compounds in lettuce during salt stress. In a greenhouse study on lettuce *(Lactuca sativa*), Levy et al. (2011) revealed that doses less than 40 µM of KIO_3 had favourable effect on superoxide dismutase (SOD) and ascorbate peroxidase (APX) and GSH and ascorbic acid (AA) under salinity stress (100 mM of NaCl). The study of Gupta et al. (2015) also reported an increase in antioxidants such as SOD, ascorbate peroxidase (APX), and glutathione reductase (GR) in soybean *(Glycine max)* under heavy metals stress (cadmium chloride). Ajiwe et al. (2019) investigated the role of iodine in the development of disease (Fusarium wilt) resistance in tomatoes. Two tomato accession were used namely: FUNAABTO/106 and FUNAABTO/123 and the iodine (KIO_3 and I_2^-) doses used were 0.5, 1, 2, 3 mM and 1, 2, 3, and 5 mM, respectively. The results showed that FUNAABTO/106 treated with 1 mM of KIO_3 had significantly lower disease severity whilst on the field, FUNAABTO/106 treated with 0.5 mM KIO_3 had the least (1.17) disease severity as well as higher yield. It was concluded that lower dose of iodine application reduced incidence and severity of wilt and increased the yield of tomato (*Solanum lycopersicum).*

Medrano Macias et al. (2021) investigated the effect of iodine base products (Q products and iodate) under normal, and salinity stress in strawberries and showed that Q base products increase APX, CAT GSH, and yield in fruits while iodate fertilizer increase GSH, APX activity as well as P and K under stress conditions. Perez-Salas and Medrano-Macías (2021) studied the effect of iodine application under salinity in tomato (*Solanum lycopersicum)* and the results showed an increase in iodine accumulation in foliar tissue when KIO_3 and KIO_3 + NaCl were used, with a positive correlation to the antioxidant potential. Additionally, a positive correlation between dry weight, fresh weight, number of leaves, stem width, as well as Ca, Na, and Cu, and a negative correlation between Mg, K, Fe, and Mn in seedlings subjected to salinity stress (NaCl and KIO_3 + NaCl). The study of Lima et al. (2023) investigated whether the application of I can improve the response to water deficit in soybean *(Glycine max)* plants, increasing the enzymatic activity of the antioxidant system as well as the photosynthetic efficiency, thus improving the productive capacity of plants. The results showed that KI enhanced the antioxidant defense system of the plants and promoted photosynthetic improvements under adverse conditions. It was concluded that iodine may directly affect plant tolerance to water deficit. These investigations demonstrate that iodine application is capable of boosting yield and quality of crops as well as inducing tolerance under abiotic challenges.

Table 2.2. Selected research publications on the impact of iodine on abiotic stress in various crops

Crops	Stress types	Effect	Reference
Tomato	Pathogen (Fusarium wilt)	Disease severity was significantly ($p < 0.05$) lower (1.00) in the greenhouse in pots containing FUNAABTO/106 treated with 1 mM KIO_3. On the field, FUNAABTO/106 treated with 0.5 mM KIO_3 had the least (1.17) disease severity.	Ajiwe et al. (2019)
Strawberry	Salt stress	The application of Q products increased (APX) and (CAT), (GSH) content and yield in fruit while the application of KIO_3 an increase in GSH and APX activity, as well as P and K concentrations, were obtained. In leaves, an increase in P, Ca, Mn and iodine accumulation was evidenced with the application of Q products and an increased concentration of ascorbic acid and iodine with KIO_3 treatments. In leaves, the application of Q products increased chlorophyll a, b and calcium.	Medrano-Macías et al. (2021)
Lettuce	Salt stress	Increase biomass and induced higher activity in the enzyme's shikimate dehydrogenase and phenylalanine ammonia-lyase as well as the lower MW phenol-degrading enzyme polyphenol oxidase. This increased hydroxycinnamic acids and derivatives in addition to total phenols, which appear to act as protective compounds against salinity	(Blasco et al. (2013)

Lettuce	Salt stress	Positive effect on biomass and raised the levels of soluble sugars while lowering the Na^+ and Cl^- concentrations as well as boosting the activity of antioxidant enzymes such as SOD, APX, DHAR and GR.	(Leyva et al. (2011)
Soybean	Heat and cadmium stress	Iodate (IO_3^-) can enhance the levels of non-enzymatic antioxidants, such as superoxide dismutase (SOD) and ascorbate peroxidase (APX), in response to severe cadmium chloride and heat stress.	Gupta et al. (2015)
Tomatoes	Salt stress	Positive correlation with the antioxidant potential with KIO_3 and KIO_3 + NaCl. negative correlation with P, N, and height. (NaCl and KIO_3 + NaCl), a positive correlation was found with dry weight, fresh weight, number of leaves, and stem width, as well as with Ca, Na and Cu, and a negative correlation with the content of Mg, K, Fe and Mn.	Pérez-Salas et al. (2021)
Soybean	Water stress	The exposure of KI (10 and 20 _mol L^{-1}) in soybeans increased plant biomass by an average of 40%. Furthermore, exposure to KI concentrations of up to 20 _M improved gas exchange (~71%) and reduced lipid peroxidation under water deficit. This was related to the higher enzymatic antioxidant activities found at 10 and 20 _M KI concentrations.	Lima et al. (2023)

2.4.3.5. *Nutrient relations*

Iodine has also been demonstrated to alter or influence other nutrients; A recent study by Dobosy et al. (2020) evaluated the translocation of iodine on the important nutrients when iodine was utilized in cabbage and tomato. The results showed a decrease in iron (Fe) and phosphorus (P), but Mg, Cu, Mn, Zn, and B did not exhibit any significant change in tomato (*Solanum lycopersicum)*. In contrary, in cabbage *(Brassica oleracea* var. *capitata)*, iron declined, and phosphorus and copper remained the same as a control treatment. Using both soil and foliar application, Smolen et al. (2011) showed that I enhanced the level of potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), and cadmium (Cd) and lowered the content of phosphorus (P), copper (Cu), and zinc (Zn) in lettuce *(Lactuca sativa*). Moreover, a hydroponic study in lettuce also observed decreased P, N, and K when using iodide and found the opposite when using iodate (Blasco et al., 2012).

A high concentration of 80 µM dm^{-3} might be accumulating more in the plant cell, which leads to toxicity in the plant cell in lettuce *(Lactuca sativa*). Smoleń et al. (2015) revealed that I concentration in lettuce *(Lactuca sativa*) was inversely linked with the level of K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Cd, and Pb. Similarly, Smoleń et al. (2011) observed that iodine in the form of iodide and iodate caused a decrease in K, Cu, Fe, S, Mo, Zn, and an increase in Na, Mn, Mg, and B in tomatoes (*Solanum lycopersicum)*. The study of Krzepilko et al. (2016) demonstrated an increase in K with higher dosages of KI applied. Other nutrients such as Ca, Zn, Fe, and Cu did not significantly alter, although Na, Mg, and Mn levels declined. Although there are scant studies on the effect of iodine and its interaction with other mineral elements, it is clear from these studies that mineral nutrients availability when I is applied can be synergistic or antagonistic and can depend on many factors such: as means of application, the dosage used, concentration growth stage, various cultivation technologies and plant species. Therefore, when conducting studies, especially under stressful conditions, these elements would be vital to consider.

2.5. CONCLUSION

With the uncertainty of environmental factors on plant growth and development, more research is required on physiology, cellular and biochemical changes to generate tolerance in plants to stress. Iodine has been found to trigger natural defensive systems to boost crop output and quality without any adverse effect on plants, given that it is applied at low concentrations. Evidence shows that basic and applied bioscience research focus has been mainly on plant metabolic response to iodine. Seed science research is still limited.

CHAPTER 3

EXPLORING THE EFFECTS OF IODATE PRIMING AND THE EFFECTIVE DOSE ON SEED

GERMINATION, VIGOUR AND SEEDLING DEVELOPMENT IN WHEAT (*Triticum aestivum*) GENOTYPES

Nkosinathi Hlatshwayo[1, *], Takudzwa Mandizvo[1], Albert Modi[2] and Tafadzwanashe Mabhaudhi[1,3]

[1] *Centre for Transformative Agricultural and Food Systems, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, P/Bag X01, Scottsville, Pietermaritzburg 3209, South Africa.*

[2] *Department of Biological and Environmental Sciences, Faculty of Natural Sciences, Walter Sisulu University, Mthatha 5100: amodi@wsu.ac.za*

[3] *Centre for Water Resources Research, School of Engineering, University of KwaZulu-Natal, Scottsville, Pietermaritzburg, South Africa,*

[4] *Centre on Climate Change and Planetary Health, London School of Hygiene and Tropical Medicine, London, United Kingdom.*

[*]Correspondence: nathi4722@gmail.com (N.H)

**Corresponding author:* nathi4722@gmail.com

Submitted to South African Journal of Botany (25 November 2024)

ABSTRACT

The study evaluated the effect of iodine priming and priming doses on seed germination and vigour parameters of three local wheat genotypes: PAN311, SST8135, and SST806. Wheat seeds were soaked in aerated iodine solutions of 0.001 and 0.01 M for 6 hours, with unprimed seeds serving as a control treatment. The experiment followed a 5 × 3 factorial design, arranged in a completely randomized with three replications. Iodine seed priming significantly improved the germination rate index (GRT), mean germination time (MGT), cold test, and seedling shoot length across the wheat genotypes. The unprimed SST806 genotype exhibited the fastest GRT at 40.12 seeds/day, while the slowest rate was observed in SST8135 treated with 0.01 M iodine (IO_3^-). The highest MGT (5.972 days) was recorded for PAN311 primed with 0.01 M, whereas the lowest MGT (5.641 days) was observed in the unprimed SST806 genotype. Correlation analysis revealed a positive relationship between most germination and vigour parameters, except for MGT and GRI, MGT and GP, AA and MGT, and MGT and CT, which were significantly but negatively correlated ($P<0.05$). in conclusion, the study highlighted the iodine priming can enhance seed performance, although its effect varies across genotypes. Further research on seed quality in various crops is needed to determine optimal iodate concentrations and their impact on seedling growth.

Keywords: Seed enhancement, genotype variation, seed development, agricultural productivity, crop establishment

3.1. INTRODUCTION

Wheat (*Triticum aestivum*) is an important staple crop after maize (*Zea mays*), with 68% carbohydrates, 15.4% proteins, 2.9% fat and 2.11% minerals (Hassan et al., 2019; Muhsin et al., 2021). It also contains vitamins (B and C) and calories. In addition to being a staple food for humans, wheat is widely used as animal feed and serves various industrial purposes, including the production of starch, straw, and alcoholic beverages (DAFF, 2010). Therefore, increasing wheat production is crucial to feed the increasing population. However, various factors such as poor seed quality, poor nutrient management, pests and diseases and climatic change have caused a decline in yield, particularly in regions like South Asia, sub-Saharan Africa, and parts of Europe and North America. (Nadew, 2018; Raza et al., 2019; Soares et al., 2019).

Seed quality is one aspect that enhances productivity and food security (Abebe & Alemu, 2017). Seed quality refers to seeds' overall health, viability, genetic purity and physical condition, which determine their ability to germinate, grow into healthy plants and produce high yields under favourable conditions (Paparella et al., 2015; Singh et al., 2020b). Seed quality can be improved through innovative seed technologies, such as using hybrid seeds and applying seed treatments like coating, priming or pelleting. Physical treatments, including magnetic exposure and plasma radiation, can enhance seed quality. (Groot, 2020b; Hanci et al., 2014; Sudhakar et al., 2020; Taylor et al., 2001).

Seed priming is cost-effective in enhancing seed germination, vigour, and quality (Hasanuzzaman & Fotopoulos, 2019). It involves soaking seeds in a specific solution allow pregermination metabolism without enabling actual germination (Sharma et al., 2015). This process optimizes seed performance during germination and early growth stages. Priming agents used include hydropriming (water), osmopriming (PEG6000), hormonal (growth-stimulating hormone), and nutrient priming (micro-macro nutrients) c. In recent years, using micronutrients as priming agents has gained traction in research, as they have been demonstrated to enhance seed germination, vigour and growth across various crops, such as chickpea (*Cicer arietinum*), wheat (*Triticum aestivum*), maize (*Zea mays*), and rice (*Oryza sativa*) (Arif et al., 2007; Farooq, Wahid, & Siddique, 2012; Saini et al., 2020; Ullah et al., 2018). However, little has been proven about the effect of iodine on enhancing seed quality.

Although iodine is not regarded advantageous for plants, it has played a crucial role in antioxidant metabolism, development and stress tolerance, notably at low concentrations (Medrano-Macías et al., 2016b). Studies highlighting the positive effects of iodine on growth have been demonstrated in various crops, including Chinese cabbage (*Brassica rapa*) (Weng et al., 2008), barley (*Hordeum vulgare)* (Borst Pauwels, 1961), beets (*Beta vulgaris)* (Borst Pauwels, 1961), and celery (*Apium graveolens)* (Dai et al., 2004). However, studies have also demonstrated the detrimental effect of iodine on various crops (Germ et al., 2020b; Jerše et al., 2017b). In recent decades, significant attention has been given to iodine for biofortification purposes in crops, mainly to boost its concentration in edible parts, combine it with other nutrients, and prevent iodine malnutrition (Gonzali et al., 2017; Li et al., 2017; Medrano-Macías et al., 2016b; Pearce et al., 2013). There is limited scientific knowledge on the effects of iodine on seed germination and vigour-related qualities, which are critical indicators of overall seed quality. Therefore, this study evaluated the effectiveness of different iodine priming concentrations and their impact on germination and vigour parameters in selected wheat genotypes.

3.2. METHODS AND MATERIALS

3.2.1. Plant material and location

The study used local wheat genotypes (SST8135, SST806 and PAN3111) sourced from Pannar Seed (-29.563633, 24.017855) and Sensako wheat breeding company (28.1856° S, 28.2149° E) based in South Africa. Genotypes were chosen based on agronomic characteristics, such as adaptability to cultivation in the dry, semi-arid parts of South Africa Table 3.1. Laboratory tests were carried out at the University of KwaZulu-Natal Seed Technology Laboratory (29°37'34.0"S 30°24'13.4"E).

Table 3.1: Characteristics, origin, and traits of wheat genotypes used in the study

Genotype	Characteristics	Breeding program/origin	Traits

SST8135	High yield potential, good disease resistance	South African breeding	Suited for high-rainfall regions, adaptable
SST806	Drought-tolerant, early-maturing	South African breeding	Suitable for low-rainfall areas, good quality
PAN3111	High protein content, rust-resistant	PANNARO breeding program	High-quality grain, suitable for bread-making

3.2.2. Experiment design, soaking methods and germination test

The study was designed as a 5 × 3 factorial experiment with 5 iodate (IO_3^-) concentration levels [0. 0.001, 0.01, 0.1, and 0. 5 M] and 3 wheat genotypes [PAN 3111, SST8135 and SST 806] levels. The experiment was set in a completely randomized design (CRD) with three replications, giving 45 experimental units. For all genotypes, except controls, seeds were immersed in IO_3^- solution for 6 h, as described by Golob et al., (2020b). During soaking, an aquarium pump was used for aeration. The seeds were then rinsed with distilled H_2O thrice. Thereafter, the treated seeds were surface-dried to match their original moisture content at 25 °C. Twenty-five treated and dry seeds (control) were placed in a moist germination paper and incubated in a germination chamber set at 22-25 °C for 10 days for standard germination (ISTA, 1993). Germination was assessed daily by counting seeds showing radicle protrusion for up to 10 days. The final germination recording was on the 11th day and was based on the normal and abnormal seedlings.

Germination parameters were calculated based on mean germination time (MGT), germination percentage (GP) and germination rate index (GRI) (Table 3.2). Seedling vigour traits studied included accelerated ageing (AA), electrolyte conductivity (EC), cold test, wet weight, dry weight, root length (RL), shoot length (SL) and vigour index (VI) (Table 3.2).

Table 3.2: Summary of formulas used to calculate seed germination indices

Studied traits	**Formula**	**Units**	**Reference**
Mean germination time (MGT)	$\mathrm{MGT} = \frac{\sum_{i=1}^{k} \mathrm{niti}}{\sum_{i=1}^{k} \mathrm{ni}}$	Days	Ellis and Robert (1981)
Final germination percentage (GP)	$\mathrm{G\%} = \frac{\sum_{i=1}^{k} \mathrm{ni}}{\mathrm{N}} \mathrm{X}\, 100$	%	AOSA (1992)
Germination rate index (GRI)	$\mathrm{GRI} = \sum_{i=1}^{k} \frac{\mathrm{ni}}{\mathrm{ti}}$	Seeds per days	Abbasi Khalaki et al. (2019)
Wet weight (WW)	Ruler	g	ISTA (1995)
Dry weight (DW)	Ruler	g	ISTA (1995)
Root length (RL)	Precision scale	cm	ISTA (1995)
Hypocotyle length (SL)	Precision scale	cm	ISTA (1995)
Vigour index (VI)	$\mathrm{VI} = \mathrm{G\%} \times (\mathrm{sl} + \mathrm{rl})$	-	Abdul-Baki et al. (1973)

ni= number of seeds germinated in the *ith* time, ***N*** =total number of seeds used; ***niti*** =the product of seeds germinated at interval ith with the corresponding time interval; ***ti*** = time taken for seeds to germinate at ith count

Accelerated aging (AA) was conducted by placing both primed and unprimed seeds on a sieve inside plastic boxes containing 50 ml of distilled water, ensuring that the seeds did not come into direct contact with the water. The boxes were sealed with lids and incubated at 41 ± 1 ºC for 48 hours. Following this, the standard germination test was performed as previously described.

Electrical conductivity (EC) was measured according to ISTA guidelines (ISTA, 2015). For each wheat genotype, three replicates of 30 seeds were weighed using a digital precision balance (Masskot, FX320, Zürich, Switzerland). The seeds were then immersed in 50 ml of deionized water at 25 ± 1 ºC for 24 hours. EC was subsequently determined using a PH9500 Benchtop pH/Conductivity meter (Apera Instruments, AI5630, Beijing, China).

The cold test was conducted following the procedure described by Bellé et al. (2014). Twenty-five (25) primed and unprimed seeds were placed on moist germination paper saturated with distilled water to evaluate germination performance. The papers were rolled, placed in sealed zipper plastic bags, and stored at 5 ºC for 7 days in a germination chamber (Standard steel, SG01, Ambala, India). After this period, the rolled papers were transferred to another germination chamber set at 23 ºC for 4 days.

Root length, shoot length (SL), wet weight (WW), and dry weight (DW) were measured according to ISTA methods (ISTA, 1995). Shoot and root lengths were measured using a ruler, while wet-weight seedlings were weighed with a precision scale (Kern ADB 100-4, Balingen, Germany). Seedlings were then oven-dried at 70 ºC for 48 hours to determine their dry weight. Iodate concentrations of 0.01 and 0.05 M (IO_3^-) were excluded from the analysis due to germination failure.

3.2.3. Data analysis

Data were subjected to analyses of variance (ANOVA) using GenStat® version 20 edition (VSN International, Hermel Hempstead, UK 2023). Fisher's unprotected test was used to separate means at the 5% significance level. Pearson's correlation coefficeient was used to assess the strength of the relationship between seed germination and vigour traits. Correlation analysis was conducted to assess the associations between the germination and vigour properties of the seeds utilizing the corrplot package in R version 4.0 (R Core Team, 2020). Additionally, Principal Component Analysis (PCA) and the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy were executed based on the correlation matrix using XLSTAT (XLSTAT 2020.5.1.1075). The loading factors obtained from PCA were employed to identify variables exhibiting strong relationships with specific principal components (PCs). Furthermore, principal component biplots were generated to investigate correlations among wheat genotypes and germination and vigour triats to assist the selection of suitable genotypes.

3.3. RESULTS

The findings showed that iodate priming significantly influenced seed quality significantly **(**Table 3.3**Table 3.3**). Iodate and genotypes and their interactions significantly affected GRI, MGT, and SL ($P<0.05$). However, AA and RL did not show significance differences on any iodate treatment, genotypes, and their interaction ($P>0.05$). Moreover, only genotypes and iodate treatment significantly affected EC ($P<0.05$). Only iodate treatments significantly affected WW and DM ($P<0.05$).

Table 3.3: Analysis of variance showing mean squares and significant tests for seed germination and vigour traits of wheat genotypes primed in different concentrations of KIO_3.

Source	**d.f**	**GRI**	**MGT**	**VI**	**EC**	**WW**	**DM**	**SL**	**RL**	**GP**
Genotype (G)	2	190.348**	0.063*	973^{ns}	31.411*	0.0241^{ns}	0.003^{ns}	0.912^{ns}	1.646^{ns}	612.15**
Iodate levels (IL)	2	13.683**	0.0425*	492821.*	77.918**	0.4103*	0.006*	17.258**	2.809^{ns}	356.15**
G × IL	4	32.500*	0.8*	58372^{ns}	5.598^{ns}	0.1115^{ns}	0.002^{ns}	4.368*	6.042^{ns}	66.37^{ns}
Error	16	7.899	0.08	66341	4.122	0.04665	0.0009	1.142	4.444	33.04
Total	26	-	-	-	-	-	-	-	-	-

		CT	AA
		GP	GP
Genotype (G)		261.33**	22.70^{ns}
Iodate levels (IL)		10.778*	46.26^{ns}
G × IL		12.111*	26.59^{ns}
Error		2.32	22.2
Total	26	-	-

d.f= degrees of freedom; **GRI**= germination rate index; **MGT** = mean germination time; **VI** = vigour index; **EC**= electrical conductivity; **WW** = Wet weight and **DM** = dry mass; **SL** = shoot length; **RL** = root length **GP**= germination percentage; **CT**= cold test; AA = accelerating ageing. *, **, ns denotes the significant differences in $P< 0.05$, $P< 0.001$, not significant, respectively.

3.3.1. Germination traits

The final GP revealed no statistically significant relationship between genotype and iodate concentration ($P>0.05$). However, the non-primed treatment in SST806 had the highest GP (96%) in all genotypes, followed by the treatment primed with 0.001 M IO_3^- concentration level in the same genotype SST806 (92 %). The lowest GP (64%) was observed in SST8135 seeds primed with 0.01 M iodate (Table 3.4). The interaction of genotypes and iodate concentration level showed significant ($P<0.05$) variation in MGT and GRI. Higher MGT (5.972 days) was recorded in PAN3111 primed with 0.01 M IO_3^-, while lower MGT (5.641 days) was recorded in unprimed treatment SST806. Additionally, the maximum GRI observed was 40.12 seed/day in unprimed SST806 genotypes, and the minimum GRI was 22.77 seed/day in SST8135 genotypes primed with 0.01 M IO_3^-.

Table 3.4: Influence of iodate seed priming on germination parameters of wheat genotypes.

Genotypes	**Iodate concentration M** $(IO_3)^-$	**Final GP (%)**	**MGT (days)**	**GRI**
PAN3111	0	89.33^{cd}	5.716^{abc}	35.89^{cd}
SST8135	0	77.33^{b}	5.901^{d}	27.54^{ab}
SS806	0	96.00^{d}	5.641^{a}	40.12^{d}
PAN3111	0.01	78.67^{bc}	5.972^{d}	26.63^{a}
SST8135	0.01	64.00^{a}	5.874^{cd}	22.77^{a}
SST806	0.01	88.00^{bcd}	5.935^{abc}	34.69^{c}
PAN3111	0.001	86.67^{bcd}	5.832^{bcd}	32.07^{bc}

SST8135	0.001	85.33^{bcd}	5.683^{ab}	34.72^{c}
SST806	0.001	92.00^{d}	5.681^{ab}	37.41^{cd}
LOS		**ns**	**0.027^{*}**	**0.018^{*}**
CV%		**6.8**	**1.5**	**8.7**

GP: final germination percentage, **MGT**:¯mean germination time, **GRI**: germination rate index. Means containing similar letters in the column are not significantly ($p<0.05$) different according to Duncan's method. LOS, level of significance; CV= coefficient of variation

3.3.2. Vigour traits

Seed vigour traits were unaffected ($P>0.05$) by the interaction of varying iodate concentrations and genotypes. However, notable exceptions were observed in CT and SL, where significant effects ($P<0.05$) were recorded (Table 3.5). The maximum AA was recorded from SST806 genotype primed with 0.001 M IO_3^- (16), while the lowest was acquired from PAN3111 primed with 0.01 M IO_3^- (7 seeds). The cold test demonstrated a significant ($P<0.05$) interaction between genotypes and iodate concentrations. The highest CT (13.33 seeds) was observed on the unprimed treatment in SST806, while the smallest CT (0.33 seeds) was recorded in PAN3111. Although EC was not significantly different, the unprimed PAN3111 genotype had the highest EC 36,63 µS g^{-1} and the SST8135 genotype primed with 0.01 M I had the lowest (19.52 µS g^{-1}**)** (Table 3.5).

The unprimed treatment had the highest wet weight and dry matter in PAN3111 and SST8135, (2.03 and 0.2467 g), while the lowest WW and DM (1.62 g and 0.14 g) were observed in SST806 primed with 0.01 M IO_3^-, respectively (Table 3.2). The interaction between iodate and genotypes significantly ($P < 0.05$) affected shoot length. The unprimed treatment of SST806 had the highest SL (13.71 cm), and the lowest (8.99 cm) was reported in treatment primed with 0.001 M in SST806. The longest (9.21 cm) and shortest RL (5.53 cm) were observed in treatment primed with 0.01 M (IO_3^-) in SST8135 and SST806, respectively. The unprimed treatment in SST806 had the greatest (2073) VI, and the lowest (1294) was recorded in SST8135 treated with 0.01 M IO_3^-.

Table 3.5: Influence of iodate seed priming on vigour parameters of wheat genotypes.

Genotypes	**IC**	**AA**	**CT**	**EC (µS g^{-1})**	**SL (cm)**	**RL (cm)**	**WW (g)**	**DM (g)**	**VI**
PAN3111	0	14.33^{a}	0.33^{a}	36.63^{c}	11.12^{bcde}	8.7^{a}	2.03^{a}	0.213^{b}	1768^{ab}
SST8135	0	8.33^{a}	8.33^{d}	29.37^{abc}	12.60^{cef}	8.1^{a}	2.0^{a}	0.247^{b}	1617^{ab}
SST806	0	16.00^{a}	13.33^{e}	33.40^{bc}	13.71^{f}	8.0^{a}	2.5^{b}	0.250^{b}	2073^{b}
PAN3111	0.01	7.00^{a}	2.33^{abc}	21.38^{a}	10.59^{abc}	8.3^{a}	1.8^{a}	0.197^{b}	1488^{a}
SST8135	0.01	12.00^{a}	2.33^{ab}	19.52^{a}	11.0^{abcde}	9.2^{a}	1.9^{a}	0.217^{b}	1294^{a}
SST806	0.01	10.67^{a}	11.00^{e}	33.20^{bc}	9.26^{ab}	5.5^{a}	1.6^{a}	0.140^{a}	1302^{a}
PAN3111	0.001	14.67^{a}	1.111^{a}	23.38^{ab}	10.6^{abcd}	6.9^{a}	1.8^{a}	0.207^{b}	1516^{a}
SST8135	0.001	12.67^{a}	5.00^{b}	21.90^{a}	10.15^{ab}	6.5^{a}	1.9^{a}	0.233^{b}	1432^{a}
SST806	0.001	15.67^{a}	11.33^{e}	26.5^{abc}	8.99^{a}	8.1^{a}	1.8^{a}	0.197^{b}	1575^{a}
LOS		**ns**	**0.007^{*}**	**ns**	**0.023^{*}**	**ns**	**ns**	**ns**	**ns**
CV%		**38.1**	**24.9**	**21.3**	**9.8**	**27.3**	**11.3**	**14.2**	**16.5**

IC: iodate concentration levels, **AA**: accelerating ageing, **CT**: cold test, **EC**: electrical conductivity, **SL**: shoot length, **RL**: root length, **WW**: Wet weight, **DM**: dry mass, **VI**: vigour index. According to Duncan's multiple range test methods, the Means containing similar letters in the column are not significantly ($p<0.05$) different. **CV**= coefficient of variation

3.3.3. Correlation between germination and vigour traits

The correlation analysis revealed that GP was significantly and positively correlated with GRI ($r = 0.92$, $p < 0.001$), VI ($r = 0.56$, $p < 0.01$), CT ($r = 0.38$, $p < 0.05$), and EC ($r = 0.38$, $p < 0.05$). Mean time to germination (MTG) was significantly negatively correlated with GP ($r = -0.57$, $p < 0.01$). Conversely, MTG demonstrated a significant negative correlation with GP ($r = -0.57$, $p < 0.01$). Furthermore, MGT was significantly and negatively correlated with AA ($r = -0.66$, $p < 0.001$) and CT ($r = -0.45$, $p < 0.05$). Germination rate index (GRI) also showed a significant positive correlation with VI ($r = 0.51$, $p < 0.01$), AA ($r = 0.55$, $p < 0.01$), and EC ($r = 0.42$, $p < 0.01$). However, GRI was negatively correlated with mean germination time (MGT) ($r = -0.83$, $p < 0.001$).

Vigour index (VI) was significantly and positively correlated with WW ($r = 0.70$, $p < 0.001$), DM ($r = 0.49$, $p < 0.01$), PH ($r = 0.66$, $p < 0.001$), and RL ($r = 0.61$, $p < 0.001$). Notably, WW exhibited the strongest positive correlation with dry mass (DM) ($r = 0.79$, $p < 0.001$) and plant height (PH) ($r = 0.77$, $p < 0.01$). Additionally, dry mass (DM) was positively correlated with plant height (PH) ($r = 0.73$, $p < 0.01$). However, no significant correlation was observed between plant height (PH) and root length (RL), not with any other trait (Figure 3.1).

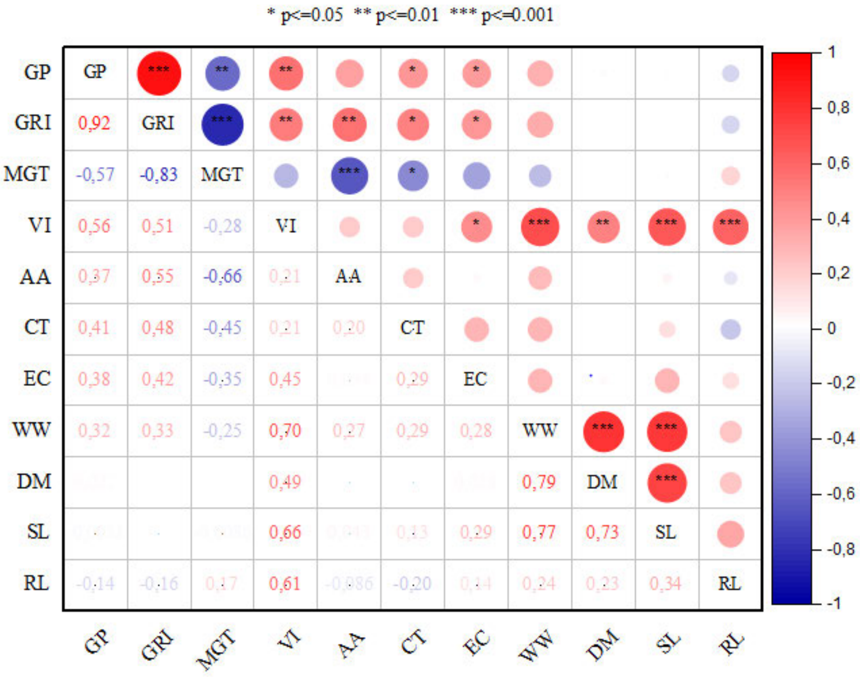


Figure 3.1: Pearson's correlation coefficient values appear at the bottom triangle and a graphical display of these values is shown on the top triangle. Negative and positive correlations are represented by the red and

blue dots, respectively. Small dots with light colours indicate lower-intensity correlations, while larger dots with darker colours indicate higher-intensity correlations. *, **, *** denotes the significant correlations at $p < 0.05$ and $p < 0.01$and $p < 0.001$ respectively.

3.3.4. Principal component analysis

Table 3.6 summarizes the relationship among various traits across four principal components (PCs). Each trait has a corresponding loading value for each principal component, reflecting its contribution. Additionally, eigenvalues and variance explained by each component indicate the importance of each principal component in capturing overall variability.

Principal Component 1 (PC1) explains 45.91% of the total variance, indicating that it captures nearly half of the trait variability. High positive loadings are observed for VI (0.408), GRI (0.361), WW (0.364), and GP (0.334), suggesting that these traits are strongly correlated and contribute positively to this component. A negative loading for MGT (-0.367) indicates an inverse relationship with the traits highly loading on PC1. Thus, PC1 appears to capture a significant axis of variation associated with vigour and growth-related traits.

PC2 accounts for an additional 27.13% of the variance, bringing the cumulative variance to 73.04%. Traits with high positive loadings include RL (0.453), DM (0.44), and SL (0.409), suggesting these traits collectively contribute to this component. Meanwhile, traits like GRI (-0.33) and GP (-0.339) have high negative loadings, indicating an inverse relationship with RL, DM, and SL. PC2 may represent variation associated with root and shoot biomass development, contrasting with PC1's focus on growth vigour.

PC3 explains 11.4% of the total variance, with a cumulative variance of 84.44%. It has prominent loadings for MGT (0.451), EC (0.421), and CT (0.391). This suggests that PC3 might capture a component of variance related to seed and metabolic factors, as traits like mean germination time (MGT) and electrolyte conductivity (EC) relate to seed and water uptake dynamics.

PC4, accounts for 6.55% of the variance (cumulative 91%), has notable loadings on CT (-0.605) and EC (0.56), suggesting it might capture variation associated with temperature and stress response traits.

Table 3.6: Summary of factor loadings, eigenvalue, percent and cumulative variation for assessed seed quality traits among 3 wheat genotypes

Trait(s)	**PC 1**	**PC 2**	**PC 3**	**PC 4**
AA	0.309	-0.139	-0.515	0.048
CT	0.214	-0.208	0.391	-0.605
EC	0.277	-0.17	0.421	0.56
SL	0.24	0.409	0.345	-0.062
RL	0.04	0.453	-0.19	0.379
WW	0.364	0.308	0.09	-0.09
DM	0.219	0.44	-0.121	-0.318

VI	0.408	0.148	0.136	0.183
GP	0.334	-0.339	0.028	0.093
MGT	-0.367	0.048	0.451	0.137
GRI	0.361	-0.33	-0.061	0.016
Eigenvalue	5.05	2.985	1.254	0.72
Variability (%)	45.905	27.134	11.4	6.55
Cumulative (%	45.905	73.039	84.439	91

PC: Principle component, **AA**: accelerating ageing, **CT**: cold test, **EC**: electrical conductivity, **SL**: shoot length, **RL**: root length, **WW**: Wet weight, **DM**: dry mass, **VI**: vigour index, **GP**: germination percentage, **MGT**: mean time germination, **GRI**: germination rate index.

Figure 3.2 illustrates the genotypes' differences in germination indices and trait profiles. The distances and angles of the vectors highlight which traits play a more significant role in distinguishing the genotypes along the principal components. SST8135 and PAN3111 share similar trait profiles, while SST806 stands out, especially in traits related to MGT and EC. Additionally, certain traits, such as DM, SL, WW, and VI, cluster together, suggesting a positive association that may reflect overall plant vigour or growth characteristics.

