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Morphophysiological responses of *Setaria viridis* to cold stress

Abstract – The objective of this work was to determine the suitability of *Setaria viridis* as a model plant in studies to validate candidate genes for cold tolerance by evaluating the response of two of its accessions to different durations of abrupt or gradual cold stress in the vegetative and reproductive stages. Plants of accessions A10.1 and Ast-1, cultivated at 25°C, were subjected to the following cold stress treatments: gradual reduction in temperature from 25 to 0°C, 5°C at a time, every 24 hours in a same chamber; or abrupt reduction in temperature, by transferring plants from a chamber at 25°C to another at 0°C. Plants were kept at 0°C for 3, 5, or 10 days, after which temperature was increased back again to 25°C; a control group remained at 25ºC. Low temperatures – reduced abruptly or gradually – caused a decrease in the gas exchange rates and shoot and root biomass of the plants, besides damage to their photochemical apparatus; the longer the cold lasted, the more pronounced the effect was. Regardless of stress duration, plants recovered and completed their life cycle. The studied accessions are tolerant to cold and, therefore, are not suitable as a model plant in studies to validate candidate genes for cold tolerance.

Index terms: abiotic stress, florescence, gas exchange, model plant, phenomics, recovery, tolerance.

Respostas morfofisiológicas de *Setaria viridis* ao estresse por frio

Resumo – O objetivo deste trabalho foi determinar a adequação de *Setaria viridis* como planta modelo em estudos de validação de genes candidatos à tolerância ao frio, ao avaliar a resposta de dois de seus acessos a diferentes durações de estresse por frio abrupto ou gradual, nas fases vegetativa e reprodutiva. Plantas dos acessos A10.1 e Ast-1, cultivadas a 25°C, foram submetidas aos seguintes tratamentos de estresse por frio: redução gradual da temperatura de 25 a 0°C, 5°C de cada vez, a cada 24 horas, em única câmera; ou redução abrupta da temperatura, pela transferência das plantas de uma câmera a 25°C para outra a 0°C. As plantas foram mantidas a 0°C por 3, 5, ou 10 dias, após os quais a temperatura foi aumentada novamente para 25°C; um grupo controle permaneceu a 25ºC. As baixas temperaturas – reduzidas gradual ou abruptamente – causaram redução nas taxas de troca gasosa e na biomassa da parte aérea e da raiz das plantas, além de prejuízos ao seu aparato fotoquímico; quanto mais o frio durou, mais pronunciado o efeito foi. Independentemente da duração do estresse, as plantas se recuperaram e completaram seu ciclo de vida. Os acessos estudados são tolerantes ao frio, e, portanto, não são adequados como planta modelo em estudos para validação de genes candidatos de tolerância ao frio.

Termos para indexação: estresse abiótico, fluorescência, troca gasosa, planta modelo, fenômica, recuperação, tolerância.

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Introduction

Setaria viridis (L.) P.Beauv. and *Setaria italica* (L.) P.Beauv. are monocot species from the family Poaceae and subfamily Panicoideae proposed as model plants in reverse genetic studies for the validation of gene function (Lata et al., 2011).

Setaria viridis has been recommended as a model genetic system for Panicoid grasses (Li & Brutnell, 2011; Martins et al., 2016; Ferreira et al., 2020) due to it relevant characteristics, such as small size when adult, short life cycle, high seed production, self-pollination, and small genome. The genetic transformation of the species, mediated by biolistics or Agrobacterium, is already routine in some laboratories. Furthermore, the whole genome of *S. viridis* has already been sequenced and annotated (Lata & Prasad, 2013), paving the way for its application in comparative genomics studies (Doust et al., 2009; Coelho et al., 2017).

In the case of *S. italica*, there are reports of the validation of genes involved in pathways that confer tolerance to dehydration, drought, and salinity in different accessions (Lata et al., 2014; Li et al., 2014, 2017). The transcription factors participating in the signaling pathways responsive to these stresses include C-repeat binding factor/dehydration responsive element binding (CBF/DREB) and inducer of CBF expression (ICE) (Lata et al., 2011). In the literature, the accessions of this plant species have been classified as very tolerant, tolerant, and sensitive to abiotic stresses such as drought and salinity, but have also been shown to have varying levels of tolerance to cold (Nadeem et al., 2020; Valença et al., 2020; Saleem et al., 2021).

In most researches on *S. italica*, a combination of different types of stresses is usually applied to the plants, making it difficult to determine the kind and level of stress the species is responding to and to establish the quality and intensity of the response to the abiotic stresses, which share metabolic pathways (Lata & Prasad, 2013; Coelho et al., 2017; Saleem et al., 2021). For *S. viridis*, some studies have shown positive results in the characterization and validation of candidate genes for tolerance to dehydration, salinity, drought, and aluminum toxicity (Kumar et al., 2013; Martins et al., 2016). Lata & Prasad (2012), for example, validated an allele-specific marker associated with tolerance to dehydration stress, whose product is in a metabolic pathway with the participation of DREB. However, there are no known reports on the characterization of the physiological responses of accessions of this species to cold stress.

The objective of this work was to determine the suitability of *S. viridis* as a model plant in studies to validate candidate genes for cold tolerance by evaluating the response of two of its accessions to different durations of abrupt or gradual cold stress in the vegetative and reproductive stages.

Materials and Methods

All experiments were carried out at the Plant Genetics and Biotechnology Laboratory of Embrapa Agroenergia, located in Brasília, Distrito Federal, Brazil (15,732ºS, 47,900ºW).

