Alma Mater Studiorum – Università di Bologna

Scienze e Tecnologie agrarie, ambientali e alimentari

XXVI ciclo

Settore Concorsuale di afferenza: 07/B1

Settore Scientifico Disciplinate: AGR/02

Evaluation of the photosynthetic efficiency of sweet sorghum under drought and cold conditions

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Esame Finale anno 2014

List of Abbreviations

$A = P_n$= Net CO2 assimilation rate

ANOVA: Analysis of variance

C: total leaf carbon content

CE: carboxylation efficiency of PEPcase

Chl.: Chlorophyll

D1: 3rd leaf stage

D2: Growing point differentiation stage

D3: booting stage

D4: Half-bloom stage

N: total leaf nitrogen content

NPQ: Non photochemical quenching

PAR: Photosynthetic active radiation

PEPcase: Phosphoenolpyruvate carboxylase

PPDK: Pyruvate phosphate dikinase

PSI and PSII: Photosystem I and II

ROS: Reactive oxygen species

RWC: Relative water content

g_s: stomatal conductance

GDD: Growing degree days

Ls: stomatal limitation

MET: Mean emergence time

SWP: Soil water potential

T_0: before chilling stress imposition

T_{48}: 48 hours of chilling exposure

T_{96}: 96 hour of chilling exposure

T_{RW48}: 48 hours of re-warming

Abstract

Sweet sorghum, a C_4 crop of tropical origin, is gaining momentum as a multipurpose feedstock to tackle the growing environmental, food and energy security demands. Under temperate climates sweet sorghum is considered as a potential bioethanol feedstock, however, being a relatively new crop in such areas its physiological and metabolic adaptability has to be evaluated; especially to the more frequent and severe drought spells occurring throughout the growing season and to the cold temperatures during the establishment period of the crop.

The objective of this thesis was to evaluate some adaptive photosynthetic traits of sweet sorghum to drought and cold stress, both under field and controlled conditions. To meet such goal, a series of experiments were carried out. A new cold-tolerant sweet sorghum genotype was sown in rhizotrons of 1 m^3 in order to evaluate its tolerance to progressive drought until plant death at young and mature stages. Young plants were able to retain high photosynthetic rate for 10 days longer than mature plants. Such response was associated to the efficient PSII down-regulation capacity mediated by light energy dissipation, closure of reaction centers (JIP-test parameters), and accumulation of glucose and sucrose. On the other hand, when sweet sorghum plants went into blooming stage, neither energy dissipation nor sugar accumulation counteracted the negative effect of drought. Two hybrids with contrastable cold tolerance, selected from an early sowing field trial were subjected to chilling temperatures under controlled growth conditions to evaluate in deep their physiological and metabolic cold adaptation mechanisms. The hybrid which poorly performed under field conditions (ICSSH31), showed earlier metabolic changes (Chl a + b, xanthophyll cycle) and greater inhibition of enzymatic activity (Rubisco and PEPcase activity) than the cold tolerant hybrid (Bulldozer). Important insights on the potential adaptability of sweet sorghum to temperate climates are given.

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General Introduction

Sweet sorghum [*Sorghum bicolo*r (L.) Moench], a C_4 crop of tropical origin, is considered as a multi-purpose crop with a high potential as bioenergy feedstock, because it is a source of either fermentable free sugars from the extracted stem's juice or lignocellulosic material from the whole plant biomass. It has also the potential to produce food and feed in various combinations. Moreover, compared to other bioenergy crops such as sugarcane and maize, sweet sorghum has several advantages for example high water, nitrogen and radiation use efficiency; broad agro-ecological adaptation; adaptability to most soil types; rich genetic diversity for breeding improvements, low input requirements, excellent forage quality, and high productivity.

Recently the interest in cultivating sweet sorghum has been renewed across the world mainly because the need to meet the targeted Renewal Energy Policies and comply with environmental issues and climate change international agreements. That is also true in temperate climates such as those of Europe. Therefore, the expansion of sweet sorghum into such areas not only will require to fulfill with the stated regulations, but also will require to cope with the prevailing environmental stresses. Frequent and severe drought stress periods is one the consequences of climate change leading to reduced plant productivity (Farooq el al. 2009). Moreover, in temperate climates, characterized by cold rainy winters and dry summer, drought spells have been intensified lately (IPCC, 2007). Another important environmental stress that sweet sorghum will face in temperate climates is the cold temperatures at the establishment period. Therefore the cultivation of sweet sorghum in temperate climates would need to put in place several actions to tackle the effects of climate change and concomitantly meet the requirements to produce energy from renewable sources. One example of such kind of actions is the SweetFuel project (FP7-KBBE-2008-2B), that aimed to develop bioethanol production in temperate and semiarid regions from sweet sorghum through genetic enhancement, identification

some stress related physiological traits, and improvement of cultural and harvesting practices. The present thesis was carried out within the framework of the aforementioned project.

Sweet sorghum in its natural habitat is well adapted to drought conditions. Such adaptability is mainly expressed by the activation of several anatomical, morphological and physiological mechanisms. A primary mechanisms of sweet sorghum adaptability to drought is to reduce canopy transpiration while maintaining a certain photosynthetic level even at very low soil water potential and/or leaf water potential (Tari et al 2013). Such improved water use efficiency has been related to the presence of leaf epicuticular wax that increasing the leaf reflectance and reduce transpiration and water pressure (Surwenshi et al. 2012). Moreover, the stomatal regulation capacity of sweet sorghum further contributes to avoid excessive water loss by transpiration (Massacci et al. 2006) but at the same time this has a direct effect on the diffusion of CO_2 to the chloroplasts, on the ability of leaves to dissipate the excess energy as latent heat, or increase mesophyll resistance, resulting in modifications in the photosynthetic apparatus functioning. Although the effects of drought stress on the photosynthetic processes have been widely reviewed in other species such as maize (*Zea mays*), studies regarding to the photosynthetic response to progressive soil drying out at different growth stages specifically at the photosystem II (PSII) electron transport activity are practically inexistent in sweet sorghum. Very few information exists on how long sweet sorghum can resist to progressive drought stress or what physiological defense mechanisms are used by young and aged plants. The analysis of PSII and electron transport chain may provide complementary and reliable information on drought-related mechanisms of sweet sorghum.

Another important environmental stress that sweet sorghum will have to cope with in temperate climates is the low temperatures at the crop establishment period. In general, sweet sorghum under cold temperatures (usually below 15 °C of soil temperature) shows poor stand establishment capacity and seedling vigor (Yu and Tuinstra 2001). Besides that low temperatures are thought to induce several physiological and metabolic alterations in the emerging seedlings.

Photosynthetic rates, leaf chlorophyll content, Rubisco function, ion uptake for example are some of the main physiological alteration due to cold. Then, it is presumable to assume that cold stress may predispose plant seedlings to a greater sensitivity at the photosynthetic level and thus poor plant survival. Hence, this may represent a limiting factor for sweet sorghum establishment at extreme northern latitudes. However the performance of different origin or genetic background sweet sorghum cultivars under sub-optimal temperatures has not yet been evaluated. A better understanding of the physiological mechanism by which sweet sorghum cope with harmful drought and low temperatures conditions, is essential for further breeding, agronomic or production programs in temperate climates.

The general objective of this study was to reveal some adaptive photosynthetic traits of sweet sorghum to cope with drought and cold conditions both under field and drought conditions.

In order to meet this objective, three related experiment were carried out:

- In the first study, it was analyzed the effect of progressive drought (suspension of irrigation until the physiological inactivity) on the PSII electron transport activity and the accumulation of leaf soluble sugars (glucose, fructose and sucrose) at different developmental stages. For such purpose a new sweet sorghum genotype was sown in twenty rhizotrons of 1 m^3 located at Cadriano experimental farm of Bologna University. Photosynthesis analysis was performed using infrared gas analyzer and direct chlorophyll *a* fluorescence emission (JIP-Test). It was found that at early developmental stages sweet sorghum, plants were able to efficiency down-regulate their photosynthetic apparatus by dissipative energy mechanism, in which soluble sugars accumulation (glucose, sucrose and fructose) played an active role.

- In an open field experiment, carried out at Cadriano experimental farm during the growing season 2012, the adaptability to cold of seven sweet sorghum hybrids (Bulldozer, Zerberus, Tarzan, Monster, ICSSH19, ICSSH31 and ICSSH58) was evaluated at four sowing dates (from end of March to middle of May). The objective of this experiment was to preliminary identify some physiological and growth traits which can be used as indicators of sweet sorghum resistance to cold. The results indicated that early spring sowing strongly affected the plant vigor (mean emergence time), which in turn provoked chlorophyll degradation (leaf nitrogen and carbon content in leaf) and reduction of photosynthetic capacity (PI_{ABS}) at the plant establishment. Although such metabolic and physiological impairment was observed in all the hybrids, Bulldozer showed the best performance at early sowing dates demonstrating its suitability for temperate zones.
- Based on the results of the previous study, the photosynthetic and biochemical adaptation mechanisms of the most contrasting hybrids was analyzed in detail under controlled environmental conditions. The trial was carried out in a growth chamber where the hybrids were subjected to a period of four days of chilling temperatures. The chilling treatment consisted in lowering the ambient temperature from 20°C/14°C to 9°C/5°C day/night respectively when the seedlings reached the 5^{th} leaf stage. Photosynthetic parameters (leaf gas exchange and both direct and modulated chlorophyll *a* fluorescence), along with changes in pigment compositions (xanthophyll cycle, Chl. a and b, luteine etc.) were measured. The hybrid which poorly performed under field conditions (ICSSH31), showed earlier changes at biochemical levels (Chl a + b, xanthophyll cycle) and greater inhibitions of enzymatic activity (Rubisco and PEPcase activity) than Bulldozer. However, after 48 hours of re-warming enhancement of photosynthetic activity in ICSSH31 was observed.

Chapter 1

Acclimation of photosynthesis by leaf soluble sugar accumulation of a sweet sorghum genotype under water deficit

Abstract

Drought is the major factor limiting photosynthesis of plants. If prolonged, it leads to oxidative stress by overproduction of reactive oxygen species (ROS). Among the plant physiological strategies to cope with drought, the accumulation of soluble sugar in leaves, especially in sugary crops, may play a crucial role against oxidative processes. Sweet sorghum [(*Sorghum bicolor* L.) Moench] is a widely recognized drought tolerant species; nonetheless, its physiological mechanisms to cope with drought during critical stages pre- and post-flowering) for sugar accumulation are still very uncertain.. The objective of this study was to evaluate the photosynthetic efficiency of a new sweet sorghum genotype selected for cold tolerance to progressive soil drying up at vegetative and reproductive developmental stages. The trial was setup in a total of 20 rhizotrons (1 m^3 each). Treatments were imposed at two vegetative and two reproductive stages. The leaf relative water content (RWC) was assumed as indicator of plant water stress status. Soil water potential (SWP),chlorophyll *a* fluorescence , leaf gas exchange, and leaf soluble sugars (glucose, sucrose and fructose) were analyzed at different ranges of relative water content (RWC) were measured. Under moderate drought stress (RWC between 70-89%), glucose and sucrose in vegetative plants increased significantly by 40 and 30%, respectively. Such increments were maintained up to severe drought condition was reached (RWC between 50-69%). At reproductive stage, significant accumulation of fructose (by 100%) was found at booting stage and under severe drought conditions only. A close relationship was found among glucose, sucrose, thermal energy dissipation (DI_o/CS_m), and density of active

reaction centres (RC_o/CS_m) at vegetative stages. Analogously fructose only was related to DI_o/CS_m and RC_o/CS_m at booting stages, suggesting an age-specific-compound sugar role on the photoprotection of the photosynthetic electron transport chain. In addition, the JIP-test analysis revealed that the acceptor side of PSI was more sensitive than the donor side of PSII, with exception of half bloom stage. In conclusion, the capacity of a new cold tolerant genotype to effectively down-regulate the PSII electron transport was enhanced by the accumulation of glucose and sucrose in leaves at vegetative stages. Whereas fructose, apparently plays such equivalent role when plants reach the blooming stage.

1.1. Introduction

As a result of global warming many areas of the world are expected to face severe drought with prolonged and recurring dry periods (Sheffield and Wood 2008, Dai 2013). Drought tolerant species, such as sweet sorghum [(*Sorghum bicolor* L.) Moench] can therefore be expected to play a strategic role to contrast the abandonment of agricultural lands due to adverse climatic conditions. Moreover, taking into account the short-term remarkable expansion of biofuel crops encouraged by aggressive biofuel policies, the expansion of sorghum cultivation areas to temperate climates and/or marginal lands can mitigate the land competition between food and non-food crops.

Because of its low input requirements (Zegada-Lizarazu and Monti 2012a), sweet sorghum is widely recognized as a well-adapted crop to arid and saline areas of sub-tropical and temperate regions (Almodares et al. 2011). Given the intrinsic high tolerance to drought of sweet sorghum, if properly selected cold resistant lines will be also available, sorghum could become a great opportunity for temperate environments. Sorghum has the ability to adopt several mechanisms to effectively contrast drought stress going into a sort of dormancy until rains return (Bennett et al. 1990). However, little is known on the ability of new breeds of sweet sorghum adapted to cool

temperate areas to maintain its drought tolerance capacity. Moreover, their photosynthetic response to progressive soil drying out is practically unknown. Furthermore, drought adaptation mechanisms may differ in new genotypes that are currently being developed for cool temperate climates such as those of northern or central Europe. Significant advancements in breeding sweet sorghum cultivars/hybrids adapted to cool temperate climates have been achieved in the framework of the SweetFuel project (www.sweetfuel-project.eu); nonetheless, such new breed lines are being developed to stand cold stress only, therefore, before introducing them to the market, their capacity to endure the increasingly frequent dry spells that occur along the growing season (e.g. young and/or mature stages) should be better understood.

The way in which drought stress influences the primary process of CO_2 fixation in C_4 plants is a complex matter (Ghannoum 2009). In general, non-stomatal (biochemical) factors become more important than stomatal limitation under severe (RWC below 70%) or prolonged drought conditions (Lawlor 2002), as well as when other stresses such as heat or high light are superimposed (Cornic 2000, Ripley et al. 2007, Takahashi and Murata 2008). The prevailing stomatal or biochemical limitations depend also on plant age, intensity and velocity at which the drying up process occurs (Saccardy et al. 1996) due to, for example, break down of chlorophyll content (Bennett et al. 1990, Younis 2000). Young grain sorghum plants showed a quicker stomatal closure than mature plants after drought stress imposition (Garrity et al. 1983, Al-Hamdanil et al. 1991). In turn, such limitations reduced the effectiveness of the chloroplasts to utilize the absorbed energy, which ultimately may inhibit the electron transport (Ott et al. 1999) within the thylakoid membranes. At the same time, the decreased CO_2 diffusion to the chloroplast may lower the ability of leaves to dissipate the excess energy as latent heat (Srivastava and Strasser 1997). This energy unbalance force the plants to activate several defense mechanisms such as, among others, the adjustment of light-harvesting antenna size (Govindjee 2000), thermal dissipation by xanthophylls cycle (Choudury and Behera 2001, Xiong et al.

2012), and cycling electrons transport around PSI (Heber and Walker 1992). Moreover, although it is generally accepted the adaptive role that carbohydrate accumulation plays on the osmotic adjustment to maintain cell turgor under drought conditions (Hare and Staden 1998), there is some evidence that soluble sugars, especially sucrose and glucose, play an antagonistic role with ROS as protective solutes against photodamage of PSI and PSII (Hare and Staden 1998, Coueé 2006, Ende and Valludru 2008, Keunen et al. 2013). Studies on antioxidant activity of soluble sugars are rapidly evolving. For example, Peshev and Van den Ende (2013) and Keunen et al. (2013) reported that soluble sugars, especially those interacting with membranes, can act as true ROS scavengers in plants; Rajagopal and Carpentier (2003) found that co-solutes such as glicinebetaine and sucrose protect the chlorophyll-protein complexes against photodamage of PSI submembrane particles .

In sweet sorghum, down regulation of photosynthesis has been found to play a major role as a protective mechanism at young stages under either short or long drought stress periods (Zegada-Lizarazu and Monti 2012b). It has been speculated that non structural sugars accumulation in the stems could contribute to prevent permanent photo-oxidative destruction of the PSII reaction centers. However, we are far from being certain on the relationship of soluble sugars accumulation in the leaves and the capacity of young or mature sweet sorghum plants to endure PSI and PSII activity upon soil drying up till prohibitive conditions for the growth. In general, PSII is considered as one of the most drought-sensitive components of the photosynthetic apparatus (Havaux and Strasser 1992), so it may be a valuable early indicator of plant stress status. A recent study on tree species showed that at severe drought conditions (e.g. terminal drought stages) PSI could show even earlier signs of damage (Huang et al. 2013). It is not known, however, whether the same mechanism occurs in sweet sorghum subjected to a rapidly developing drought stress. In sweet sorghum PSI was found to be more tolerant to heat than PSII (Yan et al. 2013). Moreover, the variable velocity of water stress evolution, due to large morpho-

physiological differences along specific growth stages and the age dependent sugar accumulation, especially in sugary crops such as sweet sorghum, may result in different photosynthetic responses. Therefore, the analysis of PSII, PSI, electron transport chain and soluble sugar accumulation may provide complementary and reliable information on drought-related mechanisms of sweet sorghum.

A valuable strategy for assessing the photochemical changes induced by a progressive drying up is through monitoring the chlorophyll-a (Chl. *a*) fluorescence. Direct Chl. a fluorescence and the JIP-test (Strasser et al. 1995, Stirbet and Govindjee 2011) have been used for many years to estimate the PSII activity trough the analysis of the O-J-I-P Kautsky curve (Misra et al. 2001, Mehta et al. 2010, Redillas et al. 2011), but only recently to evaluate the PSI content (Ceppi et al. 2012). Several studies (Schansker et al. 2003, Oukarroum et al. 2009, Ceppi et al. 2012) demonstrated that the initial O-J and the final I-P phases of the Kautsky curve, which indicate, respectively, the reduction of the acceptor side of the PSII and the re-reduction of plastocyanin and P700+ in the PSI, can be used to indirectly evaluate the damages of progressive drought stress at PSII and PSI levels. Then such information may be used as an indicator of the relative changes caused by prolonged drying out periods on sweet sorghum photosynthetic efficiency.

Improving the knowledge of the physiological and metabolic traits of sweet sorghum under progressive drought stress conditions, through easily measurable parameters, and the sequence in which they develop up to lethal or inactivating levels, could provide insights or serve as supporting parameters in breeding and crop managements programs for improving drought resistance and productivity of new sweet sorghum cultivars developed for colder temperate climates. Therefore, the objective of this study was to characterize the photosynthetic response of an early season cold adapted sweet sorghum genotype to progressively increasing drought stress at young and mature stages. Moreover, being sweet sorghum a sugary crop we speculate that the developmental-specific increment of soluble sugar components, as a response of drought, may

act as a photoprotective mechanism against marked alterations in the flow of electrons towards PSI and PSII.

1.2. Materials and Methods

1.2.1. Experimental set up

The experiment was carried out at the experimental farm of Bologna University (44°33'N, 11°21'E, 33 m a.s.l) from May 16 to September 7, 2012. The trial set up was similar to that used by Zegada-Lizarazu et al. (2012b). In brief, twenty 1-m^3 rhizotrons where arranged in two parallel lines under a prefabricated structure sheltered by a transparent polyethylene film (thickness 0.075 mm; 90% of maximum transmission of PAR). The rhizotrons were filled with sandy loam soil (pH of 7.9) with 1.9 ‰ of total N (Dumas), 9 mg kg^{-1} of assimilable P (Olsen), 108 mg kg^{-1} of exch. K (M.13.5 DM 13-9-99), and 1.27% of soil organic matter (Walkley-Black). Before sowing, 46 and 22 kg ha^{-1} of N and P, respectively, were applied in each rhizotron. During the experimental period, max and min air temperatures were 32 ± 5 and 17 ± 3.5 °C, respectively, while RH was 60 ± 13% (iMeteos, Pessl Instrument).