Traits represented by vectors that are parallel or positioned closely together indicate a strong positive association, meaning that an increase in one trait is likely associated with an increase in the other traits. Conversely, traits represented by vectors positioned nearly opposite each other (at 180 °) reveal a strong negative association, where an increase in one trait corresponds to a decrease in the other traits. Vectors oriented toward the sides, forming wider angles with one another, suggest only a weak or minimal relationship between those traits. This pattern helps identify how traits are interrelated based on their spatial orientation in the biplot.

At a concentration of 0 M, SST8135, PAN3111, and SST806 are grouped due to their high RL, DM, SL, WW and VI values. At a concentration of 0.001 M, SST806 and SST8135 are grouped based on their high values in CT, EC, AA, GP and GRI. At a concentration of 0.01 M, SST8135 and PAN3111 are grouped due to high MGT. This analysis supports selection decisions by assisting breeders or agronomists identify which genotypes excel in specific traits or understand the relationships among these traits at different priming solution concentrations.

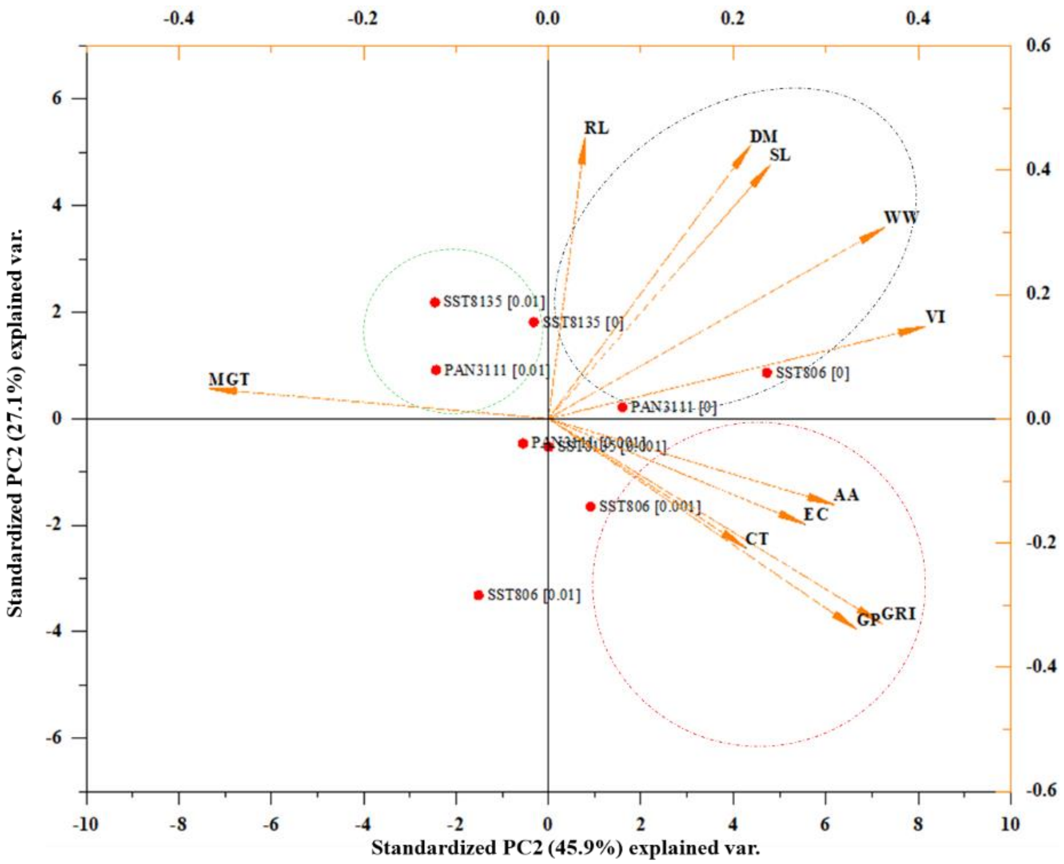


Figure 3.2: Principal component (PC) biplot of PC 1 vs PC 2 demonstrating the relationships among seed quality traits among wheat genotypes primed with different iodate concentrations.

3.4. DISCUSSION

This study evaluated the influence and effective iodate concentration on germination and vigour characteristics in wheat genotypes (*Triticum aestivum* L). Germination and seedling establishment are crucial processes that affect crop production (Groot, 2020a; Perry, 1980), used early predictors of the potential performance of crop growth (Domin et al., 2019). This study indicated substantial negative relationships between iodate content and genotypes in terms of germination traits (GP and GRI) and a positive relationship with MGT (Figure 3.1). In genotypes primed with iodate, lower GP and GRI were recorded, while the high GP and CRI were recorded in unprimed genotypes (Table 3.4). The current study findings are comparable with those of Golob et al. (2020a), who found that iodate soaking has been shown to reduce germination rate compared to the control of pumpkins (*Cucurbita pepo*). However, they are in contrast with Jerše et al. (2017c), who showed that germination was not adversely affected by iodate treatments in peas (*Pisum sativum*). Genotype PAN3111 primed with 0.01 M IO_3^- exhibited higher MGT when compared with the controls (Table 3.4). This increase in MGT may be ascribed to the priming of seeds, which stimulated and accelerated the breakdown of seed reserves, prompting the molecular, physiological and biochemical processes to supply energy to growing embryos (Farooq et al., 2006).

The reduction in germination percentage (GP) and germination rate index (GRI) observed in this study may be due to the accumulation of iodate ions (IO_3^-) within the seed reserves. This likely disrupts starch mobilization, leading to a decrease in α-amylase activity (Martínez-Ballesta et al., 2020; Sghayar et al., 2023). Wet weight (WW) and dry matter (DM) were not significantly affected by the interaction between IO_3^- priming and the wheat genotypes studied (Table 3.5). These findings aligned with the results of Golob et al. (2020a), who reported similar outcomes. However, they differ from studies on buckwheat (*Fagopyrum esculentum*) and peas (*Lathyrus oleraceus*), which documented reductions in sprout yield or mass (Germ et al., 2019; Jerše et al., 2017c). This observation suggests that, despite the accumulation of iodate in seed reserves over time, there is no detrimental effect on the wet weight of wheat seeds. Such results indicate a species-specific response to iodate treatment, highlighting the potential variability in the effects of iodate among different wheat genotypes.

The correlation analysis provided deeper insights into these relationships, demonstrating a significant positive correlation between germination percentage (GP) and both germination rate index (GRI) and vigour index (VI). Conversely, mean germination time (MGT) showed a significant negative correlation with GP and GRI. These findings highlighted the interconnected nature of germination and vigour traits, suggesting that improvements in one trait can have a cascading positive effect on others.

The results indicate that root length (RL) remained statistically unaffected by the interaction between IO_3^- priming and the wheat genotypes, suggesting that there is no significant synergistic effect of these variables on root elongation. In contrast, seedling length (SL) was significantly reduced in IO_3^--primed wheat genotypes compared to their unprimed treatments. The unprimed SST806 genotype demonstrated a significantly greater seedling length than all primed treatments (Table 3.5), highlighting the suppressive effect of iodate priming on early seedling growth. These findings are consistent with the observations of Jerse et al., (2017c), who reported a marked reduction in seedling length following iodate treatment, corroborating the notion that IO_3^- may exert inhibitory effects during early plant development. Although seed priming is generally known to enhance germination and early growth, as supported by Khalil et al., (2010), who observed improved vegetative development following nutrient priming, our data suggest that IO_3^- priming does not follow this trend. Instead, the accumulation of iodate may interfere with key physiological processes such as water uptake, enzymatic activation, or cell elongation during germination. The consistently shorter seedling lengths observed across IO_3^--treated samples suggest that, rather than conferring a developmental advantage, iodate priming may impose physiological stress or toxicity that inhibits early seedling growth.

Principal Component Analysis (PCA) supported these correlations by demonstrating the relationships among various traits across four principal components. PC1, which explained 45.91% of the total variance, highlighted strong positive loadings for VI, GRI, WW, and GP, indicating a significant axis of variation associated with vigour and growth-related traits. The inverse relationship indicated by the negative loading for

MGT on PC1 reinforces the correlation analysis findings. Furthermore, PC2 accounted for an additional 27.13% of the variance and included RL, DM, and SL, suggesting these traits contribute collectively to root and shoot biomass development.

Accelerated ageing (AA), electrical conductivity (EC), and cold test (CT) assess seed physiological quality by evaluating the metabolic status, correlating with emergence potential in varying field conditions. Electrical conductivity evaluates the membrane integrity in solution after soaking seeds (Vieira et al., 2004). Our study showed EC was not significantly affected by IO_3^- priming in wheat genotypes (Table 3.5). This could indicate that the genotypes possess robust cellular mechanisms to maintain membrane integrity despite the application of iodate, which aligns with findings by Ashraf et al., (2018) that highlight the role of certain genotypes in effectively managing oxidative stress. Additionally, the specific concentration and method of iodate application may not have been optimal for eliciting a measurable response in EC, as noted by other studies that emphasize the importance of dosage and timing in micronutrient applications (Ghobadi et al., 2017). Furthermore, it is possible that the beneficial effects of iodate, such as enhanced photosynthetic efficiency or antioxidant activity, did not translate into significant changes in EC, as these physiological improvements may occur without compromising membrane integrity (Farooq et al., 2009a). Contrary, CT and AA tests measure the tolerance of seeds to stress.

Accelerating ageing (AA) and CT determine seed vigour, longevity, and performance under stressful conditions (Milošević et al., 2010). High AA or CT suggests more vigorous seeds (Bewley & Black, 1994, 2013; Maguire, 1962). The interaction of IO_3^- priming and genotypes did not significantly impact AA, indicating that mechanisms governing general ageing resilience (e.g., protein stability, antioxidant capacity) remained intact. However, priming wheat genotypes with IO_3^- significantly reduced CT. Treatment primed with iodate revealed the lowest CT when compared with the controls (Table 3.5). This can be caused by an accumulation of ROS, including H_2O_2, HO_2 and O_2^-, which prompts lipid peroxidation (Espanany et al., 2016). Similarly, De Lamirande & Gagnon (1995) also established that CT exacerbates ROS-induced lipid peroxidation, disrupting membrane fluidity and critical physiological functions like nutrient uptake and energy metabolism.the current results suggest that priming seeds with iodate makes them more sensitive to oxidative damage when exposed to low temperature, without broadly affecting ageing-related resilience. Consequently, CT emerges as a more sensitive diagnostic tool than AA for detecting oxidative stress in seeds, particularly for stressors like iodate that disrupt redox homeostasis. Unfortunately, there is little information in the literature involving IO_3^- priming in crops on vigour tests; this may be of interest for future research.

There was no significant difference in seed vigour index between iodate-primed and non-primed genotypes (Table 3.5). This lack of difference may be attributed to the fact that RL and GP were not significantly affected by iodate priming. As a result, iodate priming did not have a harmful impact on seedling growth and may have supplied nutrients that supported normal seedling emergence and development without impairing crop growth.

The PCA results indicated a close clustering of SST8135 and PAN3111, suggesting that these genotypes exhibit similar growth characteristics, particularly in traits associated with vigour, such as RL, DM, SL, WW, and VI. This similarity indicated that both genotypes may respond similarly to environmental conditions, making them suitable candidates for cultivation in areas where rapid establishment and growth are critical (Baskin, 2014). In contrast, genotype SST806's unique performance in MGT and EC highlights its slower germination rate, which may represent a strategic adaptation to specific environmental stressors (Bewley & Black, 1994). The elevated EC values suggested enhanced metabolic activity during germination, potentially conferring better stress resilience (Finch-Savage, 2016).

3.5. CONCLUSION AND FUTURE RESEARCH

The study elucidated the effect of iodate priming on germination and vigour traits in various wheat genotypes. It revealed that iodate priming significantly influenced GP and GRI, with lower values observed in primed genotypes compared to their unprimed genotypes. Conversely, MGT was positively correlated with iodate concentration, indicating that higher concentrations may delay germination. Additionally, correlation and PCA revealed significant interrelationships among germination and vigour traits, suggesting that improvements in one trait could positively influence others. Notably, the results indicated that while EC remained unaffected by iodate treatment, the cold test (CT) showed significant differences, reflecting the potential impact of iodate on seed vigour under stress conditions. Genotype SST806 exhibited unique characteristics in MGT and EC, indicating its potential adaptability to specific environmental stressors. This study highlights that iodate priming can enhance seed performance, but its effects vary across genotypes. This underscores the importance of thoroughly understanding seed treatments to maximize agricultural productivity. Further research on seed quality in various crops is needed to determine optimal iodate concentrations and their impact on seedling growth.

Acknowledgements

The South Africa Cultivar and Technology Agency (SACTA) is acknowledged for providing financial support during the doctoral studies.

CHAPTER 4

ENHANCING DROUGHT TOLERANCE IN WHEAT (*Triticum aestivum* L.) THROUGH IODATE SEED PRIMING: EFFECTS ON GROWTH, PHYSIOLOGICAL ATTRIBUTES AND STRESS INDICES

Nkosinathi Hlatshwayo[1,*], Takudzwa Mandizvo[1], Albert Modi[2] and Tafadzwanashe Mabhaudhi[1,3]

[1] Centre for Transformative Agricultural and Food Systems, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, P/Bag X01, Scottsville, Pietermaritzburg 3209, South Africa.

[2] Department of Biological and Environmental Sciences, Faculty of Natural Sciences, Walter Sisulu University, Mthatha 5100: amodi@wsu.ac.za

[3] Centre for Water Resources Research, School of Engineering, University of KwaZulu-Natal, Scottsville, Pietermaritzburg, South Africa,

[4] Centre on Climate Change and Planetary Health, London School of Hygiene and Tropical Medicine, London, United Kingdom.

[*]Correspondence: nathi4722@gmail.com (N.H)

**Corresponding author:* nathi4722@gmail.com

To be submitted to South African Journal of Science (November 2024)

ABSTRACT

This study investigated the effects of iodine priming and iodine priming doses on the growth, physiological responses, and yield of three wheat genotypes under drought stress conditions. The study was designed as a 3 × 3 × 2 factorial experiment with 3 wheat genotypes (G) [SST8135, SST806 and PAN3111], 3 iodate doses levels (I) [0, 0.001, 0.01 IO_3^- M] and 2 water treatments (DS) [well-watered and water deficit] arranged in randomized complete block design (RCBD) with three replications. The findings revealed that iodate priming significantly enhanced drought tolerance by improving morphological and physiological attributes such as plant height stress index (PHSI), root length stress index (RLSI), dry matter stress index (DMSI), and carotenoid content, compared to untreated seeds. These improvements were most pronounced in SST 806 and SST 8135. However, drought stress reduced membrane stability index (MSI) and relative water content (RWC), although lower iodine doses alleviated these effects. Interestingly, chlorophyll a and b levels remained unaffected by both drought stress and iodine treatment. In conclusion, the study showed that iodate priming can effectively mitigate the adverse effects of drought stress on wheat growth and yield. Future research should focus on other agricultural crops to measure its impact when using other optimal iodate doses.

Keywords: Drought, iodine, seed priming, seed quality indices, physiological traits

4.1. INTRODUCTION

Drought stress is a major global challenge, particularly in sub-Saharan Africa (SSA), where it significantly hampers crop productivity and threatens agricultural sustainability (Hadebe & Modi, 2017). Wheat (*Triticum aestivum* L), a second most important staple crop after maize *(zea mays)* and provide 30–40% carbohydrates for the global population (Chen et al., 2017). However, wheat production in South Africa falls short of meeting the demands of its growing population partly due to water challenges. The country relies on imports from Australia, Argentina, Poland, Brazil, and Germany to bridge the gap (Department of Agriculture, 2016).

Wheat production shortfall is mainly due to cultivation in regions highly susceptible to drought stress, severely affecting crop establishment and yield, especially when seeds are planted in dry environments (Annandale et al., 2011; Shew et al., 2020). Seed germination and early establishment represent the critical and most sensitive stages in a plant's life cycle, rendering them particularly vulnerable to disruptions induced by drought. These disruptions encompass a range of physiological and biochemical processes that can advesly affect plant development (Iyem et al., 2021; Khan et al., 2019). Drought stress exacerbates these challenges by inducing overproduction of reactive oxygen species (ROS), leading to oxidative stress that damages cellular components through membrane degradation, protein damage, and lipid peroxidation, ultimately compromising plant health and productivity (Kerchev & van Breusegem, 2022). Given these constraints, exploring innovative strategies to enhance wheat drought tolerance is imperative, promoting sustainable production and ensuring food security in drought-prone regions like SSA.

Recently, seed priming with micronutrients (nutripriming) has gained significant attention as a promising strategy for improving early germination, seedling establishment and yield under optimal and stress conditions (Abbas Khan et al., 2021; Atar et al., 2020; Veena & Puthur, 2021). Nutripriming involves the partial hydration of seeds in a solution containing specific micronutrients rather than water. This ensures the seeds imbibe enough moisture to initiate metabolic activity without allowing full radicle emergence. In this treatment, micronutrients are required in small doses, and therefore this is a sustainable option for small-scale farmers. According to Badiri et al., (2014) and Nciizah et al., (2020), nutripriming is a cheap, simple and an effective method for improving germination, early establishment, seedling growth and sustaining steady yield. Micronutrients (zinc, selenium, boron and molybdenum) have been extensively studied for their application in seed priming or coating across various crops, including wheat, common bean *(Phaseolus vulgaris L.*) and maize *(Zea mays)* (Akhtar et al., 2019; Farooq et al., 2012; Majda et al., 2019; Mondal & Bose, 2019; Nciizah et al., 2020; Veena & Puthur, 2021).

In different crops, response to nutripriming varies in and depends on the micronutrient's concentration (Farooq et al., 2012). An effective priming treatment triggers adaptive responses, such as activating protective enzymes and accumulating osmoprotectants, which enhance seed germination and seedling vigour across various crops (Ali et al., 2018; Nadeem & Farooq, 2019). Iodine has the potential to mitigate oxidative stress by directly detoxifying ROS or by stimulating the activity of antioxidant enzymes as a priming agent. Numerous studies have demonstrated that iodine application in plants enhances the accumulation of antioxidants and osmoprotectants, which play a crucial role in stress tolerance (Blasco et al., 2008, 2011; Leyva et al., 2011; Gupta et al., 2015; Medrano-Macías et al., 2016). However, these findings predominantly focus on hydroponic systems and foliar spraying, with limited exploration of iodine seed priming—a promising yet understudied method for improving crop resilience. This raises a critical question: Can iodine priming improve the drought tolerance of wheat genotypes during the early stages of growth? Therefore, the present study evaluated the efficacy of iodine seed priming and effective doses in enhancing seedling growth, yield, and physiological attributes of wheat genotypes under water-deficit conditions. Our study hypothesized that seed priming with iodine (IO_3^-) will significantly enhance drought tolerance in wheat genotypes through improvement of growth, physiological attributes and yield.

4.2. METHOD AND MATERIALS

4.2.1. Site description and seed material

Seeds of three wheat genotypes sourced from different locations in South Africa namely: Free State

(28.1856° S, 28.2149° E) and Grey Town (-29.563633, 24.017855) were used in this study (Table 4.1). The genotypes were selected based on their agronomic traits such as tolerance to cultivation in the dry semi-arid areas of South Africa, market potential etc.

Table 4.1. Key characteristics of three wheat genotypes (PAN3111, SST8135, and SST806) sourced from different regions in South Africa.

Genotype	Region/Origin	GH	DT	YP	ONT
PAN3111	South Africa (Free State)	Semi-dwarf	Moderate	High	High protein content
SST8135	South Africa (Grey Town)	Semi-dwarf	High	Moderate	Good disease resistance
SST806	South Africa (Grey Town)	Semi-dwarf	Moderate	High	Early maturity, robust grain quality

GH: growth habit, **DT**: drought tolerance, **YP**: yield potential, **ONT**: other notable traits

4.2.2. Experimental design, priming method, and water stress

The experiment was conducted in a University of KwaZulu–Natal's Controlled Environment Research Unit (CERU), Pietermaritzburg (29°37'34.0"S 30°24'13.4"E). The environmental conditions were regulated to maintain temperatures of 33 °C during the day and 27°C at night, with relative humidity (RH) ranging from 60% to 82%, and exposure to natural daylight. These conditions closely represent those of a warm tropical climate, as outlined by Modi (2007). The study was designed as a 3 × 3 × 2 factorial experiment with 3 wheat genotypes (G) [SST8135, SST806 and PAN3111], 3 iodate concentration levels (I) [0, 0.001, 0.01 IO_3^- M] and 2 water treatments (DS) [well-watered and water deficit] arranged in randomized complete block design (RCBD) with three replications, giving 54 experimental units. During the priming process, seeds, except for controls treatment, were soaked in an aerated solution of potassium iodates for 6 hours, as described by Golob et al. (2020). An aquarium pump was used for aeration during soaking period. Afterwards, seeds were rinsed with distilled water thrice and allowed to surface dry at room temperature (25 °C) until they reached their original moisture content.

Plastic pots with a diameter and height of 17.0 x 13 cm were filled with soil from Ukulinga Research Farm, whose soil properties are indicated in Table 4.2. It is worth noting that the priming concentration levels were based on the previous study (Chapter 2). To meet crop nutrient requirements, 10% and 46 % of superphosphate and urea fertilizer were applied to the soil at the 0.1 and 0.26 g application rates, respectively. For each treatment, 10 seeds were sown in each pot filled with soil and later thinned to 3 plants. After planting, all pots were sub-irrigated to 100% field capacity for 15 days to ensure maximum emergence. Water deficit (WD) treatments were initiated by terminating irrigation for 14 days while maintaining the regular irrigation in the control treatments till the end of the experiment (Figure 5.1). Soil water was monitored using a time domain reflectometer probe (Campbell Scientific Inc. TDR100, Utah, USA). After harvesting, samples were taken to a laboratory, and agronomic and physiological traits were determined. Water use during the experiment was determined by calculating the water added from start to finish in well-watered pots.

Table 4.2: Soil properties for the Ukulinga field used in the study

Property	**Values**
Exchangeable Acidity	668 cmol/L
Total Cations	14.42 cmol/L
pH (KCI)	5.23
Phosphorus (P)	32 mg/L
Potassium (K)	668 mg/L
Calcium (Ca1)	661 mg/L
Magnesium (Mg)	534 mg/L
Zinc (Zn)	7.1 mg/L
Manganese (Mn)	28 mg/L
Copper (Cu)	2.0 mg/L
Nitrogen (N)	0.22 %
Clay	24%
Organic Carbon	2.6 %
Exchangeable Acidity	668 cmol/L

4.2.3. Data collection

4.2.3.1. Stress indices and root shoot ratio

After harvesting, the plant dry weight was measured by drying the samples in an oven (Mlab scientific ldo-080f, Mexico) at 70°C for 48 hr. Thereafter, dry matter stress index (DMSI), root length stress index (RLSI), plant height stress index (PHSI) and root shoot ratio were calculated according to Ashraf et al. (2006).

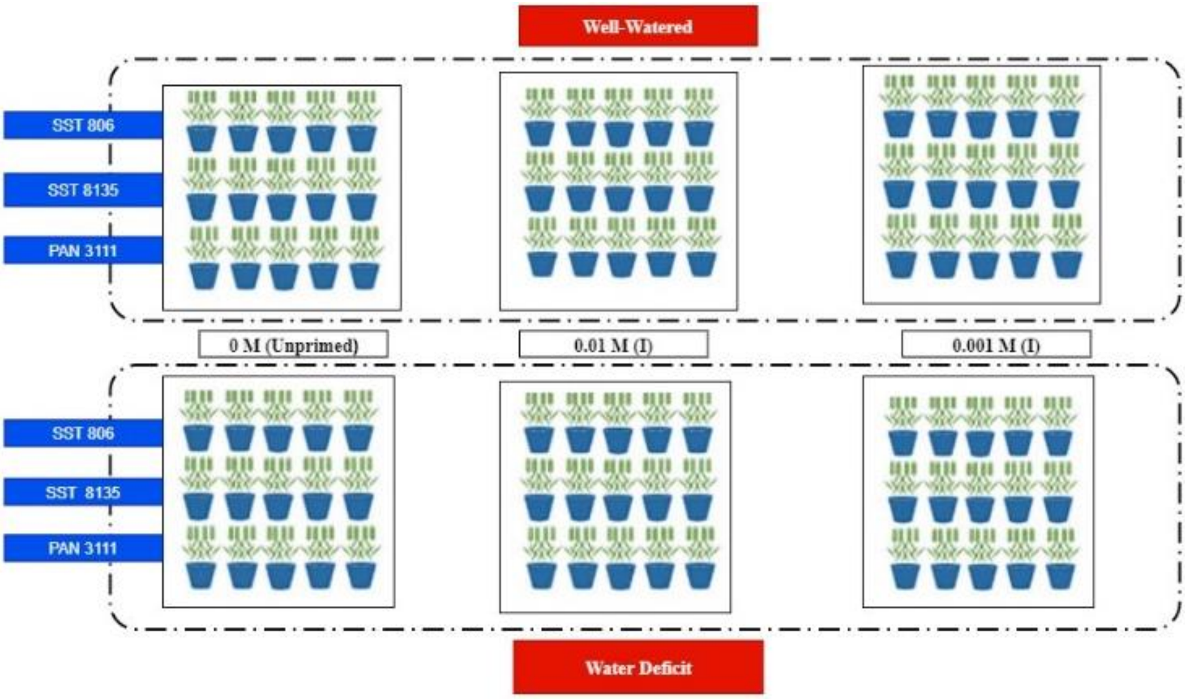


Figure 4.1: Field layout of the experiment detailing how pots were arranged to assess the impact of iodine seed priming on wheat genotypes under two water regimes (well-watered and water deficit).

$$\text{DMSI} = \frac{\text{Dry matter of stressed plant}}{\text{Dry matter of control plant}} \times 100 \qquad (1)$$

$$\text{RLS} = \frac{\text{Root length of stressed plant}}{\text{Root length of control plant}} \times 100 \qquad (2)$$

$$\text{PHSI} = \frac{\text{Plant height of stressed plant}}{\text{Plant height of control plant}} \times 100 \qquad (3)$$

$$\text{RSR} = \left(\frac{\text{Root dry weight}}{\text{Shoot dry weight}}\right) \qquad (4)$$

4.2.3.2. Membrane stability index (MSI) and membrane injury (MI)

Membrane stability index (MSI) was determined by measuring the electrolyte leakage according to Blum and Ebercon (1981) and ElBasyoni et al. (2017). Leaf samples of 0.3 g were rinsed with distilled H_2O and submerged in 6 ml of distilled H_2O for 12 hr. The conductivity of the solution (C1) was measured with a conductivity meter (Model DDS-11A; Shanghai Leici Instrument Inc., Shanghai, China). Thereafter, samples were boiled in water at 100 °C for 20 mins and then cooled to room temperature (±25 °C). The conductivity of dead tissues (C2) was again measured as indicated in Equation 5. The membrane injury (MI) was determined as the ratio of MSI of water deficit plants and MSI of well-watered plants, as shown in Equation 6 (Dhanda et al. 2004).

$$\mathrm{MSI} = \left(1 - \left(\frac{\mathrm{Conductivity\ 1}}{\mathrm{Condictivity\ 2}}\right)\right) \times\ 100 \tag{5}$$

$$\mathrm{MI} = (1 - \left(\frac{\mathrm{MSIwaterdeficit}}{\mathrm{MSIwellwatered}}\right)) \times\ 100 \tag{6}$$

4.2.3.3. Photosynthetic pigments

The method of Arnon's., (1949) was used to assess carotenoids and chlorophyll content. A leaf sample of 0.1 g was placed in liquid nitrogen and homogenized in 3 ml of 80% acetone. The mixture was centrifuged for 10 mins at 15,000 rpm and 4 °C. Following acetone extraction, the supernatant was collected, and chlorophyll a (Chl a), b (Chl b) and carotenoid were determined using a spectrophotometer (Biorad SmartSpec TM Plus, USA) at 480, 645 and 663 nm, respectively. Calculations of chlorophyll and carotenoid were calculated using the following equations:

$$\text{Chlorophyll 'a'} = 12.7\ (A663) – 2.69\ (A645); \tag{7}$$

$$\text{Chlorophyll 'b'} = 22.9\ (A645) − 8.12\ (A663); \tag{8}$$

$$\text{Total carotenoid} = [A480 + (0.114\ (A663) - (0.638\text{-}A645)] \times V/1000xW \tag{9}$$

where A = Absorbance measured at the given wavelength, V = volume of the extract (cm3); W = weight of fresh sprout (g)

4.2.3.4. Relative water content (RWC)

Relative water content (RWC) of leaves was measured by weighing (0.5g) leaf samples to estimate fresh weight (FW) and inserted in a tube containing distilled water for 6 h. Thereafter, the leaf samples were weighed to quantify turgid weight (TW) after wiping the water off the leaf surface with paper tissue. Lastly, the samples were heated in an oven (Mlab scientific ldo-080f, Mexico) at 80 ^{o}C for 48 h, to acquire dry weight (DW). All weights were determined using an analytical scale. RWC was determined using the following equation: ((FW- DW) / (TW-DW))) × 100.

4.2.4. Statistical analysis

The data were subjected to analysis of variance (ANOVA) using GenStat (Version 19, VSN International, and Hertfordshire, UK). The means were compared using the Duncan multiple range test at significance level of 0.05.

4.3. RESULTS

4.3. Seed quality indices

4.3.1. Plant height stress index PHSI)

In this study, iodate treatments significantly ($p<0.001$) improved PHSI compared to the control treatment (Table 4.3). Among the genotypes, SST806 exhibited the highest PHSI values, followed by PAN3111 and SST8135. A significant ($P<0.05$) interaction between iodate doses and genotypes was also observed with respect to PHSI. Precisely, genotype SST806 primed with 0.001M IO_3^- displayed the PHSI (93.25), whereas the lowest PHSI (40.99 cm) was recorded in the unprimed SST8135 genotype (Figure 4.2A).

4.3.2. Dry matter stress index (DMSI)

The interaction between iodate doses and genotypes had no significant ($p > 0.05$) difference but higher DMSI (70.7) was recorded in SST806 primed with the concentration of 0.001 M IO_3^- and the lowest DMSI (38.3) was observed at the highest iodate concentration (0.01 M IO_3^-) in SST806 genotypes (Figure 4.2B). Iodate doses significantly ($p <0.05$) affected DMSI genotypes primed with 0.001 M IO_3^- maintained higher DMSI (58.1), while the lowest DMSI (40.1) was observed in plants primed with 0.01 M IO_3^-. Genotypes had no significant influence on DMSI ($p > 0.05$) (Table 4.3).

4.3.3. Root length stress index (RLSI)

The interaction of genotypes and iodate showed a significant ($p <0.05$) effect; however, main effects of iodate doses and genotypes did not show any significant ($p >0.05$) difference with respect to RLSI (Table 4.3). Longest RLSI (74.79) was observed in SST806 in unprimed seeds. Contrary, the shortest RLSI (38.71) was recorded in non-stressed treatment; however, it was recorded in the SST8135 genotype (Figure 4.**2**C).

4.3.4. Membrane injury (MI)

The interaction of iodate doses and genotypes also showed an insignificant ($p >0.05$) effect regarding MI ($p >0.05$). The genotype SST8135, when primed with a 0.001 iodine concentration, exhibited a higher MI (0.6310), although it was not statistically significan t($p >0.05$). In contrast, the lowest MI (0.2289) was observed in the non-primed seeds of the genotype PAN3111 (Figure 4.2D). Iodate doses significantly affected MI of the seeds ($p <0.05$). Seeds primed with 0.01 and 0.001 M IO_3^-maintained a 23 and 98% increase, respectively, in MI compared to non-primed plants. Genotypes showed insignificant ($p >0.05$) effect in MI (Table 4.3).

Table 4.3: Summary of ANOVA showing the mean squares and the level of significance of wheat genotypes under drought stress conditions.

Source of variance	**d.f.**	**PHSI**	**DMSI**	**RLSI**	**MI**
Genotypes (G)	2	700.4^{*}	376.8^{ns}	103.9^{ns}	0.00571^{*}
Iodate doses (IL)	2	1609.4^{**}	727.5^{*}	216.8^{ns}	0.20540^{ns}
G × IL	4	400.5^{*}	192.0^{ns}	655.9^{*}	0.00848^{ns}
Residual	16	126.3	159.8	247.1	0.02821

d.f.; degrees of freedom, **PHSI**; plant height stress tolerance index, **DMSI;** dry matter stress tolerance index, **RLSI;** roots length stress tolerance index, **MI**; membrane injury index, ns; non- significant. * and ** denotes significance at 5 % and 1 % probability levels, respectively.

4.3.5. Root shoot ratio (RSR)

The interaction between genotypes and iodate doses significantly ($p < 0.05$) influenced RSR. A higher RSR was observed in non-primed seeds of PAN3111 (0.358), while the lowest RSR (0.142) was observed in seeds primed with 0.01 M IO_3^- iodate dose in SST8135 (Figure 4**.3**A). Drought stress significantly increased the RSR compared to the control ($p < 0.001$). However, iodate priming did not significantly affect the RSR ($p > 0.05$). However, genotypes significantly ($p < 0.001$) influenced RSR (Table 4.4). Genotypes PAN3111 and SST806 maintained higher (0.3111 and 0.215) RSR than SST8135, which was the lowest (0.152) (Table 4.4).

4.3.6. Membrane stability index (MSI)

Interaction between genotypes and iodate doses was significant ($p < 0.05$). Genotype SST806, when primed with 0.001M IO_3^-, exhibited the highest membrane stability index (MSI), while the lowest MSI value was recorded in SST8135 primed with 0.01M IO_3^- (Figure 4.3B). The interaction between iodate doses and drought stress was significant ($p < 0.05$). Under drought conditions, priming with 0.01 M and 0.001 M IO_3^- led to a 32 and 37% reduction in membrane stability index (MSI), respectively, compared to the control. Under well-watered conditions, seed priming with 0.001 M and 0.01 M IO_3^- had a 6% increase and 24% decrease in MSI compared to control, respectively (Figure 4**.3**B). Water deficit caused a significant decrease in MSI compared to control ($p < 0.001$). Iodate doses significantly ($p < 0.05$) affected MSI; seed primed with 0.01 and 0.001 M IO_3^- had 27% and 11% reduction in MSI compared to controls, respectively (Table 4.4).

4.3.7. Relative water content (RWC)

The data presented in Table 4.4 indicated that relative water content (RWC) in the interaction between genotypes × iodate doses, genotypes × drought stress and iodate doses × drought stress were insignificant ($p > 0.05$). Despite these insignificant ($p > 0.05$) interaction effects, certain trends were observed, genotype SST806 of unprimed seeds maintained the highest RWC in drought stress and well-watered conditions. While the lowest RWC was observed in seeds primed with 0.01 M IO_3^- in PAN3111genotypes under drought stress and in SST8135 under well-watered conditions (Figure 4.3C). While genotype effects were not significant ($p > 0.05$), both drought stress and iodate priming had a significant influence on RWC ($p < 0.001$). Drought stress led to a substantial 36% reduction in RWC compared to the control treatment. Furthermore, iodate priming at concentrations of 0.01 M and 0.001 M IO_3^- resulted in RWC reductions of 16% and 10%, respectively, relative to the control (Table 4.4). These findings suggest that, although genotype interactions were not statistically significant, drought and iodate treatments independently exert considerable effects on plant water status.

