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Setaria viridis: a biological model for studying plant responses to climate stress

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Abstract

Abiotic stresses such as water deficit, salinity, temperature fluctuations and elevated concentrations of atmospheric CO_2 are among the significant environmental events associated with global climate changes affecting plant development. In this context, the monocot species *Setaria viridis* (L.) P. Beauv. (Poaceae) is an emerging model for plant species with C_4 photosynthesis because it has many attributes, such as simple growth requirements, a short life cycle, small stature and a sequenced genome, making it ideal for studies in plant biology. Understanding the morphophysiological and molecular responses of *S. viridis* to several abiotic factors is critical for understanding the fundamental mechanisms governing the growth and adaptation of C_4 plants and developing climate-resilient crops in the context of climate change. This manuscript highlights the novel perspectives and functional mechanisms associated with how *S. viridis* can respond to abiotic stresses and its importance to the future of C_4 species, reinforcing *S. viridis* as a powerful model for understanding grass biology.

Keywords C_4 model plant · Abiotic stress · Water stress · Salinity stress · Temperature · Atmospheric CO_2

1 Introduction

The global human population is estimated to reach 9.5 billion by 2050, which demands an increased food supply and advances in food security. Between 2020 and 2021, approximately 3.1 billion people did not have access to a healthy diet. Projections for 2030 indicate that 670 million people, or 8% of the world's population, will be facing hunger (FAO 2023). Increasing the global food supply can boost the exploitation of new areas with agricultural potential. Farming region expansion impacts forest and woodland areas, resulting in environmental degradation, a systemic global problem that includes climate change impacts on ecosystems and human activities, including agriculture (Chaudhry et al. 2021; Levers et al. 2021).

Bad agricultural practices and inputs from urban and industrial pollution increase greenhouse gas emissions. According to global climate predictions, events such as drought and extreme heat will become increasingly common in response to elevated CO_2 concentrations (e.g., atmospheric CO_2 reached 410 ppm in 2019, the highest in at least 2 million years) and rising average temperatures, with the global surface temperature having already reached 1.1 °C above pre-industrial levels and projected to

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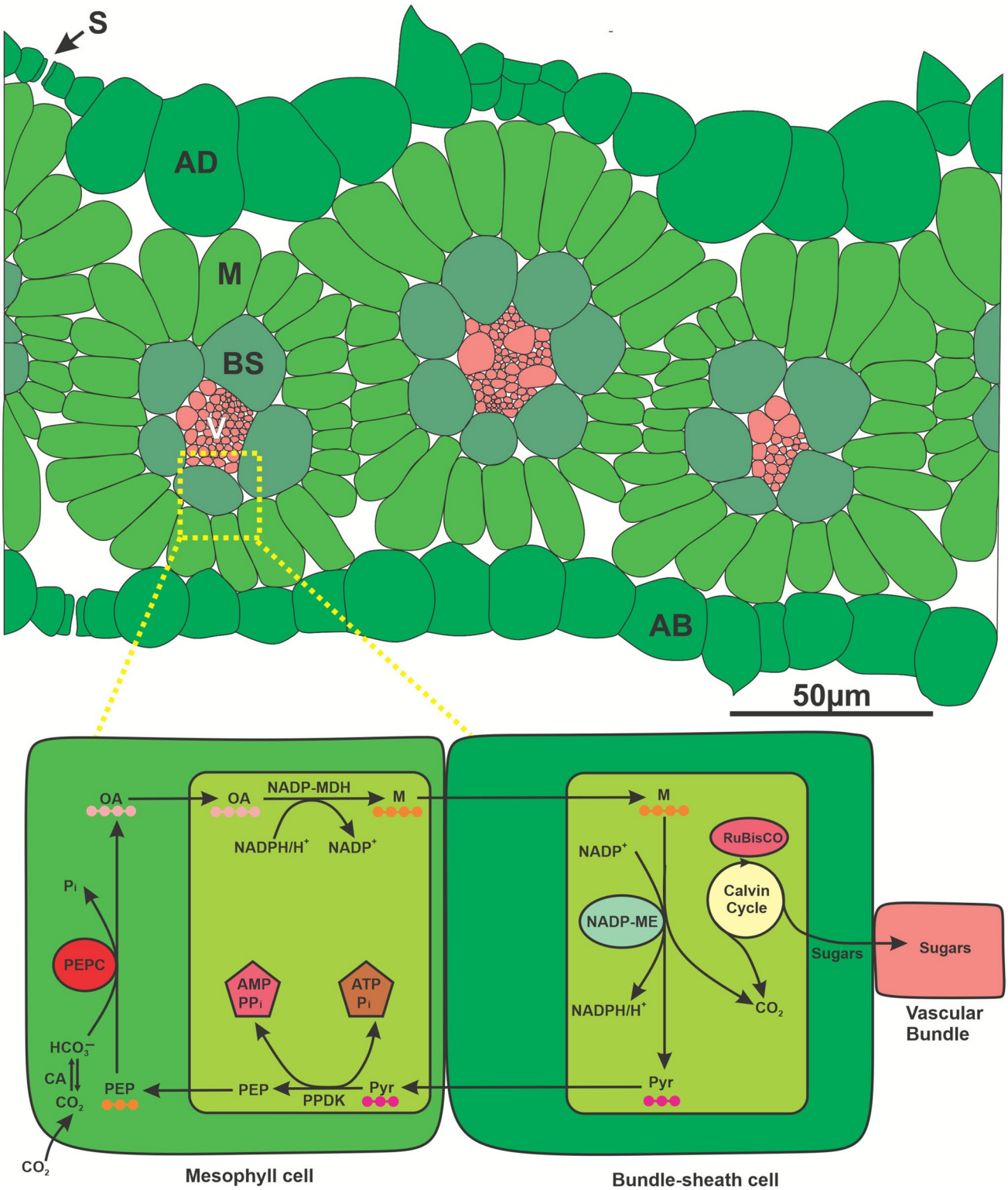
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likely reach 1.5 °C in the near term (2021–2040) (Hoegh-Guldberg et al. 2018; Siegert et al. 2020; IPCC 2023). Some consequences of climate change include land degradation, changes in precipitation patterns, desertification, and the accelerated melting of polar ice caps (Burrel et al. 2020; Defrance et al. 2020). Thus, understanding how plants cope with environmental stresses is mandatory for developing novel approaches to improve agricultural sustainability and food production (Zhang et al. 2022).

◂**Fig. 1** Schematic representation of the physiological variant of C_4 NADP-ME metabolism. In C_4 plants, phosphoenolpyruvate carboxylase (PEPC) fixes CO_2 in the cytoplasm of mesophyll cells, producing four-carbon organic compounds. These compounds are then transported to bundle sheath cells, where they are decarboxylated within the chloroplasts, generating pyruvate and releasing CO_2 near ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) carboxylation sites. The upper part of the figure shows a cross-section of a C_4 plant leaf, highlighting the Kranz anatomy. The adaxial epidermis (AD) and abaxial epidermis (AB) enclose the inner cell layers. The mesophyll cells (M) surround the bundle sheath cells (BS), which specialize in the Calvin cycle. The lower part presents a biochemical scheme of C_4 metabolism, which occurs in a compartmentalized manner between mesophyll and bundle sheath cells. In the mesophyll, PEPC fixes CO_2 in the form of bicarbonate (HCO_3^-), converting phosphoenolpyruvate (PEP) into oxaloacetate (OA), which can be reduced to malate (M) by NADP-malate dehydrogenase (NADP-MDH). Malate is then transported to bundle sheath cells, where NADP-malic enzyme (NADP-ME) releases CO_2 for the Calvin cycle, which is catalyzed by RuBisCO. The resulting pyruvate (Pyr) is transported back to mesophyll cells and regenerated into PEP by pyruvate phosphate dikinase (PPDK), completing the C_4 cycle. The sugars produced are transported to the vascular bundle for distribution throughout the plant. Abbreviations: CA - Carbonic anhydrase; P_i - Inorganic phosphate; S - Stoma; V - Vascular bundle; ATP - Adenosine triphosphate; AMP - Adenosine monophosphate; PP_i - Inorganic pyrophosphate; $NADP^+$ - Nicotinamide adenine dinucleotide phosphate (oxidized form); NADPH - Nicotinamide adenine dinucleotide phosphate (reduced form)

Since agriculture is directly related to environmental conditions, climate change affects the planting windows and cultural practices of agronomic species, contributing to a reduction in or loss of productivity (Baum et al. 2020). Such climate variations represent significant challenges for the agricultural sector, making it increasingly necessary to investigate the climatic factors that affect the performance of crop species, which may contribute to understanding the impacts of higher temperatures, alterations in CO_2 concentrations in the atmosphere, soil salinization, and drought on several crops (Zullo Junior et al. 2006; Baum et al. 2020). Clarifying plant acclimatization strategies is essential for obtaining tolerant genotypes to improve agricultural development and reduce food crisis (Diao et al. 2014; Ullah et al. 2021; Liaqat et al. 2024).

Agricultural Research, Environmental Sciences, Plant Biology, and Biotechnology are essential for developing more productive agricultural commodities under harsh conditions with improved water use efficiency (WUE) (Henkhaus et al. 2020; Stepanova 2021). For these purposes, establishing a model organism as a reference system has allowed significant advances (Provart 2016). For example, the use of *Arabidopsis thaliana* (L.) Heynh. and *Oryza sativa* L., as eudicot and monocot model plants with C_3 photosynthetic metabolism, respectively, has allowed biotechnological and breeding resources such as the comprehension of responses to abiotic stresses in the development of C_3 plants (Diao et al. 2014; Provart 2016). The success of the use of *A. thaliana* as a model plant is associated with its small genome, small size, and short life cycle, which has helped elucidating the changes in essential metabolic processes and anatomical structures under experimental and field conditions (Delatorre and Silva 2008; Brutnell et al. 2010; Provart 2016).

C_4 photosynthetic metabolism consists of the spatial division of the carbon fixation phase. In the NADP-ME type C_4 cycle, which occurs in grasses like maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), and *Setaria viridis* (L.) P. Beauv. (Poaceae), organic C_4 acids initially produced in the cytoplasm of mesophyll cells via phosphoenolpyruvate carboxylase (PEPcase) are transported to the chloroplasts of vascular sheath cells, where they undergo decarboxylation reactions (Fig. 1). During this process, the previously fixed carbon is released and refixed by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo), initiating the C_3 cycle (Raghavendra and Sage 2011; Wang et al. 2012; Hatakeyama and Ueno 2017) (Fig. 1). While there are different biochemical subtypes of C_4 metabolism, the NADP-ME type is the most widespread among grasses and is particularly relevant for studying major bioenergetic crops such as maize and sorghum. The mechanisms depicted in Fig. 1, therefore, serve as a direct representation of the specific C_4 pathway investigated in our work. C_4 metabolism improves the efficiency of carbon fixation under conditions of high temperature and low water availability, as plants can reduce stomatal conductance to avoid excessive water loss while still efficiently fixing carbon (Edwards and Voznesenskaya 2010; Wang et al. 2012; Cano et al. 2019; Danila et al. 2021). Several agricultural species of economic interest, such as maize, sugarcane, and sorghum, have C_4 photosynthetic metabolism, among other grasses (Ehleringer et al. 1997; Brutnell et al. 2010; Li and Brutnell 2011). Therefore, the C_3 plant *A. thaliana* is not a useful species for investigating the responses of the developmental aspects and metabolic pathways of plants with the C_4 system (Brutnell et al. 2010; Petricka et al. 2012; Diao et al. 2014), as their responses to abiotic stresses are distinct. Different experimental and field conditions require the use of C_4 model plant species.