The used plants were from the *S. viridis* A10.1 and Ast-1 accessions that show the most contrasting responses to most abiotic stresses (Saha et al., 2016). Seeds from both accessions underwent chemical scarification, and, after being disinfected, were spread on sterilized filter paper, left to dry for a few minutes, and then transferred to a sterilized germination medium, as described in Ferreira et al. (2020).

For germination and initial growth, plates with the seeds were kept in the A1000TC growth chamber (Conviron, Winnipeg, Manitoba, Canada) under the following conditions: 16/8 light/dark hour photoperiod, 25±2.0°C, and 150±10 µmol m^{-2} s^{-1} light intensity. After 7 days, all seedlings were transplanted individually to 0.2 L pots containing 100 g of a sterilized substrate composed of soil, vermiculite, and the Bioplant Plus commercial substrate (Bioplant, Nova Ponte, MG, Brazil) at a ratio of 2:1:1. Then, the seedlings were transferred to the PGW40 growth chamber (Conviron, Winnipeg, Manitoba, Canada), programed to provide a 16/8 hour light/dark photoperiod, air temperature of 25±2.0°C, air relative humidity of 65±5%, and photosynthetically active radiation of 400±20 µmol m^{-2} s^{-1}; CO_2 concentration varied according to the environment. Inside the growth chamber, the plants were distributed in a completely randomized design.

When the plants reached the vegetative (14 days after sowing and 7 days after transplanting) or the reproductive (28 days after sowing and 21 days after transplanting) (Martins et al., 2015) stage, they were subjected to two cold stress treatments: gradual reduction in the temperature inside the chamber from 25 to 0°C, 5°C at a time, in 24 hour cycles during 5 days;

or abrupt reduction in temperature, by transferring the plants from a chamber at 25°C directly to another at 0°C.

Once at 0ºC, plants remained at this temperature for 3, 5, or 10 days to assess plant response to the duration of cold stress. As soon as the stress period ended, the temperature in the growth chamber was increased again to 25ºC and remained at this degree until the end of the experiments, which lasted 52 days after sowing and 45 days after transplanting. Plants were harvested and weighed to determine fresh mass. Then, they were placed in an oven at 65ºC, with forced ventilation, until obtaining a constant weight.

Gas exchange in plants was determined in the infrared region, using the LI-6400XT portable gas analyzer, equipped with a measuring chamber with the 6400-02B artificial lighting system (LI-COR Biosciences, Lincoln, NE, USA). The equipment was set to: maintain the relative humidity inside the chamber between 50 and 60%, with the temperature of the block adjusted according to the temperature inside the chamber; a light intensity of 1,500 µmol m^{-2} s^{-1}; an airflow rate of 400 µmol s^{-1}; and a CO_2 concentration of 400 ppm in the reference cell. The following data were extracted by the OPEN software, version 6.3 (LI-COR Biosciences, Lincoln, NE, USA): net assimilation rate of CO_2 (*A*) in µmol CO_2 m^{-2} s^{-1}, stomatal conductance to water vapor (*gs*) in mol H_2O m^{-2} s^{-1}, transpiration rate (*E*) in mmol H_2O m^{-2} s^{-1}, and intercellular CO_2 concentration (*Ci*) in µmol CO_2 mol^{-1} air. Then, two derived parameters were calculated: intrinsic water-use efficiency (*iWUE*) in µmol CO_2 $mmol^{-1}$ H_2O (Vadez et al., 2014), using the formula $iWUE = A/E$; and apparent carboxylation efficiency (*ACE*) in mol CO_2 m^{-2} s^{-1} (Monteiro & Prado, 2006), by $ACE = A/Ci$.

Leaves from both accessions were evaluated by the chlorophyll fluorescence technique, using the Mini-PAM photosynthesis yield analyzer (Heinz Walz GmbH, Effeltrich, Germany). The equipment was set to provide an initial fluorescence light at a frequency of 0.6 kHz and an intensity level of 2, with the amplification factor of the detector electronic signal fixed at 1 and the saturation pulse light at level 12. Part of the analyzed leaf was kept in the dark for at least 30 min, with the aid of the DLC-8 leaf clip (Heinz Walz GmbH, Effeltrich, Germany). The end of the fluorescence probe was combined with the clip attached to the leaf, and, then, the obturator of the leaf clip was opened to allow the exposure of the part of the leaf to be assessed. After 2 s, enough time for the natural fluorescence signal (without the influence of actinic light) to stabilize, the start button of the equipment was pressed, triggering the routine measurements of maximum quantum yield of photosystem II (Fv per Fm) using the WinControl, version 2.08, software (Heinz Walz GmbH, Effeltrich, Germany); the value of the initial fluorescence in the dark (Fo), generated by the initial light measurement, was recorded routinely. The used equipment also triggers a pulse of saturating light and records the maximum fluorescence in the dark (Fm). From these two variables, the internal algorithm present in the WinControl software automatically calculates the value of Fv per Fm, according to the equation $FV/FM = (Fm - F_o)/Fm$.

Data on gas exchange and chlorophyll fluorescence parameters were collected throughout the stress period and up to 48 hours after the return to normal conditions. The newly expanded leaf was chosen for these measurements and used throughout the experiment. The obtained data were subjected to Shapiro-Wilk's and Cochran's t-tests for normality of errors and homogeneity of variances, respectively. Once the basic requirements were satisfied, the analysis of variance was performed and, in significant cases, the regression analysis or comparison of means was carried out by Scott-Knott's test, at 5% probability, using the SISVAR, version 5.6, software (Ferreira, 2011).

Results and Discussion

In both accessions of *S. viridis* subjected gradually to cold stress, the gas exchange rates – *A*, *gs*, and *E* – gradually reduced with decreasing air temperature until reaching insignificant values at 0°C (Figure 1). The reduction in these variables maintained a certain proportion at least up to 15°C as they depend on or are derived from other variables – *Ci*, *iWUE*, and *ACE* – that underwent small changes up to this temperature.