4.3.8. Photosynthetic pigments

Priming with iodate under drought stress significantly ($p > 0.05$) affected the

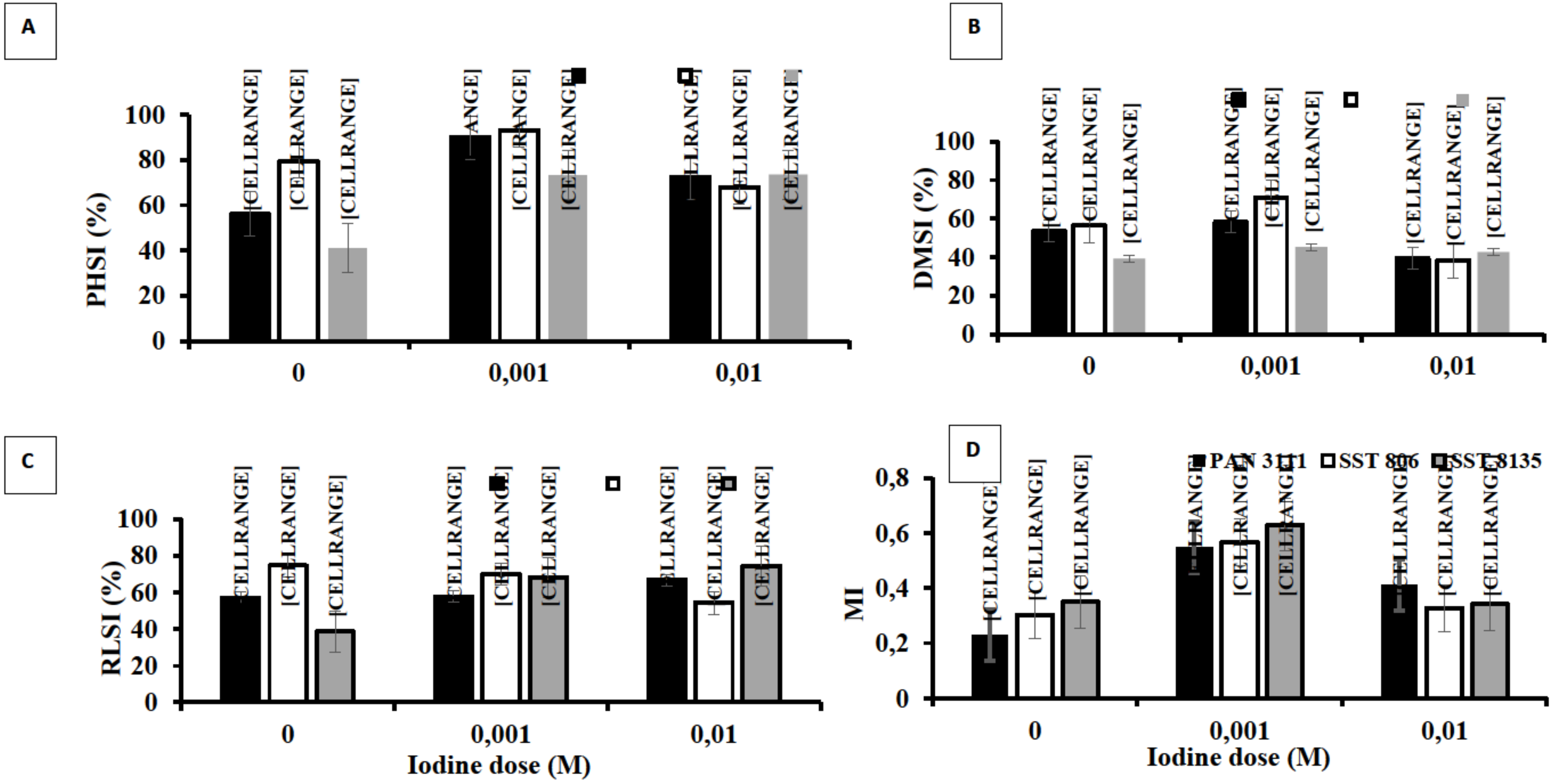


Figure 4.2. The effect of priming with different iodate doses on A) plant height stress tolerance index (PHSI), B) Dry matter stress tolerance index (DMSI), C) Roots length stress tolerance index (RLSI) and D) membrane injury index (MI) of three wheat genotypes. Means with different letter(s) are significantly different at 5% level of probability.

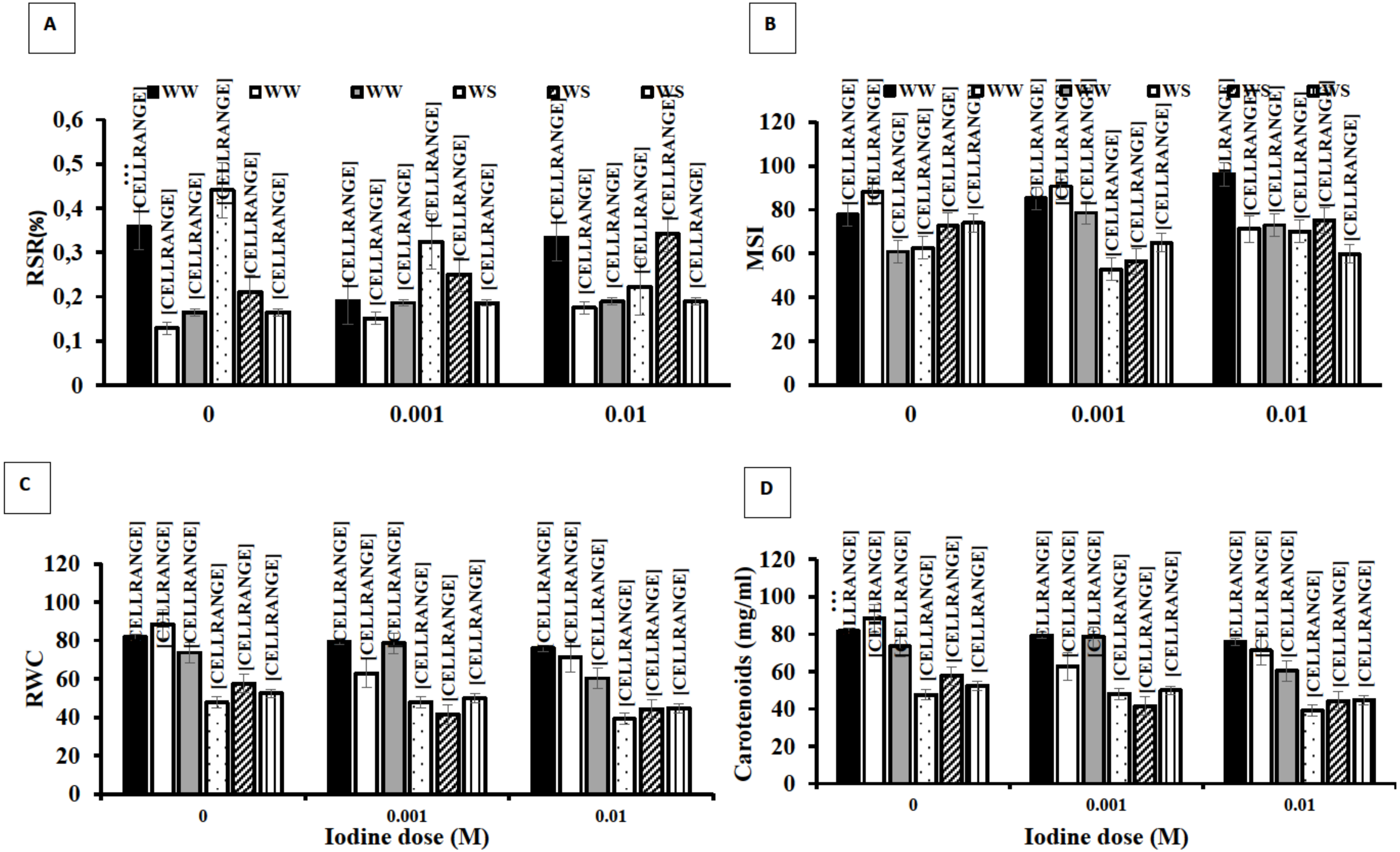
Table 4.4: Summary of ANOVA showing the mean squares and the level of significance of wheat genotypes under drought stress conditions.

Source of variance	d.f.	**RSR**	**MSI**	**RWC**	**Carotenoids**	***Chl a***	**Chl b**
Genotypes (G)	2	0.116**	180.96^{ns}	19.0^{ns}	2.5478**	1.2040^{ns}	47.62ns
Iodate doses (ID)	2	0.008^{ns}	442.54*	553.0*	0.3157^{ns}	1.0721^{ns}	45.43^{ns}
Drought Stress (DS)	1	0.076**	3846.39**	10151.3**	4.3281**	0.5994^{ns}	46.67^{ns}
G × ID	4	0.021*	274.66*	243.7^{ns}	0.0632^{ns}	1.2601*	56.73ns
G × DS	2	0.010^{ns}	10.07^{ns}	173.1^{ns}	1.4757**	1.3602^{ns}	103.39*
ID × DS	2	0.007^{ns}	315.22*	5.4^{ns}	0.2296^{ns}	1.6523*	89.55*
G × ID × DS	4	0.013^{ns}	53.02^{ns}	50.8^{ns}	0.1772^{ns}	0.9096^{ns}	61.73^{ns}
Residual	34	0.007	72.97	122.0	0.1258	0.4687	23.83

d.f.; degrees of freedom, **RSR**; root shoot ratio, **MSI**; membrane stability index, **RWC**; relative water content, ***Chl a***; chlorophyll *a*, ***Chl b***; chlorophyll *b*, ns; non- significant. * and ** denotes significance at 5 % and 1 % probability levels, respectively

photosynthetic pigments in wheat genotypes. The evaluated treatments, including genotypes, iodate priming, and drought stress, did not individually result in significant changes ($p > 0.05$) in chlorophyll content (chlorophyll a and chlorophyll b) compared to the control (Table 4.4). However, carotenoids were significantly ($p < 0.05$) effected by drought stress and genotypes, showing an increase of 56.89% under drought stress when compared with the control (Table 4.4). All carotenoids interactions were non-significant ($p > 0.05$), except for the interaction between genotypes and water stress, which was significant. Under drought stress conditions, genotype SST8135 maintained the highest carotenoids, and the lowest carotenoids were observed in PAN3111 genotypes (Figure 4.**3**D). Under well-watered conditions, a similar trend was observed in under drought conditions with respect to carotenoids.

The interaction between genotypes × iodate doses and iodate doses × drought stress significantly affected *chl a*, respectively. The highest *chl a* was maintained under non-primed seeds in genotypes SST8135, followed by seeds primed with 0.001 M IO_3^- SST806. The lowest *chl a* was detected in PAN3111 genotypes primed with 0.001 M IO_3^- followed by SST8135 genotypes primed with 0.01 M IO_3^-. Under water-stressed conditions, seeds primed with 0.01 and 0.001 M IO_3^- maintained a 12.71% increase and a 2.9% decrease in *chl a* compared to control treatment. Whereas, under full irrigation conditions, priming


A
WW
WW
WW
WS
WS
WS
RSR(%)
0,6
0,5
0,4
0,3
0,2
0,1
0
0
0.001
0.01
B
MSI
120
100
80
60
40
20
0
C
RWC
Iodine dose (M)
D
Carotenoids (mg/ml)
Iodine dose (M)
[CELLRANGE]

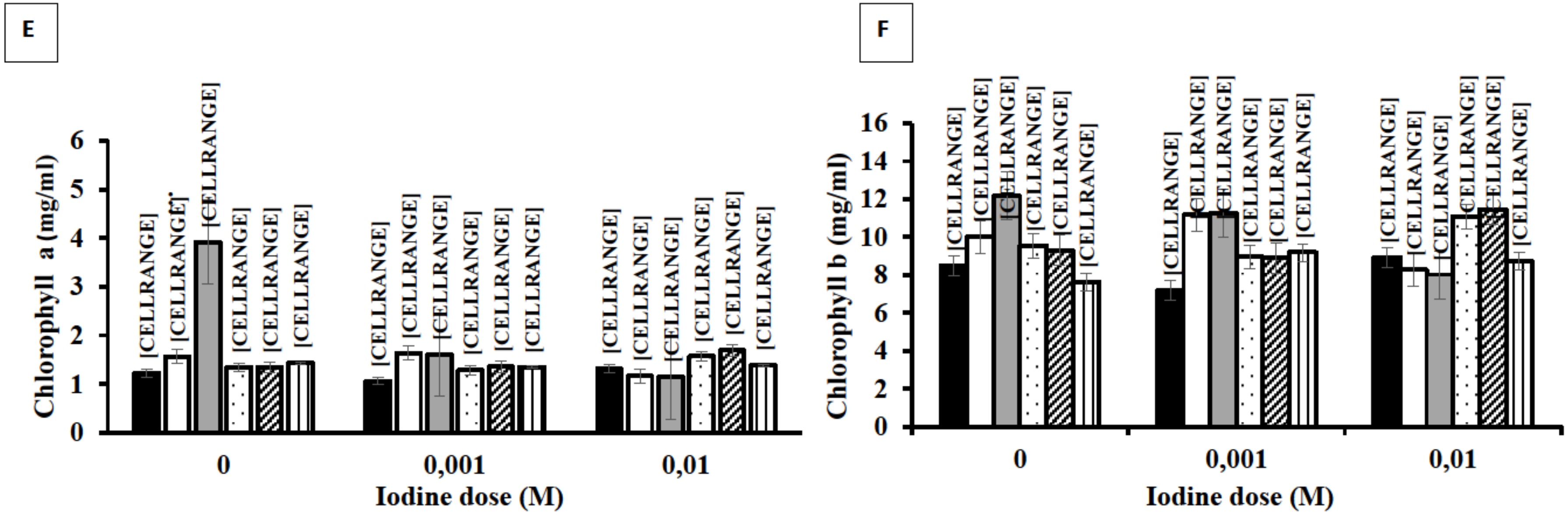


Figure 4.3. The effect of iodate priming with different doses on A) root shoot ratio (RSR), B) relative water content (RWC), C) membrane stability index (MSI), d) carotenoids, E) chlorophyll a, and F) chlorophyll b. Means with different letter(s) are significantly different at 5% level of probability.

seeds with 0.01 and 0.001 M IO_3^- maintained a decrease of 45.86 and 35.87% in chlorophyll compared with the control treatment, respectively (Figure 4.3E).

With respect to chlorophyll b, there was a significant ($p < 0.05$) difference in the interactions between genotypes × drought stress (Table 4.4). The highest *chl b* was obtain on genotype SST806 and the lowest was obtain in genotype SST8135, under drought stress. However, under well-watered conditions, the highest and lowest *chl b* was observed in SST8135 and PAN3111, respectively. Moreover, there was also a significant ($p < 0.05$) difference in the interaction iodate doses × drought stress, with respect to *chl b.* Under water stress conditions, priming with iodate using doses of 0.001 and 0.01 M IO_3^- resulted in a 2.26 and 16.26% decrease in chlorophyll b compared to control. Nonetheless, priming seeds with 0.001 and 0.01M IO_3^- resulted in a significant increase in chlorophyll b levels under well-watered conditions, with increases of 36.46 and 45.92%, respectively, when compared with the control ($p < 0.05$) (Figure 4.3F).

4.4. DISCUSSION

In this study, drought stress significantly impairs plant growth and development, particularly during early stages such as seedling establishment. However, iodate nutripriming, showed promising result in mitigating the harmful effects of abiotic stresses, thereby enhancing the utilization of crops in drylands. Research from previous studies has demonstrated that iodate can alleviate stress-induced damage in plants through various mechanisms. In general iodate application is by soil amendments, foliar sprays, and hydroponic systems (Gonzali et al., 2017; Lawson et al., 2015; Medrano-Macías et al., 2016; Pérez-Salas & Medrano-Macías, 2021). However, limited research has explored the effects of iodate on plants under drought stress, particularly during early growth stages, despite its proven efficacy under other stress conditions such as salinity. This study addresses this gap by evaluating the effectiveness of iodate priming in enhancing drought tolerance in three local wheat genotypes.

The current study indicated that soaking seeds in iodate doses significantly improves indices such as PHSI and DMSI under drought stress conditions. Similarly, decreasing iodate concentration was also associated with elevated RLSI, although there was no significant difference (Table 4.3). Moreover, the highest PHSI, DMSI and RLSI have been recorded in the lowest iodate concentration, indicating that low doses benefit growth. These findings are consistent with several studies, which collectively demonstrated that low-dose iodine supplementation enhances plant physiological performance, resulting in significantly improved growth traits and elevated productivity across diverse agricultural systems (Fuentes et al., 2022; Halka et al., 2019; Medrano-Macías et al., 2016).

The mechanisms by which iodate priming improves PHSI and RLSI are not fully understood. However, an increase in PHSI in primed seeds might be related to an increase in pre-germination processes such as increase in cell division and elongation, repair and synthesis of DNA and RNA, increased activities of reserve mobilization enzymes (α-amylase, β-amylase, acid phosphatase and dehydrogenase) (Ahmad et al., 2018; Cosgrove, 2005; Farooq et al., 2009c). These processes likely accelerate germination and seedling growth, enabling the seeds to escape the adverse effects of drought compared to the control (Hasanuzzaman & Fotopoulos, 2019; Saha et al., 2022). In primed seeds, improvement of biomass resulted from increased pre-germinative processes, leading to increased PHSI. Increases in biomass under stress conditions when iodate was applied have also been reported in strawberries (*Fragaria × ananassa)* (Medrano et al., 2021), lettuce (*Lactuca sativa)* (Blasco et al., 2013), tomato (*Solanum lycopersicum)* (Pérez-Salas & Medrano-Macías, 2021) and soybean(*Glycine max)* (Neha et al., 2015), although in these studies, the predominantly type of stress applied was mainly saline stress. The increase in biomass was due to iodine inducing ROS detoxification through activating the antioxidant defence mechanism.

Relative water content (RWC) is a crucial indicator of plant water status and defined as the water content ratio in plant tissues to their fully hydrated state (Smart & Bingham, 1974). According to Sial et al., (2017), RWC measures a plant's ability to retain water under stress conditions. Studies have shown that RWC is a valuable parameter for assessing plant health under drought stress and closely associated with yield-related traits (Chaves et al., 2002; Verslues et al., 2006; Gutierrez et al., 2010). In this study, drought stress resulted in a decline in RWC in wheat genotypes when compared with the control (Table 4.4). A well-documented response may be attributed to disrupted osmotic regulation and cellular dehydration (Farooq et al., 2009c). Furthermore, the results showed that iodate priming generally decreased RWC compared to non-primed controls aligns with studies showing that excessive oxidants like iodate can induce oxidative stress, exacerbating membrane damage and water loss (Hossain & Fujita, 2010). However, the dose-dependent efficacy of iodate at 0.001 M IO_3^-, which significantly outperformed 0.01 M IO_3^- in maintaining RWC, highlights a critical threshold for beneficial effects. This concurs with Ashraf et al., (2018), who noted that trace elements like iodine act as "biphasic agent," where low concentrations enhance antioxidant enzyme activity (e.g., catalase and peroxidase) to mitigate drought-induced ROS, while higher doses disrupt cellular redox balance.

The membrane stability index (MSI) and membrane injury (MI) are critical physiological indicators for assessing drought tolerance in plants (Blum, 1996). Our findings indicate that drought stress significantly reduces MSI, suggesting decreased membrane stability and increased susceptibility to injury. According to Yang et al., (2021), drought leads to cell dehydration, resulting in loss of turgor pressure and subsequent membrane disruptions, including decreased lipid biosynthesis and compromised cellular functions. furthermore, increasing the application of iodate at higher doses led to a more significant decrease in MSI, indicating that lower doses induce more tolerance. The application of iodate at lower concentrations (e.g., 0.001 M IO_3^-) under drought conditions enhanced MSI in wheat. This enhancement is attributed to iodate's interaction with polyunsaturated fatty acids (PUFAs) in cell membranes, which helps stabilize membrane structures against oxidative damage caused by reactive oxygen species (ROS) (Roychoudhury, 2023; Srivastava & Yadav, 2024). ROS can lead to lipid peroxidation, compromising membrane fluidity and increasing ion leakage (Seleiman et al., 2021). However, higher concentrations of iodate (e.g., 0.01 M IO_3^-) have detrimental effects, overwhelming cellular redox systems and causing ion toxicity, which exacerbates lipid peroxidation and decreases MSI. The genotypic variations play a significant role in this response. Wheat cultivars with strong antioxidant mechanisms, such as enhanced catalase and peroxidase activity, show improved MSI when primed with iodate (Raza et al., 2023). This suggests that breeding for robust antioxidant pathways may enhance drought tolerance in wheat.

Our study showed that drought stress did not affect the chlorophyll content except for carotenoids, which significantly decreased under drought stress. Carotenoids are critical antioxidants that protect chlorophyll and dissipate excess energy under stress, aligning with established patterns of drought-induced oxidative damage to the photosynthetic machinery (Farooq et al., 2009b). Notably, iodine application (0.001 M IO_3^-) reversed this trend, elevating carotenoid levels compared to control values even under water-deficit conditions. This response corroborates findings by Maglione et al. (2022), who documented analogous carotenoid enhancement in iodine-primed plants subjected to abiotic stress. The mechanism likely involves iodine-induced upregulation of carotenoid biosynthesis genes (e.g., *PSY*, *LCYB*) and/or iodine's role in stabilizing existing carotenoid molecules against ROS degradation, thereby preserving photosynthetic efficiency and membrane integrity during drought (Ashraf et al., 2018). Interestingly, genotypes SST8135 and SST806 exhibited higher carotenoid levels compared to PAN3111, suggesting a stronger photoprotective system against drought stress in these genotypes (Farooq et al., 2009; Ghobadi et al., 2017; Swapnil et al., 2021).

4.5. CONCLUSION

This our study showed that seed priming with iodate during early stages could improve plant tolerance potential to drought by increasing growth attributes such as PHSI. Moreover, iodate priming did not significantly increase physiological traits other than carotenoids. Among wheat genotypes, SST806 outperforms all genotypes, followed by genotype SST8135 and PAN3111. The growth performance and stress tolerance of genotype SST806 were mainly attributed to an increase in PHSI, RLSI, DMSI and carotenoids. Iodate priming is cost-effective for enhancing drought tolerance and increasing growth if applied correctly in wheat genotypes.

Declarations

Authors contribution statement

All authors listed have significantly contributed to the development and writing of this article.

Funding statement

This work was supported by the south African cultivar and technology agency (SACTA).

Competing interest statement

The authors declare no conflict of interest.

CHAPTER 5

ASSESSING THE EFFICACY OF IODINE APPLICATION ON MORPHOPHYSIOLOGICAL TRAITS OF WHEAT GENOTYPES UNDER DROUGHT STRESS AND VARIED GROWTH STAGES OVER TWO WINTER SEASONS (2021 AND 2022)

ABSTRACT

Iodine (I) is recognized as a biostimulant that positively influences plant growth by enhancing redox metabolism, improving antioxidant levels, and increasing tolerance to abiotic stress. However, research on iodine application under water stress conditions is limited. This study aimed to investigate the effects of iodine application methods (seed priming and foliar spraying) on two wheat genotypes (SST806 and SST8135) under well-watered and water-stressed conditions during critical growth stages (tillering and flowering). A greenhouse experiment was conducted using a 2 × 3 × 4 factorial design in a randomized complete block design (RCBD), replicated three times over two winter seasons (2021 and 2022). Physiological parameters, including photosynthesis (Pn), stomatal conductance (gs), transpiration rate (Tr), and relative water content (RWC), along with growth parameters such as plant height (PH), grain yield (GY), and its components (number of tillers (NT), spike length (SL), number of spikelets per spike (SPS), number of grains per spike (NGS), thousand grain weight (TGW), biological yield (BY), and harvest index (HI)), were evaluated. The results indicated that drought stress significantly reduced all growth, physiological, and yield components compared to control treatments. However, iodate application through seed priming and foliar spray positively influenced Pn, Tr, NT, SPS, NGS, TGW, and BY under both water-stressed and normal conditions. Notably, genotype SST806 outperformed SST8135 in terms of growth and yield. In conclusion, exogenous iodate application via seed priming or foliar spray effectively enhances drought tolerance in wheat genotypes.

Keywords: Wheat, iodine application, drought stress, gas exchange variables, morphological attributes.

5.1. INTRODUCTION

Wheat (*Triticum aestivum*) is one of the essential staple crops after maize and rice worldwide (DAFF, 2015; Soto-Gómez & Pérez-Rodríguez, 2022). It is characterised as a cereal crop that adapts easily, suitable for long-term storage, and high nutritional value (DAFF, 2015). Wheat aslo a vital food source because it offers carbohydrates, dietary proteins, fiber, and energy, along with various minerals like calcium, zinc, and lipids (Soto-Gómez & Pérez-Rodríguez, 2022). However, its production has significantly decreased over the years due to environmental stresses such as drought (Golfam et al., 2021). Drought stress is a significant environmental challenge that affects not only wheat but also agricultural production worldwide as well. Consequently, there is an imperative to develop strategies aimed at mitigating the effect of drought stress on plant growth and development. However, drought tolerance in plants is regulated by complex networks consisting of multifaceted signals including physiological, and biochemical processes (Haghpanah et al., 2024; Sehgal et al., 2018). Therefore, it is crucial to investigate the physiological mechanism and agronomic traits in plants associated with response to drought stress.

Typical physiological traits used to quantify tolerance to drought stress include relative water content, photosynthesis (P_n), transpiration rate (Tr), stomatal conductance (gs), and membrane functions (Ahmed et al., 2020; Hasanuzzaman & Fotopoulos, 2019). Moreover, agronomic traits used as indicators of drought tolerance in wheat include plant height, grain yield, spike length, 1000 kernel weight, number of productive tillers, number of spikelets per spike, and number of grains per spike (Chowdhury et al., 2021; Hasanuzzaman & Fotopoulos, 2019). However, these traits depend on the duration of the stress period, the sensitivity of plant genotypes at different growth stages and on the plant genotypes, and the severity of stress (Chowdhury et al., 2021; Khadka et al., 2020).

The use of iodine in plant growth could help improve plant tolerance and can provide an alternative option to increase yield potential under water stress conditions (Medrano-Macías et al., 2016; Riyazuddin et al., 2023). Iodine is known to stimulate the activities of both enzymatic and non-enzymatic antioxidants which can protect the cellular membrane and macromolecules from inducing oxidative stress (Riyazuddin et al., 2023). Other researchers have shown that iodine promotes growth (Blasco et al., 2013; Duborská et al., 2021; Golubkina et al., 2021; White et al., 2009). For instance, exogenous application of iodine has been demonstrated to improve yield or biomass in wheat (*Triticum aestivum*) (Cakmak et al., 2017), strawberry (*Fragaria* × *ananassa*) (Li et al., 2017), lettuce (*Lactuca sativa*) (Blasco et al., 2013), tomato (*Solanum lycopersicum*) (Borst Pauwels, 1961; Lehr et al., 1958). However, these depend on the concentration of iodine applied, and the mode of application. For example, low-concentration iodine is commonly linked with favourable effects on plant growth and productivity as well as tolerance to stress (Medrano-Macías et al., 2016; Riyazuddin et al., 2023). While higher doses are linked with toxic effects on plants particularly iodide than iodate (Gonzali et al., 2017)

Studies have reported that the performance of wheat at different stages of growth under drought stress varies, for example, Liwani et al. (2019) showed that the vegetative and flowering period in bread wheat is sensitive to drought stress. Therefore, applying iodine treatment at various growth phases may effectively safeguard crops subjected to continuous environmental stressors. However, finding the best iodine (I) concentration and delivery strategy is critical for protecting plants from environmental stressors. Promising methods to supply iodine in plants are foliar spraying and seed priming (Ikram et al., 2025; Mejía-Ramírez et al., 2023). Spraying application needs lesser amounts and has cheaper cost than chemical fertilizers. The foliar fertilization technique offers plants a rapid supply of balanced partitioning of photo assimilates (nutrients) directly to the leaves where photosynthesis occurs from source to sinks without any loss (Kuchlan et al., 2017; Niu et al., 2021). The study by Lawson et al., (2016) demonstrated that foliar application was successful in boosting crop quality in various crop including (Kohlrabi (*Brassica oleracea L. var. gongylodes L. 'Lech'*) and butterhead lettuce (*Lactuca sativa L. var. capitata cv. 'Barilla')*. On the other hand, seed priming is known as the soaking of seeds in water or solution for a specific period to induce germination without allowing radicle protrusion (Jisha et al., 2013). It is a simple, inexpensive, and effective method used to improve stand establishment, growth and productivity of numerous crops (Hasanuzzaman & Fotopoulos, 2019; Marthandan et al., 2020). So far, no data have been reported on the comparison between seed priming and foliar application at various growth stages in wheat under drought stresses. Therefore, the study was conducted with the aim of determining the agronomic and physiological responses of wheat genotypes to iodine application (seed priming and foliar spraying) under both well-watered and water stress conditions during critical growth stages (tillering and flowering). The study hypothesized that iodine treatment via seed priming and foliar spraying will positively influence agronomic and physiological traits in wheat genotypes under stress conditions.

5.2. MATERIAL AND METHODS

5.2.1. Plant materials and site description

Wheat genotypes (SST 8135 and SST806) were obtained from the local Sensako wheat breeding Company in South Africa. The relevant agronomic qualities, such as adaptation to cultivation in South Africa's dry semi-arid regions, market potential, guided the selection of genotypes. A two-year study was conducted at the controlled Environment Facility (CEF) located on the campus of the University of KwaZulu Natal, Pietermaritzburg South Africa (29°37'34.0"S 30°24'13.4"E). The CEF typically have a relative humidity that ranges from 45-55 %, and the average day and nighty temperatures were 30 °C/20 °C, respectively. Before seeding, soil samples were collected for physical and chemical analysis at KwaZulu-Natal Department of Agriculture and Rural Development, Cedara Collage of Agriculture (-29.0913°S,30.1660°E), and the findings are presented in Table 5.1. The soil in the pots were fertilized with Urea and single superphosphate at rates of 100 kg/ha (N, 46 %), 20 kg/ha (P, 10.2 %), and 0 kg/ha based on chemical property recommendations for both seasons. Ten seeds were put in a 5 L pot, which was later reduced to five.

Table 5.1. Soil physical properties used for both 2021 and 2022 were collected at Ukulinga Research Farm

Soil properties	**SD**	**P**	**K**	**Ca**	**Mg**	**EA**	**TA**	**pH**	**Zn**	**Mn**	**N**	**Cu**	**Clay**	**OC**
		mg/L	mg/L	mg/L	mg/L	-	-	KCI	mg/L	mg/L	%	mg/L	%	%
2021	1.04	32	668	1661	534	0.03	14.42	5.23	7.1	28	0.13	2	28	1.8
2022	1.07	70	594	1825	635	0.07	15.92	5.37	8.3	30	0.23	4.4	24	2.7

SD- Soil density; P- Phosphorus; K- Potassium; Ca-Calcium; Mg- Magnesium; EA- Exchange acidity; TA- Total cations; Zn-Zinc; Mn-Manganese; N-Nitrogen; Cu- Copper; OC-Organic carbon.

5.2.2. Experimental design

The 2 ×3 × 4 factorial experiment arranged in randomised complete block design (RCBD), replicated three times was used in this study. The factorial combinations consisted factor A: wheat genotype (SST 8135 and SST 806), Factor B: no iodate, iodate at 0.001 M IO_3^- M and the application to seed priming and foliar spraying), factor C: water stress levels (normal irrigation during tillering, normal irrigation during flowering, stressed at tillering (GS 35), stressed at flowering (GS 65–75) as shown in Figure 5.1.

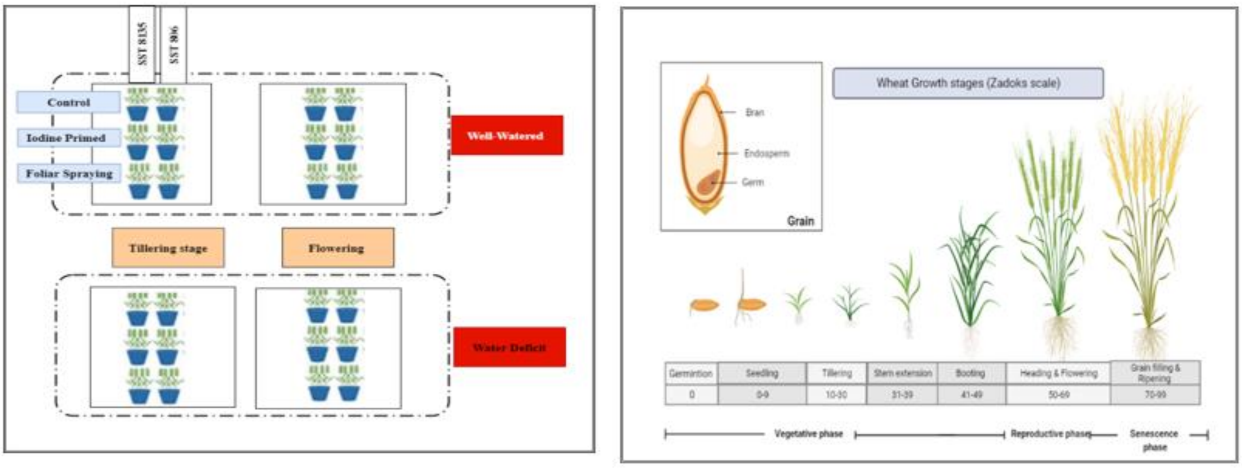


Figure 5.1. The layout of the experimental trials during both seasons (2021-2022) in the greenhouse and the Zodaks growth stages of wheat.

5.2.3. Irrigation and iodine application

Irrigation was delivered using an automated drip irrigation system put directly into each pot. For stress treatments, irrigation was delayed by removing the drippers at tillering (GS 35), and flowering stage (GS 65-75) (Poole, 2005; Zadoks et al., 1974). Drippers were reinstalled at the end of each growth stage to relieve irrigation stress (Figure 5.1). Chapter 3 of this dissertation provides more information on seed priming, including the concentration level that was found to be most effective. Priming of seeds was done by immersing seed into potassium iodate solution, for 6 h, as reported by (Golob et al., 2020). During soaking, an aquarium pump was employed for aeration. The seeds were then washed with distilled water three times. Surface drying was used to restore the seeds' original moisture content at room temperature after they had been treated. Untreated seeds were used as a standard for comparison. While for foliar spraying, a 0.05 % w/v of iodate solution was dissolved in distilled water and the amount chosen was based on the suggestion of the study by Cakmak et al. (2017). Foliar spraying was done a week early at tillering (GS 35), and flowering stage (GS 65-75) by utilizing a hand spray that with a discharge rate of 1 mL every spray equivalent to 10 mL after spraying 10 times per pot. For each stage, the foliar treatment was repeated after one week to ensure that all plants received an equal amount of foliar spray (Nawaz et al., 2017). The soil moisture condition was measured using a time domain reflectometer probe (Campbell scientific Inc. USA) which was put at a depth of 12 cm in the pots during both water regimes.