To study the photosynthetic metabolism of NADP-ME C_4 plants, the diploid green foxtail *Setaria viridis* (L.) P. Beauv. (Poaceae) has been used as a model plant (Brutnell et al. 2010; Li and Brutnell 2011; Bennetzen et al. 2012; Cesarino et al. 2020). Thus, the objective of this review is to summarize and synthesize the existing knowledge on *S. viridis* responses to key abiotic stresses, highlighting its utility as a model species for C_4 photosynthesis. By focusing exclusively on *S. viridis*, we aim to provide a coherent overview of its morphophysiological responses, offering insights for future research and for improving C_4 crop resilience under climate change conditions.

2 *Setaria viridis* as a model plant species for C_4 crops

Setaria viridis is a summer annual grass and it is known as the wild ancestor of the cultivated foxtail millet, *S. italica*, considered a cereal intended for human and animal consumption due to its high nutritional value (Vetriventhan et al. 2016; Jannah et al. 2024). As a model species, *S. viridis* provides a direct link for improving C_4 crops, as it shares crucial genetic and physiological traits with its cultivated sibling species and other major bioenergetic crops like maize and sorghum. The utility of *S. viridis* as a model for C_4 photosynthesis is supported by rapid growth, adaptability, and high seed production (Dekker 2003; Brutnell et al. 2010). Moreover, its short life cycle (~ 60 days), small genome (~ 515 Mb), and established transformation protocols have the potential to accelerate genetic and molecular studies (Dekker 2003; Brutnell et al. 2010; Li and Brutnell 2011; Bennetzen et al. 2012; Cesarino et al. 2020). *S. viridis* is also phylogenetically and metabolically close to major bioenergetic crops, such as sugarcane (*Saccharum officinale* L.), maize (*Zea mays* L.), and sorghum (*Sorghum bicolor*), and shares a similar morphology with these cereals, particularly maize and sorghum (Hodge and Doust 2017). Its root system consists of a primary root (embryonic origin) formed shortly after seed germination from the hypocotyl-radicle axis. After a few days, abundant formation of adventitious roots originating from the crown (postembryonic origin) is observed, with both structures producing lateral roots from the pericycle and endoderm cells (Sebastian et al. 2016; Costa 2018; Maqbool et al. 2022). Thus, efforts have been made to obtain a general and current understanding of the responses of this plant to environmental changes, highlighting it as a C_4 model for molecular, physiological, and anatomical studies. These data can provide information for future biotechnological studies and breeding programs (Lata et al. 2013).

The success of *S. viridis* as a model for C_4 metabolism crops is also attributed to advancements in tools for genetic studies and transgenic plant production. For example, the availability of resources such as genome sequencing, which is available for both *S. italica* (Zhang et al. 2012) and different accessions of *S. viridis* (Thielen et al. 2020); the development of efficient plant transformation protocols for the generation of *S. viridis* transgenic plants (Brutnell et al. 2010; Mookkan 2018; Martins et al. 2015a,b, 2019; Saha and Blumwald 2016; Nguyen et al. 2020; Finley et al. 2021); the phenological characterization of development stages for *S. viridis* accessions on the basis of the BBCH scale (Junqueira et al. 2020, 2025) and the application of gene editing technologies such as clustered regularly interspaced short palindromic repeats (CRISPRs) (Weiss et al. 2020; Basso et al. 2021; Liang et al. 2022) have contributed to its establishment as a model system, enabling precise genetic modifications and biotechnological approaches and providing valuable insights for further improving C_4 crop species.

In this review, we discuss the current knowledge concerning the responses of *S. viridis* under different environmental conditions predicted for the next few decades. Specifically, we provide novel perspectives and identify functional mechanisms associated with how C_4 plants can cope with climate change, particularly the morphophysiological and molecular characteristics of *S. viridis* in response to water deficit, salinity, temperature, and altered CO_2 conditions. In addition, we discuss the contrasting characteristics of different accessions in response to abiotic stresses from morphophysiological and gene expression modulation perspectives.

3 Responses of *Setaria viridis* to water deficit

Drought is one of the most extreme abiotic stresses affecting agriculture (Seneviratne et al. 2012). Water restriction affects crops by causing a series of morphological, physiological, biochemical, and genetic dysfunctions, such as restrictions in germination rates; limitations in stomatal conductance; reduced photosynthetic performance; changes in root density and length; and changes in cellular water intake (Seleiman et al. 2021) (Fig. 2).

Plants adapt to resist and survive extreme conditions via distinct morphophysiological mechanisms and strategies, and the levels of tolerance to abiotic conditions vary among species, cultivars (Bennetzen et al. 2012; El-Samad et al. 2019), and plant developmental stages (Kafi et al. 2009; Ardie et al. 2015). The use of *S. viridis* as a model to understand water deficit effects is valuable as the obtained knowledge might be translated for other C_4 grass crops (Yoo et al. 2014). Accordingly, studies have investigated the physiological and molecular responses of *Setaria* under drought or osmotic stresses (Martins et al. 2016; Saha et al. 2016; Rodrigues et al. 2019; Valença et al. 2020; Travassos-Lins et al. 2021) (Tables 1 and 2).

Studies with water deficit, simulated by treatment with polyethylene glycol (PEG) 6000 (20% w/v), which generates an osmotic potential of − 0.5 MPa, resulted in a decrease in the total germination rate and promptness index (percentage of seeds that germinated on specific days during the observation period), radical root length, coleoptile length, and number of lateral roots in *S. viridis* during the early seedling stage (Qie et al. 2014). As the osmotic stress increased, germination of *S. viridis* was inhibited at an osmotic potential of − 0.5 MPa, and under moderate salinity stress (160 mM

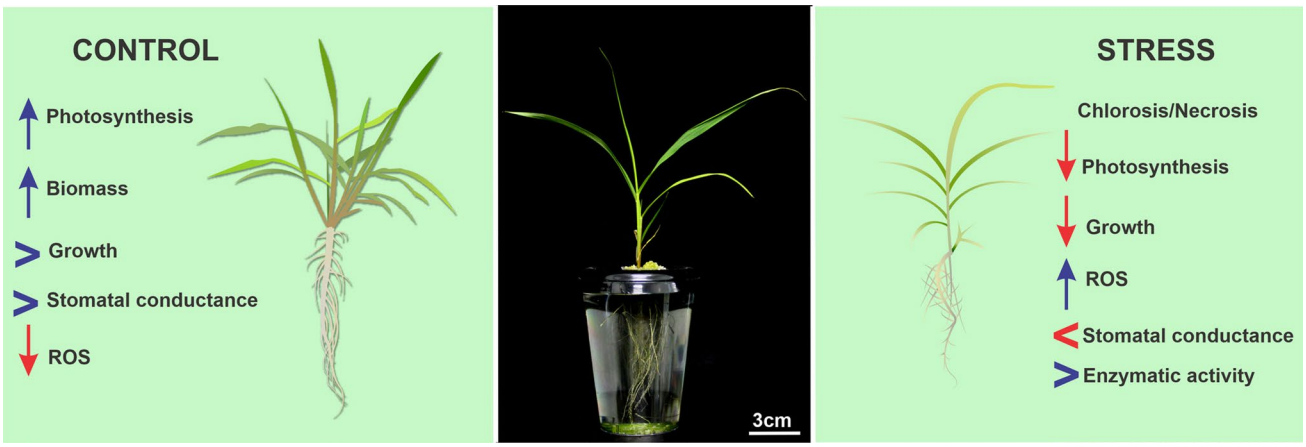


Fig. 2 Physiological and morphological effects of environmental stresses on *Setaria viridis*. An illustration of the main physiological and morphological differences between control and stressed *S. viridis* plants is presented on the basis of previous studies (Valeriano et al. 2021; Bezerra et al. 2021). The central panel presents an *S. viridis* plant growing in a hydroponic system as a reference. The left panel shows a healthy plant under control conditions, with increased photosynthesis, biomass accumulation, growth, and stomatal conductance, while reactive oxygen species (ROS) levels remain low. The right panel depicts an *S. viridis* plant under stress, exhibiting symptoms of chlorosis and necrosis. Under stress, photosynthesis and growth decrease, whereas ROS levels increase, stomatal conductance decreases, and enzymatic activity is altered in response to stress. The blue rows indicate an increase, whereas the red rows represent a decrease in the specified characteristics under the respective conditions (control or stress)

NaCl), germination was completely suppressed (Qie et al. 2014). In addition, water deficit (water withdrawal after germination) drastically inhibited growth of *S. viridis* root system, with considerable reductions in growth parameters and suppression of crown roots (Sebastian et al. 2016). Low water availability or prolonged drought also leads to seed or seedling dehydration, decreased germination percentage, delayed seedling emergence, and premature death in *S. viridis*.

3.1 Effects on growth and photosynthesis

Water limitations negatively affect the total growth of the plant, reducing the accumulation of dry biomass, limiting the development of shoots, altering the architecture of the roots and limiting the complete functionality of the plant (Novák and Lipiec 2012; Brito et al. 2013; Rodrigues et al. 2019) (Table 1; Fig. 2). Subjecting *S. viridis* to PEG 8000 (7.5% w/v) stress for 6 and 10 days caused significant reductions in biomass accumulation and leaf area (Valença et al. 2020) (Table 1). Other studies on water deficit have described a decrease in dry weight for *S. viridis* (Burbulis et al. 2013; Martins et al. 2016; Saha et al. 2016; Rodrigues et al. 2019). Compared with those under irrigated conditions, under severe stress in the field (50% field capacity), a decrease in growth, such as a decrease in height, plant density, and dry biomass, and an increase in the persistence index of *S. viridis* were observed. Under 75% field capacity, reductions in plant height, density and dry biomass ranged from 29 to 40%, 14–27% and 11–26%, respectively (Ihsan et al. 2015). The increased persistence rate of *S. viridis*, even under severe stress conditions, suggests that this species has the ability to acclimate its metabolism and growth, surviving in the field. Changes in morphological traits such as leaf curling, wilting, decreases in leaf area, and leaf number have been associated with an early morphological survival strategy in response to water deficit in *S. viridis* and other monocots. These changes result from increased WUE resulting from a lower transpiration rate, protecting leaves from photodamage (Kadioglu et al. 2012; Rauf et al. 2016; Baret et al. 2018; Banan et al. 2019). For instance, leaf curling in *S. viridis* was found to be an effective mechanism to prolong growth under stress and a necessary trait to assess tolerance, as has also been reported for maize (Baret et al. 2018; Banan et al. 2019). The slower growth of *S. viridis* allows the species to deal with water deficit more effectively, directing resources to the production of protective molecules and maintenance of the root system for water capture (Valença et al. 2020).