Ci values increased in both accessions at 10°C or lower. Since the intercellular CO_2 concentration depends on stomatal conductance and on its use in photosynthesis, this means that the non-stomatal limitation phase was strong, causing the observed increase (Brodribb, 1996). Of the derived parameters, *iWUE* and *ACE* remained practically constant until 5 and 15°C, respectively. However, the *ACE* values decreased drastically when temperature was reduced

to 10°C, which suggests a limitation of the mesophyll to CO_2 assimilation (Monteiro & Prado, 2006). Overall, *iWUE* only decreased at 0°C, except in the reproductive stage of Ast-1 when it dropped linearly from 15°C. This latter parameter can be defined as the ratio of the instantaneous rates of CO_2 assimilation and transpiration at the stomata, showing that a greater yield per unit water is one of the greatest challenges in agriculture (Condon et al., 2002).

Considering the results obtained for *ACE*, at 10°C, the functioning of the enzyme complexes linked to CO_2 assimilation/reduction, represented by *A*, was more impaired than that of *gs*, which led to an increase in *Ci*. For *iWUE*, the decrease in *A* and *E* remained proportional when temperature dropped to 5°C; in this case, as previously mentioned, the exception occurred in the reproductive stage of Ast-1, when the values of this parameter decreased from 15°C. For both studied accessions, however, at 5°C, the drop in *A* was more pronounced than that in *E*. Therefore, plant stomatal conductance underwent adjustments according to the reduction in air temperature, but was not a priori the main responsible for the drastic decrease in *A*.

Plant photosynthetic rates are closely associated with transpiration, which is mainly regulated by stomata, acting as a barrier to water loss (McAdam & Broddribb,

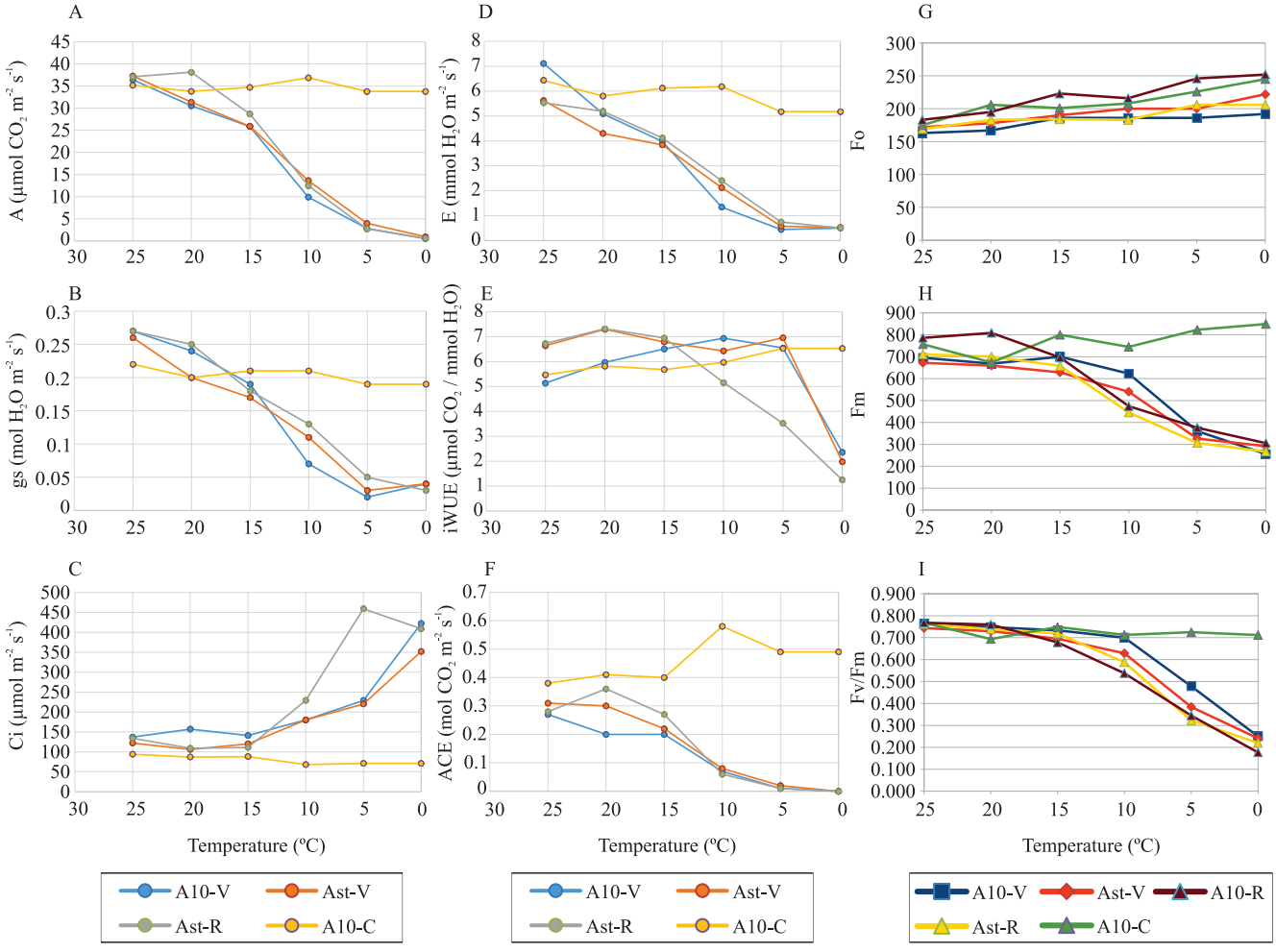

Figure 1. Gas exchange rates and derived parameters, as well as chlorophyll fluorescence parameters, for leaves of *Setaria viridis* plants grown at 25°C or subjected to cold stress applied gradually. A, net CO_2 assimilation rate (*A*); B, stomatal conductance (*gs*); C, intercellular concentration of CO_2 (*Ci*); D, transpiration (*E*); E, intrinsic water-use efficiency (*iWUE*); F, apparent efficiency of carboxylation (*ACE*); G, initial fluorescence in the dark (Fo); H, maximum fluorescence in the dark (Fm); and I, maximum quantum yield of photosystem II (Fv/Fm) in the vegetative (V) and reproductive (R) growth stages. The values represent the average of ten replicates. C, control.