5.2.4. Data collecting

5.2.4.1. Morphological and yield traits

Data were recorded for several traits: plant height (PH) was measured from the base to the tip of the spike using a measuring tape. The number of productive tillers (TN) was counted at maturity at the end of the stress period, where tillers with spikes were classified as productive and those without spikes as unproductive. After harvest, spike length (SL) was measured using a ruler. Additionally, the number of spikelets per spike (SS) and grains per spike (GPS) were counted from the tagged samples. One thousand kernel weight (TKW) was counted from randomly selected seeds and expressed as g/1000. Grain yield per pot expressed in t/ha was determined by thrashing all spikes by hand and placing them in a bag for weighing with a measuring scale (Masskot, FX320, Zürich, Switzerland). Harvest index was derived as the ratio of grain yield to total biomass and represented as the percentage (Liwani et al., 2019). Biomass was represented as the complete dry weight of plants per pot, excluding the roots, after oven drying (Mlab scientific ldo-080f, Mexico) at 70 °C for 48 hours to eliminate moisture.

$$\mathrm{HI} = \frac{\text{Grain yield}}{\text{Biomass}} \times 100 \tag{1}$$

HI = Harvest index (%), GY = Grain yield ($t\ ha^{-1}$), Biomass ($t\ ha^{-1}$)

5.2.4.2. Measurement of leaf gas exchange variables

Leaf gas exchange was measured on the fully expanded flag leaves in the morning (10:00-12:00 am) during sunny days, using the LI-6400 XT portable photosynthesis system (LI-COR Bioscience, Inc, Nebraska, USA), infrared gas analyser (IRGA) equipped with leaf chamber fluorometer (LCF) (6400-04B, LI-COR Biosciences, Lincoln, Nebraska, USA) at the end of stress period for each growth stages in both water stress and normal treatments. The measurement was taken by clamping the leaves inside the sensor head. The reference CO_2 and the artificial saturating photosynthetic active radiation (PAR) were fixed at 400 and 1000 mmol m^{-2} s^{-1}, respectively, using CO_2 injection (6400-01, LI-COR Biosciences, Lincoln, NE, USA). Leaf gas exchange variables measured included stomatal conductance (gs, mol m^{-2} s^{-1}), transpiration rate, (Tr, mmol m^{-2} s^{-1}), and the rate of photosynthesis (Pn).

5.2.5. Data analysis

The data was first assessed for normality and homogeneity of variance using the Bartlett test. Thereafter, all statistical analysis was conducted separately using Analysis of Variance (ANOVA) in the 21st edition of GenStat (VSN International, UK). Least Significant Difference and Duncan's New Multiple Range Test were used to compare the means at a 5% level of significance.

5.3. RESULTS

Agronomic trait

5.3.1. Plant height (PH)

Plant height (PH) of wheat genotypes was not significantly (P > 0.05) affected by the interaction of water stress levels and iodate application methods for both seasons (Table 5.2). Although there was no significant difference, there was a clear trend observed during both years (2021 and 2022), indicating that wheat plants stressed with drought at tillering or flowering stage had lower PH compared to wheat plants grown under well-watered conditions (Figure 5.2). This suggested that water limitation at either stage of growth reduced PH only; iodate application did not cause any effect on PH aside from iodate application methods during first season.

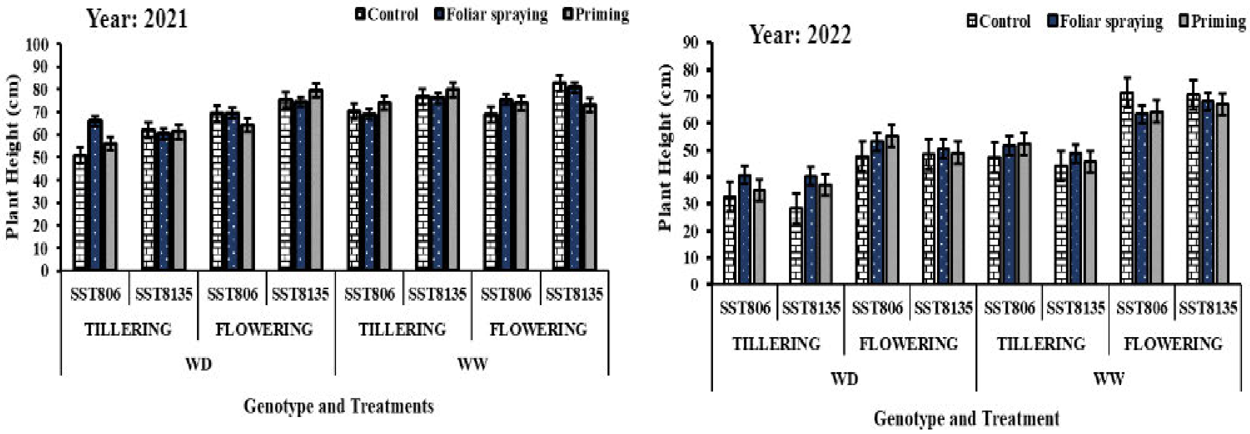


Figure 5.2. Effect of iodate application through seed priming and foliar spraying on plant height under normal and water stress conditions for two seasons (2021 and 2022). Within a column, error bars represent standard errors.

Crop physiology

5.3.2. *Stomatal conductance (gs) and transpiration rate (Tr)*

In wheat genotypes, stomatal conductance (*gs*) and transpiration rate (Tr) were significantly (P < 0.05) influenced by water stress and iodate application methods during the first season for Tr and the second season only for gs (Table 5.2). Genotypes grown from primed seeds or foliar sprayed with iodate at tillering increased Tr by 59 and 35% when compared with control under drought stress conditions, respectively. During flowering, the highest Tr was observed with no treatment supplied, while the lowest Tr was recorded for genotypes foliar sprayed with iodate during the tillering stage under normal conditions (Table 5**.3**). Conversely, genotypes grown from primed seeds or foliar sprayed with iodate at tillering or flowering stages resulted in decreases in *gs* by 7 or 45% and 157 or 987%, respectively, when compared with to controls under water deficit conditions. Similarly, genotypes grown from primed seeds or foliar sprayed with iodate during tillering and flowering stages led to decrease in *gs* by 4 or 16% and 36 or 164% when compared with controls under normal conditions, respectively (Table 5.3).

5.3.3. Rate of photosynthesis (Pn)

With respect to rate of photosynthesis *(Pn)*, there was no significant ($P > 0.05$) difference between water stress imposed at tillering and flowering stage and iodate application methods in wheat genotypes for both years. However, significant ($P < 0.05$) differences were observed between genotypes and iodate application during 2021 only (Table 5.2), particularly in genotype SST806 where applying iodate through seed priming and foliar spraying led to increased *Pn* by 7 and 5% while in genotypes SST8135, decreases in Pn by 2 and 1% when genotypes were foliar sprayed and grown from primed seeds, respectively (Table 5.3).

Table 5.2. The summary of analysis of variance showing significant difference indicated by P levels for the assessed agronomic and physiological traits in two wheat genotypes tested under well-watered and water deficit at two growth stages when exposed by iodine application.

	TRAITS																									
Source of variation	PH		SL		NT		NGS		SPS		TGW		GY		BY		HI		RWC		NP		SC		TT	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
Water treatment (W)	ns	***	***	***	***	***	*	***	***	***	***	**	***	***	***	***	***	*	ns	**	ns	ns	***	***	***	***
Iodine application (IA)	***	ns	*	**	**	**	***	ns	**	ns	ns	ns	ns	ns	ns	***	ns	***	ns	ns	***	ns	**	**	ns	ns
Genotypes (G)	***	ns	**	**	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	**	ns	ns	*	ns	ns	ns	**	*	ns	*
IA x W	ns	ns	ns	*	**	**	**	ns	**	*	*	ns	**	ns	*	***	ns	ns	ns	ns	ns	ns	ns	***	**	ns
G x W	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	*	***	***
G x IA	ns	ns	ns	ns	*	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	*	*	ns	*
G x W x IA	ns	ns	ns	**	ns	**	*	ns	ns	*	ns	ns	ns	ns	ns	*	ns	*	ns	ns	ns	ns	ns	**	ns	*
Coefficient of variation (%)	10.1	13.3	17.1	22.4	0.8	24.7	29.9	34.4	17.4	21.5	4.5	2.9	31.9	26.9	30.6	33.8	6.5	27.5	50.8	30.6	4.8	28.9	72.2	79	49.3	71.0

Table 5.3. Mean values for the physiological attributes (stomatal conductance (Gs) and ph of wheat genotype with priming and foliar application of iodate under drought stress.

	2021								2022							
	WD				WW				WD				WW			
	TILLERING		FLOWERING		TILLERING		FLOWERING		TILLERING		FLOWERING		TILLERING		FLOWERING	
Treatments	SST806	SST8135	SST806	SST8135	SST806	SST8135	SST806	SST8135	SST806	SST8135	SST806	SST8135	SST806	SST8135	SST806	SST8135
Gs																
Control	0,039^{abc}	0,037^{abc}	0,019^{a}	0,029^{a}	0,114^{bcde}	0,135^{def}	0,029a	0,035^{abc}	0,090^{a}	0,022^{a}	0,144^{a}	0,031^{a}	0,113^{a}	0,147^{a}	0,695^{c}	0,680^{c}
Seed priming	0,09^{abcd}	0,014^{a}	0,019^{a}	0,025^{a}	0,100^{abcd}	0,047^{abc}	0,031^{ab}	0,015^{a}	0,016^{a}	0,060^{a}	0,090^{a}	0,070^{a}	0,131^{a}	0,093^{a}	0,318^{ab}	0,581^{bc}
Foliar	0,184^{ef}	0,043^{abc}	0,030^{ab}	0,026^{a}	0,200^{f}	0,116^{cde}	0,031^{ab}	0,026^{a}	0,018^{a}	0,011^{a}	0,020^{a}	0,049^{a}	0,145^{a}	0,104^{a}	0,028^{d}	0,719^{cd}
Lsd $_{(p=0.05)}$	**0.07148**								**0.3299**							
Pn																
Control	22,58^{cd}	23,93^{de}	20,07^{ab}	19,79^{ab}	27,31^{f}	28,7^{fg}	18,1^{a}	19,64^{ab}	16,26^{abc}	20,9^{abcd}	20,16^{abcd}	26,12^{bcde}	31,73^{de}	30,92^{de}	25,6^{bcde}	32,95^{de}
Seed priming	24,35^{de}	23,53^{de}	18,86^{ab}	18,2^{a}	28,73^{fg}	28,61^{fg}	20,94^{bc}	19,59^{ab}	19,7^{abcd}	14,68^{ab}	9,6^{a}	14,45^{ab}	33,04^{de}	29,77^{cde}	32,65^{de}	29,16^{cde}
Foliar	24,73^{e}	22,7^{cde}	19,59^{ab}	18,92^{ab}	29,81^{g}	28,22^{fg}	20,18^{ab}	20,99^{bc}	19,8^{abcd}	15,15^{ab}	16,27^{ab}c	14,85^{ab}	27,96^{bcde}	31,76^{de}	33,22^{de}	37,04^{e}
Lsd $_{(p=0.05)}$	**1.819**								**11.547**							
TT																
Control	5,7^{abcd}	2,52^{abc}	0,8^{a}	1,13^{ab}	6,84^{bcd}	8,11^{cde}	9,8^{def}	14,59^{f}	0,54^{a}	0,44^{a}	5,06^{ab}	1,06^{a}	2,19^{ab}	2,18^{ab}	15,8^{c}	15,29^{c}
Seed	9,13^{def}	1,95^{ab}	0,67^{a}	0,91^{a}	6,7^{bcd}	3,29^{abc}	6,77^{bcd}	13,67^{f}	0,35^{a}	0,05^{a}	0,27^{a}	0,2^{a}	2,62^{ab}	1,79^{ab}	7,42^{b}	16,66^{c}

priming																
Foliar	10,1^{def}	2,98^{abc}	1,16^{ab}	0,93^{a}	13,26^{ef}	7,67^{cd}	1,45^{ab}	14,23^{f}	0,36^{a}	0,22^{a}	0,67^{ab}	1,55^{a}	2,91^{ab}	2,16 ^{ab}	2,96^{ab}	17,94^{c}
Lsd $_{(p=0.05)}$	**3.445**								**4.929**							
RWC																
Control	26.4^{ab}	30.0ab	30.9^{abc}	45.9abcd	30.7^{abc}	25.4^{ab}	29.3^{ab}	48.1^{abcd}	53.8^{abcde}	42.0^{abcde}	28.8^{abc}	58.2^{abcde}	64.0^{de}	60.0^{bcde}	58.2 ^{abcde}	71.7^{e}
Seed priming	40.2^{abc}	30.9^{abc}	69.0^{cd}	33.2^{abc}	21.1^{ab}	38.4^{abc}	22.8^{ab}	81.1^{d}	61.1^{bcde}	64.9^{de}	35.8^{abcd}	49.5^{abcde}	75.4^{e}	60.8^{bcde}	54.7^{abcde}	74.6^{e}
Foliar	33.8^{abc}	45.8^{abcd}	33.3^{abc}	58.7^{bcd}	38.7^{abc}	47.8^{abcd}	19.2^{a}	38.8^{abc}	61.9^{cde}	48.5^{abcde}	27.9^{ab}	26.1^{a}	65.4^{de}	53.5^{abcde}	56.9^{abcde}	62.3^{de}
Lsd $_{(p=0.05)}$	**31.99**								**27.62**							

5.3.4. Leaf water status (RWC)

There was no significant ($P > 0.05$) difference between water stress levels and iodate application concerning relative water content (RWC) for both years (Table 5.2). Although the results were insignificant for the interactions ($P > 0.05$), significant ($P < 0.05$) differences in RWC with water stress levels were noted for the second season. Applying drought stress during the tillering or flowering periods resulted in decreased in RWC by 14 and 67% when compared with unstressed treatments, respectively (Table 5.3).

5.3.5. Shoot length (SL)

There were no significant ($P > 0.05$) differences between water stress levels and iodate application methods regarding shoot length (SL) during the first season. However, the interaction of water stress levels and iodate application significantly ($P < 0.05$) influenced SL during the second season (Table 5.2). Under normal conditions, genotypes grown from primed seeds had the increase of 23 and 16.8% in SL at tillering and flowering stage, while foliar sprayed plants showed a 1.5 increase and 27% decrease in SL at tillering and flowering stage when compared with control, respectively. Under water-stressed conditions, untreated seeds maintained the maximum SL when compared with other applied treatments primed seeds and foliar spraying (Figure 5.3).

5.3.6. Number of grains per spike (NGS) and number of spikelets per spike (SPS)

The interaction of foliar spraying and seed priming and growth stage (tillering and flowering stages) resulted in a significant ($P < 0.05$) increase in the number of grains per spike (NGS) by 68 and 17.2%, during 2021, respectively, when compared with controls under drought stress conditions (Table 5.2). However, genotypes grown from primed seeds with iodate increased NGS by 19.5 and 2.3% at tillering and flowering stages under well-watered conditions during same year 2021, respectively (Figure 5.3). A significant difference ($P < 0.05$) was observed for spikelets per spike (SPS) between iodate application and water stress levels for both years (2021 and 2022). Genotypes grown from primed seeds and foliar sprayed wheat genotype with iodate at tillering stage increased SPS by 14 and 26% and at the flowering stages by 6 and 2% under drought stress conditions. Under normal conditions, genotypes grown from primed seeds only increased SPS by 20%. During 2022, genotypes grown from primed seeds or foliar sprayed with iodate at tillering exhibited higher increases of 26 or 28% in SPS under stressed conditions, respectively. Under normal conditions, genotypes grown from primed seeds or foliar sprayed with iodate at the flowering stage maintained high SPS by 50 and 20%, respectively (Figure 5.3).

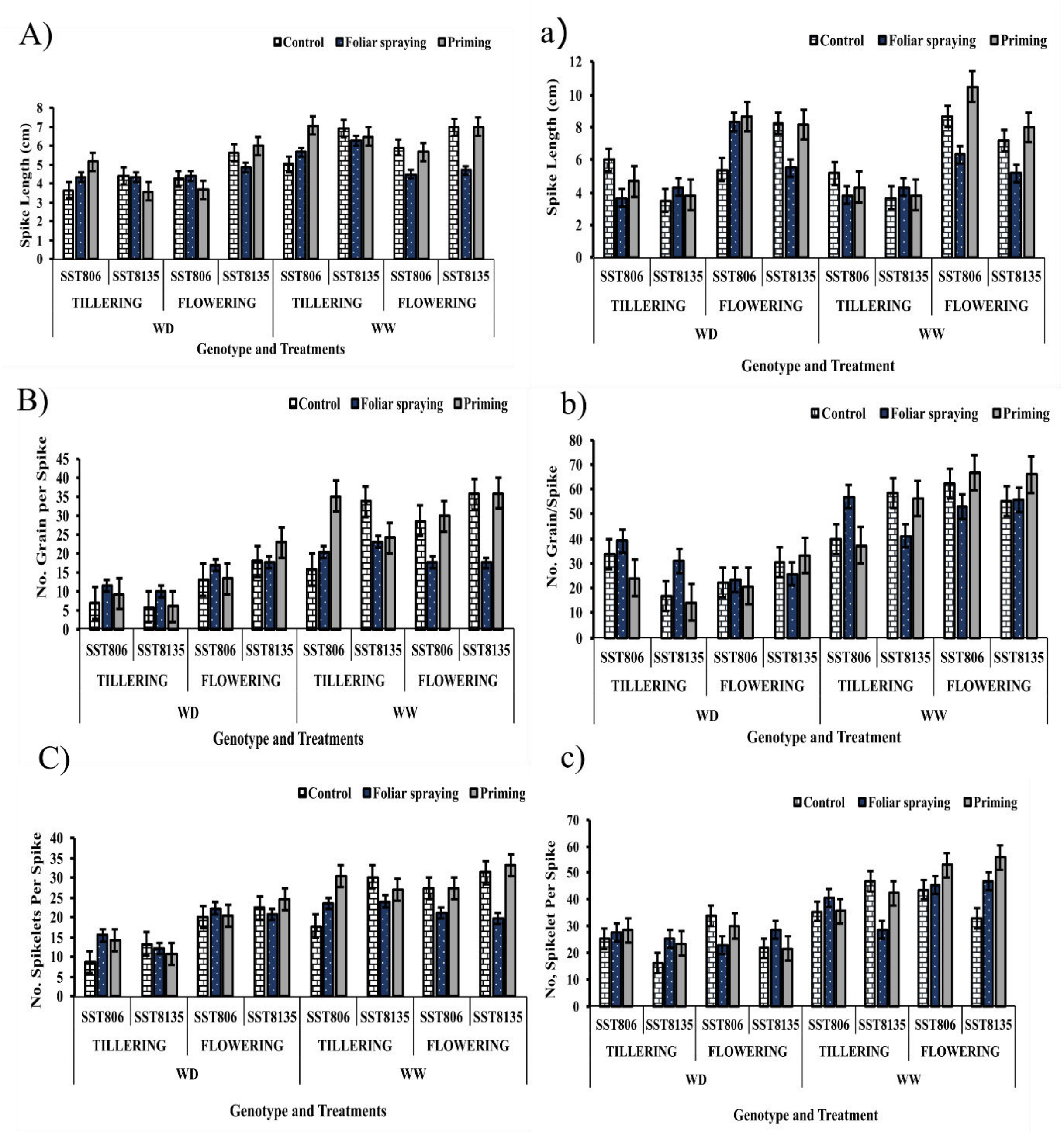


Figure 5.3. Effect of iodate application through seed priming and foliar spraying on A) spike length, B) number of grains per spike, C) spikelets per spike under normal and water stress conditions for two seasons (2021 and 2022). Within a column, error bars represent standard errors.

5.3.7. Number of productive tillers (NTP)

In wheat genotypes, the number of productive tillers (NTP) was significantly ($P < 0.05$) affected by interaction of water stress levels and iodate application methods in both years (2021 and 2022) (Table 5.2). The genotypes that experienced drought stress at tillering produced the highest NTP (2.33) when

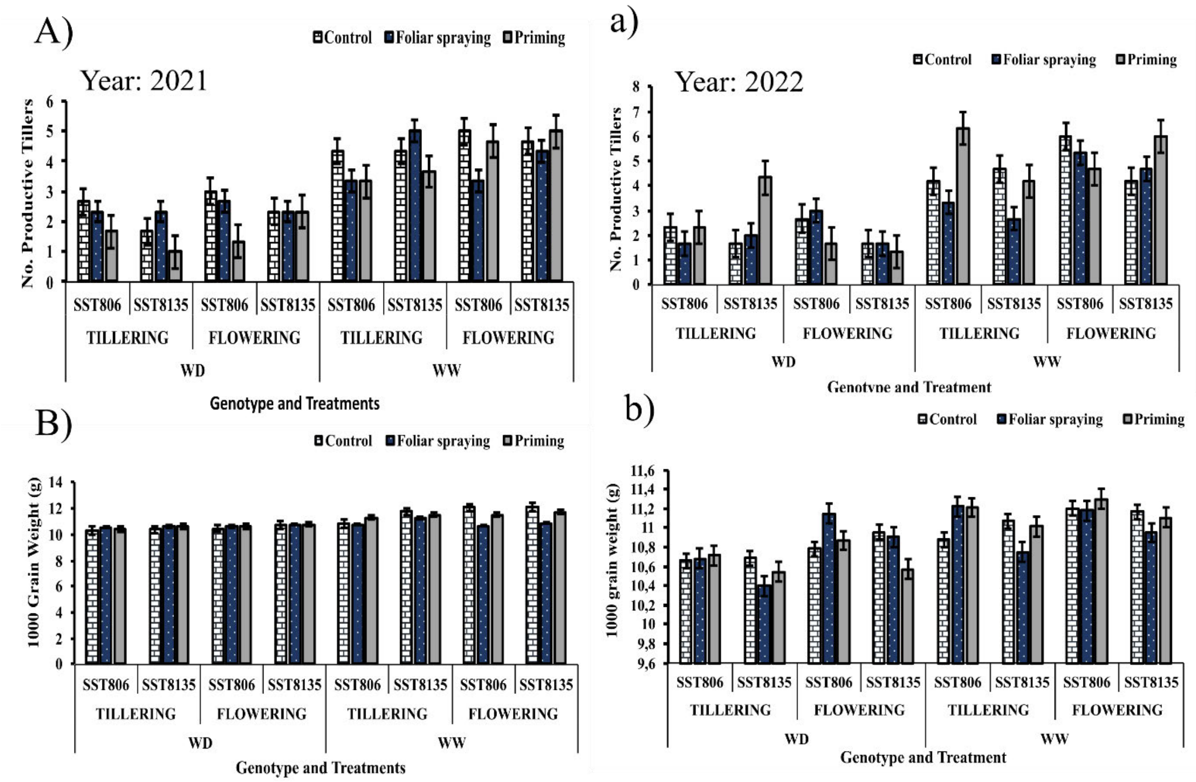


Figure 5.4. Effect of iodate application through seed priming and foliar spraying on A) number of productive tillers and B) thousand-grain weight under normal and water stress conditions for two seasons (2021 and 2022). Within a column, error bars represent standard errors.

foliar sprayed with iodate during the first season. Under normal conditions, untreated seeds and genotypes grown from primed seeds at flowering stage had higher (4.83) NTP, (Figure 5.4). In the second season, genotypes grown from primed seeds showed the highest NTP (3.33) at tillering stage, while at flowering stage, genotypes that received foliar spray exhibited the highest NTP (2.33) under drought stress conditions.

5.3.7. Thousand grain weight (TGW)

There was a significant ($P < 0.05$) difference in response to an interaction of water stress levels and iodate application methods during the first season only (Table 5.2). Genotypes grown form primed seed or foliar sprayed at tillering resulted in maximum increase of 2 and 3% in TGW, respectively, when compared with the control treatments. Similarly, Plants grown from primed seeds or foliar sprayed at flowering stage increased TGW by 1.1 %, under water-stressed conditions. Under non-stressed conditions, genotypes grown from primed seeds at tillering had a higher TGW of 11.39 g, while untreated genotypes at flowering stage exhibited a higher TGW of 11.61 g when compared with those treated with iodate application methods (Figure 5.4).

5.3.8. Grain yield (GY)

In wheat genotypes, GY in wheat was significantly ($P < 0.05$) influenced by interaction of water stress levels and iodate application methods during 2021 (Table 5.2). In 2021, seed primed and foliar sprayed at flowering stage increased GY by 50 and 15%, respectively, when compared with control under stress conditions. Similarly, primed seed and foliar spraying till flowering at flowering stage increased GY by 18.6 and 11.8% under normal conditions, respectivlely (Figure 5.5). Genotype SST 8135 maintained a significantly ($P < 0.05$) higher grain yield (GY) of 3.13 t/ha when compared with genotype SST 806 (2.07 t/ha).

5.3.9. Biological yield (BY)

An interaction of drought stress and iodine application methods (tillering and flowering) had a significant ($P < 0.05$) effect on biological yields (BY) during both seasons (Table 5.2). Foliar application at both tillering and flowering stages under drought stress conditions increased BY by 15 and 28% when compared with the control, respectively. Under normal conditions, genotypes grown from primed seeds or foliar sprayed increased BY by 20 and 16% compared to control during the flowering stage, respectively (Figure 5.5). In the second season, genotypes grown from primed seeds exhibited higher BY of 2.37 and 4.23 t/ha when water stressed at tillering and flowering stages, respectively. Similarly, genotypes grown from primed seeds had higher BY (5.65 and 3.16 t/ha) during tillering and flowering stages, under non-stressed conditions, respectively (Figure 5.5).

5.3.10. Harvest index (HI)

In both 2021 and 2022, no significant differences ($P > 0.05$) were observed between interaction treatments (Table 5.2). Despite the lack of significant differences, the application of drought stress during either the tillering or flowering stages, regardless of iodate treatment, resulted in a decrease in the harvest index (HI) when compared to unstressed genotypes. Notably, in 2022, the application of iodate via foliar sprays during drought stress at tillering and flowering stages led to increases in HI by 22% and 13%, respectively, compared to the control treatments. Under normal conditions, foliar spraying genotypes with iodate at tillering stage resulted in a 15% increase in HI (Figure 5.5).

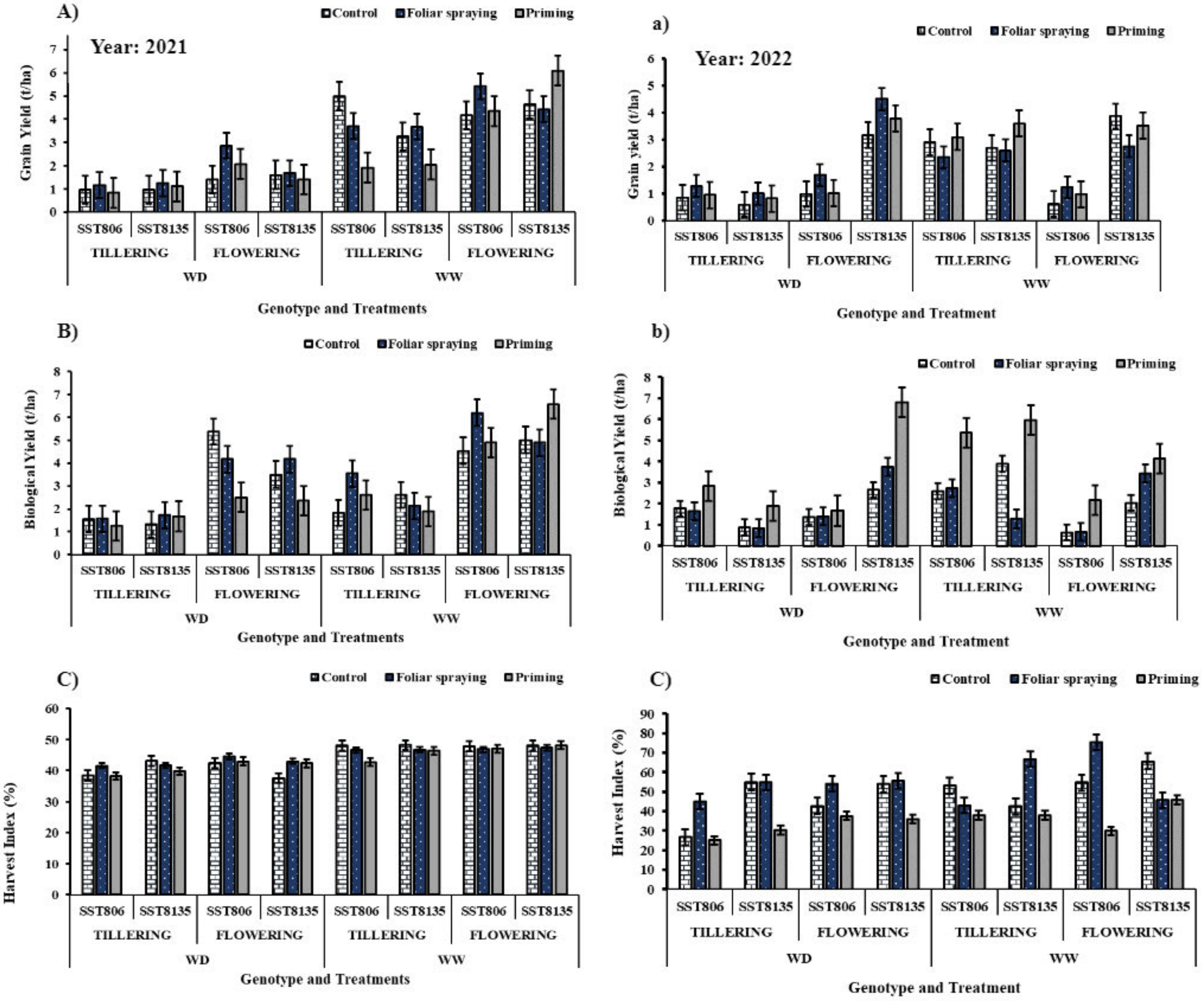


Figure 5.5. Effect of iodate application through seed priming and foliar spraying on A) grain yield, B) biological yield and C) harvest index under normal and water stress conditions for two seasons (2021 and 2022). Within a column, error bars represent standard errors.

5.3.11. Correlation among parameters

The correlation analysis (Figure 5.6A) revealed a negative correlation between physiological characteristics such as intercellular CO_2 concentration (Ci) and net photosynthesis (*Pn*). Conversely, most physiological and agronomic traits such as BY, SPS, NGS, SL, GY, TWG, Tr, and Gs exhibited significant positive correlations with one another. In the second season, all physiological and agronomic traits were positively correlated, with the exception of HI and BY, which demonstrated a significant negative correlation (Figure 5.6B).

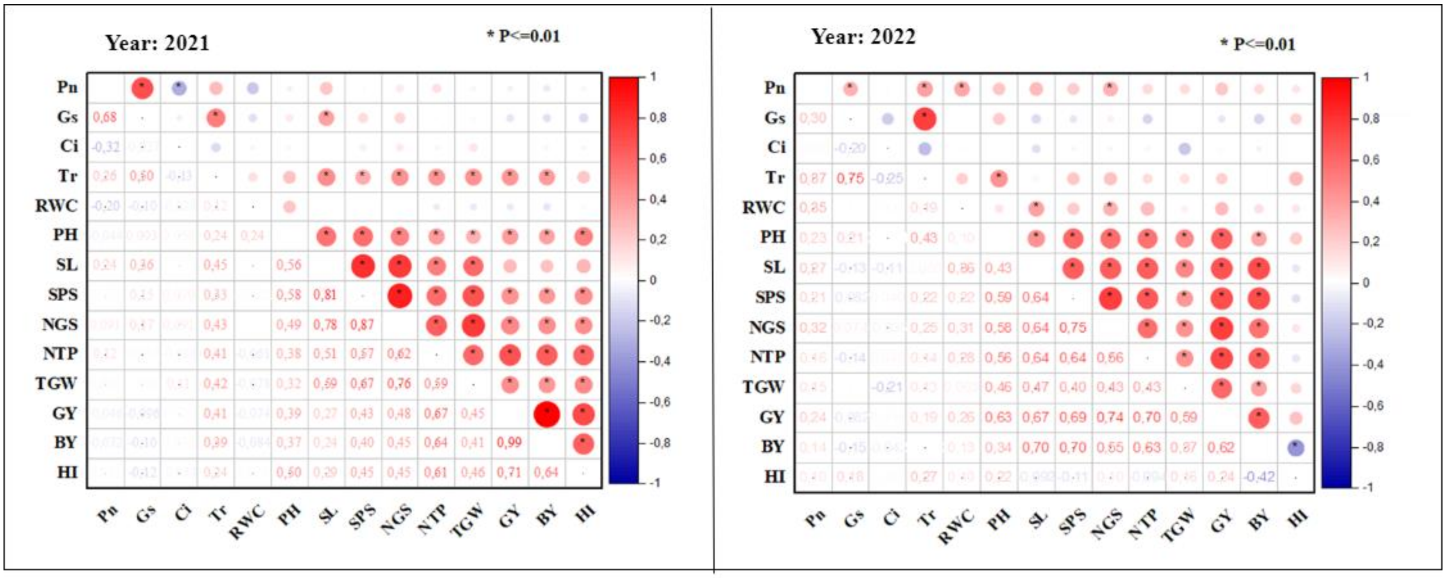


Figure 5.6. Pearson correlation coefficient of wheat grain yield on growth, physiological traits, and yield components during the two seasons (A) 2021 B) 2022. The high and lower intensity of the red colour indicates a high positive association, and the blue colour shows high negative correlation between the studied traits. Values closer to one indicate a strong correlation and values close to zero indicate a weaker interrelationship between them. (P < 0.01) indicates significant correlation between the traits.

5.4. DISCUSSION

Sustainable agricultural practices aimed at promoting plant growth and enhancing crop productivity are essential in contemporary agriculture. Various abiotic stress factors, particularly drought, significantly impact crop growth and food security on a global scale. Bio-stimulants are increasingly utilized to improve morphological traits, physiological resilience, and yield in plants through seed priming and foliar applications (Gonzali et al., 2017; Pérez-Salas & Medrano-Macías, 2021). This study aimed to evaluate the effects of iodate priming seeds and foliar spraying on the morphological and physiological responses of wheat genotypes subjected to drought stress during the tillering and flowering stages.

In this study, plant height (PH) was not significantly (P > 0.05) affected by iodate application or water stress levels; however, it was notably influenced by drought stress, suggesting that the mechanisms governing PH are primarily driven by water availability rather than iodine-mediated pathways. Drought stress alone led to a substantial decrease in PH, particularly during the tillering and flowering stages, which can be attributed to the disruption of cell division and elongation processes. During these developmental stages, plants undergo active mitotic activity and cell division, which are highly sensitive to water deficit (Cosgrove, 2005). The reduction in turgor pressure under drought conditions impairs cell expansion, while the associated metabolic stress (such as reduced nutrient uptake and photosynthetic activity) limits the energy and resources required for cell division (Cosgrove, 2005). This aligns with the findings of Zhao et al. (2020) in wheat, who reported that drought stress during critical growth stages significantly shortened plant height by inhibiting the expression of genes involved in cell cycle progression and cytoskeleton organization. Furthermore, the absence of a mitigating effect of iodate on PH under drought stress may indicate that iodine does not directly influence the hormonal or genetic regulators of cell division and elongation (e.g., auxins and expansions) or that the applied concentration was insufficient to counteract the severe physiological constraints imposed by water deficit. Lastly, the suppression of vertical growth directly compromises photosynthetic efficiency and gas exchange dynamics by restricting leaf area expansion and vascular development, which are the key determinants of light interception, CO_2 diffusion, and hydraulic conductance (Gago et al., 2020).