1.2.2. Plant material and drought treatments

Sweet sorghum (cv. ZN8M-50003/002) was seeded at a plant density of 12 plants/rhizotron on 16th May 2012. Each rhizotron was equipped with a drip irrigation system having self-regulated emitters (1.1 l h^{-1}) 0.2 m spaced. Water stress (interruption of irrigation until plant death) was imposed at 4 specific developmental stages: D1, 3rd visible leaf (leaf collar completely developed); D2, growing point of differentiation (7th leaf collar visible); D3, booting (head extended into flag leaf sheath); and D4, half-bloom. Stressed plants were compared with unstressed (field capacity of 25% v/v; $\Psi = -36$ KPa) control plants (C). The experimental layout was a complete randomized design with four replications (20 rhizotrons in total). Before

stopping irrigation, plants were irrigated every 2-3 days to field capacity. Soil water status was kept monitored (readings every 6 h) through soil moisture probes (EC-5 Decagon Devices, USA) and automatic data loggers (Em5b, Decagon Devices, USA) placed at 0.2, 0.4 and 0.6 m soil depth in each rhizotron, coupled .

1.2.3. Leaf gas exchange, RWC, and leaf soluble sugars

Net CO2 assimilation rate (Pn), stomatal conductance (g_s) was measured through a portable gas analyzer (CIRAS-2, PP Systems) on four plants per treatment every other day starting from the suspension of irrigation until plant physiological inactivity. We considered a plant as died when Pn was approximately zero for three consecutive measurements. Plants were thereafter irrigated and inspected (e.g. production of new leaves, tillers etc.) to confirm their death. During measurements, the environmental conditions inside the cuvette were 25 ± 1 °C, 1500 μmolm^{-2} s^{-1} PPFD, and 380 μmol mol^{-1} of CO_2. Leaf gas exchange was always taken at the same time (between 10 to 11 am) on the youngest fully expanded leaf, near the middle part of the leaf blade on its adaxial face.

The relative water content (RWC) was determined as given by Smart and Binghman (1974) on 4 leaf discs per treatment (1.8 cm ø) in the youngest fully expanded leaves. Leaf discs were collected weekly from the beginning of treatment. RWC was assumed as indicator of water stress level: unstressed plants, 90-100% RWC (RWC_{90-100}); mild stress, 70-89% RWC (RWC_{70-89}); severe stress, 50-69% RWC (RWC_{50-69}).

It must be noted that, in this study all the parameters were normalized to the C plants and care was taken by controlling that g_s were always above 150 μmol m^{-2} s^{-1} as recommended by Flexas and Medrano (2003) for control plants.

The leaf soluble sugars (glucose, sucrose and fructose) content was also determined at weekly intervals by high-performance liquid chromatography (HPLC, Rezex RPM-Monosacaride (300 x 78 mm), and Pb in ionic form, thermostated at 75ºC). Sugars were quantified prior calibration

curve obtained by commercial standard solutions (D(+)Glucose anhydrous, fructose pure and sucrose.

Chlorophyll *a* fluorescence emission (Chl *a*) was measured with a high time resolution fluorimeter (Handy PEA, Hansatech) on dark-adapted leaves (about 30 min). A total of 24 samples per treatment were taken between 10 to 11 am on the youngest fully expanded leaves, near the middle of the leaf blade on its adaxial face. The JIP-test as given by Strasser et al. (2000; 2010) was used to calculate indicative photosynthetic traits from fluorescence data (Table 1). The time marks were: F_o at 50 μs (O-step), F_J at 2ms (J-step), F_I at 30 ms (I-step) and maximum fluorescence at 300 ms (F_m or P-step).

Data were subjected to the analysis of variance (ANOVA). When ANOVA revealed significant differences among means, the post-hoc LSD Fisher's test was applied for separating the means into statistically different groups. For the amplitude analysis of O-J and I-P phase (JIP-test), the area beneath each fluorescence curve was calculated and statistically compared.

1.3. Results

1.3.1. Tolerance of young and mature plants to progressive water stress

The effects of increasing water stress on photosynthetic activity is shown in Figure 1.1. In general, the degree at which drought affected the photosynthetic capacity significantly varied between young and mature plants. After the suspension of water supply, the young plants (D1 and D2) continued to photosynthesize for 20 days, while mature plants for 15 (D3) and 10 (D4) days. Photosynthetic rates of young plants approximated zero at higher soil water potentials (-3.1 and -4.4 MPa in D1 and D2, respectively) than those of mature plants (-2.0 for both D3 and D4). In young plants, Pn and stomatal conductance (g_s) did not significantly change until six days from stress imposition (SWP of -0.09 MPa), while it dramatically and abruptly dropped thereafter (by about 50%) (Fig. 1.1). PSII activity traits (both PI_{ABS} and φPo) were ineffective to

predict P_n loss. Likewise, at D3 and D4 developmental stages, PSII efficiency remained unaltered when g_s and P_n shortly decreased 3 days from stress imposition (SWP of - 1 MPa)

1.3.2 Effect of water stress on energy flux

We used the pipeline leaf model (Strasser 2000) to represent the drought-induced changes in the phenomenological fluxes of fluorescence kinetic or apparent activities per cross section (CS) which is shown in figure 1.2. Energy flow changed through leaf cross section mainly with plant age and stress level. In young plants (D1 and D2), only a severe water stress affected energy flux (Fig. 1.2). The reduction of the electron transport per cross section (ET_o/CS_m; dark gray arrows) and the number of silent RCs (closed circles) was noticeable, while thermal energy dissipation (DI_o/CS_m; black arrows) progressively increased. In D3, DI_o/CS_m increased up to -1.3 MPa of (SWP) to dramatically decrease thereafter. The linear electron transport component (ET_o/CS_m) and the quantity of closed RCs were considerably affected already at mild water stress (Fig. 1.2). Under severe water stress the most of the components were drastically such as ABS/CS_m, TR_o/CS_m ETo/CSm and number of silence or closed, in some cases more than 70% of those of (C) plants. Initially, Reduction in pigment concentration (leaf greenness) and the light energy absortion, noted as ABS/CS_m (white arrows) were also observed in D3 at severe stress levels, when RWC was less than 60%. At D4, all derived indicators by the JIP analysis, with only exception of energy dissipation (DI_o/CS_m) that regularly decreased during the treatment, shortly declined immediately after the suspension of irrigation.

1.3.3 Effect of water stress on electron donor/acceptor sides of PSII and PSI

In the last years, the slowest phase (IP) of Chl. a fluorescence kinetic has been used as reliable indicator of the re-reduction of plastocyanin (PC)+ and P700+ in PSI (Schansker et al. 2003, Oukarroum et al. 2009), while the OJ rise is agreed to represent the reduction of Q_A to Q_A- (Strasser et al. 1995). In order to gain insight on the degree of inhibition of drought both at donor

side of PSII and the acceptor side of PSI in young and mature sweet sorghum plants we proceeded to calculated the areas below the double normalized OJ and IP curves (Fig. 1.4 and 1.5).

The amplitude of the O-J curve varied depending on the developmental stage and drought stress degree: young plants were inhibited (+7% curve amplitude) in the electron donor side of PSII only at severe water stress (RWC_{50-69}), whereas in D3 and D4 plants the PSII donor side was inhibited (significant reduction of Q_A to Q_A^-) at mild drought stress (RWC_{70-89}). With the increase of drought RWC fell below 70% and the proportion of reduced Q_A^- doubled.

I-P curve amplitude was taken for estimating the degree of electron acceptor side (PSI) inhibition that showed twice the values than those observed on the donor side of PSII in D1, D2 and D3 (Fig. 1.6). D3 showed the highest reduction of curve amplitude both at mild and severe drought. The ratio of the normalized variable fluorescence ($\Delta V_{OJ}/\Delta V_{IP}$) between O-J and I-P steps (Fig. 1.7) also showed that a greater reduction occurred in PSI electron acceptor side than in PSII electron donor side. Such reduction was greater in mature plants. The $\Delta V_{OJ}/\Delta V_{IP}$ ratio declined soon after starting treatment in D3 and D4, whereas in D1 and D2 a small initial increment was observed. Afterwards, when SWP was lower than -2 MPa, the $\Delta V_{OJ}/\Delta V_{IP}$ ratio slightly declined.

1.3.4 Effect of water stress on soluble sugars accumulation

In a previous work (Zegada-Lizarazu and Monti 2013) it was hypothesized the possible role of leaf sugar accumulation as photoprotective compounds of PSII. Here, we analyzed the increment of soluble sugar and their relationship with the capacity of down-regulation of PSII by energy dissipation (DI_o/CS_m) and reduction of active RCs (RC_o/CS_m).

The increment of leaf soluble sugar concentration (glucose, sucrose and fructose) at different developmental stages and leaf water status (RWC) is shown in figure 1.7. Glucose and sucrose concentrations increased in leaves of D1 and D2 plants by about 40% and 30%. as drought significantly increased when RWC fell below 80% (Fig. 1.7). Whereas no significant changes on

glucose and sucrose were observed at more mature stages. On the contrary, fructose accumulation occurred at severe drought stress (RWC< 70%) however, there were no significant increments of sugars at D4.

The relationship between leaf soluble sugar and the PSII down-regulation capacity of young and mature plants to the increasing drought was analysis by scatter plots (Fig. 1.8). Significant linear regression was found at young stages among sucrose, glucose, energy dissipation flux (DI_o/CS_m) and active reaction centers (RC_o/CS_m). While at mature stages, both JIP test parameter were linearly related to fructose at D3 stage. At D4 there was no relationship between sugar content and JIP test parameters.

1.4. Discussion

1.4.1 Effect of drought on net CO2 assimilation rate and PSII activity

As expected, the degree at which the photosynthetic apparatus tolerated the progressive increase of drought stress considerably changed with plant age. Young plants (D1 and D2) were able to maintain elevated assimilation rates and a high PSII electron transport efficiency under progressively increasing drought stress for about 10-15 days longer than mature plants, and until very severe water shortage conditions (up to SWP of -4.4 MPa). The ability of young plants to keep the photosynthetic apparatus functioning longer than mature plants is even more evident considering the faster soil drying rate of young plants compared to mature ones (0.19 and 0.12 MPa d^{-1}, respectively).

Interestingly, the four developmental stages (D1 to D4) showed peculiar responses to drought stress in term of energy absorption, electron transport, RC closure, and energy dissipation thus to presume a different impact on assimilation capacity of sweet sorghum depending on whether drought affects young or more mature plants.

As a typical initial response in C4 plants, stomatal closure counteracted the advancement of drought in all the developmental stages (Cornic, 1994; Fig.1.1). Unlike the more mature sweet sorghum plants, considerable early decrease of stomatal conductance (about 50%) and the consequent reduction of intercellular CO_2 by mild drought stress neither induced significant effect on the PSII electron transport efficiency (φPo, Fig.1.1), nor altered the energy flux component of the leaf cross section at D1 and D2 (Fig. 1.3). It suggests that at young sweet sorghum plants, mainly at three leaf stage, moderated drought is likely not to alter the balance between photo-induced inactivation of PSII complex and the repair mechanism such as degradation and re-synthesis of D1 protein (Prasad and Pardha Saradhi 2004). At very severe drought stress, however, young plants apparently enhance the PSII down-regulation capacity though dissipative mechanism (DI_o/CS_m and closing RCs) in which xhantophylls cycle are thought to be involved as consequence of trans-thilacoid lumen acidification (Demming-Adams and Adams 1996, Fig, 1.2 and 1.3). On the contrary, when the plants shifted from vegetative to reproductive stages (from D3 to D4), apparently such self-alleviation capacity against the deleterious oxidative stress in response to drought was inhibited, as evidenced by the continuative recuetion of DI_o/CS_m (Fig. 1.2 and 1.3). Such failure of energy dissipation capacity via no photochemical quenching (NPQ) was also observed by Dai et al. (2004) in wheat plants of 45 days after anthesis as consequences of natural senescence process. As consequence of such low efficiency to dissipate the energy as latent heat (Fig. 1.3), we speculate that in flowering sweet sorghum plants (final part of D3 and D4) the high oxidative condition induced by drought may have accelerated the leaf senescence (Rosenow and Clark 1995, Noodén et al. 1997, Buchanan-Wollaston 1997) as was noted by chlorophyll bleaching (not shown), high reduction of linear electron transport ET_o/CS_m, and antenna size (ABS/CS_m, Govindjee 2002; Munné-Boshc and Alegre 2000) as well as high proportion of closed RCs. (Fig. 1.2, Humbeck et al. 1996).

On the other hand, the scarce tolerance to drought of mature stages both at the donor side of PSII and the electron side of PSI was supported by the analysis of the double normalization of Chl *a* fluorescence from Fo (50μs) to F_J (2ms), namely the OJ phase, and from F_I (30ms) to F_M (300ms), the IP phase (Fig. 1.5 and 1.6). The greater amplitude of the IP than OJ curves suggests that the reduction of the Calvin-Benson cycle (Fig. 1.1) due to the soil desiccation had more marked effect on the efficiency of electron transport towards the PS I. This support the statement of Redillas et al. (2011), of that PS II is more drought tolerant in comparison to the photosystem I (PSI). Nowadays there is a mounting body of evidences that the inactivation of ferredoxin-NADP+-reductase is the responsible for a transient block on the acceptor side of PSI (Schansker et al 2006). Indeed, changes of the IP phase as consequence take place due to the loss of PSI proteins complex (Oukarroum et al. 2009, Ceppi et al 2012).). In fact, it is known that PSI subunits turnover is not as high as of that of D1, which renders the PSI more prone to photoinhibition under specific environmental condition (Sonoike 2011). Moreover, the differential response on the donor/acceptor side of PSII and PSI among developmental stages is clearly evidenced by the ratio $\Delta V_{OJ}/\Delta V_{IP}$ in figure 1.7. It may explained by the fact that drought-induced senescent leaves characterized by biochemical and molecular changes such as loss of the cyt b6/f complex, followed first by a decrease in PSI and PSII activity, then by a loss of ATP synthase (Guiamét et al. 2002, Rivero et al. 2007).

Although sucrose and hexose accumulation (mainly glucose and fructose) in sorghum leaves, have been associated with osmoregulation capacity, their complex metabolism has led to contradictory results. For example, it has been shown that glucose and sucrose accumulation in sorghum leaves, at moderated drought stress levels, accounted for 50% of the observed osmotic adjustment (Turner 1982). On the other hand, Fecade and Krieg (1992) indicated that the osmoprotectant capacity of non-structural carbohydrates was minimal, due to the prevalence of insoluble polymers such as starch. Our results, indicate that soluble sugars either at young and/or

mature stages, played an important role in delaying marked alterations of the flow of electrons in PSI and PSII, thereby in the production of exacerbated harmful levels of ROS (Couée et al. 2006; Fig. 1.4 and 1.8). Such findings support our previous hypothesis that the accumulation of soluble sugars in the stems of sweet sorghum can contribute to maintain an effective photosynthetic system, especially in aged plants (Zegada-Lizarazu and Monti 2012b). The present results show that under severe drought stress fructose is the major sugar involved in the photoprotection mechanisms, while at younger stages sucrose and glucose are the most important sugars for maintaining homeostasis *via* down-regulation of photosynthetic electron transport (Fig. 1.8).

A recent review presented an enlarged view of the traditional role of sugars under stress conditions introducing that sugars are directly involved in ROS quenching (Keunen et al. 2013). Moreover, in *Arabidopsis thaliana*, acclimation of photosynthesis was observed under moderate drought conditions along with a simultaneous increment of soluble carbohydrates, proline, and anthocyanin (Sperdouli and Moustakas 2012). Even though the relationship between sugars accumulation and ROS production cannot be explicitly derived from our results, the photosynthetic acclimation via glucose, sucrose, and fructose accumulation in the leaves, evidenced by the decrease in RC_o/CS_m and the increase of DI_o/CS_m (Fig. 1.8), supports the hypothesis that sugars play a crucial role as true ROS scavengers. It is well known that the inactivation of some RCs can help to protect the remaining active RCs (Jiang et al. 2008) through an increased DI_o/CS_m. The release of energy as heat is considered to occur at the time when maximum fluorescence is reached (t_{Fmax}), therefore heat dissipation is highly conditioned by F_m. The quenching of F_m is generally ascribed to photoinhibitory quenching (qI) (Force et al. 2003) rather than to xantophyll cycle-dependent thermal energy dissipation (Demmig-Adams and Adams 1992). Then, the long term down-regulation of PSII, which involve a mix of photoprotection and photodamage mechanisms may include leaf soluble sugars as important

players of PSII photoprotection. In our study was found that the retardation of the photoinhibion at OJ phase (up to RWC 70%) was closely related to glucose and sucrose accumulation at D1 and D2 stages, and to fructose at D3 only (Figs. 1.4, 1.5 and 1.8). Then, it is likely that sucrose accumulation may help to stabilize oxygen evolution and primary electron transport reaction centres as shown to occur in PSII sub-membrane particles under heat stress (Allakhverdiev et al. 1996). In contrast, glucose accumulation might have enhanced the NADPH production, which is a major cofactor of ROS scavenging pathways such as ascorbate-glutatione cycle (May 1998). Despite the proved antioxidative properties of fructose under low temperatures (Bogdanović et al. 2008), the seemingly preponderant protective role in the redox metabolism of mature plants observed in the present study needs to be further investigated.

1.5. Conclusion

In conclusion, the adaptation of photosynthesis to progressive soil drying up of a cold resistant sweet sorghum genotype was highly dependent on the developmental stage, and associated to specific soluble sugars. Young sorghum plants were able to keep functional its photosynthetic apparatus up to lower water potentials than mature plants. Such capacity to down-regulate its photosynthetic electron transport was endured by the glucose and sucrose accumulation in leaves, while fructose, which seems to play an analogous role, becomes preponderant only upon blooming. Moreover, at young stages PSI acceptor side was found to be more sensitive to drought than the PSII donor side thus revealing a likely change in the I-P phase according to JIP-test. It derives that PSI acceptor side can be used as prompt indicator to identify even moderate changes in leaf water status.