Water deficit induces multiple metabolic responses that affect photosynthesis, generating reactive oxygen species (ROS) and inducing the expression of key stress response genes, features that can be used to monitor plant stress (Joshi et al. 2016; Travassos-Lins et al. 2021). Water deficit conditions employing 20% PEG 8000 (−0.55 MPa) induced different physiological responses in *S. viridis* depending on the developmental stage (Rodrigues et al. 2019). Conventional chlorophyll fluorescence parameters, such as photosynthetic quenching (qP), nonphotochemical quenching (NPQ), the maximum quantum yield of PSII (Fv/Fm), and

Table 1 Morphophysiological responses and other characteristics of *Setaria viridis* under abiotic stresses. *Other *Setaria* species are listed only when included in the cited experimental studies alongside *S. viridis*

Species*	Accessions	Stress	Main remarks	References
S. viridis	A10.1 and ME034V	Water and salt	It reduced germination and limitation of post-seminal development	Acharya et al. (2017)
S. glauca, S. verticillata and *S. viridis*	–	Water, temperature, and salt	Stress causes a reduction in the germination rate, and *S. viridis* showed greater sensitivity	Amini et al. (2015)
S. viridis	ME034V	Light and temperature	There was a reduction in photosynthetic efficiency and starch accumulation in the chloroplasts of the mesophyll and sheath cells	Anderson et al. (2021)
S. viridis	–	Water and low temperature	The water potential reduced germination rate while the temperature drop delayed germination	Blackshaw et al. (1981)
S. viridis	A10.1	CO_2 and temperature	C_4 metabolism enzymes show some kinetic responses similar to C_3 metabolism	Boyd et al. (2015)
S. viridis	–	Water	Reductions in photosynthetic pigments, as well as in fresh and dry mass	Burbulis et al. (2013)
S. viridis	A10.1	CO_2	Lower CA activity reduces photosynthesis and growth, but elevated CO_2 recovers parameters	Chatterjee et al. (2021)
S. viridis	–	CO_2	Reduction of photosynthesis and vegetative aspects as it acts, number of tillers, and leaf size	Coe et al. (2018)
S. viridis	–	CO_2	Mutant plants showed lower production of tillers and biomass compared to wild ones	Danila et al. (2021)
S. italica and *S. viridis*	B_{100}	Water	The parental lineage was less responsive to water regimes concerning the original lines	Feldman et al. (2018)
S. viridis	A10.1	Salt	Germination rate and seedling development were affected. Plants showed lower pigmentation and accumulation of fresh and dry mass	Ferreira et al. (2020)
S. viridis	–	Water	Reduction of net photosynthetic rate, leaf relative water content, stomatal conductance, and water use efficiency	Luo et al. (2011)
S. viridis	A10.1	Water and aluminum	Protein Kinase, RNA Binding Protein, and SDH were the most stable genes under stress	Martins et al. (2016)

Table 1 (continued)

Species*	Accessions	Stress	Main remarks	References
S. viridis	A10.1	Salt	Stress reduces photosynthetic performance and blocks the flow of electrons	Mendes Bezerra et al. (2021)
S. glauca, *S. verticillata*, and *S. viridis*	–	Water and temperature	Osmotic stress reduced the germination rate, while the species had greater tolerance to the effects of high temperature	Mollaee et al. (2020)
S. italica and *S. viridis*	IC-4 and IC-41	Water, salt, and heat	The results point to distinct gene expression profiles as a function of cultivar, tissue, and stress	Muthamilarasan et al. (2020
S. viridis	A10.1	CO_2	Mesophyll conductance may represent a more significant limitation for CO_2 assimilation than anhydrase activity	Osborn et al. (2017)
S. viridis	A10.1	Water	Fresh mass and non-photochemical quenching (NPQ) processes were the most responsive under stress	Rodrigues et al. (2019)
S. viridis	Zha-1, A10.1, Ula-1, Ast-1, Aba-1 and Sha-1	Water and heat	Accessions from different parts of the world exhibit different behavior under stress	Saha et al. (2016)
S. viridis	A10.1	Water	Crown root development inhibition in response to stress	Sebastian et al. (2016)
S. viridis	A10.1 and Ast-1	Water	Morphophysiological aspects were affected, and the *SvNAC1* gene proved effective in stress monitoring, while *SvWRKY1* discriminated accessions	Travassos-Lins et al. (2021)
S. viridis	A10.1	CO_2 and temperature	The higher conductance of the mesophyll can potentiate the photosynthetic rate in a limited CO_2 situation	Ubierna (2018)
S. viridis	A10.1	Water	Physiological parameters were affected and partially restored after 10 days of stress	Valença et al. (2020)
S. viridis	A10.1, ME034V and Ast-1	Salt	Plant root morphology is compromised during stress and affects mainly the crown roots	Valeriano et al. (2021)

the fluorescence decay ratio (Rfd), are more affected in the reproductive stage than in the vegetative stage (Rodrigues et al. 2019). Water stress negatively affects photosynthetic efficiency, causing greater energy dissipation in the form of heat, which indicates inefficiency in light capture and damage to the photosynthetic system (Zia et al. 2024). In addition, agricultural crops and other plant species are more sensitive to water stress during the flowering and grain/seed filling periods, since this greater vulnerability of plants can be attributed to increased photosynthetic parameters, starch accumulation, greater water demand and premature senescence (Yang and Zhang 2006; Pushpavalli et al. 2015).

Table 2 Marker genes related to diverse abiotic stresses in *S. viridis*.

Accession	Stress type	Candidate molecular markers	Comments	References
A10.1	Drought	*SvP5CS* and *SvGolS2*	Genes were upregulated under stress	Duarte et al. (2019)
A10.1 and Ast-1	Drought	*SvNAC6, SvP5CS2,* and *SvDHN1*	Genes were upregulated in both accessions under stress	Travassos-Lins et al. (2021)
ME034	Heat	*SvRCA_α, GOX1, PGR5, PGRL1, PGLP1, HPR1, CA_A, HSFA6B,* and *PLGG1*	Genes were upregulated under stress	Anderson et al. (2021)
ME034	Heat	*TAP38*	Gene was downregulated under stress	Anderson et al. (2021)
A10.1	Drought	*SvDHN1* and *SvNAC6*	Genes were upregulated under stress	Rodrigues et al. (2019)
A10.1	Drought	*SvP5CS2, SvWRKY1,* and *SvDREB1C*	Genes were downregulated under stress	Rodrigues et al. (2019)
A10.1	Osmotic	*SvDREB1C*	Gene was upregulated under stress. The morphological data demonstrated a drastic reduction in shoot biomass	Valença et al. (2020)
A10.1	Osmotic	*SvP5CS2*	Gene was downregulated under stress	Valença et al. (2020)
A10.1	Drought	*SvPYL4* and *SvPYL7*	Genes were downregulated under stress	Duarte et al. (2019)
Ast-1	Drought	*SvPYL3*	Gene was downregulated under stress	Duarte et al. (2019)
Ast-1	Drought	*SvPYL1, SvPYL14,* and *SvPYL17*	Genes were upregulated under stress	Duarte et al. (2019)
A10.1	Drought	*SvSnRK2.3, SvSnRK2.4.1/4.2, SvSnRK2.5, SvSnRK2.6, SvSnRK2.9, SvSnRK2.10, and SvSnRK2.11*	Genes were upregulated under stress	Duarte et al. (2019)
A10.1	Drought	*SvSnRK2.2, SvSnRK2.7, SvSnRK2.8, SvPP2C1, and SvPP2C8*	Genes were downregulated under stress	Duarte et al. (2019)
A10.1	Salt	*SvSnRK2.3, SvSnRK2.4.1/4.2, SvSnRK2.9, SvSnRK2.10,* and SvSnRK2.*11*	Genes were upregulated under stress	Duarte et al. (2019)
A10.1	Salt	*SvSnRK2.7* and *SvSnRK2.8*	Genes were downregulated under stress	Duarte et al. (2019)
Ast-1	Salt	*SvPYL3, SvPYL5, SvPYL6, SnRK2.8, SnRK2.9, SnRK2.11, SvPP2C2.1, SvPP2C2.3.2,* and *SvPP2C2.12*	Genes were upregulated under stress	Duarte et al. (2019)
–	Cold	*BBX2, TPP*	Genes were upregulated under stress	Sun et al. (2022)
A10.1	Cold	*SvPYL1, SvPYL2, SvPYL3, SvPYL7,* and *SvPYL8*	Genes were downregulated under stress	Duarte et al. (2019)
Ast-1	Cold	*SvSnRK2*	Gene was upregulated under stress	Duarte et al. (2019)
Ast-1, Aba-1, and Sha-1	Drought and Heat	*RbcS* and *AQP genes*	Genes were downregulated under stress	Saha et al. (2016)
A10.1, Ula-1, Ast-1, Aba-1, and Sha-1	Heat	*PEPC*	Gene was downregulated under stress	Saha et al. (2016)
Ast-1, Aba-1, and Sha-1	Drought and Heat	*ZEP*	Gene was upregulated under stress	Saha et al. (2016)

Table 2 (continued)

Accession	Stress type	Candidate molecular markers	Comments	References
Zha-1, A10.1, and Ula-1	Drought and Heat	*NCED*	Gene was upregulated under stress	Saha et al. (2016)
Ast-1	Drought and Heat	*CAT*	Lower expression	Saha et al. (2016)
Ast-1, Aba-1, and Sha-1	Drought and Heat	*CS*	Gene was downregulated under stress	Saha et al. (2016)
Ast-1, Aba-1, and Sha-1	Drought and Heat	*LOX*	Gene was upregulated under stress	Saha et al. (2016)
A10.1, Ula-1, Ast-1, Aba-1, and Sha-1	Drought and Heat	*P5CR*	Gene was upregulated under stress	Saha et al. (2016)

AGT1 serine: glyoxylate aminotransferase; *AQP* genes, plasma membrane intrinsic proteins (*PIP1-1*, *PIP1-2*, and *PIP2-1*) aquaporin genes; *BBX2* CO-like transcription factor; *CA_A* carbonic anhydrase; *CAT* catalase; *CS* chlorophyll synthase; *GOX1* glycolate oxidase; *HPR1* hydroxypyrene reductase; *HSF* transcription factor; *LOX* linoleate 9S-lipoxygenase; *NADP-MDH* NAD-dependent malate dehydrogenase; *NCED* 9-cis-epoxycarotenoid dioxygenase; *P5CR* pyrroline-5-carboxylate reductase; *PEPC* phosphoenolpyruvate carboxylase; *PEPC_B* phosphoeynylpyruvate carboxylase; *PGLP1* 2-phosphoglycolate phosphatase; *PGR5* proton gradient regulation 5; *PGRL1* PGR5-like photosynthetic phenotype 1; *PLGG1* plastidic glycolate/glycerate transporter; *RbcS* ribulose bisphosphate carboxylase small subunit; *STN7* protein kinase; *SvDHN1* dehydrin 1; *SvDREB1C* dehydration-responsive element binding protein 1 class C; *SvGolS2* galactinol synthase 2; *SvNAC6* NAC protein 6; *SvP5CS* delta 1-pyrroline-5-carboxylate synthase; *SvPP2C* type 2C protein phosphatases; *SvPYL* ABA receptor PYR/PYL/RCAR protein Family; *SvRCA* rubisco activase; *SvSnRK* class III SNF-1-related protein kinase 2; *SvWRKY1* transcription factors WRKY DNA-binding domain 1; *TAP38* phosphatase; *TPP* encodes a key enzyme for trehalose biosynthesis, *ZEP* zeaxanthin epoxidase

Leaf structural characteristics and biochemical parameters are components of stomatal-independent inhibition of photosynthesis, that is, when the reduction in photosynthesis does not result in stomatal closure (Bethke and Drew 1992). Deformations in intercellular spaces around mesophyll cells and changes in the size and position of chloroplasts are observed in *S. viridis* under salt stress (250 mM NaCl), which may reduce CO_2 conductance because of irregular stomatal closure (Bezerra et al. 2021). Furthermore, the intracellular repositioning of chloroplasts after stress stimulated by the hormone abscisic acid (100 µM ABA) occurs near vascular bundle sheath cells, confirming that chloroplast movement is a response to abiotic stresses (Yamakawa et al. 2023). As a result, CO_2 diffusion and light penetration may be disturbed, followed by a decrease in photosynthetic activity (Flexas et al. 2004).

WUE (water use efficiency) in C_4 plants, such as *S. viridis*, represents the relationship between carbon fixation and the water consumed during photosynthesis. *S. viridis* is essential for understanding the responses of C_4 plants under water deficit through the identification of genotypes that are widely adapted to hostile conditions. This information will increase the understanding of *S. viridis* mechanisms and support the development of elite crops with durable drought tolerances (Tang et al. 2017). Further research can clarify C_4 metabolism mechanisms and increase water and nitrogen use efficiency (Brutnell et al. 2010) in environments with low water and nutrient availability, increasing the usefulness of arid and semiarid lands in countries in Asia, Africa, and North America (Li 1997; Huang et al. 2016; Hu et al. 2018).