Photosynthesis and gas exchange are critical for metabolic processes and biomass production (Gago et al., 2020; Pereira, 1995)**.** According to Choat et al., (2018) and Reddy et al., (2003) stomatal closure is one of the earliest responses to drought stress, aimed at minimizing water loss, which subsequently affects gs and net photosynthesis (Anet) due to both stomatal ((e.g., reduced CO_2 influx) and non-stomatal limitations (e.g., impaired Rubisco activity).Our study found that withholding water during the tillering or flowering stages decreased gs and Tr in wheat genotypes. Conversely, iodate application via seed priming and foliar spraying enhanced Tr at the tillering stage when compared with controls under water stress conditions. Furthermore, *gs* decreased under both drought and normal conditions when iodate was applied through seed priming or foliar spraying. Similar results have been shown by Lima et al. (2023) whereby, soybeans treated with 10 µM KI exhibited a higher transpiration rate, with an average increase of 73% under water deficit and an average increase of 47% in gs under normal condition, however, under water stress conditions there was no significant difference. In contrast, our findings indicated that iodate application reduced *gs* under both conditions, suggesting a consistent physiological response that may enhance water conservation but potentially limit carbon assimilation.

Our results demonstrated that iodate application through seed priming and foliar spraying increased *Pn* by 7 and 5% in genotype SST806, while in genotype SST8135, *Pn* decreased by 2 and 1%, respectively. This variation points to the activation of compensatory physiological mechanisms that offset potential growth gains and highlight how genetic diversity modulates photosynthetic responses to iodine treatments. A possible explanation is that iodate exposure induced metabolic trade-offs, such as elevated respiratory costs associated with detoxification processes or shifts in carbon allocation toward non-structural compounds (e.g., root biomass or secondary metabolites) (Gonzali et al., 2017; Pérez-Salas & Medrano-Macías, 2021). This phenomenon is consistent with previous findings in iodine-biofortified crops, where photosynthetic improvements did not translate into biomass accumulation due to reallocation of resources away from harvestable tissues (Kiferle et al., 2021). Furthermore, the absence of interactive effects between water availability and iodate on Pn suggests that iodine's physiological impact operates independently of hydraulic constraints, reinforcing the hypothesis that biomass responses were governed by intrinsic metabolic adjustments rather than carbon assimilation capacity alone. These results highlight the principle that biomass production is a integrative function of photosynthetic efficiency and whole-plant resource partitioning, as highlighted by Lambers et al., (2008), wherein Pn modulation alone is an inadequate proxy for predicting growth outcomes without accounting for sink-driven carbon allocation dynamics. Other studies have indicated that iodate concentrations of 20, 40, and 80 μM increased the photosynthetic rate in lettuce by approximately 20% (Blasco et al., 2011). Kiferle et al., (2022) also reported that proteins containing iodate might enhance the photosynthetic capacity of plants.

The absence of iodate-induced changes in relative water content (RWC) across wheat genotypes in both seasons indicates that iodate does not significantly ($P > 0.05$) influence water relations under the tested conditions, likely due to its minimal involvement in osmotic adjustment or aquaporin-mediated water transport (Kaldenhoff et al., 2014; Martinez et al., 2004). In contrast, genotype significantly influenced RWC, with SST8135 exhibiting higher RWC than SST806 under drought stress in 2022, highlighting essential genetic differences in water retention mechanisms. These differences may arise from differential aquaporin gene expression, enhancing root water uptake efficiency in SST8135, coupled with ABA-mediated stomatal regulation to reduce transpirational losses (Kaldenhoff et al., 2014). Additionally, SST8135 likely exhibits superior osmotic adjustment through solut (Ashraf & Foolad, 2007).

The greater RWC decline during flowering-stage drought compared to tillering under drought conditions aligns with findings of Lawani et al., (2018), that reproductive development imposes irreversible metabolic demands, exacerbating water stress vulnerability in rice (*Oryza sativa*). At flowering, ABA accumulation disrupts aquaporin localization in floral tissues and suppresses *MYB*-regulated pollen viability genes, impairing water allocation to reproductive structures (Farooq et al., 2009). Concurrently, resource competition between grain filling and stress defense depletes osmolytes, weakening turgor maintenance, a less severe issue during tillering due to greater sink-source flexibility and root plasticity (Farooq et al., 2009). These stage-specific constraints, combined with genotypic variations in water-use efficiency, explain the consistent RWC reduction under drought, as observed in cereals and legumes (Almeselmani et al., 2011; Jaleel et al., 2008). Our study further concurs with the studies of Almeselmani et al. (2011), Farooq et al. (2009a) and Jaleel et al. (2008) which indicated that withholding water at any stage of growth in wheat *(Triticum aestivum*) results in decreased RWC.

Drought stress significantly alters the yield potential of wheat by affecting its key yield components (number of fertile tillers, spike length, spikelet per spike, thousand kernel weight, biological yield, and harvest index) with effects varying by growth stages (Haider et al., 2020; Iqbal et al., 2019; Nawaz et al., 2012). For instance, during the vegetative phase, water deficiency primarily reduces tiller production, plant height, and leaf area expansion (Oladir et al., 1999), leading to fewer grain-bearing spikes (Veesar et al., 2007). When drought occurs during flowering, it negatively impacts spike development, resulting in shorter spikes, fewer spikelets, reduced grain numbers per spike, and lower individual grain weights (Mirbahar et al., 2009). Liwani et al. (2019b) found that some wheat varieties demonstrate adaptive responses by maintaining grain yield despite drought stress at flowering, possibly through physiological adjustments in resource allocation. These findings highlight the complex interactions between environmental stress and plant development, where wheat plants make strategic trade-offs between different yield components to optimize reproductive success under water-limited conditions. The timing of drought stress appears crucial, with early-stage stress affecting structural growth and late-stage stress influencing reproductive development (Li et al., 2023).

Our current findings showed that drought stress affected yield attributes at different growth stages which align with these studies, indicating that the effects of drought stress on morphological and physiological traits in wheat genotypes vary depending on the season and growth stage. Generally, it is suggested that drought stress has a lesser negative impact on crop yield during the vegetative phase compared to the reproductive phase (Fischer & Fukai, 2003). The observed difference is likely due to different of each growth stage. During vegetative growth, plants focus on structural development such as leaf expansion and tiller formation, which can recover after stress subsides due to inherent plasticity (Blum, 1996). In contrast, reproductive processes like pollination and grain filling are more vulnerable; drought disrupts critical metabolic pathways, leading to irreversible yield losses through reduced spikelet fertility, grain number, and kernel weight in cereals such as wheat (*Triticum aestivum*), rice (*Oryza sativa*), and maize (*Zea mays*). This is dependent upon nutrient uptake, photosynthetic capacity, and the distribution of assimilates (Blum, 1996; Fathi & Tari, 2016; Mahajan & Tuteja, 2005). Evidence from Liwani et al. (2019b) supports this: drought at tillering (vegetative) caused biomass loss but allowed partial recovery, while flowering-stage stress directly reduced grain set by up to 40%. In our study, drought stress imposed during the tillering or flowering stages led to decreases in all studied morphological and physiological traits compared to well-watered controls (Figure 5.5).

Despite these declines, the application of iodate treatments significantly reversed the negative effects, improving physiological and morphological attributes under water stress. This recovery aligns with established evidence of iodate-mediated growth enhancement in stress environments, as documented under salinity (Blasco et al., 2013; Kiferle et al., 2022; Leyva et al., 2011; Pérez-Salas & Medrano-Macías, 2021; Rodríguez-Salinas et al., 2022; Sabourmoghaddam, 2018), heavy metals stress (Neha & Singh Rita, 2016), heat stress (Hora & Holwerda, 2023) and drought stress (Lima et al., 2023) in some of the horticulture crops and vegetables, suggesting its potential as a general solution for managing different types of environmental stress. Although iodate is considered a non-essential element, it has been shown to benefit plant growth and development. Nascimento et al. (2022) reported that iodate could function as a biostimulant agent, in other elements such as silicon, iron, magnesium, selenium, and sodium. The beneficial effects of iodate are influenced by species, type of iodate applied, concentration, and the specific stress conditions.

In this study, exogenous application of iodate on wheat plants under drought stress conditions mitigated the negative effect of drought stress by improving yield and associated components such as number of productive tillers, spikelets per spike, number of grains per spike, thousand-grain weight, and biological yield. While literature on the effects of iodine applications in cereal crops under stress conditions is limited, a recent study by Lima et al. (2023) demonstrated that foliar spraying with iodine improved plant growth and biomass in soybean (*Glycine max*) under drought stress by enhancing antioxidant enzyme activity and reducing oxidative damage to plant tissues. Our findings were consistent with, Cakmak et al. (2017), which indicated that foliar spraying under normal conditions increases biomass in wheat, although yield component effects were not assessed. Kutlu et al. (2023) examined the effects of biologically active iodine (BAI) and potassium iodide (KI) on oat *(Avena sativa*) plants through seed priming, soil application, and foliar spraying under normal conditions. Their results indicated that all application methods increased grain yield when compared with the controls, although thousand-grain weight was unaffected by the application methods or iodine form. The study concluded that foliar spraying of iodine, in conjunction with basic fertilization, can enhance yield, quality, and nutritional value in oat cultivation.

Similar positive responses of iodine application have been reported in other plants such as tomatoes (*Solanum lycopersicum)* (Lim-choowong et al. 2016), strawberries *(Fragaria × ananassa)* (Li et al. 2016); wheat (*Triticum aestivum)* (Cakmak et al. 2017); berries *(Vaccinium corymbosum)* (Ramírez-Gottfried et al. 2023); lettuce *(Lactuca sativa)* (Blasco et al., 2013). These studies suggested that the beneficial effects of iodine were associated with optimal low concentrations, as excessive iodine can lead to phytotoxic effects. Therefore, maintaining an appropriate balance of iodine in the soil is essential to ensure that plants receive the necessary amount of this element. Moreover, while both methods of iodine application improved yield and yield components, seed priming proved to be more effective; this was attributed to primed seeds absorbing more water, leading to better stand establishment. Both cultivars benefited from seed priming, resulting in uniform and early stand establishment, as well as vigorous early growth. This initial growth enhanced crop growth rates and yields while reducing the likelihood of crop failure.

5.5. CONCLUSION

The iodine application proved to be effective in alleviating drought stress in wheat genotypes, as evidenced by increases in physiological and agronomic traits such as Pn and Tr. The correlation matrix indicated strong positive relationships among several physiological and agronomic parameters. The positive impact of iodine on wheat components may be linked to its ability to maintain reactive oxygen species (ROS) homeostasis, ultimately enhancing grain yield. Genotype SST806 exhibited the highest drought tolerance, followed by SST8135, indicating its potential as a parent plant for future breeding research focused on improving drought resilience in wheat. Further studies are warranted to explore the relationship between antioxidant enzyme activity, genetic variation, and grain yield in wheat genotypes exhibiting drought tolerance.

CHAPTER 6

IODINE SEED PRIMING ENHANCES YIELD AND STRESS RESILIENCE IN WHEAT *(TRITICUM AESTIVUM L.)* UNDER RAINFED AND IRRIGATED CONDITIONS

Nkosinathi Hlatshwayo[1, *], Takudzwa Mandizvo[1], Albert Modi[2] and Tafadzwanashe Mabhaudhi[1,3]

[1] *Centre for Transformative Agricultural and Food Systems, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, P/Bag X01, Scottsville, Pietermaritzburg 3209, South Africa.*

[2] *Department of Biological and Environmental Sciences, Faculty of Natural Sciences, Walter Sisulu University, Mthatha 5100: amodi@wsu.ac.za*

[3] *Centre for Water Resources Research, School of Engineering, University of KwaZulu-Natal, Scottsville, Pietermaritzburg, South Africa,*

[4] *Centre on Climate Change and Planetary Health, London School of Hygiene and Tropical Medicine, London, United Kingdom.*

[*]Correspondence: nathi4722@gmail.com (N.H)

(submitted to the Journal of agronomy MDPI 23 October 2024)

ABSTRACT

Iodine (I) serves as a crucial micronutrient in plants, influencing various physiological and biochemical processes, including antioxidant defense, nutrient uptake, and stress tolerance. This study evaluated the effects of iodine seed priming and different priming doses on yield and yield-related traits under rainfed and irrigated conditions. A field experiment was conducted at Ukulinga Research Farm, University of KwaZulu-Natal, over two consecutive winter seasons (2021/22 and 2022/23) using a factorial design in a randomized complete block design (RCBD). The three factors included water treatment (rainfed and irrigated), iodine concentration (0.001 and 0.01 M), and genotypes (PAN3111, SST806, and SST8135), replicated three times. Results indicated that genotypes treated with 0.01 M and 0.001 M iodine solutions showed significant increases in spike length (SL), with improvements of 13.7% and 11.5%, respectively, compared to the control. Yield and yield attributes significantly decreased under rainfed conditions; however, iodine seed priming mitigated drought effects by enhancing yield-related components such as spike length (SL), number of grains per spike (NGS), number of productive tillers (NTP), and grain yield (GY). Under rainfed conditions, genotype SST806 recorded the highest NGS (24.67), while PAN3111 had the lowest (22.78). The highest grain yield (134.2 kg/ha) was achieved by SST806 with 0.01 M iodine, while unprimed PAN3111 yielded the least (49.9 kg/ha). These findings suggested that iodine priming can enhance drought tolerance in wheat by improving yield and related traits.

Keywords: Wheat genotypes; drought resilience; water management; yield improvement; stress tolerance

6.1. INTRODUCTION

Wheat (*Triticum aestivum* L.) has enormous economic importance worldwide; it contributes about 20% of the total dietary calories and proteins (Shiferaw et al., 2013). It is recognised as the primary source of food, feed and industrial raw materials (Kumar et al., 2011). Wheat is the second most important rainfed crop after maize and the second most irrigated crop after rice (Molden et al., 2011). However, drought is the primary challenge of cultivating wheat, particularly in arid and semiarid regions, which can limit crop yield and reduce overall wheat production (Hussain et al., 2018; Tadesse et al., 2019). This effect can be worse in South Africa, one of the 30 driest countries in the world, with an annual rainfall < 500 mm, lower than the world average of 860 mm (Modi & Mabhaudhi, 2013). Therefore, this necessitates the need to develop approaches to improve yield as climate predictions indicate an increase in drought and temperature conditions.

When faced with limited water availability, wheat plants often experience stunted growth and reduced leaf area, negatively affecting photosynthesis and overall biomass accumulation (Zhang, 2018). This stress is particularly detrimental during the reproductive phase, where inadequate water can impair grain filling, resulting in smaller kernel sizes and lower grain weights (Farooq et al., 2018). Additionally, drought conditions disrupt the plant's ability to absorb nutrients and manage water efficiently, further compromising yield potential. As a result, the effects of drought stress on wheat yield and its related attributes pose significant challenges for agricultural productivity in dry regions.

In recent years, several researchers have shown that using mineral nutrition, such as iodine, can mitigate the negative effects of drought stress on plants (Gonzali et al., 2017; Medrano-Macías, Leija-Martínez, González-Morales, et al., 2016a; White & Broadley, 2005). Although iodine's role is not well established as that of other nutrients such as nitrogen, potassium and phosphorus, it can promote growth and development in certain plants and it can influence processes such as photosynthesis and respiration (Sharma et al., 2017). This mineral can be applied using different methods such as foliar and hydroponic application. However, iodine application through seed priming, which is an easy and cost-effective method in plants offers a new way to enhance the yield and productivity of several crops (Hasanuzzaman & Fotopoulos, 2019). Seed priming with iodine involves immersing seeds in an iodine-containing solution and then drying them back to their original moisture content before sowing (Mejía-Ramírez et al., 2023). Thus, it combines both the positive effect of seed priming with improved nutrient supply.

Nonetheless, there remains a gap in understanding the effects of iodine priming and effective dose on plant growth, particularly under drought stress. Generally, iodine application in low concentrations is known to stimulate processes necessary for tolerance and enhance crop yield under stressful environments (Dobosy et al., 2020; Gonzali et al., 2017 Huanxin et al., 2013). For instance, Kiferle et al. (2021) showed that iodine stimulates plants' metabolic processes and increases biomass and anticipated flowering in *Arabidopsis thaliana*. Moreover, Li et al. (2017) in strawberry (*Fragaria ananassa*) and Cakmak et al. (2017) in wheat also reported the positive effect of iodine at lower doses on biomass and growth. However, studies have shown that high iodine concentration can be phytotoxic and reduce agronomic performance (seed germination, plant height and yield). These studies have been reported in pumpkin (*Cucurbita pepo*) (2020), buckwheat (*Fagopyrum esculentum*) (Germ et al., 2019) and peas (*Lathyrus oleraceus*) (Jerše et al., 2017a).

In these studies, there is still a lack of information about the mechanism of seed priming with iodine and dose in crops under drought conditions. Understanding the responses of iodine seed priming in wheat is important because it can significantly enhance plant growth, yield, and resilience to environmental stresses. Farmers can optimize seed treatments by studying these responses to improve crop performance under diverse agricultural conditions (drought, salinity and nutrient-poor soils). Therefore, the study was conducted to assess the effect of iodine seed priming and effective dose in wheat genotypes planted under drought stress. This study hypothesised that iodine priming can enhance drought stress tolerance in wheat genotypes, leading to improved growth, yield, and yield-related traits under both rainfed and irrigated conditions.

6.2. MATERIAL AND METHODS

6.2.1. Plant material

Seeds of wheat genotypes SST8135 and SST806 were sourced from Sensako wheat breeding company in Pretoria, South Africa (28.1856° S, 28.2149° E), while seeds of PAN 3111 were sourced from Pannar, Durban, South Africa (-29.563633, 24.017855). Genotypes were chosen based on agronomic characteristics, such as adaptability to cultivation in the dry, semi-arid parts of South Africa (Table 6.1).

Table 6.1: Wheat genotypes used in the study, selected based on adaptability, breeding program, and crop traits.

Genotype	Characteristics	Breeding program/origin	Traits
SST8135	High yield potential, good disease resistance	South African breeding	Suited for high-rainfall regions, adaptable.
SST806	Drought-tolerant, early-maturing	South African breeding	Suitable for low-rainfall areas, good quality

PAN3111	High protein content, rust-resistant	PANARO breeding program	High-quality grain, suitable for bread-making

6.2.2. Site description and agronomic management

The two-year experiments were conducted at KwaZulu-Natal's Ukulinga Research Farm in Pietermaritzburg (29°37'S; 30°16'E; 845 m a.s.l), South Africa, during the winter seasons of 2021 and 2022. Ukulinga soils were classified as clay-loam soil (USDA taxonomic system). The soil samples taken at a depth of 0.3 m were analysed for physicochemical properties at KwaZulu-Natal Department of Agriculture and Rural Development, Cedara Agricultural College (- 29.0913°S,30.1660°E) and the results for both seasons are presented in Table 6.2. To achieve a fine seedbed, the fields were disked and rotovated. Urea and single superphosphate fertilizer were applied before planting. The application rate for each fertilizer was 100 kg/ha for N (46% N, Urea), and 20 kg/ha P single superphosphate (20% P) for both seasons 2021 and 2022. The seeds were nursed in trays and transplanted on 15 July 2021. The second season's planting was done on 15 June 2022. Weeds and pests were controlled manually by hands.

Table 6.2. Soil properties for the Ukulinga field used in the study

Property	**2021**	**2022**
Bulk density (g/mL)	1.06	1.06
Calcium (mg/L)	932	990
Magnesium (mg/L)	315	303
Phosphorus (mg/L)	17	30
Manganese (mg/L)	80	38
Potassium (mg/L)	194	194
Nitrogen (%)	0.13	0.23
Zinc (mg/L)	5.7	5.8
Copper (mg/L)	5.1	7.3
Exchange acidity (cmol/L)	0.20	0.3
Total cations (cmol/L)	7.94	8.23
pH (KCI)	4.33	4.29
Organic carbon (%)	1.8	1.7
Clay %	28	30

6.2.3. Experimental design

The study was designed as a 3 × 3 × 2 factorial experiment with 3 wheat genotypes (G) [SST8135, SST806 and PAN3111], 3 iodate concentration levels (I) [0, 0.001, 0.01 IO_3^- M] and 2 water treatments (W) [irrigated and rainfed]. The experiment was laid in a completely randomized block design (CRBD) with three replications, giving 54 experimental units. During priming, seeds were soaked except for controls in an aerated solution of potassium iodates for 6 hours (Golob et al., 2020b). An aquarium pump was used for aeration during soaking period. Afterwards, seeds were rinsed with distilled water thrice and allowed to surface dry at room temperature (25 °C) until they reached their original moisture content.

6.2.4. Trial layout and irrigation application

Each side's main plot was 212,5 m^2, and the individual sub-plot size was 3.75 m^2 (1.8 × 2.1 m) per treatment. The plant spacing used in each plot was 0.3 m inter-row and 0.1 m in-row at the seed rate of 33,33 seeds m^{-2}. The irrigated and rain-fed plots were spaced 10 m apart to prevent water sprays from reaching the rain-fed plots. The inter-plot spacing of 1 m in each plot was also made to make data collection easier. All treatments were irrigated for two weeks after transplanting; thereafter, irrigation was withdrawn for the rain-fed treatments. Irrigation scheduling for the irrigation plots was scheduled to meet 100% of the crop water requirement (ETc) based on reference evapotranspiration (ETo) and a crop factor (*Kc*) (Allen et al., 1998). The reference evapotranspiration (ETo) was obtained from the Agricultural Research Council–Institute for Soil, Climate and Water (ARC–ISCW) (25° 44' 19.4" S, 28° 12' 26.4" E) weather station weekly and calculated using FAO Irrigation and Drainage paper. The irrigation was applied using 1 m sprinkles designed to have a maximum range of 6 m radius. The application rate of the sprinkler had an estimate of 10 mm per hour, and the following formula was used to determine the run time.

$$\mathrm{ETc} = \mathrm{ETo} \times \mathrm{Kc} \qquad (1)$$

Where ETc= crop water requirement, ETo = reference evapotranspiration, and Kc= crop factor

The total amount of water applied in the irrigated areas was estimated to be around 731.8 mm. Irrigation was conducted in the mornings to ensure sufficient water availability during peak demand periods of the day.

6.2.5. Data collection

6.2.5.1. Climatic measurement

Daily climatic data collected during both seasons included temperature (Tmax and Tmin), rainfall, relative humidity RH (max and min), solar radiation and reference evapotranspiration. The data was obtained from an automatic on-station weather station (AWS) courtesy of the Agricultural Research Council–Institute for Soil, Climate and Water (ARC–ISCW). Soil moisture was monitored with a time domain reflectometer probe (Campbell scientific Inc. USA).

6.2.5.2. Yield and yield-related traits

Data for this study was recorded from randomly selected plants. Plant height (PH) was taken from the base to the tip of the plant using a meter rod, and spike length was recorded using a ruler (cm). Days to maturity (DTM) were recorded from the days of sowing to the stage when 90% of plants reached harvesting maturity. The number of productive tillers (NTP) was counted in each plot from five randomly selected plants and then averaged at physiological maturity. After harvesting each plot, the plants were sun-dried for two weeks within the respective plots. In five plants, spike length (SL), spikelets per spike (SPS) and grain number per spike (NGS) were counted on five selected plants. Thousand-grain weight (TGW) was recorded from each plot and weighed using a digital sensitive weighing balance (Masskot, FX320, Zürich, Switzerland). Biological yield (BY) was recorded using a digital weighing balance and expressed as kg/ha. Grain yield (GY) was determined by manually thrashing it from spikes and weighed using a weighing balance. The GY was converted to kg/ha (Ulfat et al., 2017). Finally, the harvest index (HI) was calculated as the ratio of grain yield to total biological yield and expressed as the percentage.

$$\text{Grain yield (kg/ha)} = \frac{\text{Grain yield}}{\text{Sample area}} \times 1000\, m^2 \tag{2}$$

$$\text{Biological yield (kg/ha)} = \frac{\text{Biological yield}}{\text{Sample area}} \times 1000\, m^2 \tag{3}$$

6.2.6. Data analysis

The data was first tested for normality and variance homogeneity using the Bartlett test over the years. All data were subjected to analysis of variance (ANOVA) in GenStat version 23rd Edition (VSN International, UK). The least significant difference and Duncan’s New Multiple Range tests were used to separate the mean at the 5% significance level. Yield and yield-related traits are presented in bar graphs as means ± standard error of means and produced in Microsoft Excel (Microsoft, Redmond, WA, USA).

6.3. RESULTS

6.3.1. Weather condition

During season 1 (2021/22), average maximum and minimum temperatures were 27.13 and 9.52 °C, respectively. Although highly variable, the highest precipitation was typically experienced during December and January 2021/2022. The lowest precipitation was recorded in July 2021 (0.07 mm), and solar radiation received for the first season ranged from 13.99 to 17.9 MJ/m^2, while for the second season, from 10.83 to 21.31 MJ/m^2 while during 2022/23, the maximum and minimum temperature ranged from 29.36 to 9.6 $^\circ$C, respectively. There was less precipitation in the first season (11.76 mm) when compared with the second season, which received 12.3 mm (Table 6.3). The recorded results suggested a higher possibility of drought stress in 2021/2022 when compared with 2022/2023. Reference evapotranspiration was also higher during 2022/2023 when compared with 2021/2022 (23.48 and 18.53 mm), respectively.

Table 6.3. The maximum and minimum temperature, maximum and minimum relative humidity, precipitation, and evapotranspiration recorded from the study site at Ukulinga Research Farm during the 2021/2022 and 2022/2023 planting seasons.

					2021/2022			
	Monthly Temperature (°C)		Monthly Relative humidity		Monthly Precipitation	Monthly Wind Speed	Monthly Evapotranspiration	Total Radiation
Months	Tx	Tn	RHx	RHn	(mm)	(ms)	(mm)	(MJ/m^2)
July	22.32	7.25	80.36	21.14	0.07	1.04	2.49	13.94
August	23.43	9.52	88.49	31.09	0.56	1.22	3.17	13.99
September	24.53	11.69	91.4	41.36	0.93	1.21	2.96	14.42
October	23.8	12.12	91.64	47.42	1.16	1.2	3.02	15.01
November	24.53	14.06	96.06	54.38	1.1	1.28	3.15	15.8
December	26.08	15.92	94.86	54.41	4.17	1.15	3.15	15.5
January	27.13	17.81	95.06	61.08	3.77	1.01	3.61	17.9
Annual average	42.96	22.1	148.04	44,4	91,1	1.16	3.08	15.2
					2022/2023			
June	22	8.46	83.06	33.66	0.14	0.79	1.94	10.83
July	23.26	9.6	85.07	30.86	0.34	0.92	2.12	11
August	23.04	8.97	89.85	34.57	0.27	1.06	2.74	12.63
September	27.23	13.12	89.81	36.12	0.69	1.22	3.09	14.31
October	26.77	14.78	92.11	47	1.31	1.26	2.98	13.94
November	24.73	14.56	94.75	57.11	2.4	1.09	3.02	15.17
December	26.41	16.04	94.09	57.72	4.45	1.04	3.14	15.37
January	29.36	16.62	93.22	47.3	2.7	1.07	4.45	21.31
Annual average	25.35	12.77	90.25	43.04	1.54	1.06	2.93	14.32

Note: Tx, maximum temperature; Tn, minimum temperature; RHx, maximum relative humidity, RHn, minimum relative humidity.

The ANOVA for determining yield and yield components effects of iodine seed priming, genotypes, water treatments and their interactions were significantly ($P < 0.05$), different for some traits during both seasons 2021/22 and 2022/23 (Table 6.4). Water treatment significantly affected all the traits of wheat genotypes studied during both seasons. Meanwhile, iodine seed priming significantly affected DTM, SL, NGS, and NTP during the studied seasons. Lastly, most interactions had no significant effect except DTM, NGS, SPS, GY and BY on the studied traits during both seasons (Table 6.4).

6.3.2. Days to maturity (DTM)

The interaction genotypes (G) × iodate treatment (I) and iodate treatment (I) × water treatment (W) significantly ($P < 0.05$) influenced the number of days to maturity. The unprimed genotypes PAN3111 recorded higher (187 days) DTM while lower DTM was recorded on genotypes SST806

(149 days) primed with the 0.01 concentration (Figure 6.1A). Under rainfed conditions, wheat genotypes primed with 0.001 and 0.01 M IO_3^- concentration had shorter (151.7 and 155 days) DTM when compared with unprimed seeds (156 days). Similarly, unprimed genotypes had a higher number of DTM (170 days) when compared with those primed with 0.001 and 0.01 IO_3^- concentration (168.7 and 167 days) under full irrigation conditions (Figure 6.1A), respectively. With regard to DTM, there was significantly ($P < 0.05$) difference water stress levels, the highest number of days to maturity was observed under full irrigation (168.59 days) compared to rainfed conditions (154.4 days). The DTM significantly differed ($P < 0.05$) on the iodine seed priming treatment. Seed primed with 0.001 and 0.01 IO_3^- concentration took fewer days (160 and 161.4 days) to mature compared to untreated treatments (163 days). Genotype PAN3111 had higher (189.9 days) DTM, followed by genotype SST806 (150 days) and SST8135 (149 days) (Figure 6.1).

Table 6.4. Mean square and significance tests from ANOVA were conducted to assess the impact of iodine seed priming on wheat genotypes under both rainfed and irrigated conditions across two growing seasons, focusing on agronomic traits.

Source of varience	Agronomic traits										
	Season 1										
	DF	DTM	PH	SL	SPS	NGS	NTP	TGW	GY	BY	HI
Genotypes (G)	2	7378.9**	1072.8**	29.3**	29.9^{ns}	33.6^{ns}	0.1^{ns}	1131.4**	60.7*	10622**	2166.4*
Iodine treatment (I)	2	36.352**	75.1^{ns}	3.7*	31.2^{ns}	64.8*	1.1^{ns}	69.35^{ns}	22.8^{ns}	215^{ns}	573.0^{ns}
Water treatment (W)	1	2702.3**	1185.3**	25.4**	763.1**	1391.3**	29.9**	2806.6**	776.3**	18013**	2533.7*
G × I	4	23.8**	87.6*	0.9^{n}s	10.4^{ns}	39.8^{ns}	0.6^{ns}	95.74^{ns}	12.7^{ns}	2918^{ns}	326.4^{ns}
G × W	2	7.6**	41.5^{ns}	1.6^{ns}	22.3^{ns}	85.6*	0.4^{ns}	53.4^{ns}	41.3^{ns}	309^{ns}	479.2^{ns}
I × W	2	34.8^{ns}	26.2^{ns}	2.3^{ns}	11.8^{ns}	14.7^{ns}	1.0^{ns}	258.64^{ns}	10.3^{ns}	55.0^{ns}	189.6^{ns}
G ×I × W	4	6.7**	9.2^{ns}	0.1^{ns}	31.2^{ns}	30.7^{ns}	0.9^{ns}	67.8^{ns}	7.6^{ns}	2279.0^{ns}	56.2^{ns}
Residual	34	3.8	28.0	1.1	13.5	19.3	0.7	78.3	14.0	1241	257.8

					Season 2						
G	2	-	14.7^{ns}	1.2^{ns}	40.2^{ns}	154.7^{ns}	0.3^{ns}	101.3^{ns}	10732.9**	48749**	390.6^{ns}
I	2	-	12.9^{ns}	3.6^{ns}	160.2^{ns}	340.7*	3.3*	89.8^{ns}	541.8^{ns}	6398^{ns}	278.7^{ns}
W	1	-	5078.9**	89.2**	2730.7**	6556.0**	27.4**	1549.9**	57954.2**	162861**	2922.5**
G × I	4	-	66.9^{ns}	1.5^{ns}	47.6^{ns}	10.4^{ns}	0.9^{ns}	42.9^{ns}	2688.3*	15999**	14.4^{ns}

G × W	2	-	32.9^{ns}	1.1^{ns}	30.9^{ns}	25.0^{ns}	0.4^{ns}	4.0^{ns}	4545.4*	13962*	345.6^{ns}
I × W	2	-	115.7^{ns}	3.9^{ns}	295.4*	50.8^{ns}	0.6^{ns}	17.4^{ns}	943.9^{ns}	348^{ns}	93.2^{ns}
G × I × W	4	-	222.9*	0.6^{ns}	40.4^{ns}	175.3^{ns}	0.1^{ns}	38.9^{ns}	2440.7*	5671^{ns}	44.7^{ns}
Residual	34	-	78.5	1.4	53.6	93.1	0.8	69.3	931.3	2478	171.4

DF, degrees of freedom; **DTM**, days to maturity; **PH**, plant height; **SL**, spike length; **SPS**, number of spikelets per spike; **NGS**, number of grians per spike; **TSW**, thousand grain weight; **GY**, grain yield per plot; *, $P < 0.05$; **, $P < 0.01$; ns, non-significant difference.

6.3.3. Plant height

The data on plant height (PH) indicated that the interaction between genotypes and iodine (G × I), as well as the individual factors related to water treatment, exhibited significant differences ($P < 0.05$) during the 2021/22 season. The genotype PAN3111 exposed to priming with 0.01 M IO_3^- concentration maintains maximum PH (74 cm). The minimum values (50.83 cm) were observed in SST 806 genotype exposed with 0.001 M IO_3^- concentration. It was observed that PAN3111 genotypes maintained a significantly ($P < 0.05$) highest PH (72.17 cm) followed by SST8135 (61.33 cm) and SST806 (52.22 cm), respectively (Figure 6.2A). The data showed significant ($P < 0.05$) differences between water treatment and G × I × W interaction during 2022/23.

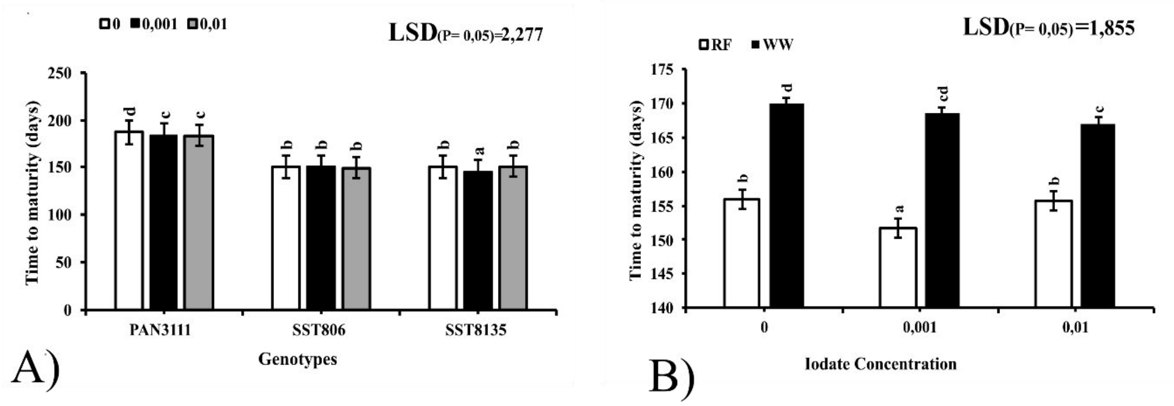


Figure 6.1. A) Time to maturity of wheat genotypes (PAN3111, SST806 and SST8135) after treatment with different iodate concentrations (0,0.001 and 0.01M). B) Time to maturity after priming with iodate under rainfed and irrigated conditions in 2021/22. The data represent means of three replications standard error and LSD. Bars with different letters indicate significant differences at $P < 0.05$.