Table 1.1. List of the JIP-Test parameters and their descriptions used in the present study

Fluorescence parameters	Description
Extracted parameter from (OJIP) transient	
F_t	Fluorescence emission from a dark-adapted leaf at the time *t*
F_o = F50µs = (O-Step)	Minimum fluorescence, when all PSII reaction centers (RCs) are open or fluorescence intensity at 50µs
F_m= F300ms = (P-step)	Maximum fluorescence, when all PSII RCs are closed or fluorescence intensity at 300 ms
F_J and F_I	Fluorescence intensities at the J-step (2 ms) and at the I-step (30 ms), respectively.
t_{Fm}	Time (in ms) to reach F_m
Derived Parameters, flux ratios or quantum yield	
$\Delta V_{O-J} = (F_J - F_o) / (F_P / F_o)$	Relative variable fluorescence at the J-step (2ms)
$\Delta V_{I-P} = (F_P - F_I) / (F_P / F_o)$	Relative variable fluorescence at the I-step (30 ms)
$W_{O-J} = (F_t - F_o) / (F_J - F_o)$	Relative variable fluorescence at time t between F_o and F_J
$W_{I-P} = (F_t - F_I) / (F_P / F_I)$	Relative variable fluorescence at time t between F_I and F_m
$\varphi P_o = (F_m - F_o) / (F_m) = F_v/F_m$	Maximum quantum yield of the primary photochemistry of a dark adapted leaf. Expresses the probability that an absorbed photon will be trapped by the PSII reaction center.
$\psi ET_o = ET_o/TR_o = (1 - V_J)$	Efficiency/probability with which a PSII trapped electron is transferred from Q_A to Q_B
$Mo = 4(F_I - F_o) / (F_m - F_o)$	Approximated initial slope (in ms^{-1}) of the fluorescence transient V = *f(t)*
Performance index	
$PI_{ABS} = (RC/ABS) (F_v/F_o) [(F_m - F_J) / (F_J - F_o)]$	Performance index (potential) for energy conservation from photons absorbed by PSII to the reduction of the intersystem electron acceptor.
Phenomenological fluxes or activities per excited cross section	
$ABS/CS_m \approx F_m$	Absorption flux per cross section (CS) at t_{Fm}
$TR_o/CS_m = (ABS/CS_m) \varphi P_o$	Trapped energy flux per CS at t_{Fm}
$ET_o/CS_m = (ABS/CS_m) \psi ET_o$	Electron transport flux per CS at t_{Fm}
$DI_o/CS_m = (ABS/CS_{m)} – TR_o/CS_m$	Dissipated energy flux per CS at t_{Fm}
Density of reaction centers	
$RC_o/CS_m = \varphi Po (ABS/CS_m) (V_J/M_o)$	Amount of active PSII RCs per CS at t = 0

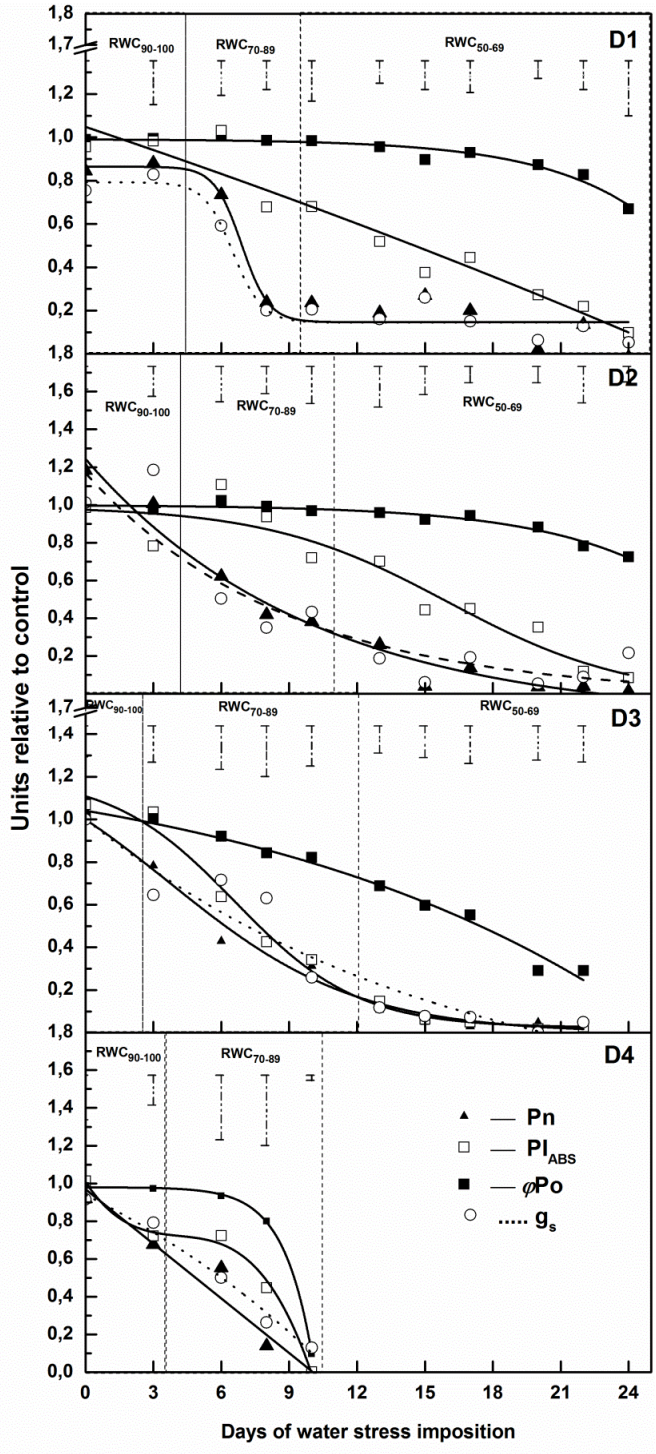

Fig. 1.1 Time-course of stomatal conductance (g_s), relative net photosynthesis (Pn), performance index (PI_{ABS}), and maximum quantum yield (φPo) at different developmental stages. Panel (D1) represents the three leaf stage, (D2) growing point differentiation, (D3) boot stage and (D4) half bloom stage. The different diagonal lines over the curves indicate the gradient of relative water content (RWC). Dashed bars above each point represent minimum difference among the means of g_s, Pn, PI_{ABS}, and φPo needed for significance (LSD test, $P \leq 0.05$).

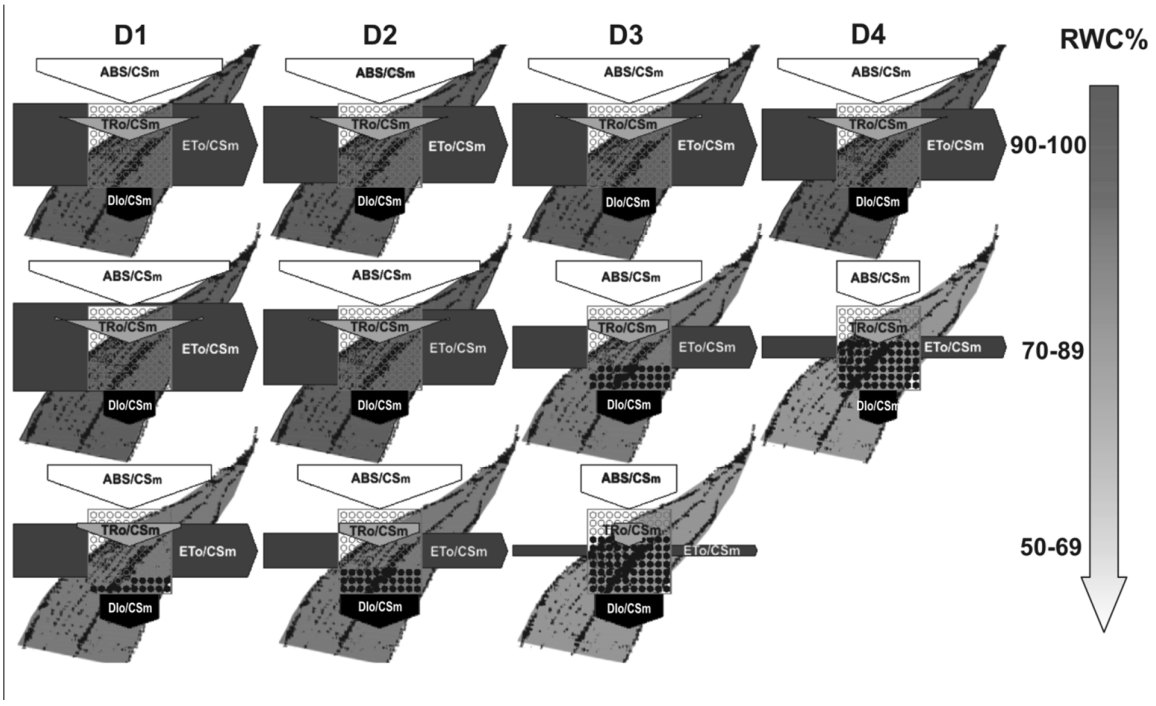

Fig. 1.2 Pipeline model of phenomenological energy fluxes per cross-section (CS) at third leaf stage (D1), growing point differentiation (D2), blooming stage (D3), and half bloom stage (D4) throughout different ranges of relative water content (RWC) as the soil dried up. The effect of progressive drought on each parameter can be seen as the relative change in the width of each arrow. The calculation of the energy fluxes are given in Table 1. Such parameters represent the stepwise flow of energy through PSII at the cross section for maximum fluorescence (CS_m) level. White arrows (ABS/CS_m) expresses the number of photons absorbed by the antenna molecules of active and inactive PSII reaction centres (RCs) over the excited CS_m of the sample. Light gray arrows (TR_o/CS_m) indicate the trapped energy flux per CS. Dark gray arrows (ET_o/CS_m) indicate the re-oxidation of reduced electron acceptors via electron transport over a CS_m of active and inactive RCs. Black arrows (DI_o/CS_m) describes the total energy dissipation measured over the sample CS_m. Active RCs are indicated by the open circles and inactive RCs (silent RCs) by the closed circles. The gray tone of the leaves indicates the pigment concentration per CS. In D4 measurements were possible only until RWC was between 70-89%.

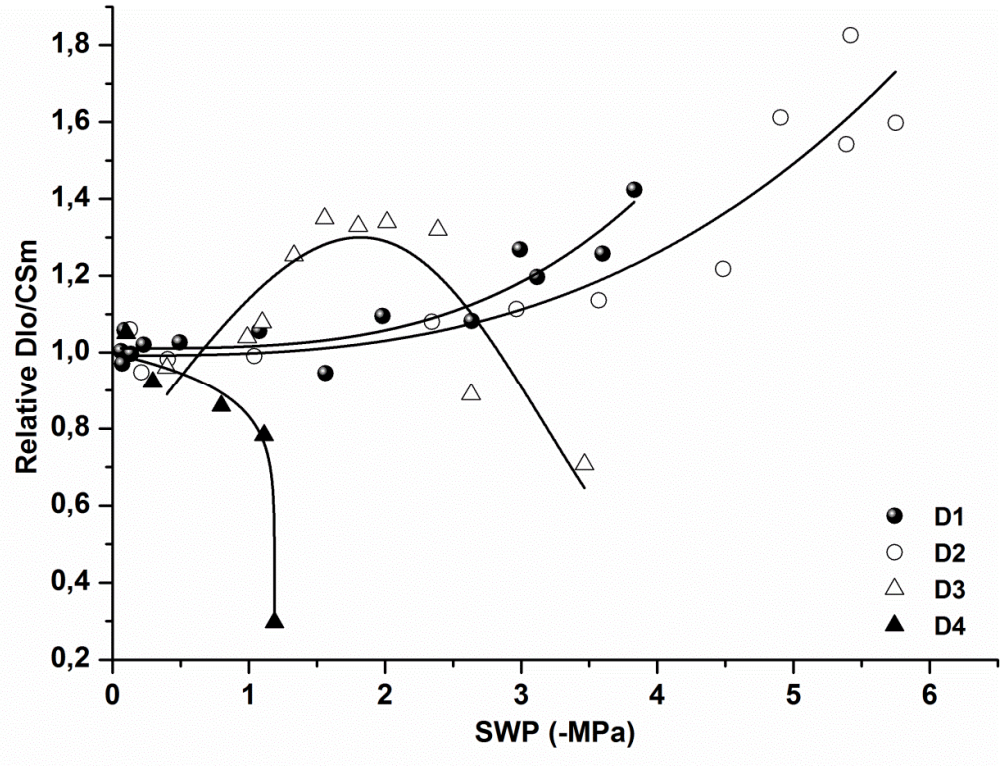

Fig. 1.3 Changes in dissipated energy flux per CS (DI_o/CS_m) relative to control versus soil water potential (SWP) at the four developmental stages as affected by decreasing soil water potentials. The trends of each developmental stage were described by different regression models. In D1, $y = a + bx^c$ $R^2 = 0.87$; D2, $y= a + bx^c$ $R^2 = 0.86$; D3, $y = ((x\text{-vrev})gmax)/ 1+e^{(x\text{-vhaf})/dx)}$ $R^2= 0.69$; D4, $y = (a+bx)^{(-1/c)}$ $R^2 = 1$.

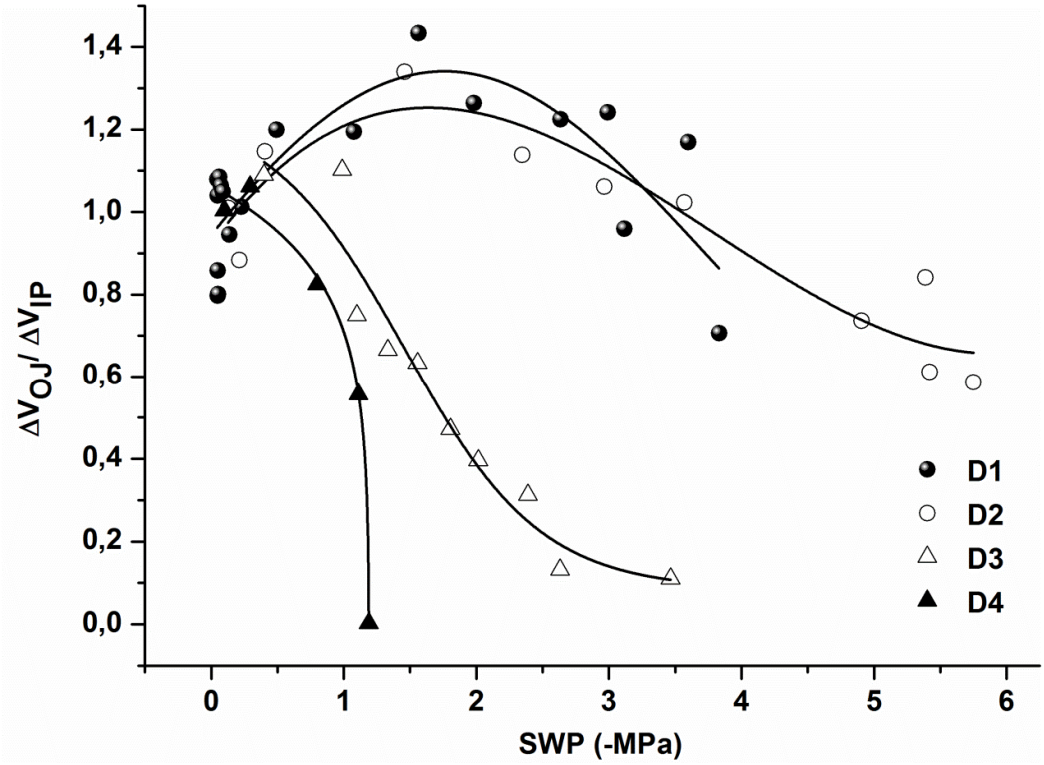

Fig. 1.4 Ratio between relative variable fluorescence at the J-step 2 ms (V_{O-J}) and relative variable fluorescence at the I-step 30 ms (ΔV_{I-P}) during the time-course of drought stress imposition. The ratio represents the PSII/PSI inhibition. The values are relative to the control. The curves were fitted by different regression models. Where, D1, $y = ((x\text{-vrev})gmax)/ 1 + e^{(x\text{-vhaf})/dx)}$ R^2 =0.51; D2, $y = a + b + cx^2\ dx^3$ R^2 = 0.8; D3, $y = (A_1\text{-}A_2) / 1 + e^{(x\text{-}x0)/dx}$ R^2 = 0.92; D4, $y = y_0 + A(abs(x\text{-}x_c)^P$ R^2 = 0.97.

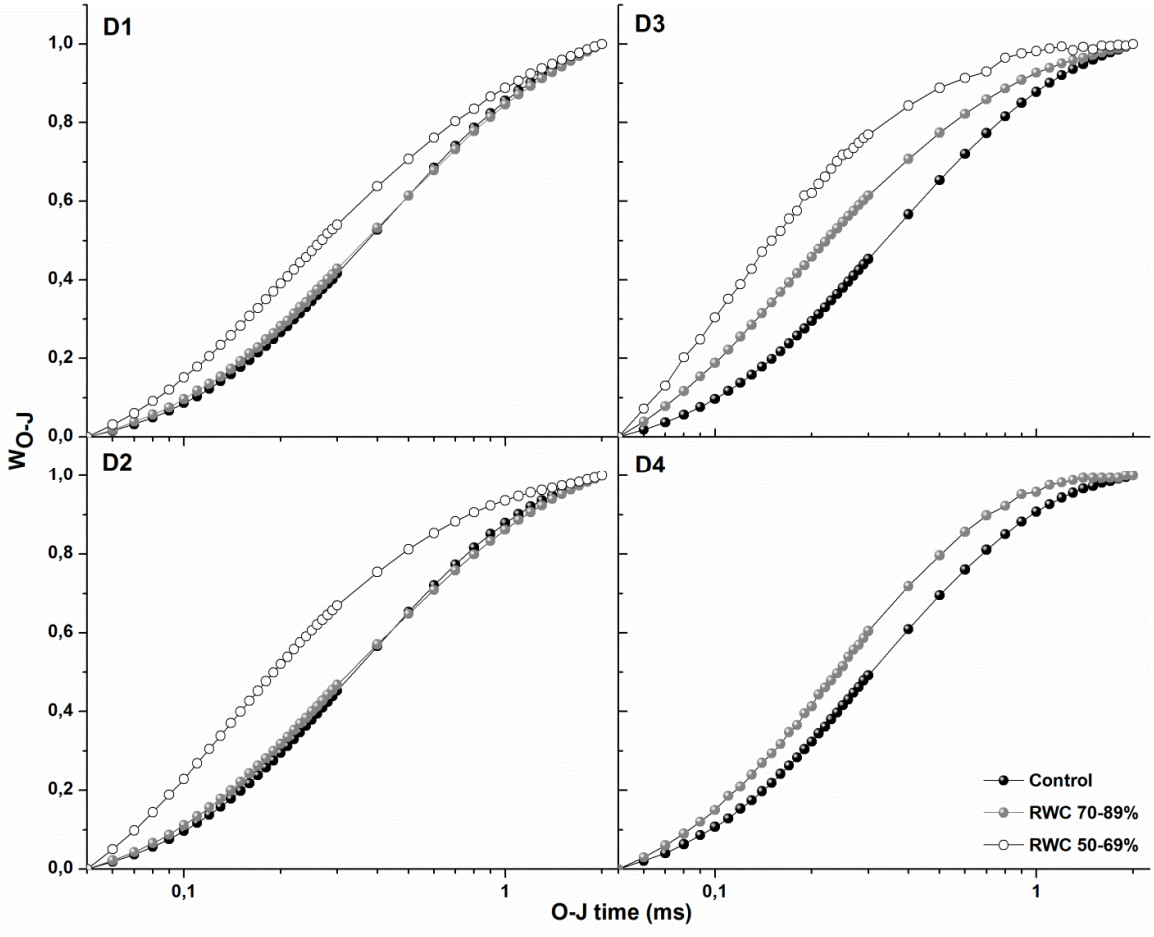

Fig. 1.5 Variable florescence from O to J step (W_{O-J}) of the chlorophyll fluorescence transient, plotted on logarithmic time scale of the four developmental stages (D1, D2, D3 and D4) at different ranges of relative water content (RWC). Control (100-90% of RWC), mild drought stress (70-89% of RWC) and severe drought stress (69-50% of RWC). The integral area below the curve was calculated to compare the amplitude of the curves which represents the accumulation Q_A reduced due to the blockage in the electron donor side of the PSII.