3.2 Contrasting responses of *S. viridis* accessions to water deficit

The environmental conditions that reduce water availability, such as high salt concentrations, osmotic stresses, and drought, are the most devastating for a plant's life cycle (Rodrigues et al. 2019; Yadav et al. 2020). Thus, phenotypic screening of root morphology based on length, diameter, surface area, volume, density, fresh mass, and dry biomass accumulation can identify tolerant genotypes (Souza et al. 2016; Wang et al. 2020). Elucidating adaptive mechanisms and root development patterns under water deficit is necessary to identify morphological markers of tolerance to such stress (Takahashi and Pradal 2021). Additionally, it is essential to investigate whether shoot and root organs possess similar sensors and signaling pathways.

Different *S. viridis* accessions have shown contrasting responses to abiotic stresses (Jia et al. 2013; Huang et al. 2014) (Table 1). For example, behavioral differences between *S. viridis* accessions subjected to heat (temperature increased from 28 to 42 °C) and water stress (water supply interrupted for 7–10 days in plants 20 days post-germination) were demonstrated by physiological and genetic parameters, which distinguished accessions Ast-1, Aba-1, and Sha-1 as sensitive compared with Zha-1, A10.1, and Ula-1 (Saha et al. 2016). Accessions A10.1 and Ast-1 have been used in water deficit studies to show contrasting responses in terms of stress tolerance (Saha et al. 2016; Travassos-Lins et al. 2021). Although the previous study (Saha et al. 2016) classified the Ast-1 accession as sensitive compared to A10.1, findings from Duarte et al.

(2019) observed that under prolonged drought conditions (45 h of water withholding), 32-day-old plants of the Ast-1 accession demonstrated slightly superior tolerance, withstanding the stress for a longer period and exhibiting better photosynthetic recovery after rehydration. This suggests that the stress responses may vary depending on stress duration or developmental stage. Importantly, accessions A10.1 and Ast-1 originated from distinct natural populations in the United States and Azerbaijan, respectively, reflecting different genetic backgrounds. The evolutionary process in various environments explains the differences in behavior in situations of water deficit (Doust and Diao 2017), highlighting the importance of genetic diversity in the stress response.

The molecular differences between these accessions should be studied to elucidate the pathways and genes involved in drought tolerance in C_4 plants, which would be useful for future biotechnological improvements in C_4 crops. For example, plants from accession A10.1 can recover after being subjected to osmotic stress (Valença et al. 2020). Some studies have focused on understanding the regulatory mechanisms that orchestrate the recovery of *S. viridis* after a long period of water restriction, predominantly the net photosynthetic rate (Luo et al. 2009, 2011). The biosynthesis of free proline is a vital defense mechanism in *S. viridis*, helping it tolerates osmotic stress and protect the photosynthetic apparatus (Luo et al. 2011). As previously reported in sorghum, the increase in the content of this amino acid as water stress becomes severe may have provided osmoprotective and antioxidant activity to native varieties, reducing tissue damage and plant death (Devnarain et al. 2016).

The A10.1 accession of *S. viridis* is considered tolerant to water restrictions (Travassos-Lins et al. 2021), and several developmental features are affected when it is exposed to salt stress (150 mM and 250 mM NaCl) (Bezerra et al. 2021). For example, A10.1 plants presented altered anatomical and ultrastructural patterns of new leaves under salt stress. In addition, salt stress caused flattening of epidermal cells and turgor loss, increased the leaf lamina thickness and intercellular space volume, and significantly decreased the number of chloroplasts. Additionally, Bezerra et al. (2021) reported that chlorophyll fluorescence peaks significantly increase under such salinity conditions, accompanied by severe damage to photosynthetic parameters, which results in drastic photoinhibition. Photosynthetic losses in such treatments were associated with negative impacts on electron transport between the photosystems, affecting ATP and NADPH production. Similarly, the range of growth parameters, such as dry mass, fresh mass, aerial tissue area, primary root length, and the number of lateral roots (Pegler et al. 2020), was reduced in *S. viridis* under salt stress (150 mM NaCl), as was also reported for maize treated with a 1 M NaCl solution (Zahra et al. 2020).

A transcriptomic study by Xu et al. (2013) revealed several genes involved in photosynthesis in *S. viridis*, such as the NADP-malic enzyme phosphoenolpyruvate carboxykinase, the pyruvate regulatory protein orthophosphate dikinase. Additionally, differential gene expression profiles of ABA signal transduction genes were observed in the A10.1 and Ast-1 accessions under drought, salinity, cold stresses, and exogenous ABA application, highlighting variations in ABA stress responses among the *S. viridis* accessions and in response to different abiotic stresses (Duarte et al. 2019). Marker genes have also been described for *S. viridis* under water deficit conditions. In comparison with four other molecular marker genes (namely *SvP5CS2*, delta-1-pyrroline-5-carboxylate synthase 2 and the three transcription factors *SvWRKY1*, *SvDREB1C*, and *SvNAC6*), *SvDHN1* gene (dehydrin 1) presented the highest levels of expression under drought conditions (Rodrigues et al. 2019). Travassos-Lins et al. (2021) evaluated root responses in two accessions of *S. viridis*, A10.1 (drought-tolerant) and Ast-1 (drought-sensitive), under controlled drought cycles via polyethylene glycol 8000 (PEG-8000) in a hydroponic system to simulate dehydration/rehydration cycles. Expression analysis of these marker genes revealed that the *SvNAC1* gene could better monitor water stress in both accessions, while the *SvWRKY1* gene could be differentiated between the two accessions evaluated, contributing to understanding water deficit responses in *S. viridis* roots (Travassos-Lins et al. 2021). In an independent study, *S. viridis* plants (A10.1) subjected to continuous osmotic stress (10 days, 7.5% PEG-8000) presented a significant reduction in shoot biomass as an effect of the water deficit caused by osmotic stress. In addition, physiological data revealed that electrolyte leakage (EL) decreased in roots subjected to continuous osmotic stress, indicating an investment in membrane protection through the expression of osmoregulator genes such as *dehydrin* (*SvLEA*) and *proline* (*SvP5CS2*). These findings suggest that *S. viridis* A10.1 plants were able to partially recover from constant osmotic stress imposed over 10 days (Valença et al. 2020).

4 *Setaria viridis* response to salt stress

Soil salinity is a drastic event for irrigated agriculture, with great potential for soil degradation (Schossler et al. 2012; Gupta and Huang 2014). Soil salinization, characterized by elevated concentrations of sodium, potassium, magnesium, calcium, chloride, sulfate, carbonate, and bicarbonate, is typical of arid and semiarid regions whose evaporation rate exceeds that of precipitation (Pedrotti et al. 2015; Kaya and Vasilevska-Ivanova 2021). Although salinized areas are reported in at least 100 countries worldwide, the lack of current and accurate data limits a precise assessment of

current environmental damage (Shahid et al. 2018). Current estimations show that approximately 900 million hectares of salt-affected land worldwide and 21 million hectares of soil are affected by exchangeable excess sodium. The agricultural occupation of these areas may become impracticable due to low production and the risk of desertification (Castro and Santos 2020).

The root system is in direct contact with the substrate, it responds to external stimuli in its architecture, which are primary adaptive strategies for plants to tolerate stress conditions (Nadeem et al. 2020). Salt perception by the plasma membrane and the underlying signaling from the root system to the shoots are topics that need to be studied. In addition, the ultrastructural study of subcellular organelles involved in the recognition and transduction of osmotic and ionic components, such as chloroplasts and mitochondria, can corroborate the elucidation of adaptive mechanisms (Wang et al. 2022). Understanding these mechanisms and their applications will increase crop tolerance to salt stress, promoting sustainable agriculture in practical settings. Pursuing these studies in *S. viridis* is important because it could result in more adaptable plant varieties, increasing agricultural productivity in arid environments and improving food security in regions vulnerable to water scarcity (Choudhary et al. 2023). Additionally, *Setaria* and other cereals have a diverse root system composed of embryonic and postembryonic roots, which allows them to improve their root acclimatization ability in response to altered environmental conditions (Pinghua et al. 2011; Orman-Ligeza et al. 2013; Costa 2018). Morphological analyses performed by Valeriano et al. (2021) revealed that *S. viridis* roots subjected to moderate saline stress (150 mM NaCl) had an average larger diameter than those subjected to severe stress (250 mM NaCl). Further investigations are needed to elucidate the anatomical changes in the root system of *S. viridis* under salt stress, analyzing whether the succulence of the roots is related to greater vacuoles and/or more cell layers and, consequently, to the greater accumulation of ions and water, as reported in sorghum (Munns and Tester 2008; Yang et al. 2020). Promoting the enhancement of this mechanism for salt stress tolerance seems essential to attenuate the effects of the photosynthetic apparatus and, consequently, guarantee the maintenance of photosynthetic activity (Bezerra et al. 2021; Valeriano et al. 2021).

The small number of crown roots can be reflected in the improved tolerance of primary roots under stress conditions, as shown in *S. viridis* and *Z. mays* under salinity (150 mM NaCl) and drought conditions (without additional irrigation), respectively, suggesting that the inhibition of a specific root type compensates for the development of other types of roots, such as primary roots (Zhan et al. 2015; Sebastian et al. 2016; Maqbool et al. 2022) (Table 1). Based on the morphology of *S. viridis* roots, mainly biomass accumulation and the relative dry mass index, together with the number, length, and diameter of the crown roots, accessions A10.1 and MEO34V are moderately sensitive to salinity, whereas Ast-1 is sensitive (Valeriano et al. 2021). Thus, root system phenotyping represents an important selection target for future research focused on improving agricultural crops such as corn and other grasses in terms of drought and salinity tolerance, allowing the use of areas with low water availability and desert regions.

Glycophytes, such as *S. viridis*, are plants that do not tolerate highly saline conditions and present limitations in growth at concentrations close to 40 mM NaCl or to 4 dSm^{-1} in terms of the electrical conductivity of the soil (Pedrotti 2015; Kataria and Verma 2018; Essemine et al. 2020). In contrast to glycophytes, halophytes are defined as plants adapted to high salt concentrations, capable of completing their life cycle in saline conditions with a salt concentration of 200 mM NaCl or greater (Flowers and Colmer 2008). Salt stress results from two main effects, osmotic and ionic, and a secondary, oxidative effect (Munns and Tester 2008; Zhu 2016). The ionic effect of salt is harmful to most cultivated crop species, which are recognized as glycophytes. Salt stress has direct and indirect impacts on the main events of plant development, limiting germination and inhibiting plant growth and productivity due to decreased water uptake and ion imbalance (Fig. 2). Under more severe stress (250 mM NaCl) conditions, these plants suffer severe restrictions, which may result in premature death (Munns and Tester 2008; Kataria and Verma 2018; Bezerra et al. 2021; Valeriano et al. 2021) (Table 1).

Studies have shown that *S. viridis* is negatively affected by salt stress. Treatments with NaCl (for saline stress) and $NaHCO_3$:Na_2CO_3 (for alkaline stress) reduce germination and inhibit plant growth, including root and seedling development (Guo et al. 2011; Ferreira et al. 2020; Pegler et al. 2020) (Table 1). In the same way, the exposure of accessions of *S. viridis* (A10.1, MEO34V and Ast-1) to excessive NaCl (150 mM and 250 mM) affects root morphological characteristics, limiting plant development; reducing dry mass accumulation, length and number of crown roots; and changing the shape and color of the roots (Valeriano et al. 2021). In addition, molecular analyses of *S. viridis* (A10.1 and Ast-1) under salt stress (200 mM NaCl) revealed that several genes involved in core abscisic acid (ABA) signaling were differentially expressed (both up- and downregulated) between the two accessions, reflecting contrasting mechanisms involved in the response to salt stress. These results indicate that ABA signaling may be a critical molecular component for physiological responses during salt stress in C_4 plants (Duarte et al. 2019).