2014). However, stomata closure may limit the entry of CO_2, reducing the availability of the essential substrate for the carbon reduction cycle (Casson & Gray, 2008). In addition, when stomatal conductance is reduced, transpiration and photosynthesis rates also decrease (Casson & Gray, 2008; McAdam & Brodribb, 2014). According to Wilkinson et al. (2001), the plant's ability to close stomata under low temperatures is related to cold tolerance. Under cold conditions, plants absorb less water, which can culminate in wilting if they do not have the mechanisms to avoid it (Centeno et al., 2018). In response to temperature drop, cold-tolerant C_4 grasses close the stomata, reducing transpiration and water loss, while sensitive plants have difficulties in controlling stomatal opening and, consequently, in regulating transpiration rates (McWilliam et al., 1982; Naidu & Long, 2004). However, the reduction in net photosynthesis rates at low temperatures does not necessarily mean intolerance to cold. For example, the photosystems of the C_4 grass *Bouteloua gracilis* (Kunth) Lag. ex Griffiths are not affected by the severe drop in photosynthesis at low temperatures, maintaining the integrity of cellular structures (Pittermann & Sage, 2000).

In the assessed *S. viridis* plants, the reduction in gas exchange rates associated with temperature drop, at least up to 15°C, seems to be related to a reduction in stomatal opening since stomatal conductance was reduced in the same proportion. The increase in the internal concentration of CO_2 at lower temperatures is an indicative of problems with the assimilation of CO_2. Indeed, the limitation of D-ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity in leaf tissues leads to low photosynthetic rates in C_4 grasses under cold stress (Pittermann & Sage, 2000).

In tropical grasses, the optimum temperature for Rubisco activity is around 30°C; however, in temperate grasses, it is close to 20°C (Hermida-Carrera et al., 2016). Therefore, it is expected that C_4 grasses of temperate climates, such as *S. viridis*, show a reduction in carbon assimilation at temperatures below 20°C. As temperature drops, a fall in Rubisco activity in the carbon reduction cycle, followed by a decline in photosynthesis, is also expected (Treharne & Cooper, 1969). Despite this, cold-tolerant species can maintain gas exchange under this condition due to a tight control over stomatal opening to keep CO_2 fixation at suboptimal temperatures (Naidu & Long, 2004).

In the present work, when the temperature dropped to 15°C, there were reductions of 14% in stomatal conductance, 22% in net CO_2 assimilation rate, and 32% in apparent carboxylation efficiency, without a significant variation in intercellular CO_2 concentration. Below 0°C, the drop was of 67, 52, and 87% in the rates of net CO_2 assimilation, stomatal conductance, and apparent carboxylation efficiency, respectively. For *Miscanthus* x *giganteus*, a cold-tolerant hybrid [*Miscanthus sinensis* Andersson × *Miscanthus sacchariflorus* (Maxim.) Hack.], grown at 14/10°C day/night, Naidu & Long (2004) reported reductions of 40% in stomatal conductance, 31% in net CO_2 assimilation rate, 19% in apparent carboxylation efficiency, and only 1% in internal CO_2 concentration. Under the same conditions, for corn (*Zea mays* L.), a species sensitive to cold, the authors observed reductions of 81, 79, and 54% in the rates of net CO_2 assimilation, stomatal conductance, and apparent carboxylation efficiency, respectively. Despite the differences in the cold stress treatments used in the present study and in that of Naidu & Long (2004), the plants evaluated here maintained gas exchange rates similar to those of miscanthus grass and higher than those of corn at 15°C, with a more significant drop only at ≤10°C.

In Fo, an increase was observed as temperature dropped (Figure 1). By convention, as the fluorescence emission by photosystem II (PSII) overlaps that of photosystem I (PSI), the Fo measurement is associated with the structural and functional integrity of the reaction centers of PSII (Campostrini, 1997; Pospísil et al., 1998; Bertamini et al., 2007). Moreover, since Fo is emitted by the chlorophylls that are part of the thylakoid membrane system, originating from PSI and PSII, the increase in this parameter can signal changes in these membranes due to low temperatures (Mishra et al., 2011) and, consequently, in the reaction centers of PSII. In normal conditions, the measurement of Fo does not change in plant leaves, but, under cold stress, there may be alterations in the structure of the membranes where the photosynthetic pigments anchor, affecting their functionality (Zhu et al., 2018). This shows that the chlorophyll fluorescence technique can be applied for the characterization of cold tolerance in plants (Adams & Perkins, 1993). In the case of *Setaria* spp., the increase in Fo may also be associated with leaf age and not only with cold stress, since the control

plants showed an increase in this parameter similar to that of the stressed plants.