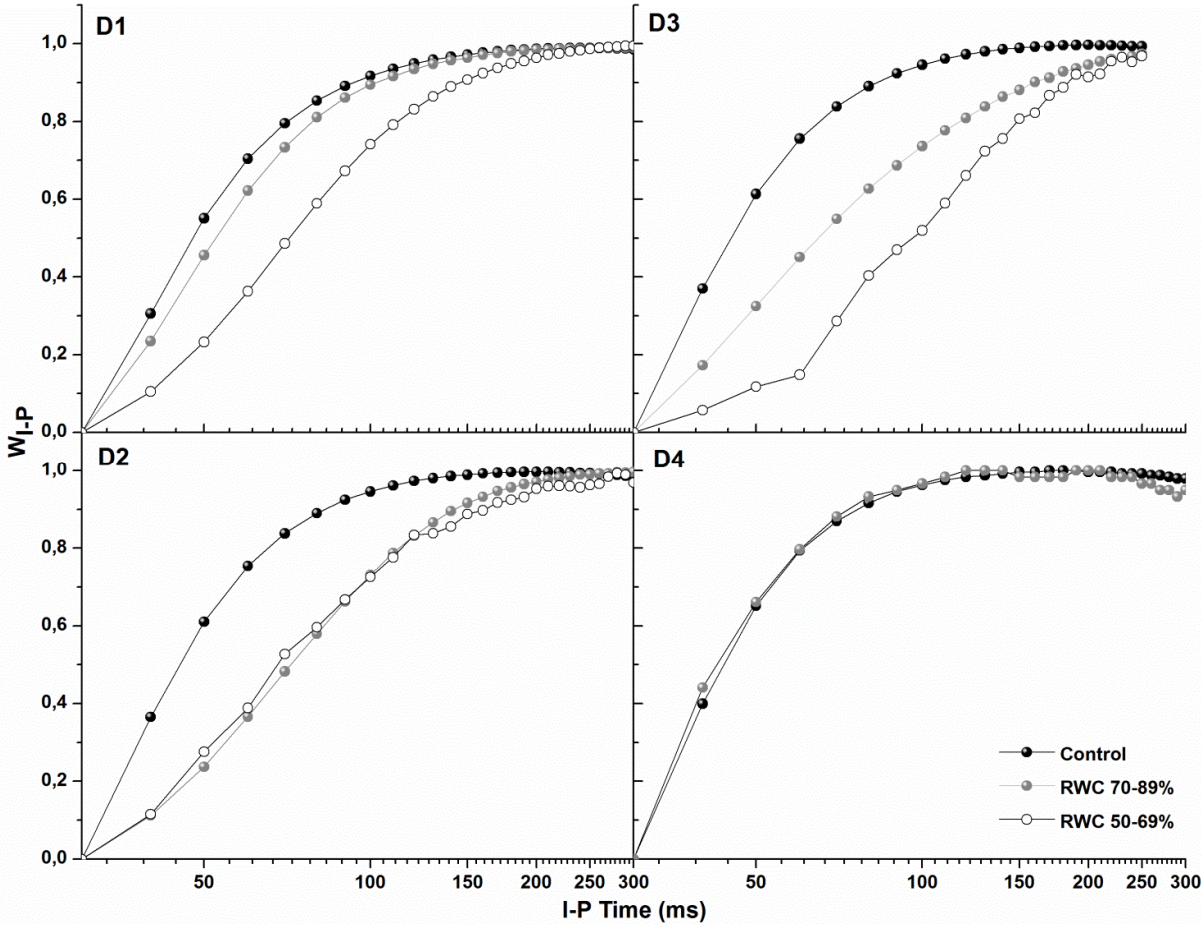

Fig. 1.6 Variable florescence from I to P step (W_{I-P}) of the chlorophyll fluorescence transient, plotted on logarithmic time scale of the four developmental stages (D1, D2, D3 and D4) at different ranges of relative water content (RWC). Control (100-90% of RWC), mild drought stress (70-89% of RWC) and severe drought stress (69-50% of RWC). The integral area below the curve was calculated to compare the amplitude of the curves which represents the block at the acceptor side of PSI and a traffic jam of electron transient formed in the electron transport chain.

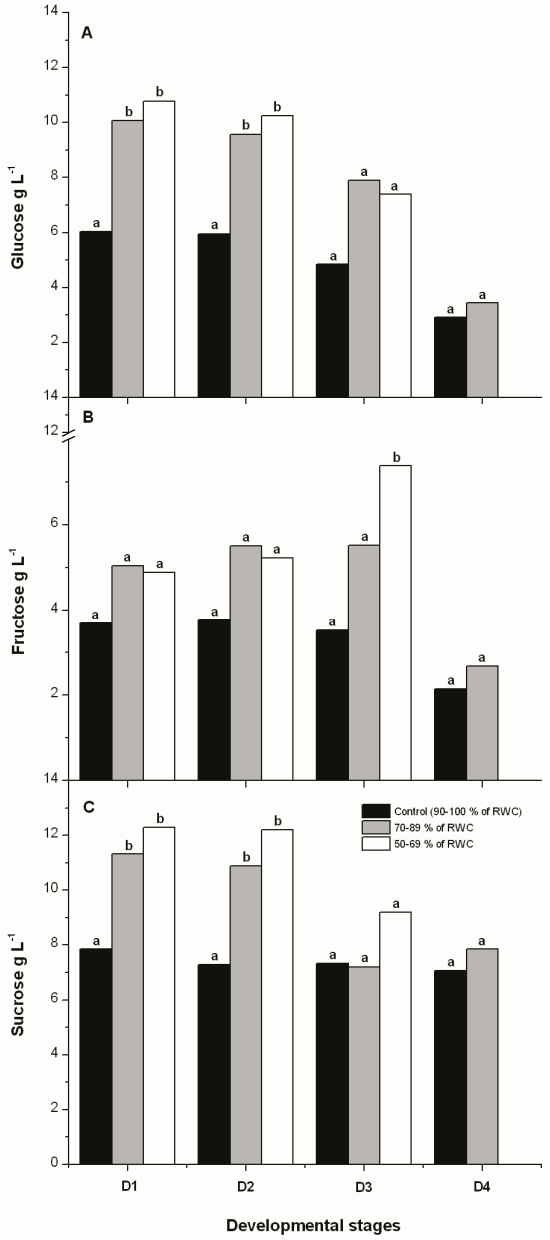

Fig. 1.7 Effect of progressive drought on glucose (Panel A), fructose (Panel B), and sucrose (Panel C) concentrations in sweet sorghum leaves at different ranges of relative water content (RWC). Different letters within each RWC range indicate significant differences (LSD test, $P \leq 0.05$).

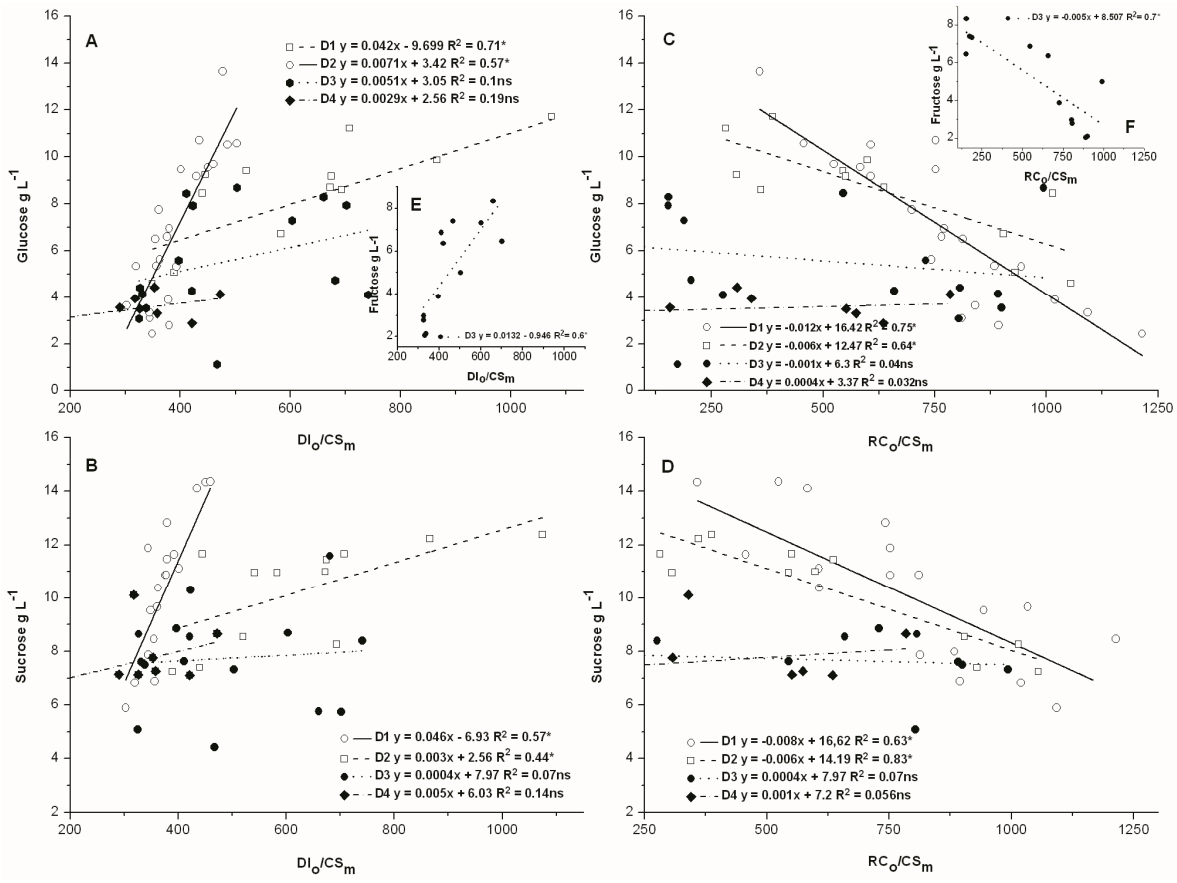

Fig. 1.8 Relationship between soluble sugars (glucose, panels A and C; sucrose, panels B and D) concentration in leaves and dissipated energy flux per CS and number of active PSII reaction centers per CS (RC_o/CS_m) at young and mature developmental stages. D1, represents the three leaf stage; D2, growing point differentiation; D3, boot stage; and D4, half bloom stage. The inset E and F show the relationship among fructose (DI_o/CS_m) and (RC_o/CS_m) at D3 (for clarity D1, D2 and D4 were not included).

Chapter 2

Evaluation of cold tolerance of seven sweet sorghum hybrids [*Sorghum bicolor* (L.) Moench)] under temperate climates

Abstract

Sweet sorghum [Sorghum bicolor (L.) Moench)], is a cold sensitive C4 specie. Low growth temperatures, characteristic of early spring in temperate climates, is traduced in poor germination and seedling establishment of sweet sorghum. Moreover, in cold-sensitive species, low temperatures during early developmental stages is thought to induce several biochemical and physiological dysfunctions such as photosynthesis and chlorophyll biosynthesis. In this study seven sweet sorghum hybrid (Bulldozer, Tarzan, Zerberus, Moster, ICSSH31, ICSSH19 and ICSSH58) were sown in the Po Valley (Northern Italy), at four sowing dates (from March to May) in order to evaluate their tolerance to early sowing both at seedling and final biomass and sugar yield (Brix degree).

Sweet sorghum establishment was evaluated by measuring mean emergence time (MET), percentage of emergence, total nitrogen and carbon leaf content, performance index (PI_{ABS}) and dry aboveground biomass and SPAD readings.

Early sowing (March 26th and April 19th) induced reduction of plant vigor and stand of plant, however no significant differences were found among hybrids. In addition, (PI_{ABS}) declined in plants sown early as consequence of low nitrogen and chlorophyll content in leaves (SPAD).

Despite the strong effect at physiological and biochemical level in all the hybrid evaluated, the hybrid Bulldozer showed a significant higher productivity suggesting a better physiological adaptation for temperate zones.

2.1. Introduction

Sweet sorghum [*Sorghum bicolor* (L.) Moench] is a tropical fast-growing crop (Doggett, 1988) considered as a suitable bioenergy feedstock (Zegada-Lizarazu and Monti 2012). Moreover, due to its good adaptability to unfavorable environmental conditions and marginal lands, it is expected to indirectly help to minimize the land use change impacts that the production of new energy crops could cause (Carillo et al 2014). In addition to that its cultivation under temperate climate conditions could further extend its production into areas where conventional bioethanol crops such as sugarcane cannot be produced. (Smith et al 1987, Smith and Buxton 1993). Nonetheless, low temperatures, characteristic of early spring in temperate climates could be restrictive for the germination and establishment of sweet sorghum (Maulana and Tesso 2013). In fact, for the successful establishment of sweet sorghum, uniform seedling emergence and vigorous initial seedling growth is required. Under adverse conditions, such as those of temperate climates, this becomes a challenging task (Wortmann and Regassa 2011). For instance, temperatures, as low as 15°C can be traduced in low germination and emergence rates, as well as in reduced growth rates after emergence (Pinthus and Rosenblum 1961, Singh 1985, Brar and Stewart 1994, Burow et al. 2011). While on the other hand, late sowing dates may reduce the length of the growing season, thus yield and carbohydrate content (Almodares et al. 1994, Almodares and Mostafafi, 2006). Therefore, in general sweet sorghum sowing is recommended when the air temperature is above 12°C (Almodares et al. 2008). Such temperature threshold is reached from mid to late spring in temperate climates, and therefore the sowing time of sweet sorghum is usually restricted the end of spring. However, there is a great potential to significantly improve quantitatively and qualitatively the productivity of sweet sorghum in temperate climates if cold tolerant cultivars are identified/selected.

Besides the reduced germination and initial growth rates low temperatures are thought to induce several physiological and metabolic alterations in the emerging seedlings (Wortmann and

Regassa 2011). Such disruptions will depend on, to mention a few, sensitivity of the species, nutrition level, growth stage, and intensity and duration of the cold spell (Ercoli et al 2004). In sorghum, exposure to cold nights (5°C) or ranges of 5-15°C is expected to lower the rate and extent of stomatal opening and hence net photosynthesis on the following day (Pasternak and Wilson 1972). Moreover, early cold-seasons stress was found to reduce leaf chlorophyll content (Maulana and Tesso 2013) and therefore this may inhibit the chlorophyll biosynthesis (Tewari and Tripathy 1998) and depress nitrogen (N) uptake (Ercoli et al. 2004). In addition to that, it was found that low temperature (below 5°C) reduces the carrying capacity of the phloem (Wardlaw and Bagnall 1981). Other study showed that even when the root seedling temperature is around 15°C the ion fluxes, particularly K^+ and $N0_3^-$ to the xylem sap were significantly reduced. Similarly, in other species such as *Secale cereale* (L.) and *Brassica napus* (L.) lowering the soil temperature from 20 to 7°C severely constrained nitrate uptake and subsequent N translocation (Laine et al. 1994).

Therefore reduced N uptake and chlorophyll degradation induced by early season cold temperatures, may result in failure/downregulation of some physiological process such photosynthesis. Rubisco being the most abundant photosynthetic protein and in a lesser extent the light harvesting complex proteins, represent a large proportion of total N in the leaves (Evans 1983, 1989a, Field and Mooney, 1986). Then it is presumable to assume that, especially in C4 plants such reduction in N and thus in Rubisco content in mesophyll chloroplasts may predispose the plant seedlings to greater cold sensitivity at the photosynthetic level and thus in poor plant survival (Kubien et al. 2003). An in deep research of such traits across different hybrids will improve our knowledge of low-temperature stress related mechanisms in physiological adaptation of sorghum to cold conditions and also will be useful for the individuation of cold tolerant cultivars and expanding the geographical growing areas of sweet sorghum. Rapid and nondestructive techniques to evaluate the photosynthetic vitality, derived

from chlorophyll *a* fluorescence (Chl. a fluor.) measurements, together with more traditional techniques such as the mean emergence time (MET) could be indicative of seedling performance under cold conditions and aid in the identification of the most adapted cultivars.

The objective of this study was to identify some indicative physiological and growth traits of the adaptability of seven sweet sorghum hybrids to cold (early sowing) under field conditions in a temperate climate.

2.2. Material and Methods

2.2.1. Experimental site and treatments

The trial was carried out at Cadriano experimental farm of Bologna University (44◦33, 24◦E, 33m a.s.l.) during the growing season 2012. The site is characterized by continental climate with a mean annual rainfall of 740 mm (Ventura et al. 2012). The soil characteristics are described in Table 2. During the growing season 2012 (from March to September) the average of daily minimum, mean and maximum temperatures were 13 °C, 20°C and 26,6 °C respectively.

Three commercial hybrids from KWS (Bulldozer, Tarzan and Zerberus), three from ICRISAT (ICSSH19, ICSSH31 and ICSSH58) and one Spanish hybrid (Monster) were sown at four sowing dates (March 26th, April 19th, April 5th and May 15th). Each sowing date was laid out in three time replicated randomized blocks with seven plots (hybrids) each of 2,6 m x 6 m of size. The distance between seeding rows was set at 0.45 m and 5 cm of distance between seeds along the rows. Fertilization was applied before sowing, with 100 kg N ha^{-1} in the form of urea, and 100 kg P_2O_5 ha^{-1}in the form of superphosphate. Thinning after the counting of germination was performed to obtain a final density of 12 plants m^{-2}. Final harvest was carried out when all the cultivars were at physiological maturity. During the whole productive cycle no supplemental irrigation was provided.

2.2.2. Vigor and plant seedling establishment

The vigor of the plants was evaluated through the calculation of the mean emergence time (MET), which is the reciprocal of the germination. Such calculation was done according to (Matthews and Khajeh- Hosseini 2006) as:

$$MET = \frac{\sum(n \times DAS)}{\sum n}$$

Where:

n = number of plants emerged (considering emergence when the cotyledon was unfolded above the soil surface). Such counting was performed always on the morning (9:00 – 10:00 am), starting after the first seedling coleoptile was visible. Counts were taken every other day until the end of the emergence.

DAS= days after sowing.

Σ n = final emergence

The plant establishment was evaluated by the percentage of plant emerged along the row (6 m). The 100% of plant emerged was taken as 120 emerged plants.

The Growing Degree Days (GDD) were calculated according to McMaster and Wilhelm (1997) as: GDD = $\left(\frac{T_{Max}+T_{Min}}{2}\right) - T_{Base}$

were: T_{Max} is the daily maximum air temperature, T_{Min} is the daily minimum air temperature and T_{Base} is the temperature below which the process of interest does not progress. We used as base temperature 13 °C (Ferraris & Charles-Edwards 1986, Barbanti et al. 2006)

2.2.3. Above ground dry biomass and leaf area at seedling establishment

The sampling for above dry biomass and leaf area were taken by manual cutting over an are of 1 m^{-2} when the 50% of the plants reached the 5^{th} full expanded leaf (moment in which sweet sorghum starts to be vigorous an competitive, Wortmann and Regassa 2011). Dry biomass was determined by oven drying to a constant mass at 105℃. Leaf area was measured with a leaf area meter (LI-3000; LI-COR, Nebraska, USA).

2.2.4. Chlorophyll a fluorescence measurement

The chlorophyll a fluorescence transient was measured by Plant Efficiency Analyzer (PEA, Hansatech, UK) when the plants were at 5^{th} leaf growth stage. A total of 12 measurements per hybrid were taken from 9:00 to 11:00 am to avoid photoinhibition by high light intensity on the youngest fully developed leaf. The leaves were previously dark adapted for at least 30 min by using specific leaf clips. Among the large number of JIP-test parameters that can be derived from the fluorescence measurements, we only used the absolute performance index (PI_{ABS}) and an indication of the photosynthetic vitality of the seedlings, since integrates the density of fully active reaction centers (RCs), the efficiency of electron transport by trapped exciton into the electron transport chain beyond the Q_A, and the probability that an absorbed photon will be trapped by RCs (Oukarroum et al. 2007, Živčák et al 2008).

Absolute performance index is calculated as follow:

$$PI_{ABS} = \frac{1-(F_o/F_M)}{M_o/V_J} \times \frac{F_M - F_0}{F_0} \times \frac{1-V_J}{V_J}$$

Where: F_0 is the minimum fluorescence intensity at time (T) = 50 □s, F_J is fluorescence intensity at the J step (T = 2ms), F_M is the maximum fluorescence intensity (T ≈ 30 ms), V_J represents the relative fluorescence at (T = 2ms) which is get by $V_J = (F_J – F=) / (FM-F=)$, M_0 represents initial

slope of fluorescence kinetics, which is derived from the equation Mo = 4* ($F_{300\mu s} - F_0$) / ($F_M - F_0$).

2.2.5. Chlorophyll content

The level of chlorophyll content in leaves was evaluated through SPAD-meter readings, following Yamamoto et al. (2002) who demonstrated that SPAD readings are closely related to the chlorophyll concentration in sorghum leaves.

2.2.6. Total Nitrogen and Carbon in plants at establishment

Leave samples were taken when the plants were at 5^{th} leaf stage, then they were dried at 60° C and finely ground with a ball mill. Total N and C were determined by micro Dumas combustion with a Costech ECS 4010 elemental analyzer (Costech Analytical Technologies). Due to experimental limitations these analysis were carried out only in the first tree sowing dates.

Statistical analysis

Data were statistically compared by two-way analysis of variance (ANOVA). Means of establishment parameters among different SDs were separated by the Fisher test ($P \leq 0{,}05$).