Plant adaptations to salinity involve multiple mechanisms that minimize Na^+ ion accumulation and maintain a high cytosolic K^+/Na^+ ratio, such as cell detoxification by

exclusion/extrusion of ions by roots and glands in leaves and tissue compartmentalization (Munns and Tester 2008; Adams and Shin 2014). Similarly, the maintenance of ionic homeostasis (K^+/Na^+) to increase salinity tolerance in whole plants is common among glycophyte and halophyte grasses (Munns and Tester 2008; Sanadhya et al. 2015; Pan et al. 2020). Studies of promising accessions of C_4 model plants, including those in *Setaria* genus, are vital for identifying features that can be manipulated to improve tolerance to environmental stresses. Although glycophytes share some mechanisms with halophytes, their survival is limited to specific salinity thresholds, whereas halophytes possess unique cellular sensory functions that enhance salt tolerance. Future research on ionic and osmotic stress receptors should therefore consider comparative analyses of phenotypes and receptor functions between the root systems of halophyte and glycophyte species (Wang et al. 2022). Insights into halophyte tolerance mechanisms could ultimately be applied to improve the ability of C_4 model species to cope with high ionic concentrations through exclusion or accumulation strategies.

5 *Setaria viridis* as a model for studying the effects of temperature

Compared with C_3 plants, C_4 plants are adapted to environmental conditions of high temperature, light, and lower water availability and have greater photosynthetic efficiency (Wang et al. 2012; Taiz and Zeiger 2013; Braga et al. 2021). However, with changes in the climate scenario, it is believed that the performance of C_4 plants and, consequently, food production will be impacted, particularly in tropical regions, as cereals such as maize, sorghum, sugarcane, and millet are vital for the nutrition of the world population (Leakey 2009a, 2009b).

Both cold and hot temperatures can affect plant cell membrane fluidity and cell wall stability, in addition to alterations at the intracellular level (Zhang et al. 2022). After sensing changes in temperature, plants trigger several stress-specific signals to cope with stress. Studies in *S. viridis,* in which plants from accessions A10.1 and Ast were subjected to a gradual decrease in temperature, revealed reduced gas exchange, yield, and WUE due to lower stomatal conductance and CO_2 assimilation (Carvalho et al. 2022).

Cooling stresses can also cause chlorophyll degradation, resulting in a considerable decrease in the maximum fluorescence (Fm) and, consequently, in the maximum quantum yield of photosystem II (Fv/Fm) (Carvalho et al. 2022). In addition, plants from accessions A10.1 and Ast presented a reduction in parameters such as stomatal conductance, gas exchange rates, maximum fluorescence (Fm), and maximum quantum yield of photosystem II (Fv/Fm) when subjected to up to 10 days of stress at 0 °C (Santos et al. 2017; Carvalho 2019). When the plants returned to 25 °C after 3 and 5 days under stress at 0 °C, some physiological parameters, such as chlorophyll fluorescence variables and leaf gas exchange, recovered to levels similar to those of the controls. The plants maintained at 0 °C for 10 days did not recover when they were returned to 25 °C. The ability of *S. viridis* to recover from cold stress demonstrates a level of stress tolerance, but the actual level of stress tolerance is unclear and needs further study.

Several genes involved in osmotic regulation, such as those related to the degradation of amino acids, lipids, and proteins, as well as those involved in sugar accumulation (raffinose synthesis) and TPS/TPP signaling (trehalose biosynthesis), are upregulated in *S. viridis* subjected to low temperatures (Sun et al. 2022). Such osmotic adaptation mechanisms in *S. viridis* can be applied to improve stress tolerance in other C_4 plants, such as maize and sugarcane, especially in challenging environments. The molecular characterization of *S. viridis* plants subjected to heat temperature revealed differential expression of several genes, such as increased expression of genes related to the *NDH* (NADPH dehydrogenase) complex in the chloroplast, as well as genes for critical components of cyclic electron flow, such as *PGR5* (proton gradient regulation 5) and *PGRL1* (photosynthetic phenotype similar to PGR5 1) genes, and some genes involved in photorespiration, such as *PGLP1* (2-phosphoglycolate phosphatase), *HPR1* (hydroxypyruvate reductase) and *PLGG1* (plastidic glycolate/glycerate transporter) (Anderson et al. 2021).

Studies on the *S. viridis* accession MEV034 revealed reduced photosynthetic rates under heat stress (Anderson et al. 2021). High light (900 µmol photons m^{-2} s^{-1}) and high temperature (40 °C) reduced the photosynthetic capacity due to thermal stress and photorespiration. Ultrastructural changes in MS and BS chloroplasts included increased chloroplast size and starch volume under high light. High temperatures slightly increase starch in sheath chloroplasts but reduce it in mesophyll chloroplasts (Anderson et al. 2021).

The developmental and molecular mechanisms of *Setaria* in response to stress still need to be elucidated. Different *S. viridis* accessions exhibit plasticity in their physiological, gene expression, and biochemical responses to abiotic stresses such as drought, salinity, and heat or cold temperatures. For example, a study by Saha et al. (2016) identified genes related to the metabolic pathways of photosynthesis, aquaporins, ABA biosynthesis and signaling, and biochemical changes in response to water deficit and heat in five *S. viridis* accessions (A10.1, Ula-1, Ast-1, Aba-1, and Sha-1), demonstrating that the Zha-1, A10.1 and Ula-1 accessions were more tolerant to water deficit and heat stress than the Ast-1, Aba-1, and Sha-1 accessions.

The intrinsic responses of *S. viridis* to combined stresses, such as drought and heat, highlight potential targets for genetic and physiological improvements in C_4 crops of key interest for food production or biofuel sources, such as sugarcane, maize and sorghum (Travassos-Lins et al. 2021). Its use as a model can aid in the development of stress-resilient C_4 crops, improving their productivity and sustainability in challenging environments.

6 Responses to altered atmospheric CO_2 levels in *Setaria viridis*

Plants harboring the Kranz syndrome show functional and spatial coordination between bundle sheath (BS) and mesophyll (MS) cells and organelles (Fig. 1) (Brown and Smith 1972; Kim et al. 1997). In terms of decarboxylation mechanism, *S. viridis* presents a NADP-dependent malic enzyme (NADP-ME) subtype of C_4 metabolism. NADP-ME is responsible for releasing CO_2 in the chloroplasts of vascular sheath cells (Bennetzen et al. 2012; Junqueira et al. 2018). Figure 1 represents the C_4 photosynthesis mechanism of the NADP-ME type, which is the characteristic photosynthetic process of the *Setaria viridis* species analyzed in this study, although there is another C_4 mechanism, of the NAD-ME type. We chose to illustrate only the NADP-ME subtype in Fig. 1 so that the figure illustrates the mechanism present in the plant studied here. The coordinated function and activity of the phosphoenolpyruvate carboxylase (PEPcase) and ribulose bisphosphate carboxylase/oxygenase (RuBisCO) enzymes in MS and BS cells, respectively, increase the efficiency of CO_2 fixation in C_4 plants (Danila et al. 2021) (Fig. 1). Owing to the greater efficiency of C_4 plants, these plants can reach a photosynthetic rate equivalent to that of C_3 plants with lower stomatal conductance and, therefore, less water loss (Feldman et al. 2018; Cano et al. 2019).

Changes in CO_2 concentration are among the main environmental factors influencing plastic responses in C_4 plants. However, leaf biochemistry, membrane permeability, aquaporin concentration, reduction in leaf area and leaf curling, structural and intercellular space changes, conductance, and internal diffusion can be affected under abiotic conditions, including drought, salinity, and temperature, resulting in lower diffusion of CO_2 in the mesophyll (Kapilan et al. 2018; Ermakova et al. 2021).

S. viridis has become increasingly employed in studies of CO_2 assimilation because it is valuable for understanding biological processes and studying various abiotic stresses in C_4 Panicoideae (Osborn et al. 2017; Doust et al. 2019; Chatterjee et al. 2021). Furthermore, the study of enzymatic activities related to carbon fixation has elucidated the importance of RuBisCO, phosphoenolpyruvate carboxylase (PEPcase), and carbonic anhydrase (CA) as contributing or limiting factors for CO_2 assimilation in C_4 plants (Boyd et al. 2015).

A recent study investigated the response of a *S. viridis* mutant with reduced carbonic anhydrase (CA) activity to different CO_2 concentrations (Chatterjee et al. 2021). Carbonic anhydrase (CA) is the enzyme responsible for converting CO_2 into bicarbonate (HCO_3^-), which is then fixed by PEPcase in C_4 metabolism. When grown at an ambient partial pressure of CO_2 (pCO_2), the mutant plants were smaller and had lighter, thinner leaves. Compared with the wild-type genotype, the BS and MS cells of the mutants were smaller, and the amount and area of chloroplasts were reduced. Therefore, the photosynthesis in the mutant with reduced CA activity still followed a C_4 pathway but with a significantly reduced assimilation rate, reinforcing the essential role of CA in C_4 photosynthesis in *S. viridis*. However, when these mutant plants were grown in elevated CO_2, their growth and photosynthetic rates were significantly recovered, a finding that highlights the CO_2 limitation imposed by the reduced CA activity.

However, it is unclear whether specific environmental conditions can limit CA activity and therefore HCO_3- production among different C_4 species (Crawford and Cousins 2021). CA activity may limit PEPcase at high temperatures and low pCO_2 without influencing mesophyll CO_2 conductance (g_m), a hypothesis recently studied in *S. viridis*. The results showed that low CA activity impaired HCO_3^- production for C_4 photosynthesis, but it had no influence on mesophyll conductance at high temperatures. In conclusion, under high temperatures, when stomatal conductance is a limiting factor for the assimilation of atmospheric CO_2, increasing leaf CA activity in C_4 species could increase the photosynthetic rate (Crawford and Cousins 2021). This aligns with the general understanding that both enzymatic activity and mesophyll and sheath conductance are key factors for maximizing CO_2 assimilation under stressful conditions (Osborn et al. 2017; Danila et al. 2021).

Studies have shown that *S. viridis* can partially restore key photosynthetic parameters, such as Fv/Fm (maximum quantum yield of photosystem II), ABS/RC (absorbed energy per reaction center), and DI0/RC (dissipated energy flux per reaction center), by the tenth day under water stress (Valença et al. 2020). This recovery capacity raises the possibility of exploring how increased CO_2 could be used to mitigate the effects of water deficit and further enhance photosynthetic recovery. This is supported by studies on other C_4 crops, where high CO_2 concentrations combined with water stress improved the biomass and yield of plants such as sorghum (Rodrigues et al. 2021). Studies combining stress and photosynthetic efficiency under altered CO_2 availability, including studies with *Setaria*, remain scarce. Future studies should assess the combined response of *S. viridis* under water deficit, salt stress, temperature, and CO_2

concentrations, revealing possible strategies to mitigate such effects in C_4 crops.