Under irrigated genotypes, SST 806 primed with 0.001 M IO_3^- concentration maintained a maximum PH (80.13 cm) whilst the lowest 62.9 cm was observed in SST8135 primed with 0.01 M IO_3^- concentration, respectively (Figure 6.2A). Under rainfed conditions, SST 806 and SST8135 were exposed with 0.01 M IO_3^- concentration maintain a maximum PH (57.7 cm) and likewise, SST 806 and SST8135 primed with 0.001 M IO_3^- concentration maintain a minimum PH (41.8 cm). The trend was in line with the observation of the first season (2021/22), where irrigated genotypes had higher PH than rainfed genotypes (Figure 6.2A).

6.3.4. Shoot length (SL)

All factor interactions showed non-significant ($P > 0.05$) differences in all the studied periods with respect to SL during both seasons. However, the statistical analysis of SL indicated that all the main factors studied showed significant differences ($P < 0.05$) during 2021/22 and 2022/23, except genotype and iodine treatment during 2022/23. PAN3111 genotype had the highest SL (8.15 cm), while the genotypes SST 806 and SST8135 had relatively smaller SL, 5.99 and 5.9 cm, respectively (Fig 2B). In terms of iodine treatment, genotypes primed with 0.01 and 0.001 M doses showed a 13.7 and 11.5%

increase in SL compared to the control, respectively (Figure 6.2B). The irrigated genotypes had a 22.9% increase in SL compared to rainfed genotypes. During 2022/23, a similar trend was observed, where irrigated genotypes had a 38% increase in SL compared to rainfed genotypes (Figure 6.2B).

6.3.5. Spikelets per spike (SPS)

With respect to SP, the study revealed non-significant differences ($P > 0.05$) in all the studied factors, except water stress treatment during both seasons and interaction I × W during the second season (Table 6.4). Higher SPS was recorded in irrigated genotypes during both seasons compared to rainfed genotypes (Figure 6.2C). Under rainfed conditions, seeds primed with 0.001 and 0.01 M IO_3^- concentrations had a 14.7 and 8.8% decrease in SPS when compared with unprimed seeds. Under irrigated conditions, genotypes primed with 0.01 and 0.001% had a 34.7 and a 12.5% increase in SPS when compared with unprimed genotypes (Figure 6.2C).

6.3.6. Number of grains per spike (NGS)

All factor interactions revealed non-significant differences ($P > 0.05$) except G × W during the first season (2021/22). The genotypes primed with 0.01 and 0.001 M IO_3^- concentration increased by 12.6 and 1.2% in NGS when compared with unprime genotypes during 2021/22, respectively (Figure 6.2D). However, genotypes primed with 0.001 and 0.01 M of IO_3^- concentrations maintained a 17.2 and 1 % decrease in NGS when compared with unprimed genotypes during 2022/23 (Figure 6.2D). Significant differences ($P < 0.05$) for the NGS were observed between iodine and water treatments except for genotypes for both seasons (Table 6.**4**). The mean values of irrigated genotypes were 43, 35% higher in NGS than rainfed seeds during 2021/22. A similar trend was shown during 2022/23, except for a 70.9% increase in NGS of irrigated genotypes when compared with rainfed genotypes (Figure 6.2D). Under rainfed conditions, genotype SST806 had the highest (24.67) NGS and the lowest (22.78) was observed in PAN3111 and SST8135, respectively. However, the highest (37.33) NGS was recorded in genotype PAN3111 and the lowest (30.5) was recorded in genotype SST806 under irrigated conditions (Figure 6.2D).

6.3.7. Number of productive tillers

For both seasons, All the factor interactions recorded non-significant differences ($P > 0.05$) with respect to NTP across both seasons. The NTP were also non-significantly influenced ($P > 0.05$) by studied factors except for water treatment during 2021/22 and iodine treatment as well as water treatment during 2022/23. water treatments had similar trends; mean values under rainfed were less than those of irrigated conditions, except those higher values were observed during 2022/23 when compared with 2021/22 (Figure 6.2E). Maximum NTP (4.39) was recorded in genotypes primed with 0.001 M IO_3^- concentration, while the minimum NTP (3.56) was observed in genotypes primed with 0.01 M IO_3^- (Figure 6.2E).

6.3.8. Thousand-grain weight (TGW)

In 2021/22, the analysis of variance showed the interaction between I × W and genotype, water treatment alone significantly influenced ($P < 0.05$) TGW. Under rainfed conditions, seeds primed with both 0.01 and 0.001 M concentrations had a 20.1% decrease in TGW compared to unprimed seeds. However, under irrigated conditions, seeds primed with 0.001 and 0.01 M IO_3^- doses had a 10 and 31% increase in TGW compared to control, respectively (Fig 3A). Genotype SST8135 had the highest (37.3 g) TGW, and the lowest (22 g) was recorded on PAN3111 genotype (Figure 6.3A). During 2022/23, the analsysis of varience showed that water treatment alone significantly affected TGW, with a similar trend as with 2021/22 results; genotypes planted under rainfed conditions had lower TGW than genotypes planted under irrigated conditions (Figure 6.3A).

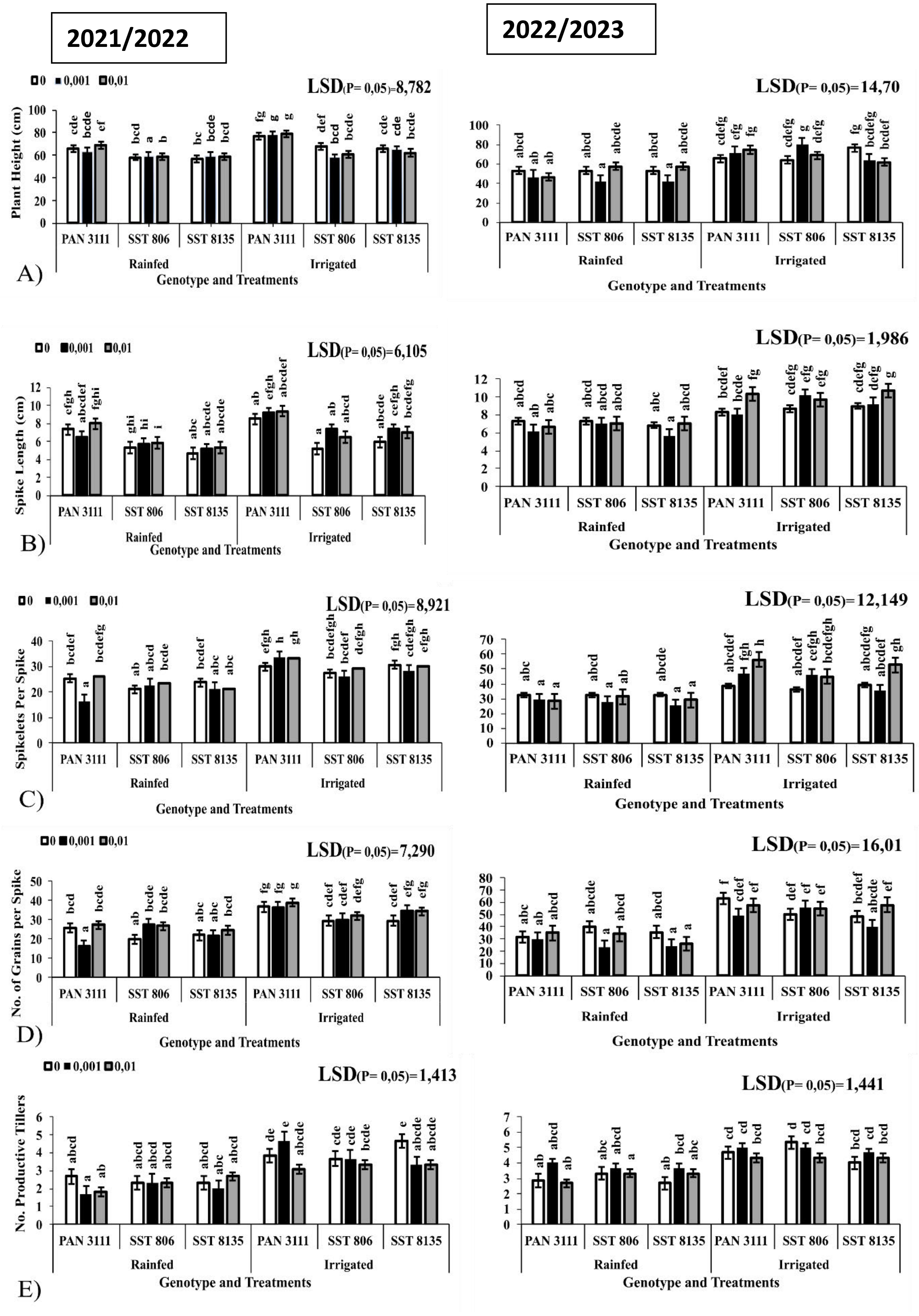


Figure 6.2. Effect of iodate priming and its priming concentrations (0,0.001 and 0.01M) on yield and yield attributes of wheat genotypes under rainfed (drought) and irrigated conditions in 2021/2022 and 2022/23. The data represent means of three replications standard error and LSD. Bars with different letters indicate significant difference at P < 0.05.

6.3.9. Grain yield (GY)

All the factor interactions had non-significant differences ($P > 0.05$) on GY during first season, 2021/2022. For both seasons, all main factors significantly influenced GY ($P < 0.05$) during except iodine treatment. The genotype SST8135 was significantly superior (9.22 kg/ha), followed by SST 806 (7.86 kg/ha), and the inferior genotype on GY was observed in PAN3111 (5.59 kg/ha) during the first season. During a second season, genotype SST806 maintained a significantly higher GY (105.7 kg/ha), while PAN3111 maintained a significantly lower GY (56.9 kg/ha) (Figure 6.3B). Rainfed genotypes had a 66.8% decrease in GY when compared with irrigated genotypes during the first season. A similar trend was also reported during the second season, except for a 57.6% decrease in rainfed when compared with irrigated genotypes (Figure 6.3B). The interaction G × I and G × W significantly influenced GY ($P < 0.05$) during 2022/23. The genotype SST 806 exposed to priming with 0.01 M IO_3^- concentration had the highest GY (134.2 kg/ha), and the lowest GY was recorded in the unprimed genotype PAN3111 (49.9 kg/ha) (Figure 6.3B). For rainfed and irrigated conditions, higher GY 63.3 and 148.1 kg/ha were recorded in the SST806 genotype, respectively. However, the lowest under rainfed conditions was recorded in genotype SST8135 (38.8 kg/ha), while under irrigated conditions, it was recorded in the PAN3111 genotype (71.3 kg/ha) (Figure 6.3B).

6.3.10. Biological yield (BY)

During the first season (2021/22), all the factor interactions had non-significant differences ($P > 0.05$) on BY. The data showed significant differences ($P < 0.05$) among genotypes and water treatments for BY. The maximum BY (425 kg/ha) was recorded in PAN3111, and the minimum BY (201 kg/ha) was recorded in genotype SST806. The highest BY (75.5 kg/ha) was recorded under irrigated conditions, and the lowest (38.9 kg/ha) was recorded under rainfed conditions. However, the data showed a significant difference ($P < 0.05$) among genotypes, water treatment and between the interaction of G × W and G × I during the second season.Under both rainfed and irrigated conditions, genotype SST806 recorded the highest BY (174 kg/ha) and (333.5 kg/ha) when compared with other genotypes, respectively (Figure 6.3C). The lowest BY (119 kg/ha) was observed in SST8135 and PAN3111 (184.2 kg/ha) under rainfed and irrigated conditions, respectively. The unprimed genotype SST 806 (289.5 kg/ha) maintained the highest BY. Conversely, the lowest BY was observed in SST8135 (120.3 kg/ha) primed with the IO_3^- concentration of 0.01 M. Irrigated genotypes had a 77.6% increase in BY when compared with rainfed genotypes (Figure 6.3C). The maximum BY was recorded in genotype SST806 (255,7 kg/ha), and the minimum BY (158 kg/ha) was observed in genotype PAN3111 (Figure 6.3C).

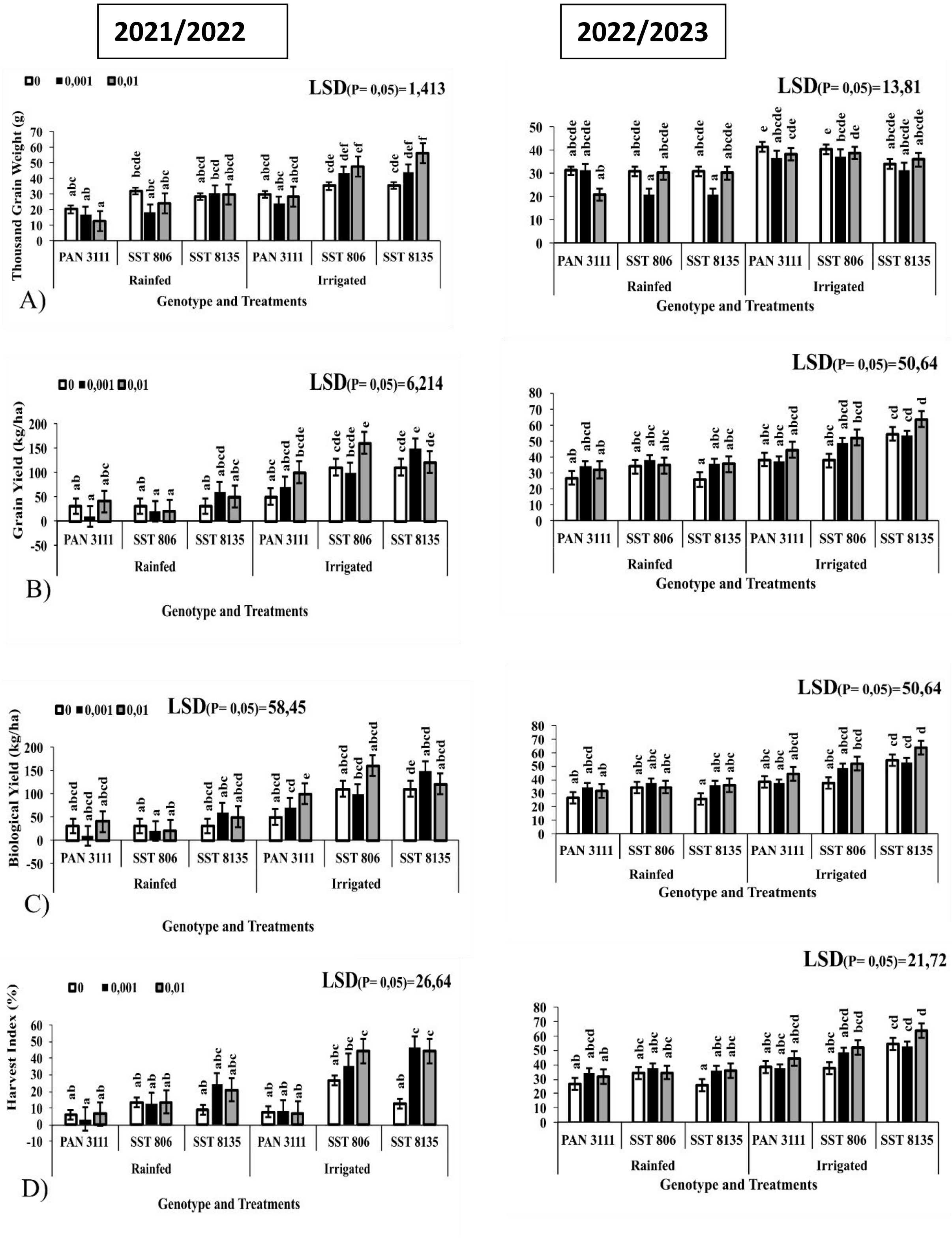


Figure 6.3. Effect of iodate priming and its iodate concentrations (0,0.001 and 0.01M) on yield and yield attributes in wheat genotypes under rainfed (drought) and irrigated conditions in 2021/2022 and 2022/23. The data represent means of three replications standard error and LSD. Bars with different letters indicate significant differences at P < 0.05.

6.3.11. Harvest index (HI)

For both seasons, all the interaction factors showed no significant (P > 0.05) differences for HI. However, the statistical analysis of HI revealed a significant difference (P < 0.05) between the factors genotype and water treatments only in the first season. SST806 had the highest HI (48,9%), while the lowest was observed in PAN3111 (13.2%). The irrigated genotypes produced an 84, 4% increase in HI when compared with rainfed genotypes (Figure 6.3D). During the second season, water treatment significantly affected HI ($P < 0.05$), and the trends were in line with the observation of 2021/22 (first season); irrigated genotypes had an increase of 44.5% in HI when compared with the rainfed genotype (Figure 6.3D).

6.4. DISCUSSION

Iodine plays a crucial role in enhancing plant growth and providing protection against abiotic stresses (Gonzali et al., 2017; Kiferle et al., 2022). Its unique properties, including antimicrobial effects and improved nutrient uptake, have led to its increasing application in agriculture, particularly for horticultural and vegetable crops, as well as in biotechnology (Ajiwe et al., 2019; Dobosy, Kröpfl, et al., 2020; 2011). Research on iodine has gained momentum due to its positive impact on plant growth, development, and productivity, acting as a bio-stimulant (Riyazuddin et al., 2022; Rodríguez-Salinas et al., 2022). Furthermore, iodine has been reported to mitigate the effects of abiotic stress (Medrano-Macías et al., 2016; Pérez-Salas & Medrano-Macías, 2021). Many authors have tried to understand how plants respond when iodine is applied under stress conditions, i.e., through salinity (Blasco et al., 2013a; Kiferle et al., 2022c; Leyva et al., 2011; Medrano Macías et al., 2021; Pérez-Salas & Medrano-Macías, 2021; Rodríguez-Salinas et al., 2022) and heavy metals (Gupta et al., 2015). Still, limited studies have focused on the utility of iodine as a priming agent under conditions of limited soil water availability.

Drought stress is a significant environmental factor affecting wheat’s growth, development, and productivity. It triggers a cascade of physiological and biochemical disruptions, including reduced photosynthetic activity, impaired water and nutrient transport, altered stomatal regulation, decreased enzyme activity, and inhibited antioxidant enzyme expression (Reddy et al., 2004). Additionally, drought stress limits osmolyte accumulation, which is critical for osmotic regulation, hindering root and shoot growth and ultimately reducing crop yield and its components (Bashir et al., 2021; Dey et al., 2022). Given these detrimental effects, the present study evaluated the impact of iodine priming and doses on yield and yield attributes of different wheat genotypes under contrasting water conditions, rainfed (drought-prone) and fully irrigated environments. The goal was to determine whether iodine priming, and the doses used can enhance the drought tolerance of wheat and mitigate the adverse effects of water scarcity on crop performance.

Our results demonstrated that the accelerated maturity observed in seeds primed with iodate (IO_3^-) at concentrations of 0.001 and 0.01 M, reducing time to maturity to 160 and 161.4 days, respectively, compared to untreated controls, can be attributed to multifaceted physiological enhancements triggered by iodine. For instance, iodate stimulates early metabolic activation by up regulating enzymes like α-amylase and dehydrogenases, accelerating reserve mobilization and energy production during germination, thereby shortening the lag phase of seedling establishment (Paparella et al., 2015). Simultaneously, it acts as a mild oxidative stressor that induces early antioxidant synthesis, reducing reactive oxygen species (ROS) damage and minimizing energy diversion toward cellular repair under environmental stresses, which preserves resources for developmental processes (Blasco et al., 2008; Kiferle et al., 2013). Furthermore, genotypes grown under rainfed conditions matured in fewer days than those cultivated under full irrigation. This finding aligns with previous studies by Boyoumi (2008), which observed faster maturation in rainfed crops. The accelerated maturity in rainfed environments can be attributed to fluctuations in rainfall, leading to reduced water and nutrient uptake. This stress condition may trigger an adaptive response in plants, promoting an "escape mechanism" where genotypes shorten their growth cycle to survive under limited water availability, a key aspect of drought tolerance (Blum, 2011).

Plant height (PH) plays a critical role in a plant's ability to allocate carbohydrates and maintain physiological functions under stress, as reduced PH under drought conditions, as observed in rainfed wheat genotypes, reflects impaired cell expansion and division due to compromised turgor pressure, accelerated senescence, and restricted nutrient uptake, consistent with mechanisms described by Hussain et al. (2008) and Bashir et al., (2021). This stress-induced reduction in PH limits photosynthetic potential and carbohydrate mobilization, ultimately constraining growth and maturity (Hasanuzzaman & Fotopoulos, 2019; Saha et al., 2022). However, seed priming with 0.01 M iodate (IO_3^-) counteracted these effects in wheat genotypes SST 806 and SST 8135 by significantly increasing PH, even under rainfed stress. Iodate achieves this by activating metabolic pathways that enhance photosynthesis and upregulate enzymes governing cell enlargement and division, thereby improving water/nutrient assimilation and turgor maintenance (Hasanuzzaman & Fotopoulos, 2019). This dual action, preserving cellular integrity while boosting resource acquisition, aligns with Hussain's framework by mitigating plasmolysis risks and sustaining growth processes, effectively overriding drought-induced growth constraints and accelerating development (Gupta et al., 2015; Kiferle et al., 2022; Paparella et al., 2015).

Yield-related traits such as SL, SPS, and NGS are crucial components for final yield. The current study showed that SL (Figure 6.2B), SPS (Figure 6.2C) and NGS (Figure 6.2D) were significantly reduced under rainfed conditions compared to normal irrigation. This is due to impaired cell elongation and division during critical growth stages, directly reducing SL by limiting turgor pressure and carbohydrate availability needed for structural development (Cosgrove, 2005). For SPS, water scarcity during tillering restricts the initiation and survival of tillers, as plants prioritize resource allocation to main stems under stress, leading to fewer viable spikes. Similarly, NGS declines due to drought-induced perturbations during reproductive phases: water deficit compromises pollen viability, stigma receptivity, and grain-set efficiency, while also triggering oxidative stress that damages developing florets. These mechanisms collectively align with findings from Eid (2009), who noted that drought disrupts meristematic activity in wheat spikes; Ofilic and Yaghasaplar (2010), who highlighted tiller abortion and floret sterility under moisture deficits; and Sharma et al., (2022), who emphasized carbon starvation and hormonal imbalances (e.g., elevated abscisic acid) as key drivers. Ultimately, these reductions in SL, SPS, and NGS diminish grain size and yield by curtailing the plant's structural capacity and reproductive success, highlighting the widespread effects of drought impact on cereal productivity. However, seed priming with iodate effectively mitigated the adverse effects of drought stress on wheat genotypes, leading to increased SL and NGS. The optimal concentration of 0.01 M iodate mitigates oxidative stress in reproductive tissues through its role in antioxidant defense systems, further protecting developing organs from environmental damage, which could contribute to increased yield (Kumar Singhal et al., 2019; Patel et al., 2020).

The reductions in spike length (SL), spikes per plant (SPS), and number of grains per spike (NGS) observed under rainfed conditions collectively impair structural yield capacity and reproductive efficiency, thereby diminishing final grain yield. However, seed priming with 0.01 M iodate counteracted these drought-induced limitations by simultaneously enhancing nutrient translocation to developing reproductive structures and activating antioxidant defense mechanisms, which mitigated oxidative stress in critical tissues (Kumar Singhal et al., 2019; Patel et al., 2020). This dual physiological action preserved spike development, grain-set efficiency, and ultimately improved yield stability under water-deficit condition.

The current study demonstrated that wheat genotypes grown under rainfed conditions exhibited a reduction in the number of tillers per plant (NTP) when compared with those cultivated under full irrigation during both seasons. This decline in NTP under rainfed conditions was likely due to limited water availability, which can inhibit plant growth and tiller formation. However, the application of iodine through seed priming significantly enhanced NTP. These findings were consistent with Liu et al., (2016), who reported that seed priming in promotes early, uniform, and vigourous seedling development, resulting in better establishment and higher yields in winter wheat genotypes. Enhanced seedling vigour facilitated by priming likely supports more efficient resource allocation and tiller formation, even under suboptimal conditions.

Iodate priming negatively influenced tiller number under rainfed conditions while positively affecting thousand grain weight (TGW) under fully irrigated conditions during the first season. This suggested that iodine seed priming may have activated mechanisms that enhance grain set and improve translocation of photosynthates into reproductive parts, leading to better grain filling (Meena et al., 2015). Furthermore, genotypes SST 8135 and SST 806 exhibited higher TGW than genotype PAN3111 during the first season. These genotypes showed superior photosynthetic efficiency and source-sink dynamics, enabling greater carbohydrate production in leaves and more efficient translocation to grains. Stronger sink strength, driven by hormonal regulation like cytokinins, supports robust nutrient storage, while optimized grain-filling duration and stress resilience extend dry matter accumulation (Farooq et al., 2009c; Prasad, 2022). Environmental conditions during the season further amplified these traits, allowing SST 8135 and SST 806 to achieve larger grains through enhanced cell expansion and starch biosynthesis. indicating genotypic variation.

In this study, the grain yield was significantly reduced under rainfed conditions when compared with fully irrigated environments. This reduction may be attributed to decreased photosynthetic capacity caused by stomatal closure, which inhibits CO_2 diffusion to chloroplasts (Chai et al., 2016; Kumar et al., 2019). However, genotypes such as SST 806 primed with 0.01 M iodate showed higher grain yields when compared with PAN3111, indicating a positive effect of iodine on yield attributable to increased SL, NTP, and NGS. Our results corroborate previous studies by Pérez-Salas et al., (2021) and Gonzali et al., (2017), which reported that low iodine concentrations can enhance plant yield such as tomatoes (*Solanum lycopersicum*). Conversely, higher concentration of iodine may be detrimental as they can reduce CO_2 assimilation by decreasing leaf size, stomatal conductance, and photosynthetic pigment content, ultimately lowering grain yield (Kiferle et al., 2019). Moreover, genotypic differences in grain yield were observed across both seasons due to variations in genetic makeup and environmental conditions.

With respect to biological yield (BY), a significantly lower BY under rainfed conditions when compared with full irrigation was observed during growth of wheat genotypes (Figure 6.3C). Previous studies have similarly reported reductions in biological yield under drought stress (Shar et al., 2021; Sial et al., 2009). Additionally, genotypes subjected to priming showed a significant decrease in BY when compared with unprimed treatments. Genotype PAN3111 exhibited lower BY while SST 806 had the highest BY during the second season; this may be due to inherent genetic differences (Liwani et al., 2019b).

The harvest index (HI) is a key factor determining a crop's efficiency in translocating assimilated resources to the grains, such as carbohydrates (Asefa, 2019; Camargo-Alvarez et al., 2023). The current study indicated that HI was influenced primarily by water treatments. Genotypes grown under rainfed conditions exhibited a lower HI than those cultivated under irrigated conditions. Several researchers have reported similar findings in wheat, demonstrating that HI is significantly affected by drought stress (Almeselmani et al., 2011; Liwani et al., 2019b). According to Khatiwada et al. (2020), reduced HI from drought stress dcrease sink size, leading to decreased demand for assimilates and retention of assimilates in vegetative organs.

The minimal influence of iodate on HI may arises from three closely physiologically linked mechanisms. First, water availability dominantly governed assimilate partitioning under rainfed conditions, as drought stress altered source-sink relationships by reducing sink size and disrupting phloem loading, thereby suppressing grain filling (Blum, 2009). Critically, stress-induced hormonal shifts (e.g., elevated abscisic acid and ethylene) retained assimilates in stems and leaves, overriding iodate-mediated improvements in nutrient absorption (Müller & Munné-Bosch, 2021; Wilkinson & Davies, 2010). Second, iodate primarily enhanced vegetative biomass as evident in genotypes like SST806 without activating grain-specific metabolic pathways (e.g., sucrose transporters or starch synthase enzymes), thus proportionally increasing total productivity without altering HI (Slewinski, 2012; Weng et al., 2018). Third, genotypic differences in stress resilience (e.g., SST 806's inherent resource optimization under duress) masked subtle iodate effects on HI, as pre-existing genetic thresholds for partitioning efficiency remained unmodified (Richards, 2006; Vadez et al., 2014). Collectively, drought stress imposed physiological constraints on sink strength and translocation, while genetic variation influenced how plants prioritize assimilate allocation, creating barriers that iodate supplementation could not overcome despite its role in nutrient uptake (Tester & Langridge, 2010).

6.5. CONCLUSION

The study has shown that seed priming with iodine significantly enhances wheat performance in terms of growth, yield, and yield-related parameters under rainfed and irrigated conditions. Key parameters such as spike length (SL), number of tillers per plant (NTP), seeds per spike (SPS), days to maturity (DTM), and grain yield (GY) were positively affected by iodine priming, particularly under limited soil moisture. Although there was some variation in grain yield among genotypes, SST 806 and PAN3111 consistently produced higher yields. The optimal iodine concentration that improved yield traits was 0.01 M. Overall; iodine priming proved to be an effective strategy for improving crop performance and grain yield in water-limited and irrigated environments. This study paves the way for further field-based research on the application of iodine in crop management.

CHAPTER 7

GENERAL DISCUSSION

Improving seed quality is crucial for increasing wheat production, as it is influenced by a variety of factors such as drought, temperature, nutrition, photoperiod, and others, which can pose various threats to successful germination and establishment, ultimately leading to reduced productivity (Farooq et al., 2009b). To counteract these negative effects and enhance wheat production, techniques are needed to promote stress tolerance and accelerated development, growth, ultimately higher yields. Nutrient seed priming, particularly with iodine, has the potential to improve germination, vigour, yield, and seed quality. Iodine can increase the concentration of osmolytes, which help plants cope with drought stress by maintaining turgor pressure and stabilising cell membranes (Kiferle et al., 2022a; Medrano-macías et al., 2018). Furthermore, iodine can enhance antioxidant activities, which protect plants from oxidative damage caused by abiotic stresses. In plants iodine can also maintain homeostasis, which is essential for optimal physiological functions (Gonzali et al., 2017).

Despite the potential benefits of iodine seed priming, research on its effects under abiotic stress conditions remains limited. Thus, more research is needed to fully understand the efficacy of iodine seed priming and its effective dose to optimize its application for improved wheat production under different environmental conditions. This study aimed to evaluate the impact of iodine priming on seed quality, growth characteristics, physiological traits, and yield of wheat genotypes subjected to drought stress. By exploring the influence of iodine on various agronomic and physiological traits, the study sought to contribute to the development of effective and sustainable strategies for enhancing wheat production.

Germination and stand establishment were the first developmental stages that can determine crop production. In a study investigating the impact of iodine priming and its effective dose on germination, seedling growth, and vigour traits (Chapter 3), it was observed that priming seeds at the dose greater than 0.01 M (IO_3^-) had detrimental effect on germination traits, specifically the germination percentage and rate, which aligns with findings from Golob et al. (2020), who observed that soaking seeds with iodate reduced seed germination and seedling mass, in pumpkins (*Cucurbita pepo*), while lower concentrations (0.001 M IO_3^-) enhanced seed reserve utilization through biochemical processes supporting embryo growth (Farooq et al., 2006). Although iodine priming showed no significant impact on most vigor traits, it is recommended that maintain iodate levels below 0.01 M to optimize germination while potentially benefiting seedling development.

The second objective was to evaluate the effect of drought stress on wheat germination, seedling growth and physiological traits. The results indicated that agronomic traits such as plant height and root length stability indices (PHSI, RLSI) and dry matter stability index (DMSI) were positively influenced by iodate priming. However, chlorophyll pigments showed no significant changes due to iodate priming, with carotenoids negatively impacted under drought stress. Similarly, the membrane stability index

(MSI) was adversely affected by increasing iodate concentrations. Furthermore, iodate priming did not significantly affect relative water content (RWC) under water stress conditions, despite high RWC being indicative of drought tolerance (Sial et al., 2017).

Various studies have showed that drought tolerance is a developmentally regulated stage specific phenomenon, so that tolerance at one of the stages of development may not be correlated with tolerance at other stages. Therefore, understanding how crops respond to drought stress at different growth stages is crucial for developing appropriate genetic and management strategies to enhance drought tolerance. It was within this context that the study aimed to assess the effectiveness of iodine application (seed priming and foliar spraying) on agronomic and physiological traits of wheat genotypes upon applying drought stress at different growth stages (chapter 5). The study hypothesized that iodate treatment would positively impact agronomic and physiological traits in wheat genotypes under drought stress conditions. The study results supported the hypothesis, revealing that iodate application partially alleviated the adverse effects of drought stress on physiological (such as photosynthesis and transpiration) and agronomic traits (including number of tillers, spikelets per spike, number of grains per spike, thousand-grain weight, and biological yield) during both tillering and flowering stages. The beneficial effects of iodate may be attributed to its protective role in maintaining reactive oxygen species (ROS) homeostasis (Blasco et al., 2008; Kiferle et al., 2013). Notably, while both application methods improved agronomic traits, seed priming was found to be more effective than foliar spraying, indicating a need for further exploration in this area

Weather parameters, including maximum and minimum air and soil temperatures, rainfall, and relative humidity, are critical climatic factors impacting plant growth and development, particularly in rainfed agricultural systems. This led to the final trial in Chapter 6, which hypothesized that iodine priming could enhance growth, yield, and yield-related traits in wheat genotypes under both rainfed and optimal conditions. The results demonstrated that iodine priming positively influenced several measured parameters, such as spike length, number of tillers per spike, spikelets per spike, days to maturity, and grain yield under water-limited conditions, although variations were observed between seasons. These findings align with previous research indicating that the effects of iodine application on crop performance depend on concentration, crop type, and the severity of stress experienced (Dávila-Rangel et al., 2019; Gonzali et al., 2017; Medrano-Macías et al., 2016).

CONCLUSION

The following conclusion can be drawn from this study:

1. The study demonstrated that iodine priming, and its iodine priming concentration affected germination traits, particularly mean germination time, while vigour traits remained largely unaffected. Notably, genotype SST806 exhibited superior seed quality compared to other genotypes. Although effective concentrations varied, it is recommended that concentrations exceeding 0.01 M may induce phytotoxic effects.

2. Seed priming with iodate during early stages showed improved tolerance to drought stress by enhancing both morphological and physiological traits, such as PHSI, RLSI, DMSI, and carotenoid levels. However, other physiological metrics, including relative water content and membrane stability index, were not significantly influenced by iodate seed priming. Among the three genotypes studied, SST806 consistently outperformed the others.
3. The application of iodine through both priming and foliar spraying positively influenced growth and yield and yield related parameters in wheat genotypes under drought stress conditions. Physiological traits, such as transpiration and photosynthetic rate, significantly effected wheat yield.
4. Under rainfed agroclimatic conditions, iodate priming resulted in variable effects on yield and yield-related traits (SL, NTP, SPS, DTM, and GY), with increases observed in one season and decreases in the next.

RECOMMENDATIONS

Below are the issues that warrant further investigation and should be addressed in the near future:

I. Future research should determine optimum length (duration) of iodine priming to prevent contamination, which may influence seed germination and vigour. Most reports about iodine application have been strictly conducted utilizing hydroponics, foliar, and soil. Using additional treatments such as seed priming is vital as it is an uncomplicated, and cost-effective for small-scale farmers.

II. Future research is required to study the influence of iodine priming at early stages in plant growth under stressful circumstances. Mainly focusing on antioxidants, as prior research indicates iodine acts as a stimulant of antioxidant activity.

III. The intergration of iodine priming with other elements should also be assessed. Understanding the plant response to the availability of iodine in relation to other elements is vital for decreasing fertilizer use in agriculture.