In Fm and Fv/Fm, however, a reduction was detected as temperature decreased below 10°C. The drop in Fm in plant leaves as temperature decreases is likely associated with an increase in the rate of chlorophyll breakdown, which exceeds the synthesis rate, as commonly found for plants subjected to cold stress (Pospísil et al., 1998; Mishra et al., 2011). This decrease in Fm and the increase in Fo led to a consequent decrease in Fv/Fm, meaning that the ability to absorb light and transfer electrons in the Z scheme of photosynthesis became impaired (Rizza et al., 2001; Mishra et al., 2011; Perboni et al., 2015).

Naidu & Long (2004) used the chlorophyll fluorescence technique to evaluate the cold tolerance of two C_4 species. According to these authors, the low values for the effective quantum yield of PSII signals photoinhibition, which is characterized by an increase in Fo and, consequently, damage to the reaction centers of the system. In the present study, the photoinhibition due to cold stress was probably milder since the increase in Fo was small; however, it is necessary consider the difference in chlorophyll content between stressed and control plants. In any case, the sharp drop in Fv/Fm (Figure 1) occurred mainly due to the decrease in Fm and not to the increase in Fo. Therefore, these results are more of an indicator of a compromise of the light capture antenna due to the breakdown of chlorophyll.

The gas exchange rates of the leaves of accessions A10.1, in the vegetative growth stage, and Ast-1, in the vegetative and reproductive growth stage, subjected gradually or abruptly to 0°C for 3 days, were recovered in 48 hours after temperature was increased to 25°C (Figure 2). Accession A10.1, in the reproductive growth stage, also managed to recover after being kept at 0°C

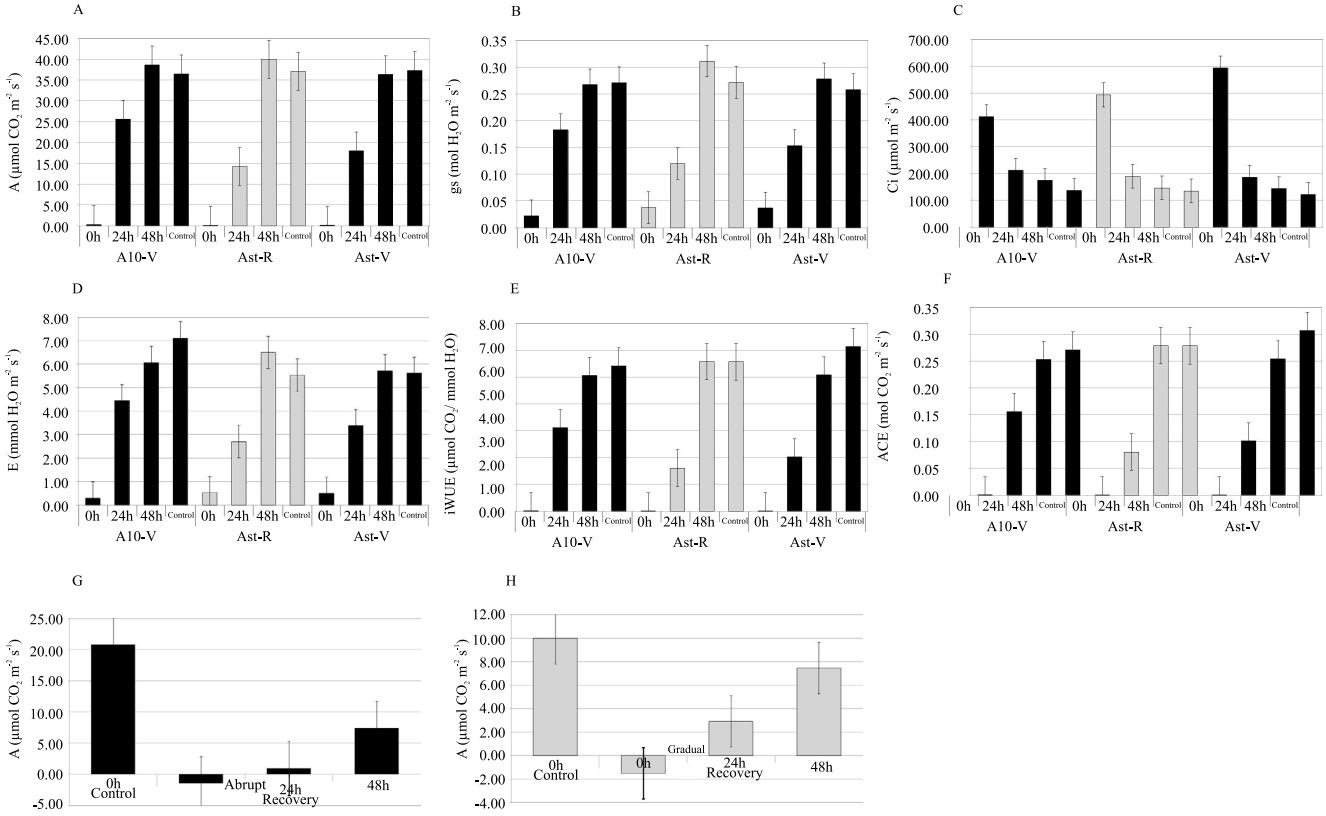

Figure 2. Gas exchange variables and derived parameters in leaves of the A10.1 and Ast-1 *Setaria viridis* accessions, in the vegetative (V) and reproductive (R) growth stages, after cold stress applied gradually. A, net CO_2 assimilation rate (*A*); B, intercellular concentration of CO_2 (*Ci*); C, transpiration (*E*); D, stomatal conductance (*gs*); E, intrinsic water-use efficiency (*iWUE*); F, apparent carboxylation efficiency (*ACE*); and G and H, net CO_2 assimilation rate (A) in leaves of accession A10.1 in the reproductive growth stage during and after subjected to cold stress applied gradually or abruptly. The values represent the average of five or ten replicates, and the bars represent the standard error of the mean.

for 5 days, regardless of being subjected gradually or abruptly to 0°C. However, under 0°C for 10 days, there was no recovery of the measuring leaf, meaning that the most lasting stress caused irreversible damage considering the gas exchange rates.