S. viridis has been used as a C_4 model plant for studying distinct stress conditions affecting agriculture, such as water deficit, salinity, temperature, and atmospheric CO_2. Studies have already been performed to characterize the effects of water and thermal stress on *S. viridis* individually, but understanding the morphological, physiological, and molecular alterations when different stresses are concurrently applied is key for the development of agronomic traits of interest. One of the important gaps and questions that remain unsolved, as is also true for crop plants with C_4 metabolism, includes identifying, characterizing, and describing the anatomical variations in the root system among the different accessions of *S. viridis*. While this manuscript has explored the responses of *S. viridis* to water deficit, salinity, and temperature, a notable gap remains in understanding its responses to different light conditions (high light and shading) and elevated CO_2 levels, both in isolation and in combination with other stresses. Studies involving diverse accessions of *S. viridis* are interesting because *S. viridis* can be used in biotechnological approaches on C_4 to respond to global environmental changes, offering more productive crops and food security in the face of world population growth and climate change.

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Declarations

Conflict of interest There are no conflicts of interest.

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References

Acharya BR, Roy Choudhury S, Estelle AB, Vijayakumar A, Zhu C, Hovis L, Pandey S (2017) Optimization of phenotyping assays for the model monocot *Setaria viridis*. Front Plant Sci 8:1–13. https://doi.org/10.3389/fpls.2017.02172

Adams E, Shin R (2014) Transport, signaling, and homeostasis of potassium and sodium in plants. J Integr Plant Biol 56:231–249. https://doi.org/10.1111/jipb.12159

Amini V, Zaefarian F, Rezvani M (2015) Interspecific variations in seed germination and seedling emergence of three *Setaria* species. Braz J Bot 38:539–545. https://doi.org/10.1007/s40415-015-0158-6

Anderson CM, Mattoon EM, Zhang N (2021) High light and high temperature reduce photosynthesis via different mechanisms in the C_4 model *Setaria viridis*. Commun Biol 4:1–19. https://doi.org/10.1101/2021.02.20.431694

Ardie SW, Khumaida N, Nur A, Fauziah N (2015) Early identification of salt tolerant foxtail millet (*Setaria Ardie italica* L. Beauv). Procedia Food Sci 3:303–312. https://doi.org/10.1016/j.profoo.2015.01.033

Banan D (2019) Phenotypic and genetic variation in the architectural responses of a C_4 grass to drought stress. University of Illinois at Urbana-Champaign, Thesis

Baret F, Madec S, Irfan K, Lopez J, Comar A, Hemmerlé M, Dutartre D, Praud S, Tixier MH (2018) Leaf-rolling in maize crops: from leaf scoring to canopy-level measurements for phenotyping. J Exp Bot 69:2705–2716. https://doi.org/10.1093/jxb/ery071

Basso MF, Duarte KE, Santiago TR, de Souza WR, Garcia BO, da Cunha BDB, Kobayashi AK, Molinari HBC (2021) Efficient genome editing and gene knockout in *Setaria viridis* with CRISPR/Cas9 directed gene editing by the non-homologous end-joining pathway. Plant Biotechnol 38:227–238. https://doi.org/10.5511/plantbiotechnology.21.0407a

Baum ME, Licht MA, Huber I (2020) Impacts of climate change on the optimum planting date of different maize cultivars in the central US Corn Belt. Eur J Agron 119:1–11. https://doi.org/10.1016/j.eja.2020.126101

Bennetze JL, Schmutz J, Wang H et al (2012) Reference genome sequence of the model plant *Setaria*. Nat Biotechnol 30:555–561. https://doi.org/10.1038/nbt.2196

Bethke PC, Drew MC (1992) Stomatal and non-stomatal components to inhibition of photosynthesis in leaves of *Capsicum annuum* during progressive exposure to NaCl salinity. Plant Physiol 99:219–226

Bezerra ACM, Valença DC, Junqueira NEG et al (2021) Potassium supply promotes the mitigation of NaCl-induced effects on leaf photochemistry, metabolism and morphology of *Setaria viridis*. Plant Physiol Biochem 160:193–210. https://doi.org/10.1016/j.plaphy.2021.01.021

Blackshaw RE, Stobbe EH, Shaykewich CF et al (1981) Influence of soil temperature and soil moisture on green foxtail (*Setaria viridis*) establishment in wheat (Triticum aestivum). Weed Sci 29:179–184.

Boyd RA, Gandin A, Cousins AB (2015) Temperature response of C_4 photosynthesis: biochemical analysis of Rubisco, Phosphoenolpyruvate Carboxylase and Carbonic Anhydrase in *Setaria viridis*. Plant Physiol 169:1850–1861. https://doi.org/10.1104/pp.15.00586

Braga MF, Ferreira EA, Cabral CM, Freitas IC, Maciel JC, Freitas MSS, Aspiazu I, Santos JB, Fernandes LA, Frazão LA, Sampaio RA (2021) Review: Growth of C_3 and C_4 plants in response to different CO_2 concentrations. Res Soc Dev 10:1–14. https://doi.org/10.33448/rsd-vl0i7.16701

Brito MEB, Filho GDA, Wanderley JAC et al (2013) Growth, physiological and yield of sweet corn under water stress. Biosci J 29:1244–1254

Brown WV, Smith BN (1972) Grass evolution, the Kranz syndrome, 13C/12C ratios and continental drift. Nat 239:345–346. https://doi.org/10.1038/239345a0

Brutnell TP, Wang L, Swartwood K et al (2010) *Setaria viridis:* a model for C_4 photosynthesis. Plant Cell 22:2537–2544. https://doi.org/10.1105/tpc.110.075309

Burbulis N, Jonytiene V, Blinstrubiene A, Vainoriene R (2013) Growth and photosynthetic pigments responses of *Setaria viridis* (L.) P. Beauv. to drought stress. Rural Devel 6:66–69

Burrell AL, Evans JP, De Kauwe MG (2020) Anthropogenic climate change has driven over 5 million km^2 of drylands towards desertification. Nat Commun 11:1–11. https://doi.org/10.1038/s41467-020-17710-7

Cano FJ, Sharwood RE, Cousins AB, Ghannoum O (2019) The role of leaf width and conductances to CO_2 in determining water use efficiency in C_4 grasses. New Phytol 223:1280–1295

Carvalho CGI (2019) Resposta morfofisiológica de *Setaria viridis* ao estresse de frio, e prospecção e caracterização de genes responsivos ao frio, diferencialmente expressos em *Elaeis guineensis*. Dissertação, Universidade Federal de Lavras.

Carvalho CGI, Santos ML, Vieira LR et al (2022) Morphophysiological responses of *Setaria viridis* to cold stress. Pesq Agropec Bras 57:e02424. https://doi.org/10.1590/S1678-3921.pab2022.v57.02424

Castro FC, Santos AM (2020) Salinity of the soil and the risk of desertification in the semiarid region. Mercator 19:1–12. https://doi.org/10.4215/rm2020.e19002

Cesarino I, Dello Ioio R, Kirschner GK, Ogden MS, Picard KL, Rast-Somssich MI, Somssich M (2020) Plant science's next top models. Ann Bot 126:1–23. https://doi.org/10.1093/aob/mcaa063Chatterjee

Chatterjee J, Coe RA, Acebron K et al (2021) A low CO_2-responsive mutante of Setaria viridis reveals that reduced carbonic anhydrase limits C_4 photosynthesis. J Exp Bot 72:3122–3136. https://doi.org/10.1093/jxb/erab039

Chaudhry S, Sidhu GPS, Paliwal R (2021) Restoring Ecosystem Services of Degraded Forests in a Changing Climate. Handbook of Ecological and Ecosystem Engineering 353–375. https://doi.org/10.1002/9781119678595.ch19

Choudhary P, Shukla P, Muthamilarasan M (2023) Genetic enhancement of climate-resilient traits in small millets: a review. Heliyon 9:e14502. https://doi.org/10.1016/j.heliyon.2023.e14502

Coe RA, Chatterjee J, Acebron K et al (2018) High-throughput chlorophyll fluorescence screening of *Setaria viridis* for mutants with altered CO_2 compensation points. Funct Plant Biol. https://doi.org/10.1071/fp17322

Costa MCA (2018) Development of *Setaria viridis* with emphasis on the establishment of the root meristem. Dissertation, Universidade de São Paulo.

Crawford JD, Cousins AB (2021) Limitation of C_4 photosynthesis by low carbonic anhydrase activity increases with temperature but does not influence mesophyll CO_2 conductance. J Exp Bot 73:927–938. https://doi.org/10.1093/jxb/erab464

Danila FR, Thakur V, Chatterjee J et al (2021) Bundle sheath suberisation is required for C_4 photosynthesis in a *Setaria viridis* mutant. Commun Biol 4:1–10. https://doi.org/10.1038/s42003-021-01772-4

Defrance D, Ouedraogo I, Linka O, Sultan B (2020) Impact of climate change inn west Africa on cereal production per capita in 2050. Sustain 12:1–19. https://doi.org/10.3390/su12187585

Dekker J (2003) The foxtail (*Setaria*) species-group. Weed Sci 51:641–656

Delatorre CA, Silva AA (2008) *Arabidopsis thaliana*: uma pequena planta um grande papel. Rev Cienc Agrar 31:58–67

Devnarain N, Crampton BG, Chikwamba R et al (2016) Physiological responses of selected African sorghum landraces to progressive water stress and re-watering. S Afr J Bot 103:61–69. https://doi.org/10.1016/j.sajb.2015.09.008

Diao X, Schnable J, Bennetzen JL, Li J (2014) Initiation of *Setaria* as a model plant. Front Agr Sci Eng 1:16–20. https://doi.org/10.15302/J-FASE-2014011

Doust A, Diao X (2017) Genetics and Genomics of *Setaria.* Plant Genetics and Genomics: Crops and Models. https://doi.org/10.1007/978-3-319-45105-3

Doust AN, Brutnell TP, Upadhyaya HD, Van Eck J (2019) *Setaria* as a model genetic system to accelerate yield increases in cereals, forage crops, and bioenergy grasses. Front Plant Sci. https://doi.org/10.3389/fpls.2019.01211

Duarte KE, Souza WR, Santiago TR et al (2019) Identification and characterization of core abscisic acid (ABA) signaling components and their gene expression profile in response to abiotic stresses in *Setaria viridis*. Sci Rep 9:1–16. https://doi.org/10.1038/s41598-019-40623-5

Edwards GE, Voznesenskaya EV (2010) C_4 photosynthesis: Kranz forms and single-cell C_4 in terrestrial plants C_4 Photosynthesis and Related CO_2 Concentrating Mechanisms. Springer 4:29–61

Ehleringer JR, Cerling TE, Helliker BR (1997) C_4 photosynthesis, atmospheric CO_2, and climate. Oecologia 112:285–299

El-Samad HMA, Shaddad MAK, Ragaey MM (2019) Drought strategy tolerance of four barley cultivars and combined effect with salicylic acid application. Am J Plant Sci 10:512–535. https://doi.org/10.4236/ajps.2019.104037

Ermakova M, Osborn H, Groszmann M et al (2021) Expression of a CO_2-permeable aquaporin enhances mesophyll conductance in the C_4 species *Setaria viridis*. Elife 10:1–16. https://doi.org/10.7554/eLife.70095

Essemine J, Qu M, Lyu M-JA, Song Q, Khan N, Chen G, Wang P, Zhu X-G (2020) Photosynthetic and transcriptomic responses of two C_4 grass species with different NaCl tolerance. J Plant Physiol 253:153244. https://doi.org/10.1016/j.jplph.2020.153244

FAO (2023) Rising global food insecurity: Assessing policy responses. A report prepared at the request of the Group of 20 (G20). Rome, FAO.