2.3. Results and discussions

2.3.1. Effect of the sowing date on vigor and seedling establishment

The effect of the sowing dates on the vigor and seedling establishment (Fig. 2.2) shows that all hybrids followed a similar pattern with the highest percentage of emergence when the environmental conditions were close to the optimum. In fact the mean emergence time decrease as the season progressed from close to 16 days at the earliest sowing time (end of March) to about only 10 days at the latest sowing time. The lower emergence percentage at early sowing dates, especially that of ICSSH31 (56%), could be related to the low nighttime temperatures

(below 5°C) that usually occur during early spring in the experimental site (Fig. 2.1). Besides that, the low precipitation registered during March and beginning of April may have contributed to such low percentage of emergence. These results are in contrast to the ones obtained by Patanè et al. (2012) in a Mediterranean climate. They found that at early spring sowing times, the average MET was 16 days and the percentage of emergence was 79.2%. Besides the different cultivars used in both studies, one of the reasons for the contrasting results with those of Patanè et al. could be that the nighttime temperatures in their study never decreased below 10^{o}C as it happened in the present one.

2.3.2. Effects of sowing times on some biochemical and physiological characteristics of seedling (5th leaf stage) establishment

Early sowing dates had significant effects on the metabolic and physiological functions of all hybrids (Fig. 2.3). The several episodes of temperature below 5°C, negatively influenced the chlorophyll content of the leaves, however, no significant differences were observed among hybrids.

The loss of chlorophyll pigments and/or the inhibition of its re-synthesis evidenced from our data are in agreement with those of Maulana and Tesso (2013). They found chlorophyll deficiency in sorghum seedlings due to sub optimal growing temperatures. In addition, in our study it was demonstrated that the loss of leaf greenness was linked to lower total nitrogen content as the mean emergence time increased (Figs. 2.3 and 2.5). Such N deficiency could be related, besides the harmful effect of nocturnal low temperatures, to the fact that root hydraulic properties and water ion transport are strongly controlled by temperature (Knennedy and Gonsalves 1988, BassiriRad el al. 1991, Sabala and Sabala 2002). For instance, , it was demonstrated that the hydraulic conductivity of sorghum root seedlings was two times lower at 15°C than at 35°C. Moreover, the ion flux (NO_3^- and K^+) released into the xylem sap was significantly decreased (BassiriRad et al. 1991). Furthermore, considering the large number of

nights when the temperature fell below 5°C (Fig. 2.1) it could be speculated that solutes translocation was constrained, with the concomitant reduction of N allocation to the leaves (Wardlaw and Bagnall 1981, Ercoli 2004). Consequently, such impairment possibility contributed to the low aboveground dry biomass produced by the seedlings at early sowing times (Fig. 2.8). Such lower biomass production could be the result of an extended duration of the meristematic cycle and hence arrested the leaf growth (Rymen et al. 2007) and therefore decreased CO_2 assimilation rates. Moreover, it should be highlight that besides the lesser amount of radiation during the seedling establishment (Table 2.3) at early sowing times, there were almost no precipitations for about 20 days before and 10 days after the sowing. Therefore this may have further contributed to the low biomass accumulation registered.

Although leaf gas exchange was not evaluated in this study, the sowing dates had noticeable effects on the photosynthetic efficiency at the PSII level. The PSII electron transport activity, showed a positive linear response to the N concentration in leaves (R^2=0.53) in all the hybrids (Fig. 2.7), indicating that N deficient seedlings as those sown early in the season, had lower CO_2 assimilation capacity and inhibited primary photosynthetic activity. These results are in agreement with those of Sage (1987), Terashima and Evans (1988), and Lu et al. (2001). It remains, however, to elucidate how these photosynthetic acclimation processes to early sowing (cold) are beneficial for some hybrids to resume growth and arrive to higher productivity than when sown under optimum sowing temperatures.

2.3.3. Effect of sowing dates on the total dry biomass

Even though the accumulation of dry biomass, chlorophyll contentment (Fig 2.8) and other physiological processes were increased as the sowing date was delayed, all the activated defense mechanisms and acclimation constrains due to cold during the early sowing dates were reverted towards the end of the growing season. Only in the case of ICSSH31 and ICSSH19 such constrains were linearly maintained till the plants matured (Fig. 2.8). So these results indicate

that the reduced physiological and metabolic activity due to cold is reversible depending on the cultivars. For example in contrast to ICSSH31, Bulldozer seedlings, that had very low biomass, chlorophyll content, and PSII electron transport activity at the first sowing date, were able to produce the highest biomass at the end of the growing season. These contrasting physiological and metabolic adaptation mechanisms should be further studied for establishing adequate breeding programs and/or the introduction of cultivars with the highest cold adaptability and therefore productivity potential in temperate climates.

2.4. Conclusion

Seedling cold acclimation resulted in the loss of chlorophyll pigments and/or the inhibition of its re-synthesis, which was linked to low total nitrogen content. Moreover, the low N content in the leaves reduced significantly the PSII electron transport activity thus the CO_2 assimilation capacity as indicated by the reduced biomass accumulation up to the 5^{th} leave stage. Despite that, most of the hybrids tested here were able to revert such situation reaching at the end of the growing season higher productivity levels than when sown late in the season. The exception to that were ICSSH31 and ICSSH19 hybrids. These contrasting physiological and metabolic adaptation mechanisms should be further studied for establishing adequate breeding programs and/or the selection of cultivars with adequate cold adaptability traits that would improve survival and productivity potential.

Bulldozer demonstrated the highest capacity to revert the cold effects of early sowing and reach the highest biomass productivity at the end of the season, therefore could be considered one the best fitted hybrids to temperate climates. Multi-location trials would confirm such capacity.

Table 2.1 Soil characteristics of the experimental site in the top layer (0-50 cm)

Soil Characteristics	Unit	Value
Sand	%	24
Silt	%	47
Clay	%	29
pH in water solution		7.95
Total calcareous	%	1
Organic matter	%	1.06
Total N	%	0.93
P_2O_5 availability	mg kg^{-1}	113
K_2O availability	mg kg^{-1}	174
Bulk density	g cm^{-3}	1.2

Table 2.2 Meteorological data during the sweet sorghum growing season 2012

Months	T. min (°C)	T. med (°C)	T. max (°C)	Accumulated precipitations (mm)	Net Radiation (W m^{-2})	PAR (mol m^{-2} s^{-1})
March	3,26	11,4	19,8	0.2	67,16	448,2
April	7,61	12,87	18,34	92	73,47	456,63
May	11,22	17,64	23,73	89,6	114,03	403,87
Jun	16,91	24,01	30,66	16,8	147,43	369,4
July	18,58	26,54	33,67	0	118,77	281,32
August	18,49	26,89	34,49	6,4	98,7	277,58
September	14,94	20,03	25,52	117,8	62,46	326,9
Mean	13,00	19,91	26,6		97,43	366,27
Total				322,8		

Table 2.3 Two-way ANOVA of the productive components harvested at physiological maturity.

Source of variation	Total biomass	Stem Biomass	Leaf biomass	Brix
SD	*	***	ns	*
Hybrid	***	***	***	***
SD *x* Hybrid	P=0.055	**	ns	ns

ANOVA Significance level: ***<0.001, **<0.01 *<0.05 ns= not significant

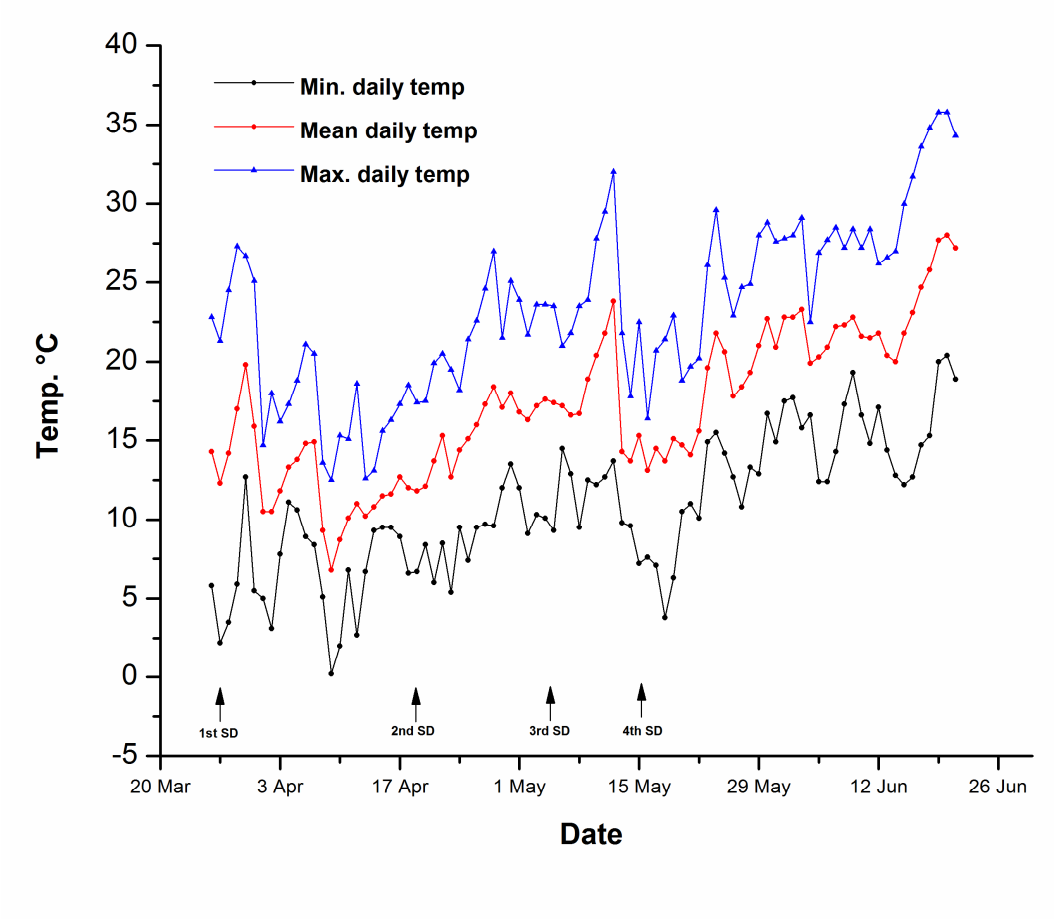

Fig. 2.1 Mean daily temperatures (minimum, mean and maximum) during the establishment period. Arrows indicate the date of sowing.

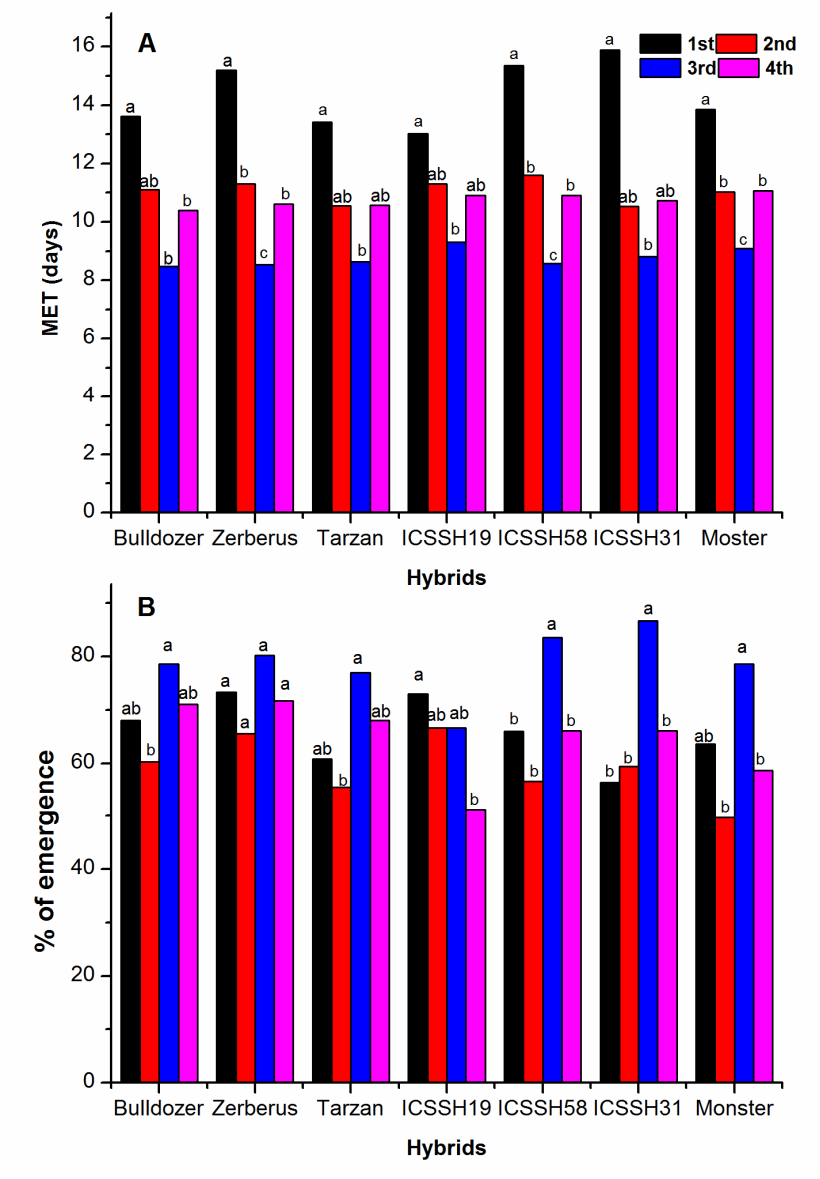

Fig. 2.2 Mean emergence time (MET; Panel A) and percentage of emergence (Panel B) of the seven hybrids in the four SDs.

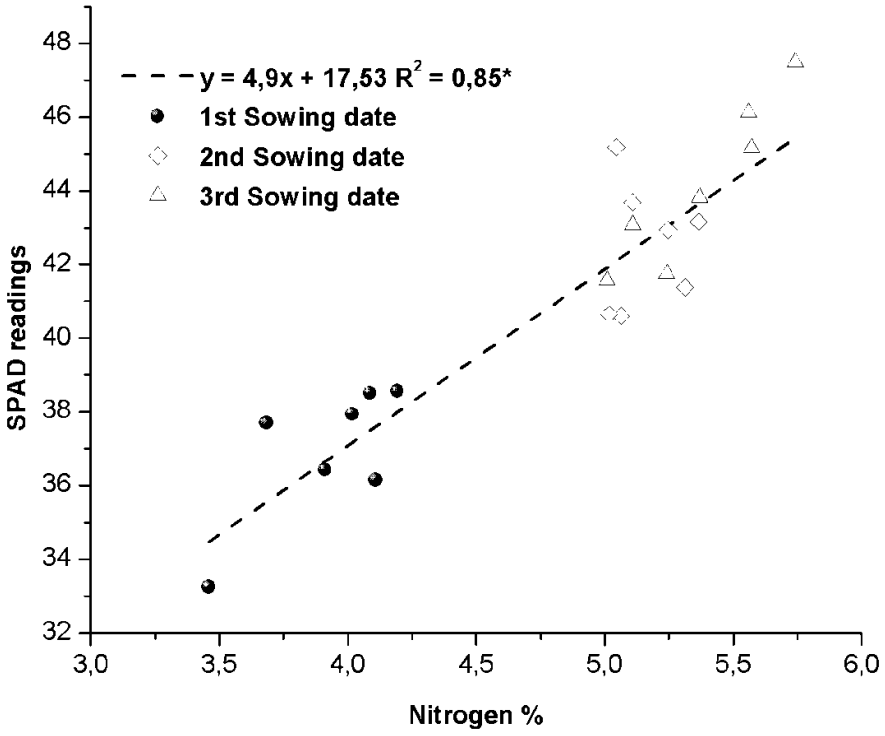

Fig. 2.3 Relationship between total leaf N content and SPAD readings, at the end of establishment (5^{th} leaf stage).

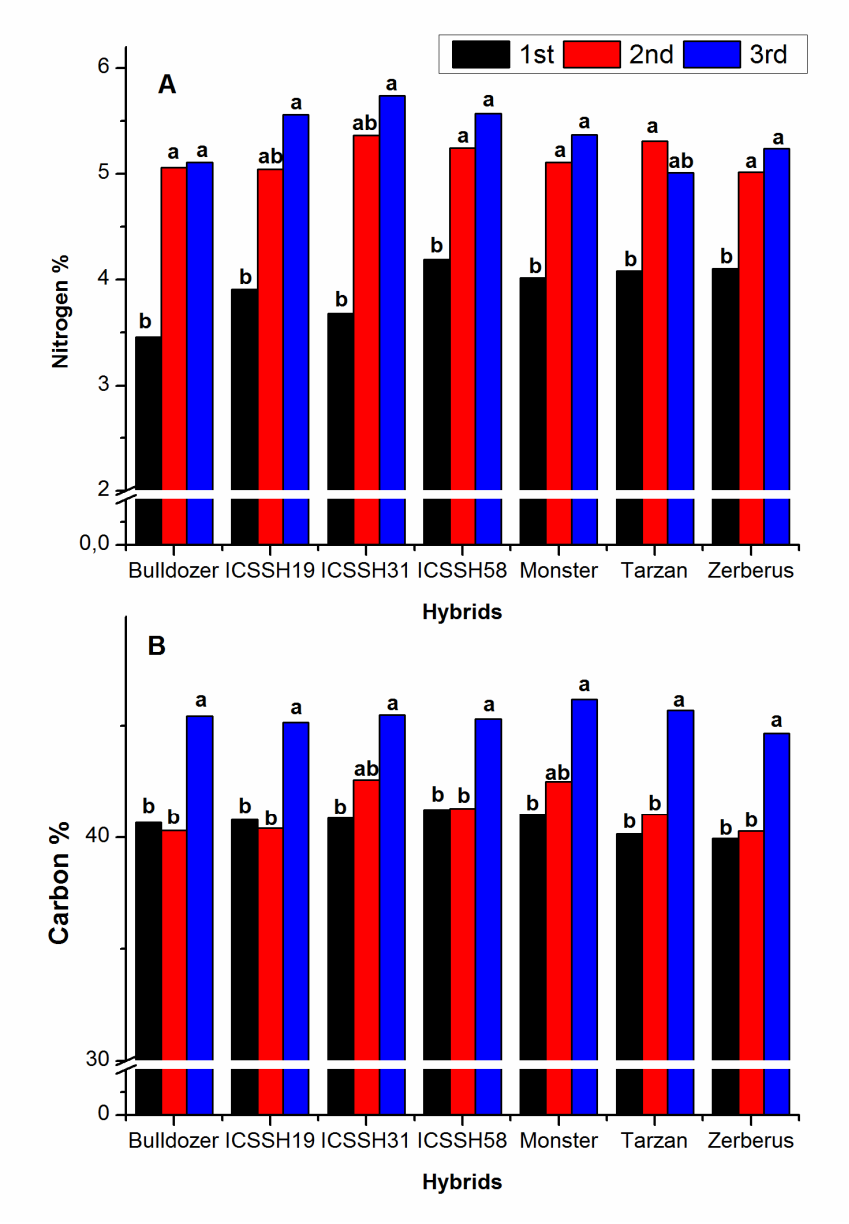

Fig. 2.4 Total leaf N content (Panel A) and total leaf C content (Panel B)

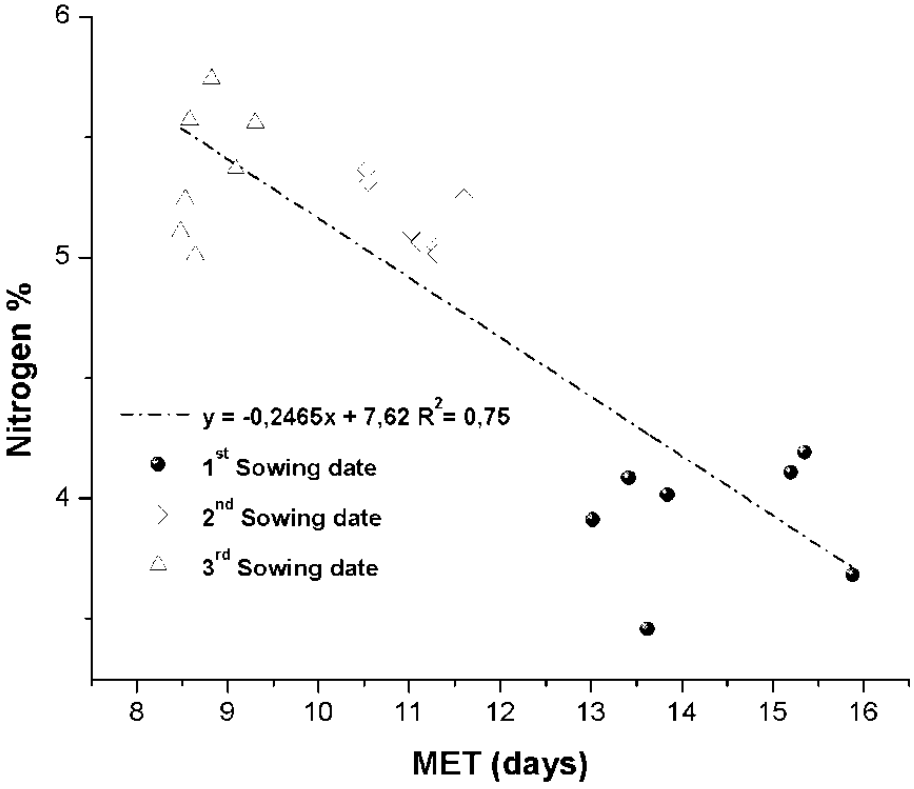

Fig. 2.5 Relationship between mean emergence time (MET) and total leaf N content.