IV. Increasing yield requires a comprehensive understanding of the physiological mechanism involved after iodine priming, and this may assist breeders in selecting tolerant genotypes to different conditions. Therefore, future research should also be centred on physiological, biochemical, and cellular response after iodine seed priming.

V. Some research has revealed that foliar spraying is more effective; thus, investigations should also evaluate the effective methods of iodine application at different phenological stages, mainly looking at the chlorophyll fluorescence and accumulation of osmolytes in different crops.

REFERENCES

Abbas Khan, R., Noreen, Z., Khan, A., Ahmed Qadri, T., Rashid Abbas Khan, C., & Ahmed Qadri Nutri, T. (2021). 5. Nutri-Priming as an Efficient Means to Improve Germination and Growth of Mung bean (Vigna radiata L.) Grown Under NaCl Stress. *Pure and Applied Biology (PAB), 10*(1), 34–45.

Abbasi Khalaki M, Ghorbani A, Dadjou F. (2019). Influence of Nano-Priming on Festuca ovina Seed Germination and Early Seedling Traits under Drought Stress, in Laboratory Condition. *ECOPERSIA*, *7*(3), 133-139

Abdul-Baki, A. A., & Anderson, J. D. (1973). Vigour Determination in Soybean Seed by Multiple Criteria 1. *Crop Science*, *13*(6), 630-633.

Abebe, G., & Alemu, A. (2017). Role of improve seeds towards improving livelihood and food security at Ethiopia. *International Journal of Research -GRANTHAALAYAH*, *5*(2), 338-356.

Adams, S. R., & Langton, F. A. (2005). Photoperiod and plant growth: A review. In *Journal of Horticultural Science and Biotechnology*. *80*(1), 2-10.

Ahanger, M. A., Gul, F., Ahmad, P., & Akram, N. A. (2018). Environmental stresses and metabolomics—Deciphering the role of stress responsive metabolites. *Plant Metabolites and Regulation under Environmental Stress*, 53–77.

Ajiwe, S. T., Popoola, A. R., Afolabi, C. G., Oduwaye, O. A., Ganiyu, S. A., Fajinmi, O. B., Chikaleke, V. A., Imonmion, J. E., Adigun, J. A., Taiwo, B. F., & Uzoemeka, I. P. (2019). Effect of iodine biofortification on incidence and severity of Fusarium wilt and yield of tomato (*Solanum lycopersicum* L.). *Nigerian Journal of Biotechnology*, *36*(1), 146.

Akgün, İ., Duran, F., & Kaya, M. (2015). Effect of different zinc application on yield, yield components and some quality traits of bread and durum wheat varieties. *Agrosym*, 594-601.

Akhtar, M., Yousaf, S., Sarwar, N., & Hussain, S. (2019). Zinc biofortification of cereals—role of phosphorus and other impediments in alkaline calcareous soils. *Environmental Geochemistry and Health*, *41*(5), 2365–2379.

Akinci, Ş., & Lösel, D. M. (2006). Plant Water-Stress Response Mechanisms, *Water stress*, *15*, 42.

Akram, H. M., Iqbal, M. S., Saeed, M., Yar, A., Ali, A., Sahi, K. A., & Nadeem, M. A. (2004). Drought tolerance studies of wheat genotypes. *Pak. J. Biol. Sci*, *7*(1), 90–92.

Akram, M. (2011). Growth and yield components of wheat under water stress of different growth stages. *Bangladesh Journal of Agricultural Research*, *36*(3), 455–468.

Ali, A. S., & Elozeiri, A. A. (2017). Metabolic Processes During Seed Germination. In *Advances in Seed Biology*, 2017, 141-166.

Ali, N., Farooq, M., Hassan, M. A., Arshad, M. S., Saleem, M. K., & Faran, M. (2018). Micronutrient seed priming improves stand establishment, grain yield and biofortification of bread wheat. *Crop and Pasture Science*, *69*(5), 479–487.

Allen, R. G., Pereira, L. S., Raes, D., & Smith, M. (1998). Crop evapotranspiration: Guidelines for computing crop requirements. *Irrigation and Drainage Paper No. 56, FAO*.

Almeselmani, M., Abdullah, F., Hareri, F., Naaesan, M., Ammar, M. A., & ZuherKanbar, O. (2011). Effect of drought on different physiological characters and yield component in different varieties of Syrian durum wheat. *Journal of Agricultural Science*, *3*(3), 127.

Annandale, JG., Stirzaker, R., Singels, A., van der Laan, M., & Laker MC. (2011). Irrigation scheduling research: South African experiences and future prospects. *Water South Africa (SA), 37*(5), 751–764. https://www.ajol.info/index.php/wsa/article/view/72843

AOSA Association of Official Seed Analysts: (1992). *Seed Evaluation Handbook. Contribution No.35*.

Areington, C. A. (2016). *The utility of Brachylaena discolor as a bioindicator of air pollution within selected industrial areas in KwaZulu-Natal.* Thesis for MSc. University of KwaZulu-Natal, Westville.

Arif, M., Waqas, M., Nawab, K. H. A. L. I. D., & Shahid, M. (2007). Effect of seed priming in Zn solutions on chickpea and wheat. *Arfrican Crop Sceince Conference Proceeding*, 237–240.

Arzani, A., & Ashraf, M. (2017). Cultivated ancient wheats (Triticum spp.): A potential source of health-beneficial food products. *Comprehensive Reviews in Food Science and Food Safety, 16*(3), 477–488.

Ashraf, M., & Foolad, M. R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany, 59(2), 206-216.*

Ashraf, M. Y., Akhter, K., Hussain, F., & Iqbal, J. (2006). Screening of different accessions of three potential grass species from cholistan desert for salt tolerance. *Pakistan Journal of Botany 38(5), 1589-1597.*

Atar, B., Uygur, V., & Sukusu, E. (2020). Effects of priming with Copper, Zinc and Phosphorus on seed and seedling composition in Wheat and Barley. *Turkish Journal of Agricultural and Natural Sciences, 7*(1), 104–111.

Augustine, R., & Kalyanasundaram, D. (2020). Agronomic biofortification of food crops with micronutrients. *Plant Archives*, *20*(2), 1383–1387. http://www.plantarchives.org/SPL ISSUE 20-2/222__1383-1387_.pdf

Avercheva, O. v., Berkovich, Y. A., Erokhin, A. N., Zhigalova, T. v., Pogosyan, S. I., & Smolyanina, S. O. (2009). Growth and photosynthesis of Chinese cabbage plants grown under light-emitting diode-based light source. *Russian Journal of Plant Physiology, 56, 14-21.*

Badiri, A., Mirshekari, B., Hadavi, E., & Hamidi, A. (2014). Effect of seeds priming with micronutrients on growth, seed yield and mucilage of plantain. *International Journal of Plant, Animal and Environmental Sciences, 4*(3), 335–342.

Bashir, S. S., Hussain, A., Hussain, S. J., Wani, O. A., Zahid Nabi, S., Dar, N. A., Baloch, F. S., & Mansoor, S. (2021). Plant drought stress tolerance: Understanding its physiological, biochemical and molecular mechanisms. *Biotechnology & Biotechnological Equipment*, *35*(1), 1912–1925.

Baskin, C. C. (2014). *Seeds: Ecology, biogeography, and evolution of dormancy and germination*. Academic Press. San Diego, 150-162.

Batool, N., Ilyas, N., Noor, T., Saeed, M., Mazhar, R., Bibi, F., & Shahzad, A. (2014). Evaluation of drought stress effects on germination and seedling growth of Zea mays L. *International Journal of Biosciences (IJB)*, *5*(4), 203-209.

Bayoumi, T. Y., Eid, M. H., & Metwali, E. M. (2008). Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. *African Journal of Biotechnology, 7*(14). 2341–2352.

Bayu, W., Rethman, N., Hammes, P., Pieterse, P., Grimbeek, J., & van der Linde, M. (2006). Water stress affects the germination, emergence, and growth of different sorghum cultivars. *SINET: Ethiopian Journal of Science* 28(2), 119-128.

Bean, E. W. (1980). Factors affecting the quality of herbage. In, Hebblethwaite, PD (ed.). *Seed Production, ed. 28th* Easter School in Agricultural Science, Mniversity of Nottingham: 93–604.

Bellé, C., Kulczynski, S. M., Basso, C. J., Edu Kaspary, T., Lamego, F. P., & Pinto, M. A. B. (2014). Yield and quality of wheat seeds as a function of desiccation stages and herbicides. *Journal of Seed Science*, *36*(1), 63–70.

Bewley, J. D., & Black, M. (1994). *Seeds: physiology of development and germination. Plant Cell, 9 (7), 1055.*

Biemond, C. (2013). *‘’*Seed quality in informal seed systems” in *PhD thesis* (Wageningen: Wageningen University).

Blasco, B., Leyva, R., Romero, L., & Ruiz, J. M. (2013a). Iodine effects on phenolic metabolism in lettuce plants under salt stress. *Journal of Agricultural and Food Chemistry*, *61*(11), 2591–2596.

Blasco, B., Leyva, R., Romero, L., & Ruiz, J. M. (2013b). Iodine effects on phenolic metabolism in lettuce plants under salt stress. *Journal of Agricultural and Food Chemistry*, *61*(11), 2591–2596.

Blasco, B., Rios, J. J., Cervilla, L. M., Sánchez-Rodrigez, E., Ruiz, J. M., & Romero, L. (2008). Iodine biofortification and antioxidant capacity of lettuce: Potential benefits for cultivation and human health. *Annals of Applied Biology*, *152*(3), 289–299.

Blasco, B., Ríos, J. J., Leyva, R., Cervilla, L. M., Sánchez-Rodríguez, E., Rubio-Wilhelmi, M. M., Rosales, M. A., Ruiz, J. M., & Romero, L. (2011). Does iodine biofortification affect oxidative metabolism in lettuce plants. *Biological Trace Element Research*, *142*(3), 831–842.

Blasco, B., Rios, J. J., Leyva, R., Melgarejo, R., Constán-Aguilar, C., Sánchez-Rodríguez, E., Rubio-Wilhelmi, M. M., Romero, L., & Ruiz, J. M. (2011). Photosynthesis and metabolism of sugars from lettuce plants (Lactuca sativa L. var. longifolia) subjected to biofortification with iodine. *Plant Growth Regulation*, *65*(1), 137–143.

Blasco, B., Ríos, J. J., Sánchez-Rodríguez, E., Rubio-Wilhelmi, M. M., Leyva, R., Romero, L., & Ruiz, J. M. (2012). Study of the interactions between iodine and mineral nutrients in lettuce plants. *Journal of Plant Nutrition, 35(13), 1958-1969.*

Blokhina, O., Virolainen, E., & Fagerstedt, K. v. (2003). Antioxidants, oxidative damage and oxygen deprivation stress: A review. In *Annals of Botany, 91(2), 179-194*.

Blum, A. (1996). Crop responses to drought and the interpretation of adaptation. *Plant Growth Regulation*, *20*, 135–148.

Blum, A. (2011). Plant breeding for water-limited environments. 27-28. (Springer-Verlag).

Blum, A., & Ebercon, A. (1981). Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop Science*, *21*(1), 43-47.

Boelt, M. I. B. (2018). *Zinc seed priming improves salt resistance in maize*. *Journal of Agronomy and Crop Science*, *204*(4), 390–399.

Borst Pauwels, G. W. F. H. (1961). Iodine as a micronutrient for plants. *Plant and Soil*, *14*(4), 377–392.

Caffagni, A., Arru, L., Meriggi, P., Milc, J., Perata, P., & Pecchioni, N. (2011). Iodine fortification plant screening process and accumulation in tomato fruits and potato tubers. *Communications in Soil Science and Plant Analysis*, *42*(6), 706–718.

Cakmak, I., Prom-u-thai, C., Guilherme, L. R. G., Rashid, A., Hora, K. H., Yazici, A., Savasli, E., Kalayci, M., Tutus, Y., Phuphong, P., Rizwan, M., Martins, F. A. D., Dinali, G. S., & Ozturk, L. (2017). Iodine biofortification of wheat, rice and maize through fertilizer strategy. *Plant and Soil*, *418*(1–2), 319–335.

Candan, N., Cakmak, I., & Ozturk, L. (2018). Zinc-biofortified seeds improved seedling growth under zinc deficiency and drought stress in durum wheat. *Journal of Plant Nutrition and Soil Science*, *181*(3), 388–395.

Carmona, V. M. V., Filho, A. B. C., de Almeida, H. J., Silva, G. C., & dos Reis, A. R. (2020). Agronomic biofortification of beet plants with zinc via seed priming. *Revista Caatinga*, *33*(1), 116–123.

Chaves, M. M., Pereira, J. S., Maroco, J., Rodrigues, M. L., Ricardo, C. P. P., Osório, M. L., Carvalho, I., Faria, T., & Pinheiro, C. (2002). How plants cope with water stress in the field? Photosynthesis and growth. *Annals of Botany*, *89 (7)*, 907–916.

Chen, K., & Arora, R. (2013). Priming memory invokes seed stress-tolerance. *Environmental and Experimental Botany*, *94*, 33-45.

Chen, Y. E., Cui, J. M., Su, Y. Q., Zhang, C. M., Ma, J., Zhang, Z. W., Yuan, M., Liu, W. J., Zhang, H. Y., & Yuan, S. (2017). Comparison of phosphorylation and assembly of photosystem complexes and redox homeostasis in two wheat cultivars with different drought resistance. *Scientific Reports*, *7*(1), 12718.

Cho, L. H., Yoon, J., & An, G. (2017). The control of flowering time by environmental factors. *Plant Journal*, *90*(4), 708–719.

Choat, B., Brodribb, T. J., Brodersen, C. R., Duursma, R. A., López, R., & Medlyn, B. E. (2018). Triggers of tree mortality under drought. *Nature*, *558*(7711), 531–539.

Collins, W. J. (1978). The effect of defoliation on inflorescence production, seed yield and hard-seededness in swards of subterranean clover. *Australian Journal of Agricultural Research*, *29*(4), 789-801.

DAFF. (2010). Wheat Production guideline. *Department of Agriculture, Forestry and Fisheries*.

Dai, J. L., Zhu, Y. G., Zhang, M., & Huang, Y. Z. (2004). Selecting iodine-enriched vegetables and the residual effect of iodate application to soil. *Biological Trace Element Research*, *101*, 265-276.

Dávila-Rangel, I. E., & Leija-Martínez, P. Medrano-Macías, J. Fuentes-Lara, L.O. González-Morales, S. Juárez-Maldonado, A. Benavides-Mendoza, A. (2019). Iodine Biofortification of Crops. In *In Nutritional Quality Improvement in Plants. Springer, Cham*, 79–113.

De Lamirande, E., & Gagnon, C. (1995). Impact of reactive oxygen species on spermatozoa: A balancing act between beneficial and detrimental effects. *Human Reproduction*, *10*(SUPPL. 1), 15–21.

Department Agriculture, F. and F. (2016). Production Guideline. *Production Guidlines for Wheat*, 1–25.

Dey, P., Datta, D., Pattnaik, D., Dash, D., Saha, D., Panda, D., Bhatta, B. B., Parida, S., Mishra, U. N., & Chauhan, J. (2022). Physiological, biochemical, and molecular adaptation mechanisms of photosynthesis and respiration under challenging environments. In *Plant perspectives to global climate changes* 79–100.

Dey(née Pathak), G., & Mukherjee, R. K. (1984). Iodine treatment of soybean and sunflower seeds for controlling deterioration. *Field Crops Research*, *9(1)*, 205–213.

Dezfuli, P. M., Sharif-zadeh, F., & Janmohammadi, M. (2008). Influence of priming techniques on seed germination behavior of maize inbred lines (Zea mays L .). *Journal of Agricultural and Biological Science*.

di Girolamo, G., & Barbanti, L. (2012). Treatment conditions and biochemical processes influencing seed priming effectiveness. *Italian Journal of Agronomy*, *7*(2), 25–25.

Dlamini, J. (2015). Maize Growth and Yield As Affected By Different Soil Fertility. Thesis for MSc. *University of Pretoria*, *July*.

Dobosy, P., Endrédi, A., Sandil, S., Vetési, V., Rékási, M., Takács, T., & Záray, G. (2020). Biofortification of Potato and Carrot With Iodine by Applying Different Soils and Irrigation With Iodine-Containing Water. *Frontiers in Plant Science*, *11*, 593047.

Dobosy, P., Kröpfl, K., Óvári, M., Sandil, S., Németh, K., Engloner, A., Takács, T., & Záray, G. (2020). Biofortification of green bean (Phaseolus vulgaris L.) and lettuce (Lactuca sativa L.) with iodine in a plant-calcareous sandy soil system irrigated with water containing KI. *Journal of Food Composition and Analysis*, *88*, 103434.

Dobosy, P., Vetési, V., Sandil, S., Endrédi, A., Kröpfl, K., Óvári, M., Takács, T., Rékási, M., & Záray, G. (2020). Effect of irrigation water containing iodine on plant physiological processes and elemental concentrations of cabbage (brassica oleracea l. var. capitata l.) and tomato (solanum lycopersicum l.) cultivated in different soils. *Agronomy*, *10*(5), 1–15.

Domin, M., Kluza, F., Góral, D., Nazarewicz, S., Kozłowicz, K., Szmigielski, M., & Slaska-Grzywna, B. (2019). Germination energy and capacity of maize seeds following low-temperature short storage. *Sustainability*, *12*(1), 46.

Duborská, E., Urík, M., & Šeda, M. (2021). Iodine biofortification of vegetables could improve iodine supplementation status. *Plants*, *10*(7), 1352.

Edwards, B. R., Burghardt, L. T., Zapata-Garcia, M., & Donohue, K. (2016). Maternal temperature effects on dormancy influence germination responses to water availability in Arabidopsis thaliana. *Environmental and Experimental Botany*, *126*, 55-67

Eid, M. H. (2009). Estimation of heritability and genetic advance of yield traits in wheat (Triticum aestivum L.) under drought condition. *International Journal of Genetics and Molecular Biology*, *1*(7), 115–120.

ElBasyoni, I., Saadalla, M., Baenziger, S., Bockelman, H., & Morsy, S. (2017). Cell membrane stability and association mapping for drought and heat tolerance in a worldwide wheat collection. *Sustainability (Switzerland)*, *9*(9), 1606.

Elias, S., Garay, A., Schweitzer, L., & Hanning, S. (2006). Seed Quality Testing of Native Species. *Native Plants Journal*, *7*(1), 15-19.

Ellis, R. H., & Roberts, E. H. (1981). The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology, 9*(2), 373–409.

Elmore, R. W., & Jackobs, J. A. (1984). Yield and Yield Components of Sorghum and Soybeans of Varying Plant Heights when Intercropped 1. *Agronomy Journal*, *76*(4), 561-564.

Espanany, A., Fallah, S., & Tadayyon, A. (2016). Seed priming improves seed germination and reduces oxidative stress in black cumin (Nigella sativa) in presence of cadmium. *Industrial Crops and Products*, *79*, 195-204.

Farooq, M., Basra, S. M. A., Cheema, M. A., & Afzal, I. (2006). Integration of pre-sowing soaking, chilling and heating treatments for vigour enhancement in rice (Oryza sativa L.). *Seed Science and Technology*, *34*(2), 499-506.

Farooq, M., Usman, M., Nadeem, F., Rehman, H. ur, Wahid, A., Basra, S. M. A., Siddique, K. H. M., Farooq, M., Usman, M., Nadeem, F., Rehman, H. ur, Wahid, A., Basra, S. M. A., & Siddique, K. H. M. (2019). Seed priming in field crops: potential benefits, adoption and challenges. *Crop and Pasture Science*, *70*(9), 731–771.

Farooq, M., Wahid, A., and, K. S.-J. of soil science, & 2012, undefined. (2012). Micronutrient application through seed treatments: a review. *Journal of Soil Science and Plant Nutrition*, *12*(1), 125–142.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. M. A. (2009a). *Plant drought and molecular approaches*. Haworth Press, New York., 23-25.

Farooq, M., Wahid, A., & Siddique, K. H. M. (2012). Micronutrient application through seed treatments - a review. In *Journal of Soil Science and Plant Nutrition*, 12, (1), 125–142).

Fathi, A., & Tari, D. B. (2016). Effect of Drought Stress and its Mechanism in Plants. *International Journal of Life Sciences*, *10*(1), 1.

Finch-Savage, W. E. , & B. G. W. (2016). Seed vigour and seed quality: a new perspective on an old problem. *Seed Science Research*, *26*(1).

Finch-Savage, W. E., & Bassel, G. W. (2016). Seed vigour and crop establishment: Extending performance beyond adaptation. *Journal of Experimental Botany*, *67*(3), 567–591.

Fischer, K. S., & Fukai, S. (2003). How rice responds to drought. *Breeding Rice for Drought-Prone Environments*, *1*, 32-36.

Food and Agriculture Organization of the United Nations. (2019). *Food outlook – Biannual report on global food markets*. FAO. Https://Www.Fao.Org/3/Ca4526en/Ca4526en.Pdf.

Fuentes, J. E. G., Castellanos, B. F. H., Martínez, E. N. R., Ortiz, W. A. N., Mendoza, A. B., & Macías, J. M. (2022). Outcomes of foliar iodine application on growth, minerals and antioxidants in tomato plants under salt stress. *Folia Horticulturae*, *34*(1), 27–37.

Fuge, R., & Johnson, C. C. (2015). Iodine and human health, the role of environmental geochemistry and diet, a review. *Applied Geochemistry, 63,* 282-302.

Germ, M., Stibilj, V., Šircelj, H., Jerše, A., Kroflič, A., Golob, A., & Maršić, N. K. (2019). Biofortification of common buckwheat microgreens and seeds with different forms of selenium and iodine. *Journal of the Science of Food and Agriculture*, *99*(9), 4353–4362.

Germ, M., Stibilj, V., Šircelj, H., Jerše, A., Kroflič, A., Golob, A., Maršić, N. K., Marši´cmarši´c, N. K., & Maršić, N. K. (2019). *Biofortification of common buckwheat microgreens and seeds with different forms of selenium and iodine*. *99*(9), 4353–4362.

Ghobadi, M. E., Ghobadi, M., & Zebarjadi, A. (2017). Effect of waterlogging at different growth stages on some morphological traits of wheat varieties. *International Journal of Biometeorology, 61*(4), 635–645.

Golob, A., Krofliˇ, A., Jerše, A., Marši, N. K., Šircelj, H., Kroflič, A., Jerše, A., Maršić, N. K., Šircelj, H., Stibilj, V., & Germ, M. (2020). Response of Pumpkin to Different Concentrations and Forms of Selenium and Iodine, and their Combinations. *Plants, 9*(7), 1–12.

Golob, A., Novak, T., Maršić, N. K., Šircelj, H., Stibilj, V., Jerše, A., Kroflič, A., & Germ, M. (2020). Biofortification with selenium and iodine changes morphological properties of Brassica oleracea L. var. gongylodes) and increases their contents in tubers. *Plant Physiology and Biochemistry*, *150*, 234–243.

Golubkina, N., Moldovan, A., Kekina, H., Kharchenko, V., Sekara, A., Vasileva, V., Skrypnik, L., Tallarita, A., & Caruso, G. (2021). Joint Biofortification of Plants with Selenium and Iodine: New Field of Discoveries. *Plants, 10(7), 1352.*

Gómez-Merino, F. C., & Trejo-Téllez, L. I. (2018). The role of beneficial elements in triggering adaptive responses to environmental stressors and improving plant performance. *Biotic and Abiotic Stress Tolerance in Plants*, 137–172.

Gonzali, S., Kiferle, C., & Perata, P. (2017). Iodine biofortification of crops: agronomic biofortification, metabolic engineering and iodine bioavailability. *Current Opinion in Biotechnology*, *44*, 16–26.

Grass, L., & Burris, J. S. (1995). Effect of heat stress during seed development and maturation on wheat (*Triticum durum)* seed quality. I. Seed germination and seedling vigor . *Canadian Journal of Plant Science*, 75(4), 821-829.

Groot, S. P. C. (2020). Seed Science and Technology. Volume 48 Issue 1 (2020). *Seed Science and Technology*, *48*(1), 133–142.

Gupta, N., Bajpai, M., Majumdar, R., & Mishra, P. (2015). Response of iodine on antioxidant levels of Glycine max L. Grown under Cd2+ stress. *Advances in Biological Research*, *9*(1), 40-48.

Gurusinghe, S., & Bradford, K. (2014). Galactosyl-sucrose oligosaccharides and potential longevity of primed seeds. *Seed Science Research*, *11*(2), 121–134.

Hadebe, S., & Modi, A. (2017). Drought tolerance and water use of cereal crops: A focus on sorghum as a food security crop in sub-Saharan Africa. *Journal of Agronomy and Crop Science*, *203*(3), 177–191.

Haider, I., Raza, M. A. S., Iqbal, R., Aslam, M. U., Habib-ur-Rahman, M., Raja, S., Khan, M. T., Aslam, M. M., Waqas, M., & Ahmad, S. (2020). Potential effects of biochar application on mitigating the drought stress implications on wheat (Triticum aestivum L.) under various growth stages. *Journal of Saudi Chemical Society*, *24*(12), 974–981.

Halka, M., Smoleń, S., Ledwożyw-Smoleń, I., & Sady, W. (2019). Comparison of Effects of Potassium Iodide and Iodosalicylates on the Antioxidant Potential and Iodine Accumulation in Young Tomato Plants. *Journal of Plant Growth Regulation*, *39*(0123456789), 282–295.

Hampton, J. G., Boelt, B., Rolston, M. P., & Chastain, T. G. (2013). Effects of elevated CO2 and temperature on seed quality. *Journal of Agricultural Science*, *151*(2), 154-162.

Hampton, John G and TeKRONY, D. M. (1995). ISTA Vigour Test Committee. In *Handbook of Vigour Test Methods.International Seed testing Association; Zurich, Switzerland.Understanding Seed Vigour*.

Hanci, F., Cebci, E., & Polat, Z. (2014). The Effects of Trichoderma harzianum on Germination of Onion (*Allium cepa L* .) Seeds. *Tarım Bilimleri Araştırma Dergisi*, *7*(1), 45–48.

Hasanuzzaman, M., & Fotopoulos, V. (2019). Priming and Pretreatment of Seeds and Seedlings. In *Priming and Pretreatment of Seeds and Seedlings*.

Hasanuzzaman, M., Fujita, M., Oku, H., Nahar, K., & Hawrylak-Nowak, B. (2018). Plant nutrients and abiotic stress tolerance. *Plant Nutrients and Abiotic Stress Tolerance*, 1–590.

Hassan, M. U., Chattha, M. U., Ullah, A., Khan, I., Qadeer, A., Aamer, M., Khan, A. U., Nadeem, F., & Khan, T. A. (2019). Agronomic biofortification to improve productivity and grain Zn concentration of bread wheat. *International Journal of Agriculture and Biology*, 615-620.

Hendriks, J. H. M., Kolbe, A., Gibon, Y., Stitt, M., & Geigenberger, P. (2003). ADP-Glucose Pyrophosphorylase Is Activated by Posttranslational Redox-Modification in Response to Light and to Sugars in Leaves of Arabidopsis and Other Plant Species. *Plant Physiology*, *133*(2), 838-849.

Hernández-Díaz, J. A., Garza-García, J. J. O., García-Gaytán, V., León-Morales, J. M., Zamudio-Ojeda, A., & García-Morales, S. (2021). Beneficial elements improve the growth, biomass production, and photosynthetic pigments of calendula officinalis l. Seedlings. *Horticultural Science and Technology*, *39*(4), 413–423.

Hora, K., & Holwerda, H. T. (2023). Loss of yield or lower fruit quality due to iodine deficiency in tomato and sweet pepper crops under heat stress can be prevented by application of potassium nitrate with iodine. *Acta Horticulturae*, *1372*, 251–258.

Huanxin, W., Chunlai, H., Tianhong, X. I. A., Liting, B. A. O., & Huiping, L. I. U. (2013). *Iodine biofortification of vegetable plants — An innovative method for iodine supplementation*. *58*(17), 2066–2072.

Hussain, M., Malik, M.A., Farooq, M., Ashraf, M.Y. and Cheema, M. A. (2008). Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. *Journal of Agronomy and Crop Science*, *194 (3)*, 193-199.

Ibrahim, E. A. (2019). Seed priming to alleviate salinity stress in germinating seeds. *Journal of Plant Physiology*, *192*, 38-46.

Ibrahim, E. A.A. (2019). Fundamental Processes Involved in Seed Priming. *In Priming and Pretreatment of Seeds and Seedlings: Implication in Plant Stress Tolerance and Enhancing Productivity in Crop Plants, 63-115.*

Ilyas, A., Yasin Ashraf, M., Hussain, M., Ashraf, M., Ahmed, R., & Kamal, A. (2015). Effect of micronutrients (Zn, Cu AND B) on photosythetic and fruit yield attributes of citrus reticulata blanco var. kinnow. *Pak. J. Bot*, *47*(4), 1241–1247.

Imran, M. (2012). *Nutrient seed priming improves abiotic stress tolerance in Zea mays L. and Glycine max L.* 1–183.

Incrocci, L., Carmassi, G., Maggini, R., Poli, C., Saidov, D., Tamburini, C., Kiferle, C., Perata, P., & Pardossi, A. (2019). Iodine Accumulation and Tolerance in Sweet Basil (*Ocimum basilicum L* .) With Green or Purple Leaves Grown in Floating System Technique. *Frontiers in Plant Science*, *10*(December), 1–15.

International Seed Testing Association. (2015). International Rules for Seed Testing. *International Rules for Seed Testing*.

Iqbal, R., Raza, M. A. S., Saleem, M. F., Khan, I. H., Zaheer, M. S., Ahmad, S., Haider, I., Aslam, M. U., & Toleikiene, M. (2019). Foliar applied iron and zinc improves the growth, physiological and yield related traits of wheat under drought. *International Journal of Biosciences*, *14*, 376–387.

ISTA. (1993). International rules for seed testing. *12. ISTA (1993) International Rules for Seed Testing. Zurich, Switzerland.*, *12. ISTA (*(12. ISTA (1993) International rules for seed testing. Zurich, Switzerland.), 345.

Iyem, E., Yildirim, M., & Kizilgeci, F. (2021). Germination, seedling growth and physio-biochemical indices of bread wheat (Triticum aestivum l.) genotypes under peg induced drought stress. *Agriculture and Forestry*, *67*(1).

Jaleel, C. A., Manivannan, P., Lakshmanan, G. M. A., Gomathinayagam, M., & Panneerselvam, R. (2008). Alterations in morphological parameters and photosynthetic pigment responses of Catharanthus roseus under soil water deficits. *Colloids and Surfaces B: Biointerfaces*, *61*(2), 298–303.

Jerše, A., Kacjan-Maršić, N., Šircelj, H., Germ, M., Kroflič, A., & Stibilj, V. (2017a). Seed soaking in I and Se solutions increases concentrations of both elements and changes morphological and some physiological parameters of pea sprouts. *Plant Physiology and Biochemistry*, *118*, 285–294.

Jisha, K. C., Vijayakumari, K., & Puthur, J. T. (2013). Seed priming for abiotic stress tolerance: An overview. In *Acta Physiologiae Plantarum*, 35*(5),* 1381–1396).

Jogawat, A. (2019). Osmolytes and their Role in Abiotic Stress Tolerance in Plants. *Molecular Plant Abiotic Stress: Biology and Biotechnology*, 91–104.

Kabiri, R., Nasibi, F., & Farahbakhsh, H. (2014). Effect of exogenous salicylic acid on some physiological parameters and alleviation of drought stress in Nigella sativa plant under hydroponic culture. *Plant Protection Science*, *50*(1).

Kamithi, K., Wachira, F., & Kibe, A. (2016). Effects of Different Priming Methods and Priming Durations on Enzyme Activities in Germinating Chickpea (Cicer arietinum L .). *American Journal of Natural and Applied Sciences*, *1*(1), 1-9.

Kasim, W. A. E., Saad-allah, K. M., & Hamouda, M. (2016). *Seed Priming with Extracts of two Seaweeds Alleviates the Physiological and Molecular Impacts of Salinity Stress on Radish (Raphanus sativus),* 18(3), 653–660.

Kerchev, P., & van Breusegem, F. (2022). Improving oxidative stress resilience in plants. *The Plant Journal*, *109*(2), 359–372.

Khabiri, E., & Jafari, M. (2012). The effect of different light levels on the growth of wheat Gascogne. *International Research Journal of Applied and Basic Sciences*, *3*(12), 2358-2363.

Khalil, S. K., Khan, S., Rahman, A., Khan, A. Z., Khalil, I. H., Amanullah, Wahab, S., Mohammad, F., Nigar, S., Zubair, M., Parveen, S., & Khan, A. (2010). Seed priming and phosphorus application enhance phenology and dry matter production of wheat. *Pakistan Journal of Botany*, *42*(3), 1849–1856.

Khan, M. N., Zhang, J., Luo, T., Liu, J., Ni, F., Rizwan, M., Fahad, S., & Hu, L. (2019). Morpho-physiological and biochemical responses of tolerant and sensitive rapeseed cultivars to drought stress during early seedling growth stage. *Acta Physiologiae Plantarum*. , *41*, 1-13.

Khatiwada, A., Neupane, I., Sharma, B., Bhetwal, N., & Pandey, B. (2020). Effects of drought stress on yield and yield attributing characters of wheat: A review. *Agriways*, *8*(2), 115–121.

Kiferle, C., Ascrizzi, R., Martinelli, M., Gonzali, S., Mariotti, L., Pistelli, L., Flamini, G., & Perata, P. (2019). Effect of Iodine treatments on Ocimum basilicum L.: Biofortification, phenolics production and essential oil composition. *PLoS ONE*, *14*(12).

Kiferle, C., Gonzali, S., Beltrami, S., Martinelli, M., Hora, K., Holwerda, H. T., & Perata, P. (2022a). Improvement in fruit yield and tolerance to salinity of tomato plants fertigated with micronutrient amounts of iodine. *Scientific Reports*, *12*(1), 14655..

Kiferle, C., Martinelli, M., Salzano, A. M., Gonzali, S., Beltrami, S., Salvadori, P. A., Hora, K., Holwerda, H. T., Scaloni, A., & Perata, P. (2021). Evidences for a Nutritional Role of Iodine in Plants. *Frontiers in Plant Science*, *12,* 616868.

Kiliç, H., & Yağbasanlar, T. (2010). The effect of drought stress on grain yield, yield components and some quality traits of durum wheat (Triticum turgidum ssp. durum) cultivars. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *38*(1), 164-170.

Kiniry, J. R., & Ritchie, J. T. (1985). Shade-Sensitive Interval of Kernel Number of Maize 1 . *Agronomy Journal*, *77*(5), 711-715.

Krzepiłko, A., Prażak, R., & Skwaryło-bednarz, B. (2019). *Agronomic biofortification as a means of enriching plant foodstuffs with iodine*, *72*(2), 1–9.

Krzepiłko, A., Święciło, A., Zych-Wężyk, I., Agronomy, I. Z.-W.-, & 2021, undefined. (2021). The antioxidant properties and biological quality of radish seedlings biofortified with iodine. *Agronomy*, *11*(10), 2011.

Krzepiłko, A., Zych-Wężyk, I., Molas, J., Skwaryło-Bednarz, B., Święciło, A., & Skowrońska, M. (2016). The effect of iodine biofortification on selected biological quality parameters of lettuce and radish seedlings. *Acta Scientiarum Polonorum, Hortorum Cultus*, *15*(3), 3–16.