Feldman MJ, Ellsworth PZ, Fahlgren N et al (2018) Components of water use efficiency have unique genetic signatures in the model C_4 grass *Setaria*. Plant Physiol 178:699–715. https://doi.org/10.1104/pp.18.00146

Ferreira TMM, Santos ML, Lopes CL et al (2020) Effect os falinity stress in *Setaria viridis* (L.) P. Beauv. accession A10.1 during seed germination and plant development. Cien Agrotec 44:1–16

Finley T, Chappell H, Veena V (2021) *Agrobacterium*-mediated transformation of *Setaria viridis*, a model system for cereals and bioenergy crops. Curr Protoc 1(5):e127. https://doi.org/10.1002/cpz1.127

Flexas J, Bota J, Loreto F et al (2004) Diffusive and metabolic limitations to photosynthesis under drought and salinity in C_3 plants. Plant Biol 5:1–11. https://doi.org/10.1055/s-2004-820867

Flowers TJ, Colmer TD (2008) Salinity tolerance in halophytes. New Phytol 179(4):945–963. https://doi.org/10.1111/j.1469-8137.2008.02531.x

Guo R, Zhou J, Hao WP et al (2011) Germination, growth, photosynthesis and ionic balance in *Setaria viridis* seedlings subjected to saline and alkaline stress. Can J Plant Sci 91:1077–1088. https://doi.org/10.4141/cjps10167

Gupta B, Huang B (2014) Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. Int J Genom. https://doi.org/10.1155/2014/701596

Hatakeyama Y, Ueno O (2017) Intracellular position of mitochondria in mesophyll cells differs between C_3 and C_4 grasses. J Plant Res 130:885–892. https://doi.org/10.1007/s10265-017-0947-z

Henkhaus N, Bartlett M, Gang D et al (2020) Plant science decadal vision 2020–2030: reimagining the potential of plants for a healthy and sustainable future. Plant Direct 4:1–24. https://doi.org/10.1002/pld3.252

Hodge JG, Doust AN (2017) Morphological development of *Setaria viridis* from germination to flowering. In: Genetics and Genomics of *Setaria*. Springer. 161–175.

Hoegh-Guldberg O, Jacob D, Taylor M (2018) Impacts of 1.5ºC global warming on natural and human systems. Global warming of 1.5°C. An IPCC Special Report 135–311.

Hu H, Mauro-Herrera M, Doust AN (2018) Domestication and improvement in the model C_4 grass, *Setaria*. Front Plant Sci 9:1–13. https://doi.org/10.3389/fpls.2018.00719

Huang P, Feldman M, Schroder S et al (2014) Population genetics of *Setaria viridis*, a new model system. Mol Ecol 23:4912–4925. https://doi.org/10.1111/mec.12907

Huang P, Shyu C, Coelho CP et al (2016) *Setaria viridis* as a model systm to advance millet genetics and genomics. Frent Plant Sci 7:1–9. https://doi.org/10.3389/fpls.2016.01781

Ihsan MZ, El-Nakhlawy FS, Ismail SM (2015) Water use efficiency, growth and yield of wheat cultivated under competition with *Setaria*. Planta Daninha 33:679–687. https://doi.org/10.1590/s0100-83582015000400006

IPCC (2023) Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva. https://doi.org/10.59327/IPCC/AR6-9789291691647

Jannah RM, Ratnawati S, Willy S, Ardie S (2024) Digital phenotyping for robust seeds variability assessment in *Setaria italica* (L.) P. Beauv. J Seed Sci 46:e202446011. https://doi.org/10.1590/2317-1545v46281586

Jia G, Shi S, Wang C et al (2013) Molecular diversity and population structure of Chinese green foxtail [*Setaria viridis* (L.) Beauv.] revealed by microsatellite analysis. J Exp Bot 64:3645–3656. https://doi.org/10.1093/jxb/ert198

Joshi R, Wani SH, Singh B (2016) Transcription factors and plants response to drought stress: current understanding and future directions. Front Plant Sci 7:1–15. https://doi.org/10.3389/fpls.2016.01029

Junqueira NEG, Ortiz-Silva B, Leal-Costa MV et al (2018) Anatomy and ultrastructure of embryonic leaves of the C_4 species *Setaria viridis*. Ann Bot 121:1163–1172. https://doi.org/10.1093/aob/mcx217

Junqueira NEG, Bezerra ACM, Cattem MVO et al (2020) Phenology of the genetic model *Setaria viridis* (Poaceae) according to the BBCH-scale of development. Bot J Linn Soc 192:224–241. https://doi.org/10.1093/botlinnean/boz070

Junqueira NEG, Bezerra ACM, Travassos-Lins J et al (2025) Phenological growth stages according to the BBCH-scale as a powerful tool for differentiating among CRISPR/Cas9 mutated lineages of the model plant *Setaria viridis* (L.) P. Beauv. (Poaceae). Botany. https://doi.org/10.1139/cjb-2024-0022

Kadioglu A, Terzi R, Saruhan N, Saglam A (2012) Current advances in the investigation of leaf rolling caused by biotic and abiotic stress factors. Plant Sci 182:42–48. https://doi.org/10.1016/j.plantsci.2011.01.01

Kafi M, Zamani Gh, Ghoraishi SGh (2009) Relative salt tolerance of south Khorasan millets. Desert 14:63–70

Kapilan R, Vaziri M, Zwiazek JJ (2018) Regulation of aquaporins in plants under stress. Biol Res 51:4. https://doi.org/10.1186/s40659-018-0152-0

Kataria S, Verma SK (2018) Salinity stress responses and adaptive mechanisms in major glycophytic crops: the story so far. Salinity Responses Tolerance Plants 1:1–39. https://doi.org/10.1007/978-3-319-75671-4_1

Kaya Y, Vasilevska-Ivanova R (2021) Wild Sunflowers: The Primary Genetic Resource for Sunflower Breeding - Wild Germplasm for Genetic Improvement in Crop Plants. Academy Press. 153–185.

Kim IS, Pak JH, Seo B, Song SD (1997) Ultrastructural aspects of Kranz anatomy in *Digitaria sanguinalis* and *Setaria viridis* (Poaceae). J Plant Biol 40:102–109

Lata C, Gupta S, Prasad M (2013) Foxtail millet: a model crop for genetic and genomic studies in bioenergy grasses. Crit Rev Biotechnol 33:328–343. https://doi.org/10.3109/07388551.2012.716809

Leakey ADB (2009) Rising atmospheric carbon dioxide concentration and the future of C_4 crops for food and fuel. Proc R Soc B 276:2333–2343. https://doi.org/10.1098/rspb.2008.1517

Leakey ADB, Ainsworth EA, Bernacchi CJ (2009) Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from Face. J Exp Bot 60:2859–2876. https://doi.org/10.1093/jxb/erp096

Levers C, Romero-Muñoz A, Baumann M et al (2021) Agricultural expansion and the ecological marginalization of forest-dependent people. Proc Naltl Acad Sci 118:1–9. https://doi.org/10.1073/pnas.2100436118

Li Y-M (1997) Breeding for foxtail millet drought tolerant cultivars (in Chinese). In: Li Y (ed) Foxtail millet breeding. Chinese Agr Press, Beijing, pp 421–446

Li P, Brutnell TP (2011) *Setaria viridis* and *Setaria italica*, model genetic systems for the Panicoid grasses. J Exp Bot 62:3031–3037. https://doi.org/10.1093/jxb/err096

Liang Z, Wu Y, Ma L et al (2022) Efficient genome editing in *Setaria italica* using CRISPR/Cas9 and base editors. Front Plant Sci 13:815946. https://doi.org/10.3389/fpls.2021.815946

Liaqat W, Altaf MT, Barutçular C et al (2024) Sorghum: a star crop to combat abiotic stresses, food insecurity, and hunger under a changing climate: a review. J Soil Sci Plant Nutr 24:74–101. https://doi.org/10.1007/s42729-023-01607-7

Luo Y, Zhao X, Zhou R et al (2009) Photosynthetic performance of *Setaria viridis* to soil drought and rewatering alternations. Sci Cold Arid Reg 676:0404–0411

Luo Y, Zhao X, Zhou R et al (2011) Physiological acclimation of two psammophytes to repeated soil drought and rewatering. Acta Physiol Plant 33:79–91. https://doi.org/10.1007/s11738-010-0519-5

Maqbool S, Hassan MA, Xia X et al (2022) Root system architecture in cereals: progress, challenges and perspective. Plant J 110:23–42. https://doi.org/10.1111/tpj.15669

Martins PK, Ribeiro AP, Cunha BADBD, Kobayashi AK, Molinari HBC (2015a) A simple and highly efficient Agrobacterium-mediated transformation protocol for *Setaria viridis*. Biotechnol Rep (Amst) 18:41–44. https://doi.org/10.1016/j.btre.2015.02.002

Martins PK, Nakayama TJ, Ribeiro AP, Cunha BADBD, Nepomuceno AL, Harmon FG, Kobayashi AK, Molinari HBC (2015b) *Setaria*

viridis floral-dip: a simple and rapid *Agrobacterium*-mediated transformation method. Biotechnol Rep (Amst) 4:61–63. https://doi.org/10.1016/j.btre.2015.02.006

Martins PK, Mafra V, Souza WR et al (2016) Selection of reliable reference genes for RT-qPCR analysis during developmental stages and abiotic stress in *Setaria viridis*. Sci Rep 6:1–10. https://doi.org/10.1038/srep28348

Martins PK, da Cunha BADB, Kobayshi AK, Molinari HBC (2019) *Setaria viridis* as a model plant for functional genomic studies in C_4 crops. Methods Mol Biol 1864:49–65. https://doi.org/10.1007/978-1-4939-8778-8_4

Mollaee M, Darbandi EI, Aval MB, Chauhan BS (2020) Germination response of three *Setaria* species (*S. viridis*, *S. verticillata*, and *S. glauca*) to water potential and temperature using non-linear regression and hydrothermal time models. Acta Physiol Plant 42:1–14. https://doi.org/10.1007/s11738-020-03133-w

Mookkan M (2018) Particle bombardment-mediated gene transfer and GFP transient expression in *Seteria viridis*. Plant Signal Behav 3(4):e1441657. https://doi.org/10.1080/15592324.2018.1441657

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Muthamilarasan M, Singh RK, Suresh BV et al (2020) Genomic dissection and expression analysis of stress-responsive genes in C_4 panicoid models, *Setaria italica* and *Setaria viridis*. J Biotechnol 318:57–67. https://doi.org/10.1016/j.jbiotec.2020.05.007

Nadeem F, Ahmad Z, Ul HM et al (2020) Adaptation of Foxtail Millet (*Setaria italica* L.) to Abiotic Stresses: a special perspective of responses to Nitrogen and Phosphate Limitations. Front Plant Sci 11:1–11. https://doi.org/10.3389/fpls.2020.00187

Nguyen DQ, Van Eck J, Eamens AL et al (2020) Robust and reproducible *Agrobacterium*-mediated transformation system of the C_4 genetic model species *Setaria viridis*. Front Plant Sci 13(11):281. https://doi.org/10.3389/fpls.2020.00281

Novák V, Lipiec J, Lo Pinto R (2012) Water extraction by roots under environmental stresses. Pollution and water resources, Columbia University seminar proceedings: Impact of anthropogenic activity and climate changes on the environment of central Europe and USA (151st ed.).