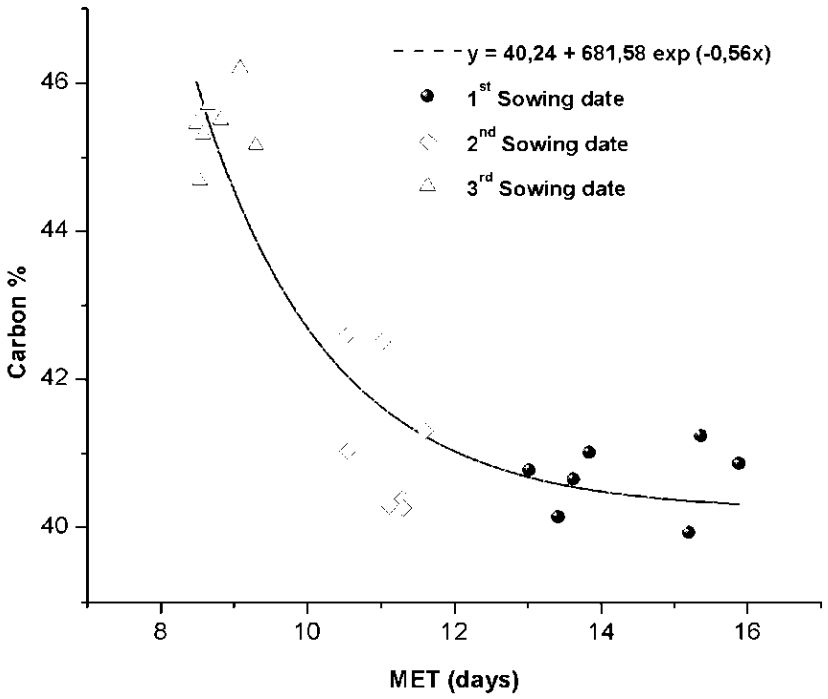

Fig. 2.6 Relationship between mean emergence time (MET) and total leaf N content.

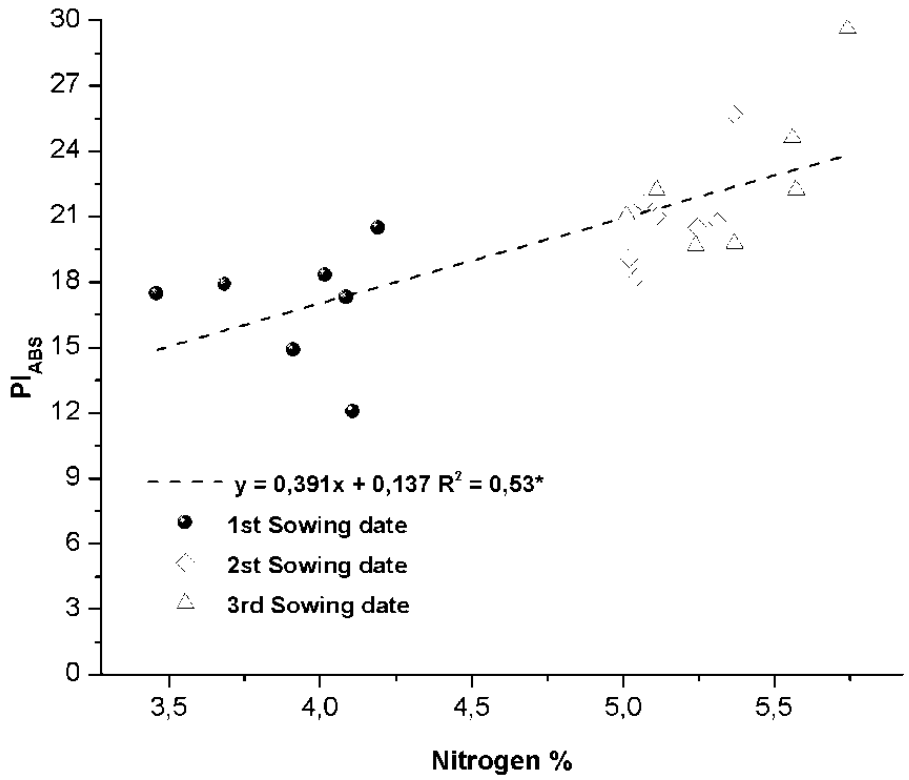

Fig. 2.7 Relationship between absolute performance index (PI_{ABS}) and total leaf N content.

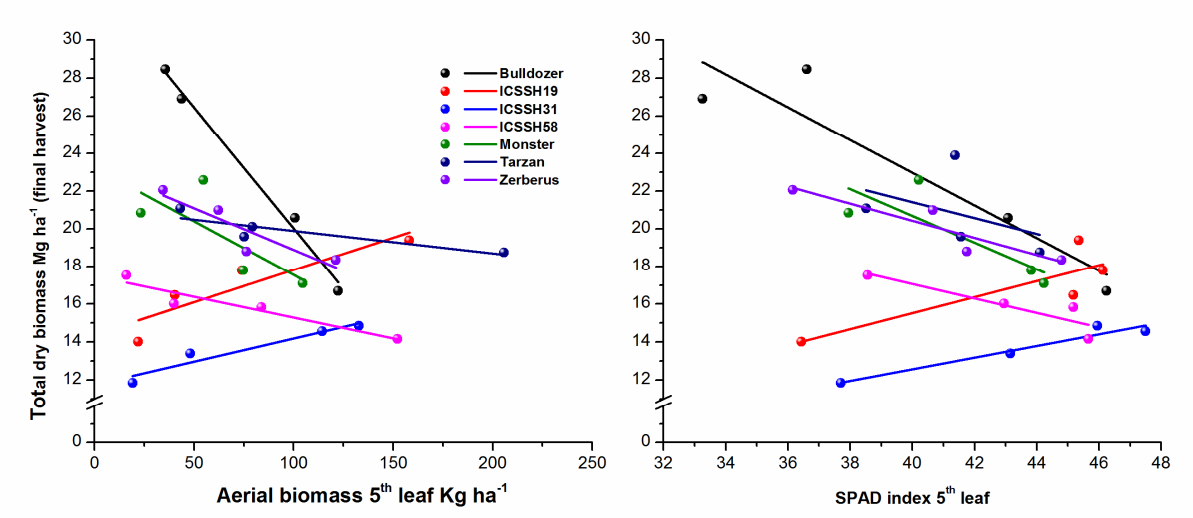

Fig. 2.8 Relationship establishment above ground biomass and total dry biomass at harvest (Panel A). Relationship between SPAD readings and total dry biomass at harvest (Panel B)

Chapter 3

Photosynthetic and biochemical adaptability to chilling stress of two contrasting sweet sorghum hybrids

Abstract

Chilling is a major constraining factor determining the geographical distribution of many thermophilic plants, especially in chilling-sensitive C4 species. In temperate regions sweet sorghum, is often exposed to chilling during early developmental stages resulting in poor growth and low photosynthetic performance. In cold-sensitive species such as maize, chilling temperatures induce reduction of photosynthetic capacity due to inactivation of Rubisco and PEPcase. On the other hand, chilling predispose the plants to oxidative stress triggering several strategies to self-detoxify through several strategies such as heat energy dissipation, xantophylls cycle pool, antioxidants scavengers like β-carotene and tocopherols etc. Such mechanisms to cope with long chilling events are rather scant in sweet sorghum.

The objective of this work was to analyze the sensitivity of the photosynthetic apparatus of two sweet sorghum hybrids during four day to chilling temperatures and the recover after two days re-warming. The experiment was carried out under growth chamber conditions. The growth temperature were set at 20°C/14°C, when the plants reached the 5th leaf stages the temperature was lowered to 9°C/5°C for 4 days and then reestablished to the initial one. Direct and modulated Chl. a fluorescence, A/C_i response, xantophylls cycle, Chl. a+b content, luteine and β-carotene were measured/quatified before, during and after chilling period.

Chilling temperatures provoked engagement of zeaxanthin after 48 hours of chilling in ICSSH31, and Chl. a+b degradation. Our results indeed, revealed that Rubisco activity was significantly inhibited by chilling that PEPcase in both hybrids. Finally, less Rubisco activity

was observed in ICSSH31 before the treatment in relation to Bulldozer. In conclusion, despite both varieties were able to self-regulate the electron transport components and enzymatic activity, greater metabolic dysfunction (chlorophyll a+b degradation and re-synethesis, xanthophyllscycle de-epoxidation) may justify the low performance of ICSSH31 field conditions.

3.1. Introduction

Recently, sweet sorghum [*Sorghum bicolor* (L.) Moench] has gained particular interest due to its outstanding characteristics of rusticity and capacity to store large amounts of fermentable carbohydrates in the stems for bioethanol production. Nonetheless, being a native tropical crop, it is particularly sensitive to cold and chilling temperatures during germination and early plant growth stages. Therefore its expansion into nontraditional growing areas such as those of central northern Europe is severely limited. Chilling stress is one of the main environmental factors affecting cold-sensitive crops (Theocharis et al. 2012). .

The photosynthetic system of sweet sorghum, as the majority of other C_4 plants, is particularly sensitive to cold temperatures (Tari et al. 2013). In fact, several C4-photosynthetic functions were found to fail even when chilling temperatures lasted only few days (Leipner et al. 2000), and the regain of functionality depends on several factors, such as, the duration and growth stage of its occurrence (Ercoli et al. 2003) as well as the cultivar genetic background. Yet information on the way sweet sorghum cultivars react to short-duration chilling temperatures at biochemical and photosynthetic levels and at which degree such metabolic disturbance could be reversible is still missing.

It is thought that short-duration chilling temperatures affect the enzymatic activation state which plays a fundamental role in the photosynthetic compensation (Holaday et al 1992). Therefore the scarce photosynthetic performance of C4 plants in cold areas is related to the high activation energy of their principal enzymes involved in CO2 fixation such as phosphoenolpyruvate

carboxylase (PEPcase) and pyruvate orthophosphate dikinase (PPDK) which can hardly work at temperatures below 12° C (Edwars et al. 1985, Du and Wasano 1999, Wang et al. 2008). The reason lies in the fact that low temperature can dissociate PPDK and PEPcase and hence compromise the CO_2 pump capacity (Leegood and Edwars. 1996). In sorghum a chilling temperature of 10°C induced the loss of two fundamental C4-pathway enzymes: NADP-malate dehydrogenase and pyruvate PPDK rather than an incomplete enzymatic activation (Taylor et al. 1974). While in maize (*Zea mays*) and miscanthus (*Miscantus x giganteous)*, two closely related species to sorghum with the same NADP-malic enzyme (NADP-ME) pathway, showed that cold sensitivity differences between these species were mainly due to contrasting Rubisco properties and/or PPDK activities. Moreover, miscanthus showed reduced susceptibility to photoinhibition than maize. Photosynthetic rates were also significantly reduced (about 90% at 14 ^{o}C) while those of miscnathus were only slightly reduced at 10°C (Naidu and Long 2004)

Other studies showed that low photosynthetic rates under cold conditions were closely correlated with transgenic reduction of Rubisco activity (Kubien et al. 2003). Up to now, however, the main process limiting C4 photosynthesis under low temperature conditions remains unclear, especially for sweet sorghum cultivars of diverse genetic origin. Nonetheless, the capacity of Rubisco to maintain its activation energy, PEP regeneration by PPDK and PEP carboxylase activity continue to be the most generally accepted mechanisms controlling the photosynthetic activity under chilling temperatures (Kingston-Smith el al. 1997, Du et al. 1999, Pittermann and Sage 2000, 2001, Chintapalli et al. 2003, Kubien and Sage 2003, Sage et al. 2011).

Reduction of photosynthetic activity by stomatal limitation could also take place under circumstances of chilling stress, as was observed by Aguilera et al. (1999) in maize, demonstrating that it is governed by a genetic component. Contrarily, Mustárdy et al. (1982) stated for the same species that low temperature increased stomatal conductance, being more markedly in the sensitive maize varieties. In sorghum low night temperature of 5°C under filed

conditions reduced stomatal aperture and as consequence its photosynthetic activity the next day (Pasternak and Wilson 1972).

A poor adaptation to chilling temperatures may cause the formation of reactive oxygen species (ROS) as consequence of the excessive energy pressure, resulting in photoinhibition and, if persisting for long time, in photodamage (Williams et al. 2013). Nonetheless, depending on the chilling stress conditions e.g. duration and growth stage at which occurs, the efficiency of PSII electron trasport can be a reversible process regulated from min to hours via xanthophylls cycle pool and the associated dissipation of energy light by non-photochemical process (Adams and Demming-Adams 1995, Streb et al. 2003b). Adjustment of photosynthesis involves, besides energy dissipation, changes in the level of proteins (and RNA coding these proteins) related to the biochemistry of photosynthesis, electron transport, and chlorophyll bindings proteins and thus leaf chlorophyll content (Krapp and Stitt 1995, Smeekens 2000, Paul and Foyer 2001). On the other hand, the capacity of the plants to efficiently down-regulate their photosynthetic apparatus under a sudden drop of temperature will depend on, in some cases, the previous acclimation. Thus, the acclimated plants to chilling will show a faster recovering of their photosynthetic functionality by a greater antioxidant defense such as scavenging enzymes and de-epoxidated xanthophyll pool (Leipner et al 2000).

The objective of this work will be analyze the response of the photosynthetic apparatus of two sweet sorghum hybrids which were which revealed comparatives cold sensitivity under field conditions. From the field experiment results, we hypothesize a more efficient down-regulation capacity in hybrid Bulldozer with respect to ICSSH31 under chilling conditions, as well as greater recovering capacity after re--warming.

3.2. Material and Methods

3.2.1. Growth conditions and treatments

Two sweet sorghum hybrids (Bulldozer and ICSSH31) were subjected to chilling temperatures under controlled environmental conditions in a growth chamber. Based on biomass productivity, these hybrids were identified as cold tolerant (Bulldozer) and cold sensitive (ICSSH31) in a previous field trial. Three seeds were planted in 28 pots of 700 cm^3 volume each. Pots were filled with sandy soil. Throughout the experimental period the plants were watered as required every 2 days. Fertilization was applied as 100 Kg ha^{-1} of total nitrogen, 100 Kg ha^{-1} of P_2O_2 and 100 Kg ha^{-1} K_2O through a soluble solution (20-20-20) with microelements (Bo, Fe, Mg and Zn) distributed in three times through the whole experimental cycle. The growing temperature was kept at 20°C and 14°C day/night respectively during the growing cycle. When the plants reached the 5^{th}- 6^{th} leaf stage chilling treatments started . The plants were subjected to a 96 h-period of chilling temperatures (9°C and 5°C day/night respectively, which are the mean chilling periods occurring in Po Valley). After the chilling period was completed, normal growing temperatures (20°C/14°C) were reestablished (re-warming) for about 48 hours. Relative humidity and irradiance was set up at 60% and 300 μmol m^{-2} s^{-1} of PAR, respectively. The photoperiod for the day/night cycles was established as 13 and 11 h, respectively.

3.2.2. Chlorophyll a fluorescence transient measurements

Direct chlorophyll a fluorescence transients were measured with a Plant Efficiency Analyzer (Handy PEA, Hansatech, UK). Measurements were taken at 24 h before chilling stress imposition (T_0); at (48 and 96 h after chilling stress imposition (T_{48} and T_{96}, respectively); and at 48 h after terminated the chilling treatment (T_{48RW}). Eight measurements per hybrid and per stress period were taken on the 5^{th} and 6^{th} fully expanded leaves. Before taken the measurements, leaves were dark adapted with leaf-clips for 30 minutes in order to allow the complete oxidation

of quinone pool in the electron transport system. The dark adapted leaves were illuminated with 1 s pulse of continuous red light (650 nm peak wavelength, 3000 μmol photons m^{-2} s^{-1} maximum light intensity), given by an array of three light-emitting diodes. The OJIP test parameters (Strasser et al., 2000; 2004) were calculated from the recorded original data: (i) minimal fluorescence intensity at 50μs (F_o, when all reaction centers of PSII are open); (ii) the maximum fluorescence intensity (F_m, when all reaction centres of PSII are closed); and (iii) fluorescence intensities at 300 ms (K-step) and 2 ms (J-step).

3.2.3. Modulated chlorophyll fluorescence measurements

For the quenching analysis, Chlorophyll a fluorescence emission from the adaxial surface of the 5^{th} and 6^{th} leaf was measured , immediately after direct Chl a fluor was done with a modulated Fluorescence Monitoring System (FMS 2; Hansatech Instruments Ltd., Norfolk, UK). A PAR/temperature leaf-clip was used which was designed for measurements carried out under ambient light conditions. In order to completely oxidize all Q_A molecules (for F_0 measurement), 5 s duration of ca. 6 μmol (photons) m^{-2} s^{-1} of far-red radiation (with peak emission at 732 nm) was applied. The maximum fluorescence (F_m) was induced by applying a short pulse (0.8 s) of saturating radiation (5000 μmol m^{-2} s^{-1}; Rosenqvist and Van Kooten, 2003). To determine the maximum fluorescence under actinic illumination (F'_m), various pulses of saturating light (5,000 μmol m^{-2} s^{-1}, 0,8 s duration) were applied until reaching the steady-state (F_s). The minimum fluorescence (F'_0) was determined by turning off the actinic light and immediately applying a 2 s far-red pulse. From the recorded data the following parameters were calculated following the protocol and nomenclature used by Dewez and Perreault (2013): The operational quantum yield determined as the ratio $\Phi'_M II = (F'_m - F_s) / F'_m$ (Genty et al. 1989); the photochemical quenching value, which represent the photochemical energy conversion at PSII (Schreiber et al. 1986) is indicated as $q_P = (F'_m / F_s) / (F_m/F_0)$; the yield of non-photochemical energy conversion via PSII (non-regulated pathway) was indicated by $\Phi'_{NO} = 1 / (((F_m - F'_m) / F'm) + 1 + (q_P(F'_0/F)) /$

($Fm/F_0 - 1$)); the yield of PSII non-photochemical energy dissipation (via regulated pathway) is noted as $\Phi'_{NPQ} = 1 - \Phi'_{M}II - \Phi'_{NO}$ (Kramer et al. 2004). The total energy dissipation via PSII is represented by the sum of $\Phi'_{M}II - \Phi'_{NO} + \Phi'_{NPQ} = 1$.