The chlorophyll fluorescence variables – Fm and Fv/Fm – affected by gradual cold recovered when subjected to 0°C for 3 days 48 hours after temperature was increased to 25°C (Figure 3). However, for plants from accession A10.1, subjected to 0°C gradually or

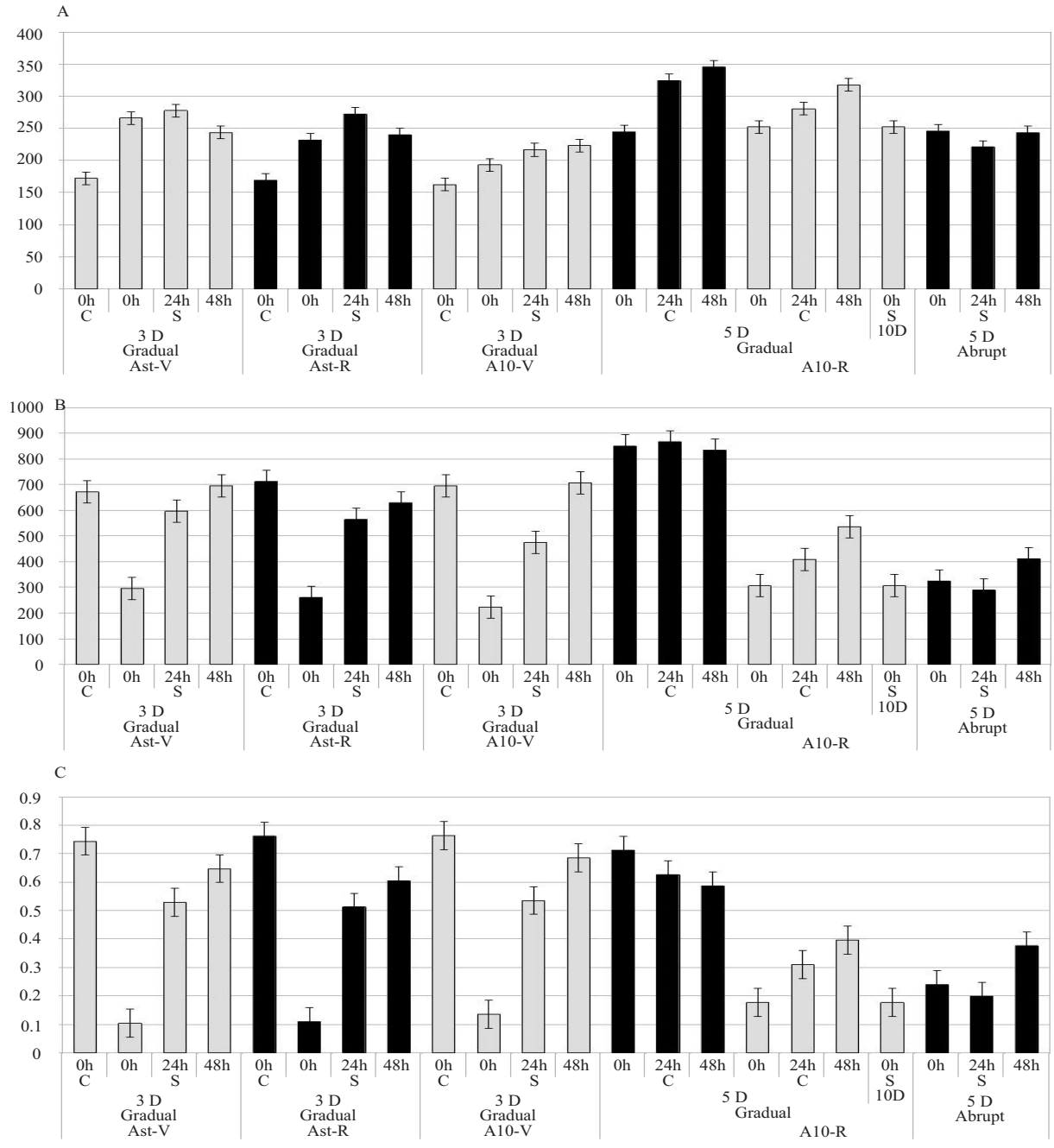

Figure 3. Chlorophyll fluorescence parameters in leaves of the A10.1 and Ast-1 *Setaria viridis* accessions, in the vegetative (V) and reproductive (R) growth stages, during and after cold stress applied gradually or abruptly. A, initial fluorescence in the dark (Fo); B, maximum fluorescence in the dark (Fm); and C, maximum quantum yield of photosystem II (Fv/Fm). C, control; and S, stressed. The values represent the average of five or ten replicates, and the bars represent the standard error of the mean.

abruptly, for 5 or 10 days, in the reproductive growth stage, none of the variables returned to the control levels. Therefore, the obtained data are indicative that there was irreversible photochemical damage to the leaf, which preceded any biochemical change. Since the same leaf per plant was evaluated during the periods of stress and recovery, it can be concluded there was a combined effect of stress and leaf age.

In the literature, the chlorophyll fluorescence technique has been used to assess responses to cold stress in other plant species. In canola (*Brassica napus* L.), for example, after a certain period, the same level of quantum yield was found for plants under the cold and control treatments (Perboni et al., 2015). However, in oat (*Avena sativa* L.) plants subjected to cold stress, there was a lower drop in Fv/Fm and a better recovery after stress in the cultivars tolerant to low temperatures (Rizza et al., 2001). In this case, the more severe the stress, the higher the negative impact on the recovery of the chlorophyll fluorescence variables.