Orman-Ligeza B, Parizot B, Gantet PP et al (2013) Post-embryonic root organogenesis in cereals: branching out from model plants. Trends Plant Sci 18:459–467. https://doi.org/10.1016/j.tplants.2013.04.010

Osborn HL, Alonso-Cantabrana H, Sharwood RE et al (2017) Effects of reduced carbonic anhydrase activity on CO_2 assimilation rates in *Setaria viridis*: a transgenic analysis. J Exp Bot 68:299–310. https://doi.org/10.1093/jxb/erw357

Pan J, Li Z, Dai S et al (2020) Integrative analyses of transcriptomics and metabolomics upon seed germination of foxtail millet in response to salinity. Sci Rep 10:1–16. https://doi.org/10.1038/s41598-020-70520-1

Pedrotti A, Chagas RM, Ramos VC et al. (2015) Causas e consequências do processo de salinização dos solos. REGET. 19:1308–1324

Pegler JL, Nguyen DQ, Grof CPL et al (2020) Profiling of the Salt Stress Responsive MicroRNA Landscape of C_4 Genetic Model Species *Setaria viridis* (L.) Beauv. Agron 10:1–25. https://doi.org/10.3390/agronomy10060837

Petricka JJ, Winter CM, Benfey PN (2012) Control of *Arabidopsis* root development. Annu Rev Plant Biol 63:563–590. https://doi.org/10.1146/annurev-arplant-042811-105501

Pinghua L, Thomas PB (2011) *Setaria viridis* and *Setaria italica*, model genetic systems for the Panicoid grasses. J Exp Bot 62:3031–3037. https://doi.org/10.1093/jxb/err096

Provart NJ (2016) 50 years of *Arabidopsis* research: highlights and future directions. New Phytol 209:921–944. https://doi.org/10.1111/nph.13687

Pushpavalli R, Zaman-Allah M, Turner NC et al (2015) Higher flower and seed number leads to higher yield under water stress conditions imposed during reproduction in chickpea. Funct Plant Biol 42:162–174. https://doi.org/10.1071/fp14135

Qie L, Jia G, Zhang W et al (2014) Mapping of quantitative trait locus (QTLs) that contribute to germination and early seedling drought tolerance in the interspecific cross *Setaria italica*×*Setaria viridis*. PLoS ONE 9:1–8. https://doi.org/10.1371/journal.pone.0101868

Raghavendra AS, Sage RF (2011) Advances in Photosynthesis and Respiration: C_4 Photosynthesis and Related CO_2 Concentrating Mechanisms. Netherlands (Eds.): Springer. pp. 317. https://doi.org/10.1007/978-90-481-9407-0

Rauf S, Al-Khayri JM, Zaharieva M et al. (2016) Breeding Strategies to Enhance Drought Tolerance in Crops. Advances in Plant Breeding Strategies: Agronomic, Abiotic and Biotic Stress Traits. 397–445. https://doi.org/10.1007/978-3-319-22518-0_11

Rodrigues TS, Travassos-Lins J, Cattem MV et al (2019) Evaluation of *Setaria viridis* physiological and gene expression responses to distinct water-deficit conditions. Biotechnol Res Innov 3:42–58. https://doi.org/10.1016/j.biori.2020.03.001

Rodrigues TS, Arge LWP, Guedes FAF et al (2021) Integrative Analysis of *Sorghum bicolor* green prop roots under elevated CO_2 and water deficit conditions. BioRxiv. https://doi.org/10.1101/2021.03.12.435150

Saha P, Blumwald E (2016) Spike-dip transformation of *Setaria viridis*. Plant J 86:89–101. https://doi.org/10.1111/tpj.13148

Saha P, Sade N, Arzani A et al (2016) Effects of abiotic stress on physiological plasticity and water use of *Setaria viridis* (L.). Plant Sci 251:128–138. https://doi.org/10.1016/j.plantsci.2016.06.01

Sanadhya P, Agarwal P, Agarwal PK (2015) Ion homeostasis in a salt-secreting halophytic grass. AoB Plants 7:1–15. https://doi.org/10.1093/aobpla/plv055

Santos ML, Silva VNB, Vieira LR et al (2017) Use of *Setaria viridis* (A10.1) as model plant for validation of genes for cold tolerance. In: XVI Brazilian Congress of Plant Physiology.

Schossler TR, Machado DM, Zuffo AM (2012) Salinidade: Efeitos na fisiologia e na nutrição mineral de plantas. Encicl Biosf 8:1563–1578

Sebastian J, Yee MC, Viana WG et al (2016) Grasses suppress shoot-borne roots to conserve water during drought. Proc Natl Acad Sci USA 113:8861–8866. https://doi.org/10.1073/pnas.1604021113

Seleiman MF, Al-Suhaibani N, Ali N et al (2021) Drought stress impacts on plants and different approaches to alleviate its adverse effects. Plants 10:259. https://doi.org/10.3390/plants10020259

Seneviratne SI, Nicholls N, Easterling D (2012) Changes in climate extremes and their impacts on the natural physical environment. Manag Risks Extreme Events Disasters Adv Climate Change Adaptation. https://doi.org/10.1017/cbo9781139177245.006

Shahid SA, Zaman M, Heng L (2018) Soil Salinity: Historical Perspectives and a World Overview of the Problem. In: Guideline for Salinity Assessment, Mitigation and Adaptation Using Nuclear and Related Techniques. Springer, Cham. https://doi.org/10.1007/978-3-319-96190-3_2

Siegert M, Haywood A, Lunt D et al. (2020) What ancient climates tell us about high carbon dioxide concentrations in Earth's atmosphere, Grantham Institute Briefing Note 13, Imperial College Londo. https://doi.org/10.25561/79292

Souza TC, Magalhães PC, Castro EM (2016) Corn root morphoanatomy at different development stages and yield under water stress. Pesqui Agropecu Brasil 51:330–339. https://doi.org/10.1590/s0100-204x2016000400005

Stepanova NA (2021) Plant biology research: what is next? Front Plant Sci 12:749104. https://doi.org/10.3389/fpls.2021.749104

Sun S, Liu Q, Dai X, Wang X (2022) Transcriptomic analysis reveals the correlation between End-of-Day Far Red Light and Chilling

Stress in *Setaria viridis*. Genes. https://doi.org/10.3390/genes13091565

Taiz L, Zeiger E (2013) Fisiologia vegetal. 5.ed. Porto Alegre: Artemed, 954p.

Takahashi T, Pradal C (2021) Root phenotyping: important and minimum information required for root modeling in crop plants. Breed Sci 71:109–116. https://doi.org/10.1270/jsbbs.20126

Tang S, Li L, Wang Y et al (2017) Genotype-specific physiological and transcriptomic responses to drought stress in *Setaria italica* (an emerging model for Panicoideae grasses). Sci Rep 7:10009. https://doi.org/10.1038/s41598-017-08854-6

Thielen PM, Pendleton AL, Player RA et al (2020) Reference genome for the highly transformable *Setaria viridis* ME034V. Genome Rep 10:3467–3478. https://doi.org/10.1534/g3.120.401345

Travassos-Lins J, Rocha CCO, Rodrigues TS, Alves-Ferreira M (2021) Evaluation of the molecular and physiological response to dehydration of two accessions of the model plant *Setaria viridis*. Plant Physiol Biochem 169:211–223. https://doi.org/10.1016/j.plaphy.2021.11.015

Ubierna N, Gandin A, Cousins AB (2018) The response of mesophyll conductance to short-term variation in CO_2 in the C_4 plants *Setaria viridis* and *Zea mays*. J Exp Bot 69:1159–1170. https://doi.org/10.1093/jxb/erx464

Ullah A, Bano A, Khan N (2021) Climate change and salinity effects on crops and chemical communication between plants and plant growth-promoting microorganisms under stress. Front Sustain Food Syst 5:1–16. https://doi.org/10.3389/fsufs.2021.618092

Valença DC, Moura MS, Travassos-Lins J et al (2020) Physiological and molecular responses of *Setaria viridis* to osmotic stress. Plant Physiol Biochem 155:114–125. https://doi.org/10.1016/j.plaphy.2020.07.019

Valeriano FR, Moura SM, Travassos-Lins J et al (2021) Evaluation of *Setaria viridis* responses to salt treatment and potassium supply: a characterization of three contrasting accessions. Braz J Bot 44:821–836. https://doi.org/10.1007/s40415-021-00773-1

Vetriventhan M, Upadhyaya HD, Dwivedi SL, Pattanashetti SK, Singh SK (2016). Finger and foxtail millets. pp. 291–319. https://doi.org/10.1016/b978-0-12-802000-5.00007-1

Wang C, Guo L, Li Y, Wang Z (2012) Systematic comparison of C_3 and C_4 plants based on metabolic network analysis. BMC Syst Biol 6:1–14. https://doi.org/10.1186/1752-0509-6-S2-S9

Wang H, Liang L, Liu S et al (2020) Maize genotypes with deep root systems tolerate salt stress better than those with shallow root system during early growth. J Agron Crop Sci 206:1–11. https://doi.org/10.1111/jac.12437

Wang D, Yang N, Zhang C, He W, Ye G, Chen J, Wei X (2022) Transcriptome analysis reveals molecular mechanisms underlying salt tolerance in halophyte *Sesuvium portulacastrum*. Front Plant Sci 13:973419. https://doi.org/10.3389/fpls.2022.973419

Weiss T, Wang C, Kang X et al (2020) Optimization of multiplexed CRISPR/Cas9 system for highly efficient genome editing in *Setaria viridis*. Plant J 104:828–838. https://doi.org/10.1111/tpj.14949

Xu J, Li Y, Ma X et al (2013) Whole transcriptome analysis using next-generation sequencing of model species *Setaria viridis* to support C_4 photosynthesis research. Plant Mol Biol 83:77–87. https://doi.org/10.1007/s11103-013-0025-4

Yadav S, Modi P, Dave A, Vijapura A, Patel D, Patel M (2020) Effect of abiotic stress on crops. Sustainable Crop Production 1:21. https://doi.org/10.5772/intechopen.88434

Yamakawa S, Kato Y, Taniguchi MOT (2023) Intracellular positioning of mesophyll chloroplasts following to aggregative movement in *Setaria viridis* analysed three-dimensionally with a confocal laser scanning microscope. Flora 306:1–9. https://doi.org/10.1016/j.flora.2023.152364

Yang J, Zhang J (2006) Grain filling of cereals under soil drying. New Phytol 169:223–236. https://doi.org/10.1111/j.1469-8137.2005.01597.x

Yang Z, Li JL, Liu LN et al (2020) Photosynthetic regulation under salt stress and salt-tolerance mechanism of sweet *sorghum*. Front Plant Sci. https://doi.org/10.3389/fpls.2019.01722

Yoo SY, Park JY, Lee DW (2014) Chlorophyll a fluorescence imaging analysis in air-drying stressed sorghum (*Sorghum bicolor* L.) leaf. Int J Adv Agric Sci Thecnol 2:58–66. https://doi.org/10.1590/1807-1929/agriambi.v22n10p673-678

Zahra N, Raza ZA, Mahmood S (2020) Effect of salinity stress on various growth and physiological attributes of two contrasting maize genotypes. Agric Agrib Biotechnol 63:1–10. https://doi.org/10.1590/1678-4324-2020200072

Zhan A, Schneider H, Lynch JP (2015) Reduced lateral root branching density improves drought tolerance in maize. Plant Physiol 168:1603–1615. https://doi.org/10.1104/pp.15.00187

Zhang G, Liu X, Quan Z et al (2012) Genome sequence of foxtail millet (*Setaria italica*) provides insights into grass evolution and biofuel potential. Nat Biotechnol 30:549–556. https://doi.org/10.1038/nbt.2195

Zhang H, Zhu J, Gong Z, Zhu JK (2022) Abiotic stress responses in plants. Nat Rev Genet 23:104–119. https://doi.org/10.1038/s41576-021-00413-0

Zhu JK (2016) Abiotic stress signaling and responses in plants. Cell 167:313–324. https://doi.org/10.1016/j.cell.2016.08.029

Zia A, Gulzar S, Ruban AV (2024) Enhanced photochemical efficiency of PSII in *Prosopis juliflora* suggests contribution to invasion advantage over native C_3 xero-halophytes under salt stress. Funct Plant Biol. https://doi.org/10.1071/FP23272

Zullo Junior J, Pinto HS, Assad ED (2006) Impact assessment study of climate change on agricultural zoning. Meteorol Appl. https://doi.org/10.1017/S135048270600257X

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