3.2.4. Gas exchange measurements

The photosynthetic responses to increasing intercellular CO_2 concentration (A/C_i) were evaluated with a portable infrared gas analyzer (CIRAS-2, PPSystems, UK) in the same leaves used for chlorophyll a fluorescence analysis measurement. The A/C_i curves were measured using a light intensity of 2000 μmol m^{-2} s^{-1} quanta. The intercellular CO_2 concentration (C_a) was set at different CO_2 levels (0, 100, 200, 400, 900, 1400, 1700, 2000 μmol m^{-2} s^{-1}). The values of photosynthesis were recorded after the steady state was reached (CV < 0.5%). Air temperature and VPD were not controlled in order to measure the leaf gas exchange with the same experimental conditions. The biochemical parameters derived from A/C_i curves were: stomatal limitation of photosynthesis (Ls; Long and Bernacchi, 2003) as Ls = [(A' – A)/A'] * 100 where A' indicates the net CO2 assimilation at Ci = 370 μmol mol^{-1}; A is the net CO2 assimilation under regular C_i when C_a = 370 μmol mol^{-1} obtained by linear regression between C_i vs C_a. The carboxylation efficiency (CE) of the enzyme phosphoenolpyruvate carboxylase (PEPc) was calculated as the initial slope of every A/C_i curve (C_i< 100 μmol m^{-2} s^{-1}) of all individual curves. The photosynthesis at CO_2-saturated rate noted as V_{pr} was calculated as the asymptote of each dataset of A/C_i curve fitted as non-rectangular hyperbola

3.2.5. Leaf pigment analysis

After chlorophyll a fluorescence and leaf gas exchange were measured on the chosen sampling times (T_0, T_{48}, T_{96}, and T_{48RW}), the whole 5th leaves were cut and immediately immersed in liquid nitrogen and stored at -80° C until subsequent analyses.

Chlorophyll and carotenoids were extracted and analyzed by high-performance liquid chromatography (HPLC; model LC-10AS with a detector SPD-10AV, Shimadzu, Kyoto, Japan) as described by Baraldi et al. (2008). To refer the Xantophyll de-epoxidation (conversion of violaxanthin (V) to zeaxanthin (Z) via antheraxanthin (A)), was calculated as the ratio (Z + A)/(V + A + Z) according to Müller et al. (2006).

3.2.6. Analysis of Data

ANOVA General Linear Model (GLM) was performed for statistical comparison between hybrids at every measuring time. Dunnett's test was used to compare the treatment against the control ($P \leq 0,05$). While for the mean comparison between verities of all the set of data, the Tukey's test ($P \leq 0,05$) was used for each point of the cycle.

A nonlinear regression model of the *A*/Ci curves was used to determine V_{pr} (asymptote) and a linear regression model to calculate the CE of PEPc (initial slope; Ci< 100 ml l-1) of the same curves,

3.3. Results

3.3.1. JIP-test analysis

The photosynthetic behavior at the electron transport chain was evaluated by the JIP-test analysis through selected biophysical parameters (Fig. 3.1). In general, the normalized values to the control (T_0) varied depending on time of exposure to chilling temperatures with the largest effects at 96 hours after initiated the treatment. For most of the parameters evaluated, ICSSH31 showed significantly greater inhibition than Bulldozer. After 48 hours of chilling, the PI_{ABS} in ICSSH31 and Bulldozer decreased by 41% and 23%, respectively, while the maximum quantum yield (φP_o) by about 6.4% and 3.1%. In both crops the energy dissipation (DI_o/CS) increased, but in the case of ICSSH31 it was almost double than in Bulldozer. The total electron carriers per

RC (Sm) increased by more than 20% in both cultivars. The efficiency of the electron transfer from the reduced intersystem until PSI electron acceptors ($\delta RE_{o,}$ $\psi RE1_o$ and $\varphi RE1_o$) increase by 25,9%, 16%, 8,65 in ICSSH31 and by 27.12%, 16.9%, 4.9% in Bulldozer. Although no significant differences between cultivars were found, PI_{ABS} and φP_o after 96 hours of chilling were 74% and 19.8% lower than in the control situation in ICSSH31, such decrements in bulldozer were in the range of 45% and 10%. On the contrary, significant differences between cultivars on the light energy dissipation (DI_o/CS_m) were found ($p < 0.05$). DI_o/CS_m in ICSSH31 increased by 124%, while in Bulldozer the increment was only 60.9%. A significant difference was also found between both hybrids in the reduction of quantum yield of the electron transport from Q_A to Q_B ($\varphi ET2_o$). The maximum quantum yield (φP_o) was also greatly reduced after 96 hours of chilling (ICSSH31 by 20.8% and Bulldozer by 10.3%). The functionality of the electron transport chain was ameliorated after 48 hours of re-warming in both hybrids, as is clearly evidenced by most of the biophysical parameters evaluated, as for example the light energy dissipation parameters (DI_o/CS and φD_o).

Figure 3.2 shows the changes in the initial and last phase of the JIP-test (O-J phase), denoted as W_{O-J} and (I-P phase) denoted as W_{I-P}, induced by the different periods of exposition to chilling and rewarming temperatures (T_{48}, T_{96} and T_{48RW}). In general, the curve amplitudes become more negative as the exposure time to chilling temperatures increased. However, it is worth to point out that only ICSSH31 showed a significant electron transport recovering at T_{48RW} as it evidenced by the positive amplitude of W_{O-J} in Figure 3.2, Panel B).

3.3.2. Quenching analysis

Either 48 or 96 hours of exposure to chilling temperatures provoked significant changes ($p < 0.05$) in the efficiency of light energy capture by PSII (Fig. 3.3). However, the responses were not significantly different between both hybrids. Photochemical quenching (q_p) was already

reduced by 70% and 80% in Bulldozer and ICSSH31 after 48 hours of chilling and such level of inhibition was maintained even after 96 hours of chilling (Fig. 3.3, panel A). The operational quantum yield ($\Phi'M_{II}$) resembled the response of q_p (Fig. 3.3 panel C). There were only significant changes in F_0 and F_m after 96 hour of treatment in relation to the control (T_0). In the case of non-photochemical energy conversion of PSII via non-regulated pathway (Φ'_{NO}) and photochemical energy dissipation of PSII via regulated pathway (Φ'NPQ), both parameters increased in both cultivars and reached their maximum values at 96 and 48 hours after chilling, respectively. Such increments were higher, though not significantly different, in ICSSH31 than in bulldozer. After 48 hours of re-warming all parameters showed variable degrees of recovered functionality.

3.3.3. Changes in pigment concentration

After 96 hours of exposition to chilling temperatures a peak in the maximal de-epoxidation state of the xanthophyll pool (A+Z/V+A+Z) was reached in both cultivars (Fig. 3.4; panel A). However, significant differences between bulldozer and ICSSH31 were found only at 48 hours of chilling. As for ICSSH31 the ratio (A+Z/V+A+Z) increased 4,6 folds in comparison to T_0, whereas in Bulldozer the value of the ratio increased only 2,5 folds with respect to T_0 after 48 hours of chilling stress. Significant differences were also found between Bulldozer and ICSSH31 ($p < 0.05$) at T_{48}. After 48 hours of re warming the de-epoxidation state of the xhantophyll pool turned back to pre-chilling values in both hybrids. Luteine concentration was similar in both cultivars and remained almost constant through the chilling treatment period (Fig 3.4; panel B). A significant reduction of chlorophyll a+b after 96 hour of chilling and almost complete recover after 48 of re-warming was observed in ICSSH31. On the other hand chlorophyll a+b concentration remained unchanged in Bulldozer during the whole chilling cycle. Beta carotene concentrations in both cultivars decreased up to 96 hours of chilling. Afterwards, normal beta carotene concentrations were observed at 48 hours of re-warming.

The operational quantum yield ($\Phi'M_{II}$) was related in a negative exponentially mode to the conversion state of the xanthophyll cycle [(Z+A)/(V+A+Z)] along the whole chilling cycle (Fig. 3.5). In addition, the maximum chlorophyll fluorescence emission measured by modulated chlorophyll fluorescence showed a negative linear response to the changes in the xanthophyll state as shown in the inlet of figure 3.5.

3.3.4. A/C_i response to chilling temperature

The responses of net CO_2 assimilation (*A*) to the variation of the internal CO_2 partial pressure (C_i) are shown in Figure 3.6. The degree at which net photosynthesis varied to the increasing of internal CO_2 depended on the time of chilling exposure as well as depending on the hybrid. The fundamental differences between hybrids were found at the beginning of the treatment (T_0), where ICSSH31 showed lesser Rubisco activity (V_{pr}) that Bulldozer. Besides, after 48 hour of chilling treatment, both CE and V_{pr} fell slightly more in ICSSH31 that in Bulldozer.

The reduction of the efficiency of PEPc assessed by the analysis of the *A*/C_i curves is shown in Figure 3.7, panel A. After 48 hours of chilling the CO2 saturated photosynthetic rate (V_{pr}), representing the Rubisco activity, dropped in both cultivars. The V_{pr} decreased in both cultivars by about 45% with respect to T_0, nonetheless, the lowest value was observed in ICSSH31 (6.4 μmol m^{-2} s^{-1}). At T_{96}, the V_{pr} remained unchanged in ICSSH31. In contrast, Bulldozer continued to decrease up to 4.9 μmol m^{-2} s^{-1} After 48 hours of re-warming, V_{pr} in Bulldozer turned back to pre-chilling values (about 20 μmol m^{-2} s^{-1}) while in ICSSH31 the recovered V_{pr} overpassed the pre-chilling value by about 7 μmol m^{-2} s^{-1}.

As in V_{pr}, the PEPc efficiency (CE) was strongly reduced by the exposure time to chilling temperatures (Figure 3.7; panel B). After 48 hours of chilling, CE decrements were significantly higher in ICSSH31 than in Bulldozer ($p< 0.05$). At T_{96} CE values continued to decrease but no

longer significant differences between cultivars were evident. At TR_{W48} CE values turned back close to pre-chilling values.

The relationship between some JIP- test parameters and V_{pr} are shown in figure 3.8. The reduction of V_{pr} caused by the chilling treatment provoked an enhancement on the efficiency of the reduced intersystem electron acceptor to the PSI electron acceptors ($\delta RE1_o$). Even though both cultivars followed the same pattern, increment rates of $\delta RE1_o$ per decrease in V_{pr} were higher in bulldozer than in ICSSH31 (Fig 3.8, Panel A). On the contrary, the quantum yield of the electron transport flux from Q_A to Q_B ($\varphi ET2_o$) and V_{pr} were positively correlated, but with a more pronounced slope in the case of ICSSH31 (slope of -0.012) than in the case of Bulldozer (slope of -0.005).

The quantum yield for energy dissipation (φDI_o) and V_{pr} were also significantly correlated. The rates of energy dissipation increments where faster in ICSSH31 (slope of - 0,0135) than in Bulldozer (slope of -0.0047). The photochemical quenching (q_P) was also significantly and linearly correlated with V_{pr}. In the case of ICCSH31 such relationship seems to be more sensitive (slope of -0.046) than in Bulldozer (slope of -0.012).

The efficiency of PEPcase was also linearly related to $\delta RE1_o$, $\varphi ET2_o$, φDI_o, and qP. Such relationships followed the same patterns as in the case of V_{pr}. However, significant differences between cultivars were found only in the $\delta RE1_o$ and CE PEP correlation.

3.4. Discussion

3.4.1. Effect of chilling on PSII electron transport and pigment composition

The photosynthetic performance, in terms of quantum efficiency, was strongly constrained in both sweet sorghum hybrids by chilling temperatures (Fig. 3.1, 3.2 and 3.3). Nevertheless, the

degree at which they responded was variable depending on their genetic background and time of exposure. In the case of ICSSH31, the hybrid that poorly performed under field conditions, showed an earlier sensitivity to cold stress, evidenced by a higher accumulation of zeaxhantin induced by 48 hours of chilling exposition ($T_{48;}$ Fig. 3.4, Panel A). Such response is thought to be a common and effective mechanism to self-alleviate the excessive incoming energy pressure which takes place under high light or under environmental stress condition known as photoinhibition (Adams et al. 1995). Yet, it is not well understood whether such response is an intrinsic protective mechanism against photodamage or it is an acclimation response to cold stress. For instance, it was shown, that in annual crops of different chilling sensitivity such as barley [*Hordeum vulgare L.*], wheat [*Triticum aestivum L.*], maize [*Zea mays L.*], sorghum [*Sorghum bicolor L.*] etc. photoinhibition (assessed by $_{\varphi}P_o$ or what is the same F_v/F_m) induced by low light and temperature conditions, was up to 166-fold grater in sensitive species in relation to the tolerant ones. Nonetheless, while the photoinhibition during chilling generally occurred more rapidly in chilling-sensitive plants, this was not related directly to chilling sensitivity (Hetherington et al. 1989). In contrast, other study in maize plants which were grown at suboptimal (15°C) temperature conditions, exhibited a greater de-epoxidized xanthophyll and a faster recovering capacity of those growth at 25°C when were subjected to chilling temperatures of 6°C for 4 days (Leipner et al. 2000), supporting the fact that photoprotection represent an acclimation response rather than an intrinsic characteristic of the variety.

Besides the photoinhibition at electron acceptor of PSII (Fig. 3.1), the JIP-test analysis revealed other significant difference between the hybrids. It was observed that the efficiency of the electron transport acceptor around PSI was enhanced in ICSSH31 by short-term low temperature (T_{48}; Fig. 3.1). The fact that the increment in the electron flux to oxygen in circumstances where CO_2 assimilation is restricted, is seen as necessary condition to allow the PSII electron acceptors to be maintained in a partially oxidized state in order to minimizing the possibility of

photoinactivation and damage of PSII (Farage et al. 2006). Nonetheless, such strategy could only be achieved in condition that the reactive oxygen species generated by the reduction of oxygen were quickly scavenged, if not they could damage the thylakoid membrane and other cellular components (Asada 1999a,1999b, Baker 2002, Farage et al. 2006). Our results seems to be in agreement whit those observed in maize growing at low temperatures in field which showed a rise in the ratio of quantum efficiencies of the electron transport and CO_2 fixation in contrast to leaves grown at optimal temperatures (Fryer et al. 1998). Therefore, Bulldozer and in a greater degree ICSSH31, could have amplified the rate of oxygen reduction by PSI with the aim of maintaining the electron transport and help to prevent the complete reduction of PSII electron acceptors and hence limit further injuries of PSII reaction centers (Fig 3.1; Ort and Baker 2002).

The cold treatment provoked the degradation of chlorophyll a and b in ICSSH31, which was more evident after 96 hours of chilling exposure. However such effect was not observed in Bulldozer. This characteristic of losing prematurely and in greater degree chlorophyll pigments, mainly Chl. a and b was attributed to sensitive genotypes of *Zea maize* in response to chilling stress with respect to tolerant ones (Haldimann 1998). Beside pigment bleaching, low growing temperature in maize was found to induce modifications on the thylakoid composition in the mesophyll and bundle sheath cells, since low temperature could cause failure in the accumulation of in a number of polypeptides encoded by chloroplasts genome (Nie and Baker 1991; Nie et al. 1995).

The ending of the chilling period (T_{48RW}) was followed by a partial the recovering of the photochemical efficiency of the electron transport in both varieties. Even though this process has been stated to be accompanied by an increment in the thylakoid plastoquinone A content as well as an apparent size in the intersystem electron donor pool to PSI (Gray et al. 1997), our results showed that in ICSSH31 the donor side of PSII electron transport chain capacity was enhanced (evaluated by the variable fluoresncen W_{O-J} in relation to T_0; Fig 3.2), exceeding the initial

condition. This feature could have took place due to ROS scavenging system is increased under cold conditions as well as dissipation via non-photochemical quenching (Fig. 3.3, Panel B and D; Streb and Feirabend, 1999; Streb et al. 1999; 2003 a,b). Consequently, it could be said that, at PSII electron transport level, in both verities of sweet sorghum the mayor defense against photoinhibition processes was the dissipative non-photochemical quenching mediated by the de-epoxidation of xanthophyll pigments.

3.4.2. Effect of chilling on stomatal conductance, PEPc and Rubisco activity and (A/Ci curves)

The CO_2 assimilation rate in both sweet sorghum verities strongly fell by the effect of low temperatures (Fig. 3.6 and 3.7). Such depletion was even more pronounced than that of the PSII electron transport (Fig. 3.1, 3.2 and 3.3). It is widely accepted that the main restriction to cold temperatures on the photochemical events in C4 species is the enzymatic activity of the C4 and Benson-Calvin cycles as well as from a decrease in metabolite transport (Leegood & Edwards 1996). However the degree at which enzymatic activity is reduced by low temperature may differ among sensitive/tolerant genotypes being still a matter of study. In this study the short-duration of chilling exposure (T_{48}; Fig. 3.7 Panel A and B) significantly reduced the PEPc efficieny (CE) and Rubisco activity (V_{pr}), calculated from the initial slope and the asymptote of the *A* in function of CO2 internal partial pressure (Ci), respectively according to von Caemmerer and Farquar (2000). These results are in accordance with previous studies on photosynthetic response to low-temperature tolerance in maize and *Miscanthus x giganteus*, two species which share the same enzymatic pathway with sweet sorghum (C_4 NADP-ME type). The last revealed that ribulose biophosphate carboxylase/oxygenase (Rubisco) and/or pyruvate orthophosphate dikinase (PPDK) played a more preponderant role which determines the low temperature tolerance to that of PEPcase effiency (Naidu and Long 2004). Here, in relation to the no stressed situation Rubisco activity was significantly more impaired than PEPcase by chilling in both

verities, supported by fact that C4 plants are inherently more prone to undergo Rubisco limitation as declines the temperature (Wang et al. 2008). However, after 48 hours of chilling stress, ICSSH31 suffered a greater reduction of enzymatic activity (Rubisco and PEPc) than Bulldozer. It is worthy to note, however, that ICCSH31 showed minor Rubisco activity than Bulldozer before the commencement of the chilling treatment (Fig. 3.7 Panel A), so in biochemical terms, this could indicate a lesser intrinsic Rubisco content which explain the poorer photosynthetic performance of ICSSH31 in contrast to Bulldozer (Kubien et al. 2003).

The reduction on the enzymatic activity was followed by an increment in stomatal aperture (Fig. 3.7; Panel C). Such phenomenon was previously observed by Mústardy et al. (1982) in of *Zea mays* genotypes of different chilling sensitivity, such that chilling reduce the stomatal limitation, apparently as consequence of water uptake limitation (Sowinski and Królikowski 1995), as well as increased leakage of electrolytes (Janowiak and Markowski 1994), but care should be taken since we did not determined water relation in this study.

Despite to the fact that, ICSSH31 showed a less photosynthetic activity before the commencement of the chilling cycle in comparison to Bulldozer, we should note that after 96 hours of chilling temperatures ICSSH31 apparently underwent a stimulant effect on its photosynthetic machinery (Fig. 3.7 Panel A). It is likely that temperature compensation may have been achieved by ICSSH31, since short-duration of chilling exposure in some cases, may induce an increment in amounts of enzyme involved in CO_2 fixation. As a result of this mechanism of enzymatic modulation, which is metabolically expensive and inefficient, is likely to be one of the reason that explain its poor performance at final harvest time (Somero 1978).

3.5. Conclusions

Despite of having showed a differential response to the chilling treatment, both sweet sorghum varieties were able to successfully adapt their photosynthetic apparatus through coordinated mechanism involving either enzymatic compensation, energy dissipation via non-photochemical quenching mediated by xanthophyll cycle pool, and possibly reducing stomatal limitation. The lower performance of the variety ICSSH31 observed under field conditions could be a result of the greater metabolic expense to self-regulate its photosynthetic machinery by re-synthesis pigments (chlorophyll a, b, lutein and beta-carotene), increment of Rubisco content and anticipated engagement of zeaxanthin in thermal energy dissipation.

Fig 3.1 Radar plots showing the changes in the constellation of JIP-test parameters to the onset of chilling treatment (before, during and after chilling exposure) in Bulldozer and ICSSH31 hybrids.