Since the effects of cold stress on the leaf gas exchange and chlorophyll fluorescence parameters did not differ between the two evaluated accessions, the effects on biomass and inflorescence were only characterized for accession A10.1 (Table 1). Independently of the development stage, cold stress at 0°C for 5 days reduced plant height and inflorescence dry weight. However, in the vegetative stage, the number of inflorescences increased and the average length of inflorescences decreased, whereas, in the reproductive stage, the dry weight of the whole plant decreased. Therefore, cold stress was more harmful to plants in the reproductive stage.

In this stage, the analyzed variables were not affected by the form of stress application, i.e., gradual or abrupt (Figure 4 and Figure 5). However, there was a significant effect of the increase of the duration of stress from 5 to 10 days, when these variables did not recover.

In general, plants kept under low temperatures show a small growth since cold stress reduces their metabolic activity and impairs cell division and elongation (Nievola et al., 2017). As a result, the plant accumulates less biomass and is reduced in size (Aghaee et al., 2011; Mertz et al., 2009). However, if cold stress is applied only in the vegetative stage, the plant can recover, as observed for *S. viridis* in the present study.

Under cold stress, the biomass of the *S. viridis* plants did not show a significant reduction in the vegetative growth stage, but reduced in 25% in the reproductive stage. When evaluating a tolerant and a sensitive rice (*Oryza sativa* L.) cultivar at 15/10°C day/night during two weeks, Aghaee et al. (2011) found losses in the shoot and root biomass of 46.71 and 36.74% and of

Table 1. Biomass production and inflorescence variables of the A10.1 *Setaria viridis* plant accession subjected to cold stress in the vegetative (V) and reproductive (R) growth stages[(1)].

Variable	Treatment	A10-V	A10-R
Number of inflorescences	Control	14.70b	14.70a
	Stressed	19.90a	13.60a
Shoot size (cm)	Control	59.13a	38.13a
	Stressed	45.60b	22.14b
Average length of the inflorescence (cm)	Control	3.86a	3.58a
	Stressed	3.36b	4.00a
Inflorescence dry weight (g)	Control	3.31a	1.88a
	Stressed	2.48b	1.08b
Shoot dry weight (g)	Control	3.02a	3.02a
	Stressed	3.10a	2.48a
Root dry weight (g)	Control	1.71a	1.76a
	Stressed	1.56a	1.41a
Plant dry weight (g)	Control	8.05a	6.67a
	Stressed	7.15a	4.98b

[(1)]Means followed by equal letters, in the columns, do not differ by Scott-Knott's test, at 5% probability.