Kuchlan, P., Kuchlan, M. K., & Husain, S. M. (2017). Effect of foliar application of growth activator, promoter and antioxidant on seed quality of soybean. *Legume Research*, *40*(2), 313-318.

Kumar, P., Yadava, R. K., Gollen, B., Kumar, S., Verma, R. K., & Yadav, S. (2011). Nutritional Contents and Medicinal Properties of Wheat: A Review. *Life Sciences and Medicine Research*, *22*, 1–10.

Kumar Singhal, R., Pradesh, U., Vivek Kumar, I., Bandana Bose, I., Rajesh Kumar Singhal, C., Kumar, V., & Bose, B. (2019). Oat Breeding View project Multiple Abiotic Stress Tolerance in Higher Plants: Addressing the Growing Challenges View project Improving the yield and yield attributes in wheat crop using seed priming under drought stress. *Journal of Pharmacognosy and Phytochemistry*, *8*(2), 214–220.

Kutlu, I., Gulmezoglu, N., & Smoleń, S. (2023). Comparison of biologically active iodine and potassium iodide treatments in increasing grain iodine content and quality of oats. *Journal of Plant Growth Regulation*, *42*(5), 2776–2786.

Lawson, P. G., Daum, D., Czauderna, R., Meuser, H., & Härtling, J. W. (2015). Soil versus foliar iodine fertilization as a biofortification strategy for field-grown vegetables. *Frontiers in Plant Science*, *6*(JUNE), 1–11.

Lawson, P. G., Daum, D., Czauderna, R., & Vorsatz, C. (2016). Factors influencing the efficacy of iodine foliar sprays used for biofortifying butterhead lettuce (Lactuca sativa). *Journal of Plant Nutrition and Soil Science*, *179*(5), 661–669.

Ledwożyw-Smoleń, I., Smoleń, S., Rożek, S., Sady, W., & Strzetelski, P. (2020). Iodine biofortification of potato (Solanum tuberosum l.) grown in field. *Agronomy*, *10*(12), 1916.

Lehr, J. J., Wybenga, J. M., & Rosanow, M. (1958). *Iodine as a micronutrient for Tomatoesi H8B03*. *Plant Physiol.* 33, 421–427.

Leyva, R., Sánchez-Rodríguez, E., Ríos, J. J., Rubio-Wilhelmi, M. M., Romero, L., Ruiz, J. M., & Blasco, B. (2011). Beneficial effects of exogenous iodine in lettuce plants subjected to salinity stress. *Plant Science*, *181*(2), 195-202.

Li, R., Liu, H. P., Hong, C. L., Dai, Z. X., Liu, J. W., Zhou, J., Hu, C. Q., & Weng, H. X. (2017). Iodide and iodate effects on the growth and fruit quality of strawberry. *Journal of the Science of Food and Agriculture*, *97*(1), 230-235.

Lima, J. de S., Andrade, O. V. S., Santos, L. C. dos, Morais, E. G. de, Martins, G. S., Mutz, Y. S., Nascimento, V. L., Marchiori, P. E. R., Lopes, G., & Guilherme, L. R. G. (2023a). Soybean Plants Exposed to Low Concentrations of Potassium Iodide Have Better Tolerance to Water Deficit through the Antioxidant Enzymatic System and Photosynthesis Modulation. *Plants*, *12*(13), 2555.

Liu, E. K., Mei, X. R., Yan, C. R., Gong, D. Z., & Zhang, Y. Q. (2016). Effects of water stress on photosynthetic characteristics, dry matter translocation and WUE in two winter wheat genotypes. *Agricultural Water Management*, *167*, 75–85.

Liwani, U., Magwaza, L. S., Odindo, A. O., & Sithole, N. J. (2019). Growth, morphological and yield responses of irrigated wheat (Triticum aestivum L.) genotypes to water stress. *Acta Agriculturae Scandinavica Section B: Soil and Plant Science*, *69*(4), 369–376.

Liwani, U., Magwaza, L. S., Sithole, N. J., Odindo, A. O., & Tsilo, T. J. (2018). Physiological responses of irrigated wheat (Triticum aestivum L.) genotypes to water stress. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science*, *68*(6), 524–533.

Lutts, S., Benincasa, P., Wojtyla, L., K. S., Pace, R., Lechowska, K., Quinet, M., & Garnczarska, M. (2016). Seed priming: new comprehensive approaches for an old empirical technique. In *New challenges in seed biology-basic and translational research driving seed technology*, 1–46.

Lyons, G. (2018). Biofortification of cereals with foliar selenium and iodine could reduce hypothyroidism. *Frontiers in Plant Science*, *9*(June), 1–8.

Lyons, G., & Cakmak, I. (2012). Agronomic Biofortification of Food Crops with Micronutrients. In *Fertilizing Crops to Improve Human Health: a Scientific Review*, 1, 97–122.

Madakadze, R. M., & Kwaramba, J. (2004). Effect of Preharvest Factors on the Quality of Vegetables Produced in the Tropics. *Production Practices and Quality Assessment of Food Crops Volume 1*, 1–36.

Maglione, G., Hanaka, A., Majewska, M., Hawrylak-Nowak, B., Vitale, E., Costanzo, G., Polimeno, F., Arena, C., & Vitale, L. (2022). *Iodine Enhances the Nutritional Value but Not the Tolerance of Lettuce to NaCl. Horticulturae*, *8*(7), 662.

Mahajan, S., & Tuteja, N. (2005). Cold, salinity and drought stresses: An overview. *Archives of Biochemistry and Biophysics*, *444*(2), 139–158.

Majda, C., Khalid, D., Aziz, A., Rachid, B., Badr, A. S., Lotfi, A., & Mohamed, B. (2019). Nutri-priming as an efficient means to improve the agronomic performance of molybdenum in common bean (*Phaseolus vulgaris L.*). *Science of the Total Environment*, *661*, 654–663.

Mao, H., Wang, J., Wang, Z., Zan, Y., Lyons, G., & Zou, C. (2014). Using agronomic biofortification to boost zinc, selenium, and iodine concentrations of food crops grown on the loess plateau in China. *Journal of Soil Science and Plant Nutrition*, *14*(2), 459–470.

Marthandan, V., Geetha, R., Kumutha, K., Renganathan, G., Karthikeyan, A., & Ramalingam, J. (2020). Seed priming: a feasible strategy to enhance drought tolerance in crop plants. *International Journal of Molecular Sciences*, *21*(21), 8258.

Masipa, T. S. (2017). The impact of climate change on food security in South Africa: Current realities and challenges ahead. *Jàmbá : Journal of Disaster Risk Studies*, *9*(1), 1-7.

Medrano, J., Nohemi, E., Mart, R., Alfredo, W., Mendoza, A. B., Mart, P., Medrano Macías, J., López Caltzontzit, M. G., Rivas Martínez, E. N., Narváez Ortiz, W. A., Benavides Mendoza, A., & Martínez Lagunes, P. (2021). Enhancement to salt stress tolerance in strawberry plants by iodine products application. *Agronomy*, *11*(3), 1–16.

Medrano-Macías, J., Leija-Martínez, P., González-Morales, S., Juárez-Maldonado, A., & Benavides-Mendoza, A. (2016). Use of iodine to biofortify and promote growth and stress tolerance in crops. *Frontiers in Plant Science*, *7*(AUG2016), 1–20.

Medrano-Macías, J., Leija-Martínez, P., Juárez-Maldonado, A., Rocha-Estrada, A., Benavides-Mendoza, A., Medrano-Macías, J., Leija-Martínez, P., Juárez-Maldonado, A., Rocha-Estrada, A., & Benavides-Mendoza, A. (2016). Effect of iodine application on antioxidants in tomato seedlings. *Revista Chapingo. Serie Horticultura*, *22*(2), 133–143.

Medrano-macías, J., Mendoza-villarreal, R., Fuentes-lara, L. O., Fuentes-lara, O., Robledo-torres, V., Ramírez-godina, F., & Ángel, M. (2018). *The Use of Iodine , Selenium , and Silicon in Plant Nutrition for the Increase of Antioxidants in Fruits and Vegetables and Benavides-Mendoza*.

Meena, R. P., Tripathi, S. C., Chander, S., Chhokar, R. S., & Sharma, R. K. (2015). Seed priming in moisture-stress conditions to improve growth and yield of wheat (Triticum aestivum). *Indian Journal of Agronomy*, *60*(1), 99–103.

Mejía-Ramírez, F., Benavides-Mendoza, A., González-Morales, S., Juárez-Maldonado, A., Lara-Viveros, F. M., Morales-Díaz, A. B., & Morelos-Moreno, Á. (2023). Seed Priming Based on Iodine and Selenium Influences the Nutraceutical Compounds in Tomato (Solanum lycopersicum L.) Crop. *Antioxidants*, *12*(6), 1265.

Milošević, M., Vujaković, M., & Karagić, D. (2010). Vigour tests as indicators of seed viability. *Genetika*, *42*(1), 103-118.

Mirbahar, A. A., Markhand, G. S., Mahar, A. R., Abro, S. A., & Kanhar, N. A. (2009). Effect of water stress on yield and yield components of wheat (Triticum aestivum L.) varieties. *Pak. J. Bot*, *41*(3), 1303–1310.

Moaveni, P. (2011). Effect of water deficit stress on some physiological traits of wheat (Triticum aestivum). *Agricultural Science Research Journal*, *1*(1), 64–68.

Modi, A., & Mabhaudhi, T. (2013). *Water-Use and Drought Tolerance of Selected Tradtional Crops* (Issue WRC Report No. 1771/1/13).

Molden, D., Vithanage, M., De Fraiture, C., Faures, J. M., Gordon, L., Molle, F., & Peden, D. (2011). 4.21-Water availability and its use in agriculture. *Treatise on Water Science*, 707–732.

Mondal, S., & Bose, B. (2019). Impact of micronutrient seed priming on germination, growth, development, nutritional status and yield aspects of plants. In *Journal of Plant Nutrition*. 42(19), 2577–2599. Taylor & Francis.

Muhsin, M., Nawaz, M., Khan, I., Chattha, M. B., Khan, S., Aslam, M. T., Iqbal, M. M., Amin, M. Z., Ayub, M. A., Anwar, U., Hassan, M. U., & Chattha, M. U. (2021). Efficacy of Seed Size to Improve Field Performance of Wheat under Late Sowing Conditions. *Pakistan Journal of Agricultural Research*. *34*(1)*,* 247–254.

Munné-Bosch, S., & Peñuelas, J. (2003). Photo- and antioxidative protection, and a role for salicylic acid during drought and recovery in field-grown Phillyrea angustifolia plants. *Planta*. *217(5),* 758-766.

Murchie, E., & Lawson, T. (2013). Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *Journal of Experimental Botany*, *64,* 3983–3998.

Nadeem, F., & Farooq, M. (2019). Application of Micronutrients in Rice-Wheat Cropping System of South Asia. *Rice Science*, *26*(6), 356–371.

Nadew, B. B. (2018). Effects of Climatic and Agronomic Factors on Yield and Quality of Bread Wheat (Triticum aestivum L.) Seed: A Review on Selected Factors. *Advances in Crop Science and Technology*, *6*(2), 356.

Nascimento, V. L., Souza, B. C. O. Q., Lopes, G., & Guilherme, L. R. G. (2022). On the Role of Iodine in Plants: A Commentary on Benefits of This Element. *Frontiers in Plant Science*, *13, 836835.*

Nawaz, F., Ahmad, R., Waraich, E. A., Naeem, M. S., & Shabbir, R. N. (2012). Nutrient uptake, physiological responses, and yield attributes of wheat (Triticum aestivum L.) exposed to early and late drought stress. *Journal of Plant Nutrition*, *35*(6), 961–974.

Nawaz, F., Ashraf, M. Y., Ahmad, R., Waraich, E. A., Shabbir, R. N., & Hussain, R. A. (2017). Selenium supply methods and time of application influence spring wheat (Triticum aestivum L.) yield under water deficit conditions. *Journal of Agricultural Science*, 155(4), 643-656.

Nciizah, A., Rapetsoa, M., Wakindiki, I., Heliyon, M. Z.-, & 2020, undefined. (2020). Micronutrient seed priming improves maize (Zea mays) early seedling growth in a micronutrient deficient soil. *Heliyon*, *6*(8), 04766.

Neha, G., Bajpai, M., & Majumdar, R. (2015). Response of iodine on antioxidant levels of Glycine max L. grown under Cd2+ stress. *Advances in Biological Research*, *9*(1), 40–48.

Neha, G., & Singh Rita, M. (2016). *Effect of iodine on nonenzymatic antioxidant levels of Soybean (Glycine max L.) grown under heavy metal and heat stress*. *4*.

Niu, J., Liu, C., Huang, M., Liu, K., & Yan, D. (2021). Effects of foliar fertilization: a review of current status and future perspectives. *Journal of Soil Science and Plant Nutrition*, *21*, 104–118.

Odindo, A. O. (2007). *Cowpea seed quality in response to the production site and water stress*. Ph.D. thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Oladir, G., Saeed, M., & Cheerna, M. A. (1999). Effect of water stress on growth and yield performance of four wheat cultivars. *Pakistan Journal of Biological Sciences*, *2*, 236–239.

Pal, A., Ali, M., & Pal, A. (2017). Effect of seed priming on germination behavior, oxidative stress and antioxidant enzyme activities in groundnut (Arachis hypogaea L) under salinity stress. *International Journal of Agriculture Sciences*, *9*(36), 479–485.

Paparella, S., Araújo, S. S., Rossi, G., Wijayasinghe, M., Carbonera, D., & Balestrazzi, A. (2015). Seed priming: state of the art and new perspectives. In *Plant Cell Reports* (Vol. 34, Issue 8, pp. 1281–1293).

Patel, P. R., Parmar, G. M., & Parmar, S. K. (2020). Manipulation of Source-Sink Relationship in Pearl Millet Through Growth Retardants. *International Journal of Current Microbiology and Applied Sciences*, *9*(3), 2963–2973.

Pawar, V. A., & Laware, S. L. (2018). Seed Priming A Critical Review. *International Journal of Scientific Research in Biological Sciences*, *5*(5), 94–101.

Pearce, EN., Andersson, M., & Zimmermann, MB. (2013). Global iodine nutrition: Where do we stand in 2013? In *Thyroid*, *23*(5), 523-528.

Peltonen-Sainio, P., Rajala, A., & Jauhiainen, L. (2011). Hidden viability risks in the use of farm-saved small-grain seed. *Journal of Agricultural Science*, *149*(6), 713-724.

Peñaloza, P., Ramirez-Rosales, G., McDonald, M. B., Bennett, M. A., & Peñaloza, P., Ramirez-Rosales, G., McDonald, M.B. and Bennett, M. A. (2005). Lettuce (Lactuca sativa L.) seed quality evaluation using seed physical attributes, saturated salt accelerated aging and the seed vigour imaging system. *Electronic Journal of Biotechnology, 8 (3),* 0-0.

Pérez-Salas, & Medrano-Macías. (2021). Use of Iodine as a Tolerance Inducer in Tomato Seedlings Under Salinity Stress. *Científica Univ. Autónoma Coahuila, 13, 14-22.*

Perry, D. A. (1980). The concept of seed vigour and its relevance to seed production techniques. In *Seed production*, 585–591.

Pham Thi, A. T., Vieira Da Silva, J., & Mazliak, P. (1990). The role of membrane lipids in drought resistance of plants. *Bulletin de La Société Botanique de France. Actualités Botaniques*, *137*(1), 114.

Poole, N. (2005). Cereal Growth Stages: The Link to Crop Management. *Grains Research and Development Corporation. Barton, Australia, ISBN 1-875477-40- https://grdc.com.au/__data/assets/pdf_file/0031/364594/Cereal-growthstages.pdf*.

Praharaj, S., Skalicky, M., Maitra, S., Bhadra, P., Shankar, T., Brestic, M., Hejnak, V., Vachova, P., Hossain, A., Camargo, D., & Iriti, M. (2021). Zinc biofortification in food crops could alleviate the zinc malnutrition in human health. *Molecules, 26*(12), 3509.

Rady, M. M., Belal, H. E. E., Gadallah, F. M., & Semida, W. M. (2020). Selenium application in two methods promotes drought tolerance in Solanum lycopersicum plant by inducing the antioxidant defense system. *Scientia Horticulturae, 266*, 109290

Rakshit, A., Singh, H. B., Singh, A. K., Singh, U. S., & Fraceto, L. (2020). New Frontiers in Stress Management for Durable Agriculture. In Springer (Ed.), *New Frontiers in Stress Management for Durable Agriculture*,35-37.

Ramzan, Y., Hafeez, M. B., Khan, S., Nadeem, M., Saleem-ur-Rahman, Batool, S., & Ahmad, J. (2020). Biofortification with Zinc and Iron Improves the Grain Quality and Yield of Wheat Crop. *International Journal of Plant Production*, *14*(3), 501-510.

Rashid, A., & Ryan, J. (2004). Micronutrient constraints to crop production in soils with mediterranean-type characteristics: A review. In *Journal of Plant Nutrition*. *27*(6), 959-975.

Raza, A., Razzaq, A., Mehmood, S. S., Zou, X., Zhang, X., Lv, Y., & Xu, J. (2019). Impact of climate change on crops adaptation and strategies to tackle its outcome: A review. In *Plants*, *8*(2), 34.

Reddy, A. R., Chaitanya, K. V., & Vivekanandan, M. (2004). Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. In *Journal of Plant Physiology*,161(11), 1189–1202.

Reddy, T. Y., Reddy, V. R., & Anbumozhi, V. (2003). Physiological responses of groundnut (Arachis hypogaea L.) to drought stress and its amelioration: A review. *Acta Agronomica Hungarica*, *51*(2), 205–227.

Rehman, A., & Farooq, M. (2016). Zinc seed coating improves the growth, grain yield and grain biofortification of bread wheat. *Acta Physiologiae Plantarum, 38,* 1-10.

Reis, S., Pavia, I., Carvalho, A., Moutinho-pereira, J., Correia, C., & Lima-brito, J. (2018). *Seed priming with iron and zinc in bread wheat: effects in germination, mitosis and grain yield*. *Protoplasma*, *255,* 1179-1194.

Riyazuddin, R., Singh, K., Iqbal, N., Nisha, N., Rani, A., Kumar, M., Khatri, N., Siddiqui, M. H., Yasheshwar, Kim, S. T., Attila, F., & Gupta, R. (2022). Iodine: an emerging biostimulant of growth and stress responses in plants. In *Plant and Soil*, *486*(1), 119-133.

Roach, T., & Krieger-Liszkay, A. (2014). *Regulation of Photosynthetic Electron Transport and Photoinhibiti...: Ingenta Connect*. Current Protein and Peptide Science, *15*(4), 351-362.

Rodríguez-Salinas, P. A., Carballo-Méndez, F. de J., Rodríguez-Ortiz, J. C., Niño-Medina, G., Olivares-Saénz, E., & Garza-Alonso, C. A. (2022). Iodine increases the concentration of phenolic compounds and photosynthetic pigments in three cultivars of Ficus carica L. subjected to saline stress. *Revista Mexicana de Ciencias Agrícolas, 28,* 309–318.

S. Lisar, S. Y., Motafakkerazad, R., M., M., & M. Rahm, I. M. (2012). Water Stress in Plants: Causes, Effects and Responses. In *Water Stress, 25(1), 33*.

Sabourmoghaddam, N. (2018). *Benefit of iodine soil application for alleviating detrimental effects of salinity stress in strawberry*. *Journal of Plant Biological Sciences*, *10*(2), 57-72.

Sadeghian, S. Y., & Yavari, N. (2004). Effect of water-deficit stress on germination and early seedling growth in sugar beet. *Journal of Agronomy and Crop Science*, *190*(2), 138-144.

Saha, D., Choyal, P., Mishra, U. N., Dey, P., Bose, B., MD, P., Gupta, N. K., Mehta, B. K., Kumar, P., Pandey, S., Chauhan, J., & Singhal, R. K. (2022). Drought stress responses and inducing tolerance by seed priming approach in plants. *Plant Stress*, *4*, 100066.

Saini, D. K., Devi, P., & Kaushik, P. (2020). Advances in genomic interventions for wheat biofortification: A review. *Agronomy*, *10*(1), 1–28.

Sarkar, B. K., Yang, W. Y., Zhen, W. U., Tang, H., & Ding, S. (2009). Variations of water uptake, lipid consumption, and dynamics during the germination of sesamum indicum seed: a nuclear magnetic resonance spectroscopic investigation. *Journal of Agricultural and Food Chemistry, 57(18), 8213-8219.*

Sehgal, A., Sita, K., Siddique, K. H. M., Kumar, R., Bhogireddy, S., Varshney, R. K., HanumanthaRao, B., Nair, R. M., Prasad, P. V. V., & Nayyar, H. (2018). Drought or/and heat-stress effects on seed filling in food crops: Impacts on functional biochemistry, seed yields, and nutritional quality. In *Frontiers in Plant Science*, *9*, 1705.

Seleiman, M. F., Al-Suhaibani, N., Ali, N., Akmal, M., Alotaibi, M., Refay, Y., Dindaroglu, T., Haleem Abdul-Wajid, H., & Leonardo Battaglia, M. (2021). Drought stress impacts on plants and different approaches to alleviate its adverse effects. *Plants*, *10*(2), 259.

Sershen, Perumal, A., Varghese, B., Govender, P., Ramdhani, S., & Berjak, P. (2014). Effects of elevated temperatures on germination and subsequent seedling vigour in recalcitrant Trichilia emetica seeds. *South African Journal of Botany*, *90*, 153-162.

Shar, P. A., Shar, A. H., Memon, S., Soomro, A. A., Naich, S. A., Rind, N. A., Laghari, A., Rind, K. H., Meghwar, P., & Otho, S. A. (2021). Morpho-physiological responses in wheat (Triticum aestivum L) influenced by normal and water stress conditions. *Journal of Agriculture and Applied Biology*, *2*(1), 1–10.

Sharma, K. K., Singh, U. S., Sharma, P., Kumar, A., & Sharma, L. (2015). Seed treatments for sustainable agriculture-A review. *Journal of Applied and Natural Science, 7(1), 521.*

Sharma, N., Acharya, S., Kumar, K., Singh, N., & Chaurasia, O. P. (2018). Hydroponics as an advanced technique for vegetable production: An overview. *Journal of Soil and Water Conservation*, *17*(4), 364-371.

Sharma, P., Aggarwal, P., & Kaur, A. (2017). Biofortification: A new approach to eradicate hidden hunger. *Food Reviews International*, *33*(1), 1–21.

Sharma, V., Kumar, A., Chaudhary, A., Mishra, A., Rawat, S., B., B. Y., Shami, V., & Kaushik, P. (2022). Response of Wheat Genotypes to Drought Stress Stimulated by PEG. *Stresses*, *2*(1), 26–51.

Shew, A. M., Tack, J. B., Nalley, L. L., & Chaminuka, P. (2020). Yield reduction under climate warming varies among wheat cultivars in South Africa. *Nature Communications 2020 11:1*, *11*(1), 1–9.

Shiferaw, B., Smale, M., Braun, H. J., Duveiller, E., Reynolds, M., & Muricho, G. (2013). Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. *Food Security*, *5*(3), 291–317.

Sial, M. A., Dahot, M. U., Arain, M. A., Markhand, G. S., Mangrio, S. M., Naqvi, M. H., Laghari, K. A., & Mirbahar, A. A. (2009). Effect of water stress on yield and yield components of semi-dwarf bread wheat (Triticum aestivum L.). *Pak. J. Bot*, *41*(4), 1715–1728.

Sial, M. A., Mangrio, S. M., Bux, H., Channa, A. W., & Shaikh, M. (2017). Effect of water stress on some physiological traits of bread wheat genotypes. *Pakistan Journal of Agriculture, Agricultural Engineering and Veterinary Sciences*, *33*(1), 1–11.

Siddique, M. R. B., Hamid, A., & Islam, M. S. (2000). Drought stress effects on water relations of wheat. *Botanical Bulletin of Academia Sinica*, *41*, 35-39.

Singh, H., Jassal, R. K., Kang, J. S., Sandhu, S. S., Kang, H., & Grewal, K. (2015). *Seed priming techniques in field crops - A review*. *36*(4), 251–264.

Singh, V. K., Singh, R., Tripathi, S., Devi, R. S., Srivastava, P., Singh, P., Kumar, A., & Bhadouria, R. (2020b). Seed priming: state of the art and new perspectives in the era of climate change. In *Climate Change and Soil Interactions*, 143-170.

Sirohi, G., Upadhyay, A., Shankar Srivastava, P., & Srivastava, S. (2015). PGPR mediated zinc biofertilization of soil and its impact on growth and productivity of wheat. *Journal of Soil Science and Plant Nutrition*, *15*(1), 202-216.

Smoleń, S., Kowalska, I., Czernicka, M., Halka, M., Kęska, K., & Sady, W. (2016). Iodine and selenium biofortification with additional application of salicylic acid affects yield, selected molecular parameters and chemical composition of lettuce plants (Lactuca sativa L. var. capitata). *Frontiers in Plant Science*, *7,* 1553.

Smoleń, S., Ledwożyw-Smoleń, I., & Sady, W. (2016). The role of exogenous humic and fulvic acids in iodine biofortification in spinach (Spinacia oleracea L.). *Plant and Soil*, *402,* 129-143.

Smoleń, S., Rozek, S., Ledwozyw-Smoleń, I., & Strzetelski, P. (2011). Preliminary evaluation of the influence of soil fertilization and foliar nutrition with iodine on the efficiency of iodine biofortification and chemical composition of lettuce. *Journal of Elementology 16(4),* 613–622.

Smoleń, S., Sady, W., Ledwozyw-Smoleń, I., Strzetelski, P., Liszka-Skoczylas, M., & Rozek, S. (2014). Quality of fresh and stored carrots depending on iodine and nitrogen fertilization. *Food Chemistry 159, 316-322*.

Smoleń S., WierzbińSka J., Liszka-Skoczylas M., & Rakoczy, R. (2011). The effect of iodine form on yield quantity and biological quality of tomato cultivated in hydroponics system NFT. *Environ. Prot. Nat. Resour., 48,* 59–66.

Smoleń, S., Wierzbińska, J., Sady, W., Kołton, A., Wiszniewska, A., & Liszka-Skoczylas, M. (2015). Iodine biofortification with additional application of salicylic acid affects yield and selected parameters of chemical composition of tomato fruits (Solanum lycopersicum L.). *Scientia Horticulturae*, *188,* 89-96.

Smoleń, Sylwester., Skoczylas, Ł., Rakoczy, R., Ledwożyw-Smoleń, I., Kopeć, A., Piątkowska, E., Biezanowska-Kopeć, R., Koronowicz, A., Kapusta-Duch, J., Sady, W., & Pysz, M. (2015). Mineral composition of field-grown lettuce (Lactuca sativa L.) depending on the diversified fertilization with iodine and selenium compounds. *Acta Scientiarum Polonorum. Hortorum Cultus = Ogrodnictwo , 14*, 97–114.

Soares, J. C., Santos, C. S., Carvalho, S. M., Pintado, M. M., & Vasconcelos, M. W. (2019). Preserving the nutritional quality of crop plants under a changing climate: importance and strategies. In *Plant and Soil*, *443(1),* 1-26.

Sudhakar, N., Karthikeyan, G., RajhaViknesh, M., Saranya, A. S., & Shurya, R. (2020). Technological Advances in Agronomic Practices of Seed Processing, Storage, and Pest Management: An Update. In *Advances in Seed Production and Management*, 359–398.

Swapnil, P., Meena, M., Singh, S., & Dhuldhaj, U. (2021). Vital roles of carotenoids in plants and humans to deteriorate stress with its structure, biosynthesis, metabolic engineering and functional aspects. *Current Plant Biology*, *26*, 100203.

Taylor, A. G., Eckenrode, C. J., & Straub, R. W. (2001). Seed coating technologies and treatments for onion: Challenges and progress. In *HortScience, 36*(2), 199-205.

Toole, E. H., Hendricks, S. B., Borthwick, H. A., & Toole, V. K. (1956). Physiology of Seed Germination. *Annual Review of Plant Physiology*.

Ulfat, A., Majid, S. A., & Hameed, A. (2017). Hormonal seed priming improves wheat (Triticum aestivum L.) field performance under drought and non-stress conditions. *Pak. J. Bot*, *49*(4), 1239–1253.

Ullah, A., Farooq, M., Hussain, M., Ahmad, R., & Wakeel, A. (2019). Zinc seed priming improves stand establishment, tissue zinc concentration and early seedling growth of chickpea. *Journal of Animal and Plant Sciences*, *29*(4), 1046–1053.

Ullah, A., Farooq, M., Rehman, A., Arshad, M. S., Shoukat, H., Nadeem, A., Nawaz, A., Wakeel, A., & Nadeem, F. (2018). Manganese nutrition improves the productivity and grain biofortification of bread wheat in alkaline calcareous soil. *Experimental Agriculture*, *54*(5), 744–754.

Varier, A., Vari, A. K., & Dadlani, M. (2010). The subcellular basis of seed priming. *Current Science*, *99*(4), 450–456.

Veena, M., & Puthur, J. T. (2021a). Seed nutripriming with zinc is an apt tool to alleviate malnutrition. *Environmental Geochemistry and Health 2021*, 1–19.

Veesar, N. F., Channa, A. N., Rind, M. J., & Larik, A. S. (2007). Influence of water stress imposed at different stages on growth and yield attributes in bread wheat genotypes Triticum aestivum L. *Wheat Information Service*, *104*, 15–19.

Ventura, L., Donà, M., Macovei, A., Carbonera, D., Buttafava, A., Mondoni, A., Rossi, G., & Balestrazzi, A. (2012). Understanding the molecular pathways associated with seed vigor. *Plant Physiology and Biochemistry*, *60*, 196–206.

Venturi, S. (2011). Evolutionary Significance of Iodine. *Current Chemical Biology, 5(3), 155-162.*

Verslues, P. E., Agarwal, M., Katiyar-Agarwal, S., Zhu, J., & Zhu, J.-K. (2006). Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *Wiley Online Library*, *45*(4), 523–539.

Vieira, R. D., Scappa Neto, A., Bittencourt, S. R. M. de, & Panobianco, M. (2004). Electrical conductivity of the seed soaking solution and soybean seedling emergence. *Scientia Agricola*, *61*, 164-168.

Vieira, R. D., TeKrony, D. M., & Egli, D. B. (1992). Effect of Drought and Defoliation Stress in the Field on Soybean Seed Germination and Vigor. *Crop Science*, *32*(2), 471-475.

Waller, J. C., Hussain, S., Khan, F., Hussain, H. A., & Nie, L. (2016). Physiological and Biochemical Mechanisms of Seed Priming-Induced Chilling Tolerance in Rice Cultivars. *Frontiers in Plant Science*, *7* , 1–14.

Wang, H. L., Lee, P. du, Chen, W. L., Huang, D. J., & Su, J. C. (2000). Osmotic stress-induced changes of sucrose metabolism in cultured sweet potato cells. *Journal of Experimental Botany 51(353), 1991-1999.*

Waqas, M., Korres, N. E., Khan, M. D., Nizami, A.-S., Deeba, F., Ali, I., & Hussain, H. (2019). Advances in the Concept and Methods of Seed Priming. *Priming and Pretreatment of Seeds and Seedlings*, 11–41.

Weng, H., Hong, C., & Yan, A. (2008). *Mechanism of Iodine Uptake by Cabbage : Effects of Iodine Species and Where It is Stored*. *Biological Trace Element Research*, *125*, 59–71.

Weng, H. X., Weng, J. K., Yong, W. bin, Sun, X. W., & Zhong, H. (2003). Capacity and degree of iodine absorbed and enriched by vegetable from soil. *Journal of Environmental Sciences, 15(1), 107-111.*

Weng, H. X., Yan, A. L., Hong, C. L., Xie, L. L., Qin, Y. C., & Cheng, C. Q. (2008). Uptake of different species of iodine by water spinach and its effect to growth. *Biological Trace Element Research*, *124*(2), 184–194.

White, P. J., & Broadley, M. R. (2005). Biofortifying crops with essential mineral elements. *Trends in Plant Science*, *10*(12), 586–593.

White, P. J., White, P. J., & Broadley, M. R. (2009). Biofortification of crops with seven mineral elements often lacking in human diets–iron, zinc, copper, calcium, magnesium, selenium and iodine. *New phytologist, 182(1),* 49–84.

Wojciechowska, R., Strzetelski, P., & Kołton, A. (2013). Npływ aplikacji dolistnej różnymi formami jodu na wybrane parametry fluorescencji chlorofilu a w liściach dwóch odmian bazylii (The effect of different forms of foliar iodine application on chlorophyll a fluorescence in leaves of two basil cultivars). In *Episteme czasopismo naukowo - kulturalne* ,613–622.

Wójcik, P., & Wójcik, M. (2021). Preharvest iodine sprays at high rates are more effective in biofortification of apples than soil application. *Plant and Soil*, *465*(1–2), 317–334.

Yadav, S., Modi, P., Dave, A., Vijapura, A., Patel, D., & Patel, M. (2020). Effect of abiotic stress on crops. *Sustainable Crop Production*, *3*(17), 5-16.

Yamori, W., Takahashi, S., Makino, A., Price, G. D., Badger, M. R., & von Caemmerer, S. (2011). The Roles of ATP Synthase and the Cytochrome b 6/f Complexes in Limiting Chloroplast Electron Transport and Determining Photosynthetic Capacity. *Plant Physiology* , *155*(2), 956–962.

Yan, A., & Chen, Z. (2020). The Control of Seed Dormancy and Germination by Temperature, Light and Nitrate. *Botanical Review*, *86*(1), 39-75.

Yang, X., Wang, X., Wang, L., & Wei, M. (2012). Control of light environment: A key technique for high-yield and high-quality vegetable production in protected farmland. *Agricultural Sciences, 3(7), 923-928.*

Yunze, S., & Shuangsheng, G. (2014). Effects of photoperiod on wheat growth, development and yield in CELSS. *Acta Astronautica, 105(1), 24-29.*

Zadoks, J. C., Chang, T. T., & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed Research*, *14*(6), 415-421.

Zewide, I., & Sherefu, A. (2021). Review Paper on Effect of Micronutrients for Crop Production. *Journal Nutrition and Food Processing*, *4*(7), 1-8.

Zhang, H. ; W. Y. ; L. X. ; & Z. J. (2018). Effects of water stress on wheat growth, development, and yield. *Journal of Agronomy and Crop Science*, *204*(2), 123–135.

Zhao, W., Liu, L., Shen, Q., Yang, J., Han, X., Tian, F., & Wu, J. (2020). Effects of water stress on photosynthesis, yield, and water use efficiency in winter wheat. *Water (Switzerland)*, *12*(8), 2127.

Zhu, Y. G., Huang, Y. Z., Hu, Y., & Liu, Y. X. (2003). Iodine uptake by spinach (Spinacia oleracea L.) plants grown in solution culture: Effects of iodine species and solution concentrations. *Environment International*, *29*(1), 33–37.

Zulfiqar, U., Maqsood, M., Hussain, S., & Anwar-ul-Haq, M. (2020). Iron Nutrition Improves Productivity, Profitability, and Biofortification of Bread Wheat under Conventional and Conservation Tillage Systems. *Journal of Soil Science and Plant Nutrition*, *20*, 1298-1310.