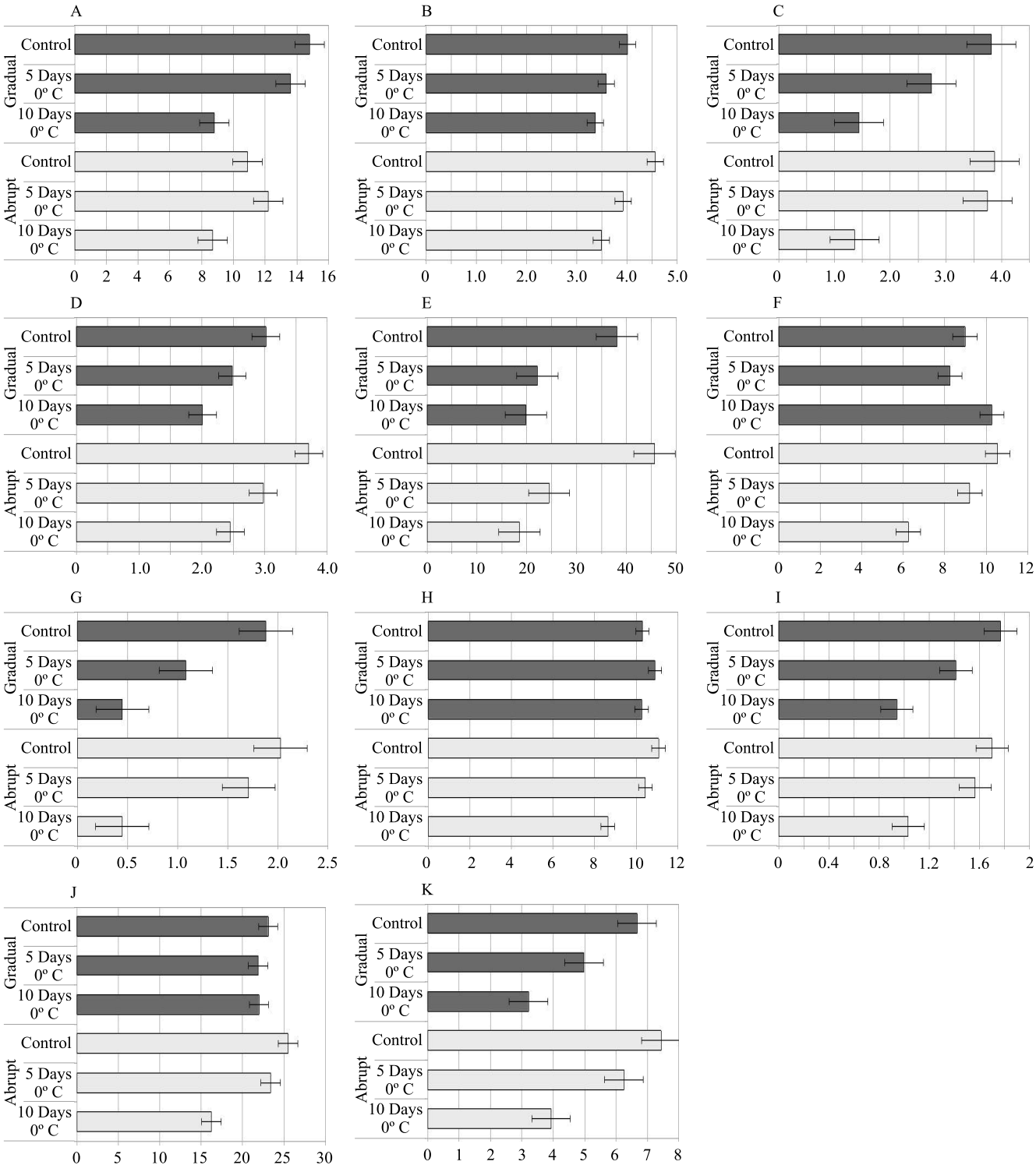

Figure 4. Biomass yield parameters of the A10.1 *Setaria viridis* accession at the end of the plant life cycle and after the gradual or abrupt application of cold stress in the reproductive stage. A, number of inflorescences; B, size of inflorescences (cm); C, inflorescence fresh weight (g); D, inflorescence dry weight (g); E, size of the main stem (cm); F, shoot fresh weight (g); G, shoot dry weight (g); H, root fresh weight (g); I, root dry weight (g); J, plant fresh weight (g); and K, plant dry weight (g). The values represent the average of five or ten replicates, and the bars represent the standard error of the mean.

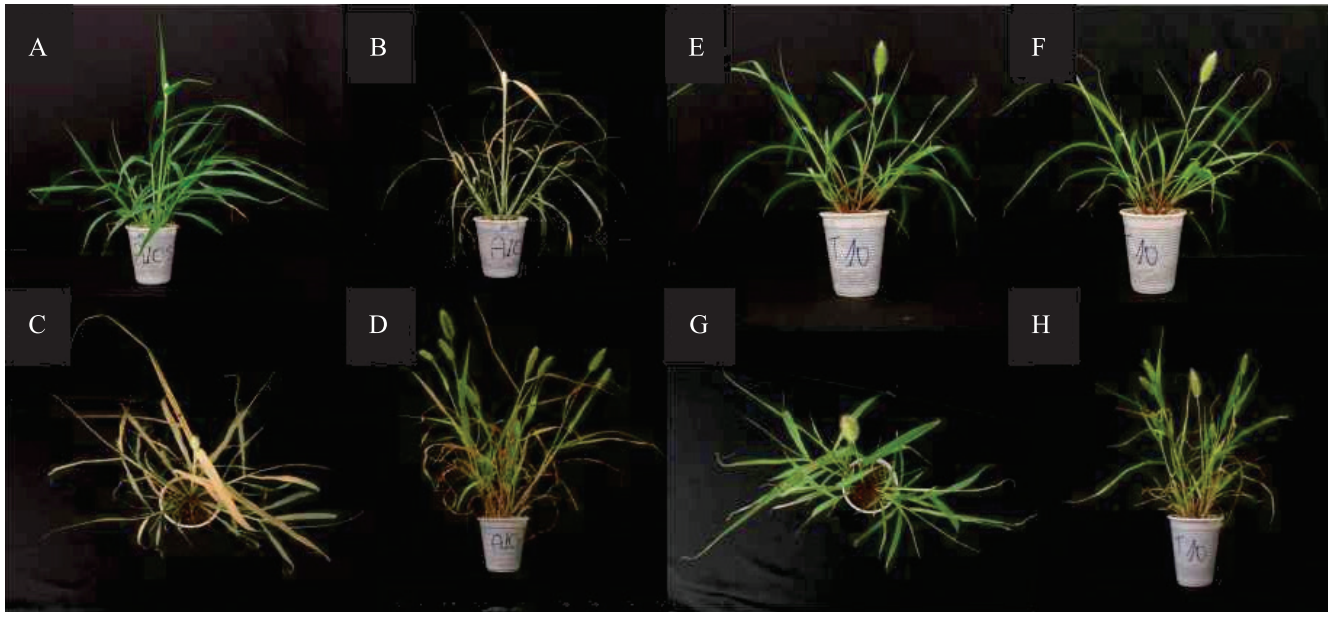

Figure 5. Reproductive growth stage of a *Setaria viridis* plant before (A and E) and after cold stress for 10 days (B, C, F, and G) and at the end of the plant life cycle after recovery (D and H). Photos by Calil Gibran Iraiore Carvalho.

72.58 and 78.01%, respectively. Therefore, tolerant plants have a lower shoot/root ratio, suggesting the existence of a mechanism that provides a better organ growth adjustment under stress. The same authors reported a respective shoot/root ratio of 3.47 and 7.48 for the tolerant and sensitive cultivars. These values are higher than those of 1.19 and 1.75 found, in the present study, for the shoot/root ratio under cold stress in the vegetative and reproductive growth stages, respectively, which is an indicative that *S. viridis* maintained a good shoot/root ratio regardless of the growth stage in which cold stress was applied.

Conclusions

1. The A10.1 and Ast-1 *Setaria viridis* accessions are cold tolerant and, therefore, are not suitable as a model plant in studies to validate candidate genes for cold tolerance.

2. The A10.1 and Ast-1 accessions are tolerant to cold stress, regardless of whether applied gradually or abruptly.

3. Accessions A10.1 and Ast-1 recover from cold stress even when plants are damaged.

4. Accessions A10.1 and Ast-1 recover with difficulty when cold stress is applied in the reproductive stage or for a longer duration.

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