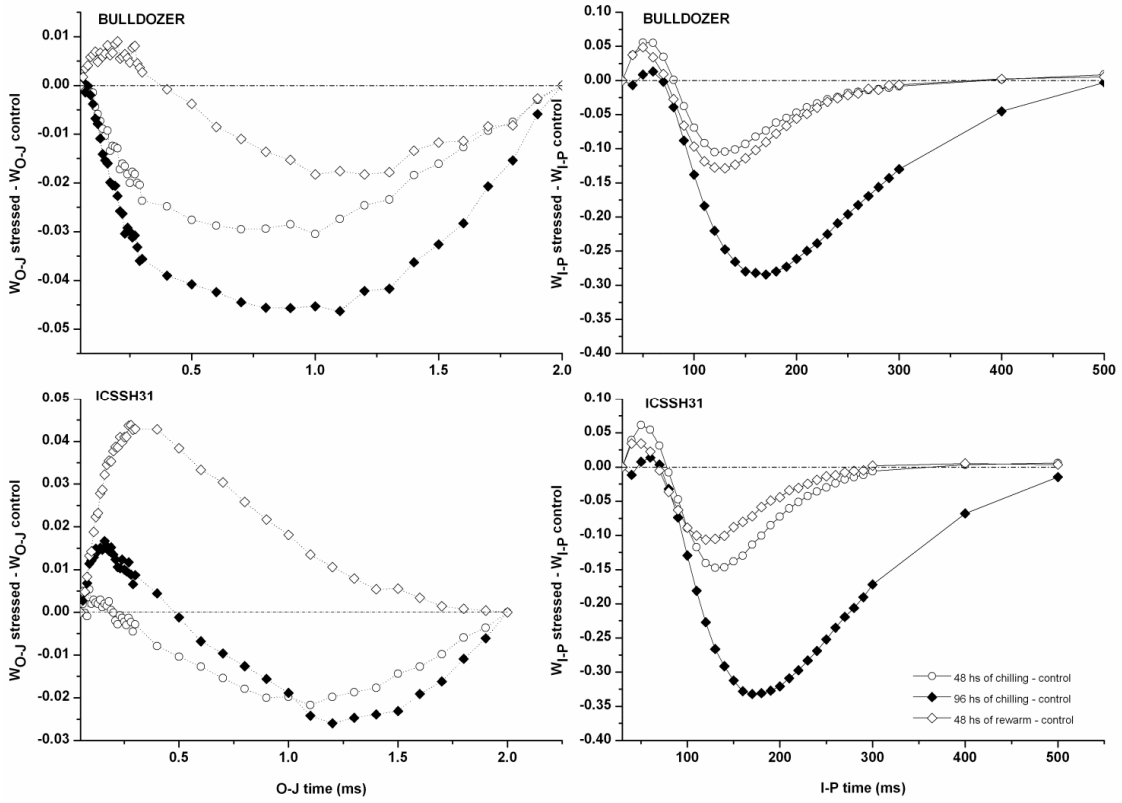

Fig. 3.2 Relative changes in O-J and I-P phase of the JIP-test at different time-exposure of chilling.

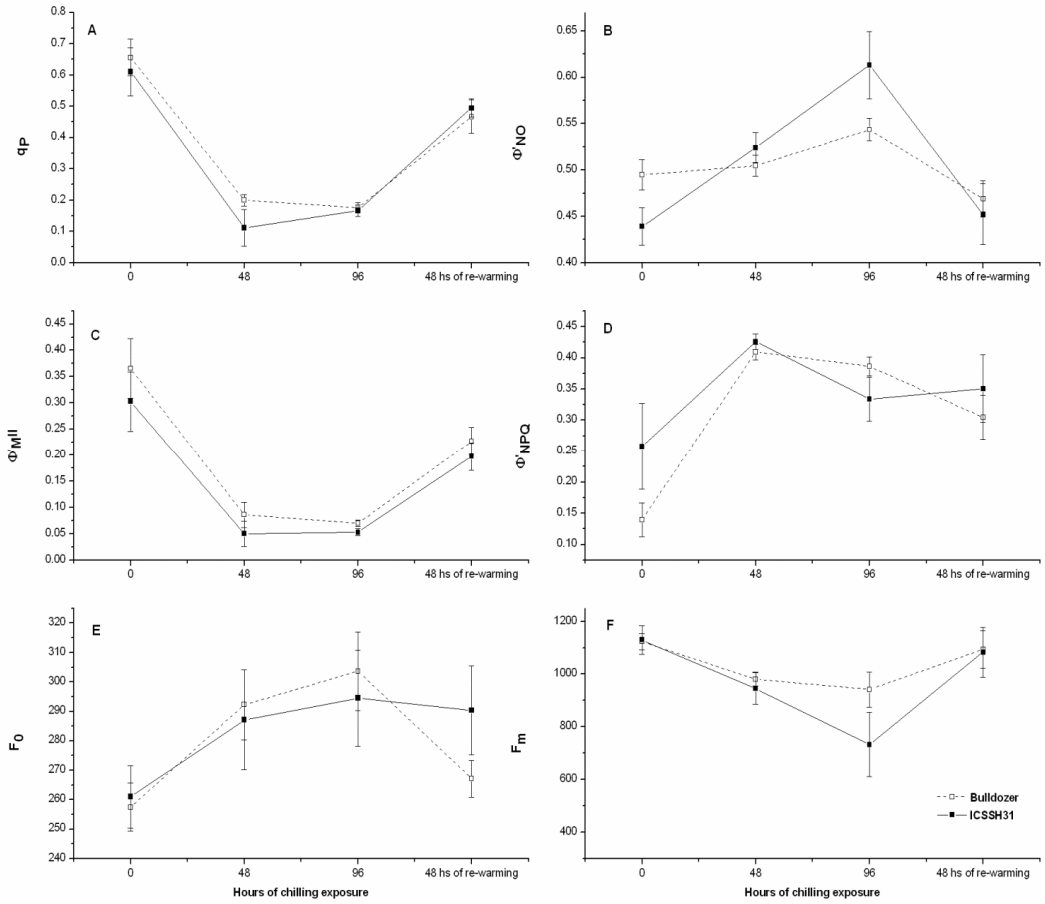

Fig. 3.3 Changes in modulated Chl. a fluorescence parameters to at different time-exposures of chilling in Bulldozer and ICSSH31 hybrids. In panel A q_P values indicate the photochemical quenching ($q_P = (F'_0/ F) / (F_m/F_0)$). In panel B, the yield of non-photochemical energy conversion via PSII (non-regulated pathway) is represented by $\Phi'_{NO} = 1 / (((F_m - F'_m) / F'_m) + 1 + (q_P (F'_0/F)) / (Fm/F_0 - 1))$; in panel C, the operational quantum yield expressed by $\Phi'MII = (F'_m - F_s) / F'_m$. Panel D, the non-photochemical energy dissipation of PSII (via regulated pathway) is denoted by $\Phi'_{NPQ} = 1 - \Phi'_{M}II - \Phi'_{NO}$. In panel E the minimum chlorophyll fluorescence when all the reaction centers are open noted as F_0 (chlorophyll fluorescence emission at time = 50μs) and panel F, F_m represents the maximum chlorophyll fluorescence emission when all the reaction centres are closed (chlorophyll fluorescence emission at time ≈ 300 ms).

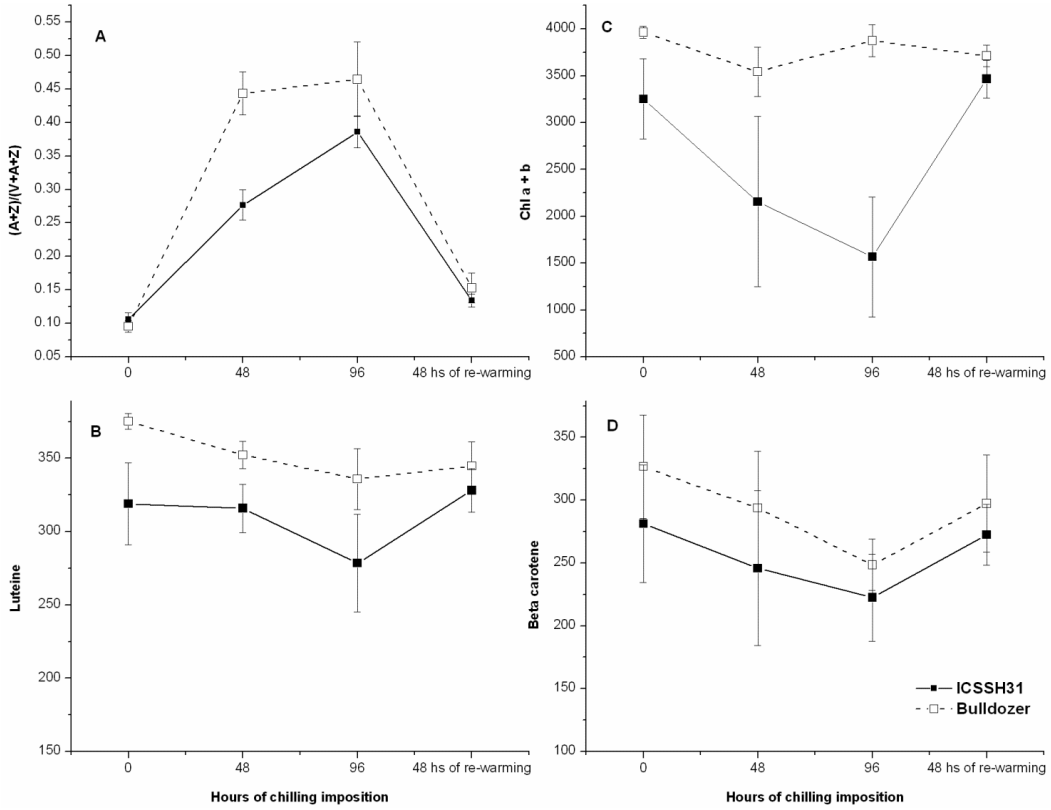

Fig. 3.4 Changes in de-epoxidised form of antheraxanthin and zeaxanthin (A+Z) and leaf pigment concentration (Chlorophyll a+b, Luteine and β-carotene) under different time-exposures of chilling in Buldozer and ICSSH31 hybrids.

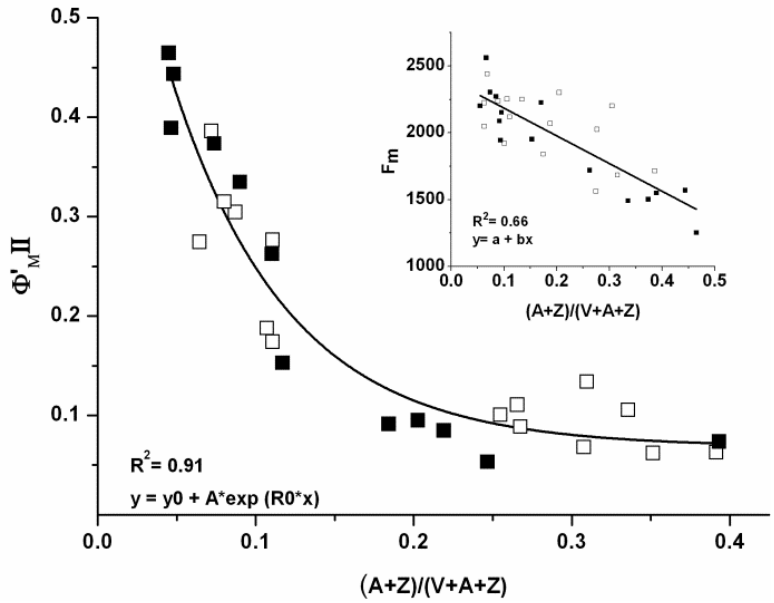

Fig. 3.5 Relationship between the operational quantum yield (Φ'MII = (F'_m – F_s) / F'_m) and changes in de-epoxidised form of antheraxanthin zeaxanthin and violanzanthine (A+Z)/ (V+A+Z) before, during and after chilling treatment. Inset shows the changes in xhantophyll cycle and maximum fluorescence emission F_m.

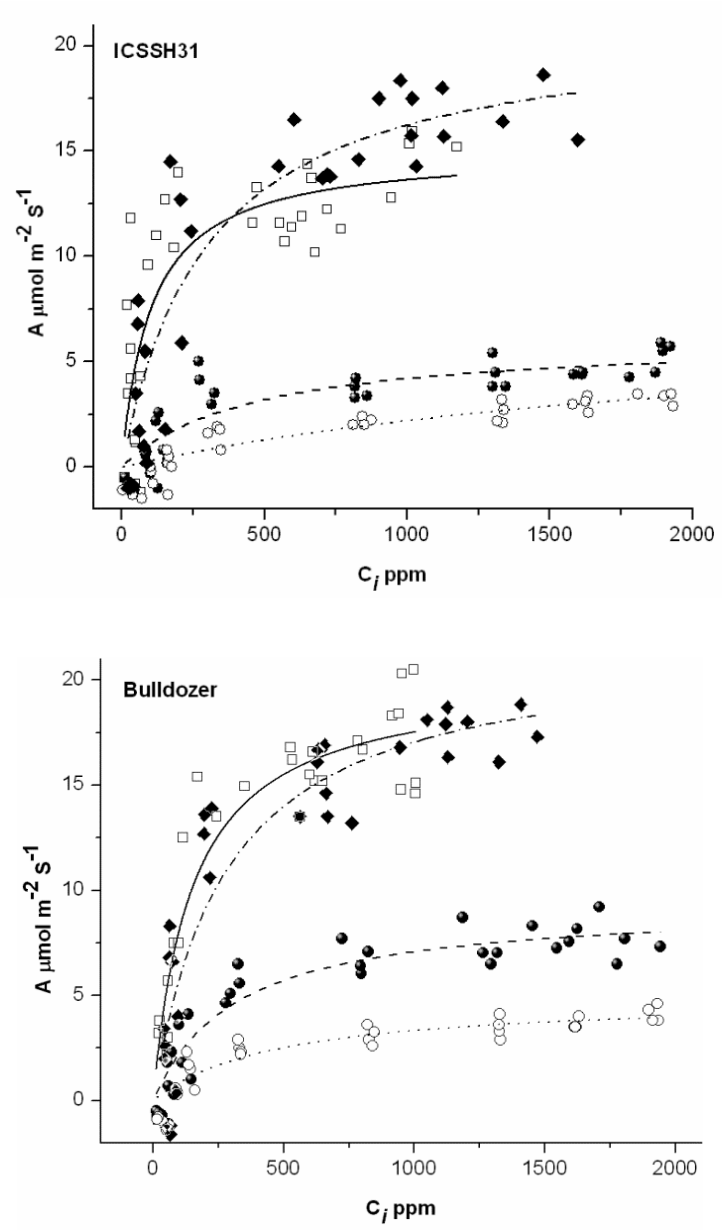

Fig 3.6 Net photosynthetic CO2 assimilation (A), as function of intercellular CO_2 parcial pressure (C_i) in two sweet sorghum hybrid (Bulldozer, Panel A) and ICSSH31 (Panel B). The irradiance was set up at 2000 µmol quanta. Each points represent the average of 4 measurements (n=4) ± SE were taken on the 5th full expanded leaf of different plants. The reference CO_2 concentration were increased to 0, 100, 200, 400, 900, 1400, 1700 and 2000 µmol mol^{-1}.

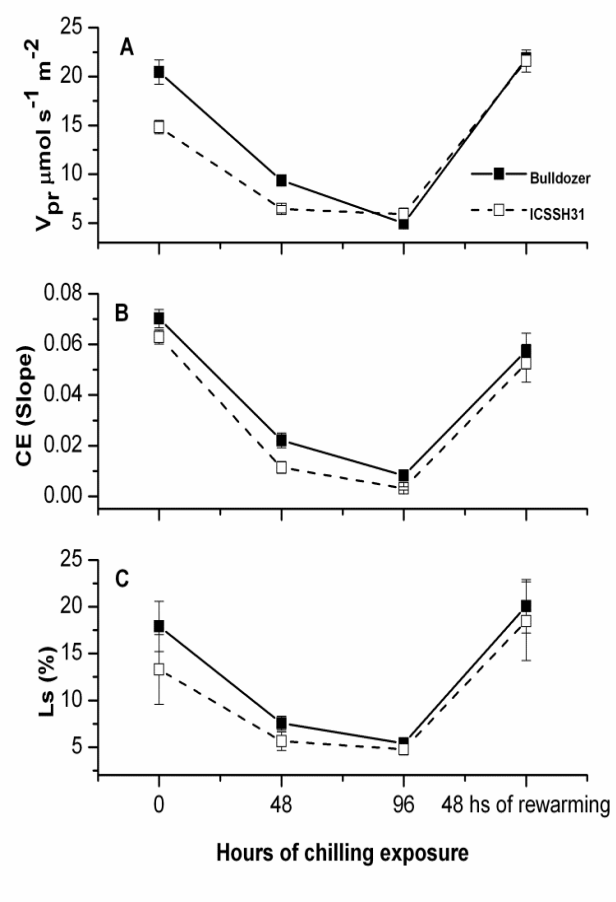

Fig 3.7 Parameters derived from A/C_i curves showing the changes in enzymatic efficiency to the time exposure to chill temperature. In panel A the CO_2 saturated photosynthetic rate (V_{pr}) expressing the PEP regeneration capacity which is proportional to the Rubisco activity. In panel B changes in PEPc efficiency, calculated by the initial slope of A/C_i with Ci < 100 µmol m^{-2} s^{-1}. Panel C shows the percent of the stomatal limitation (Ls).

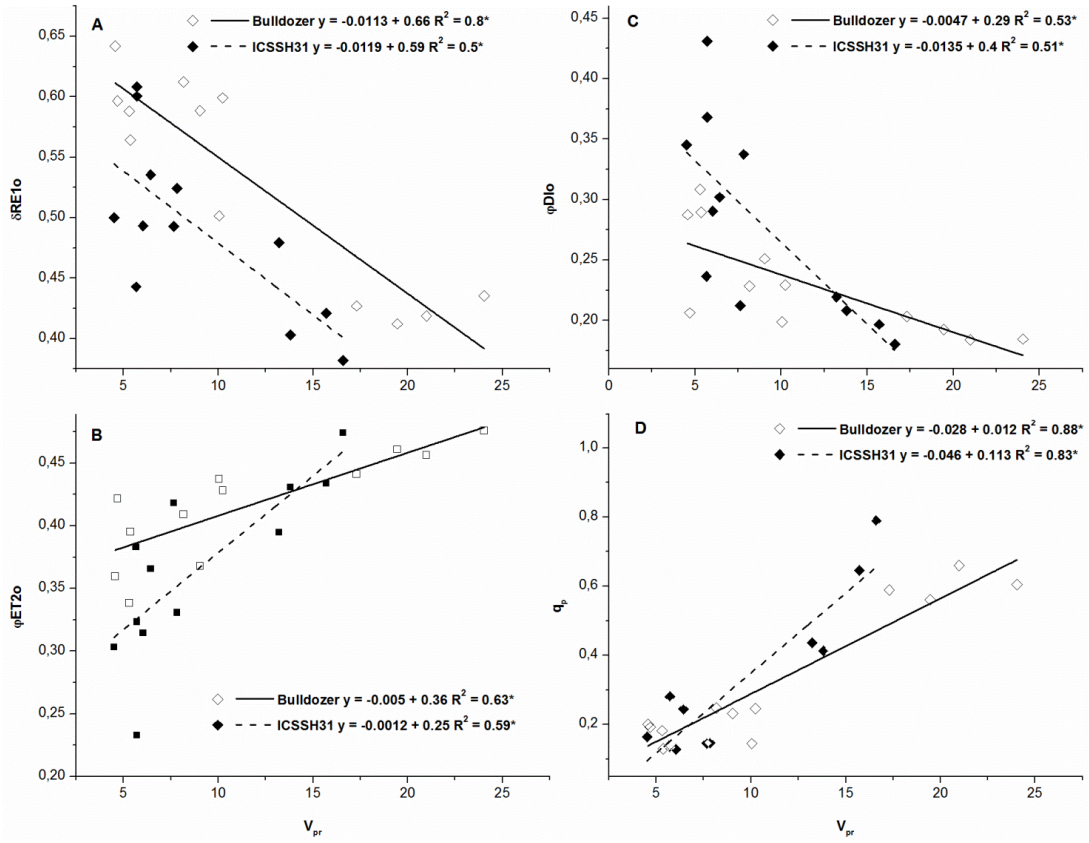

Fig. 3.8 Relationship among PEP regeneration capacity (V_{pr}) and: the efficiency of the reduced intersystem electron acceptor to the PSI end acceptor ($\delta RE1_o$; panel A), the quantum yield of the electron transport flux from Q_A to Q_B ($\varphi ET2_o$; panel B), the quantum yield for energy dissipation (φDIo; panel C) and the no photochemical quenching (q_P).

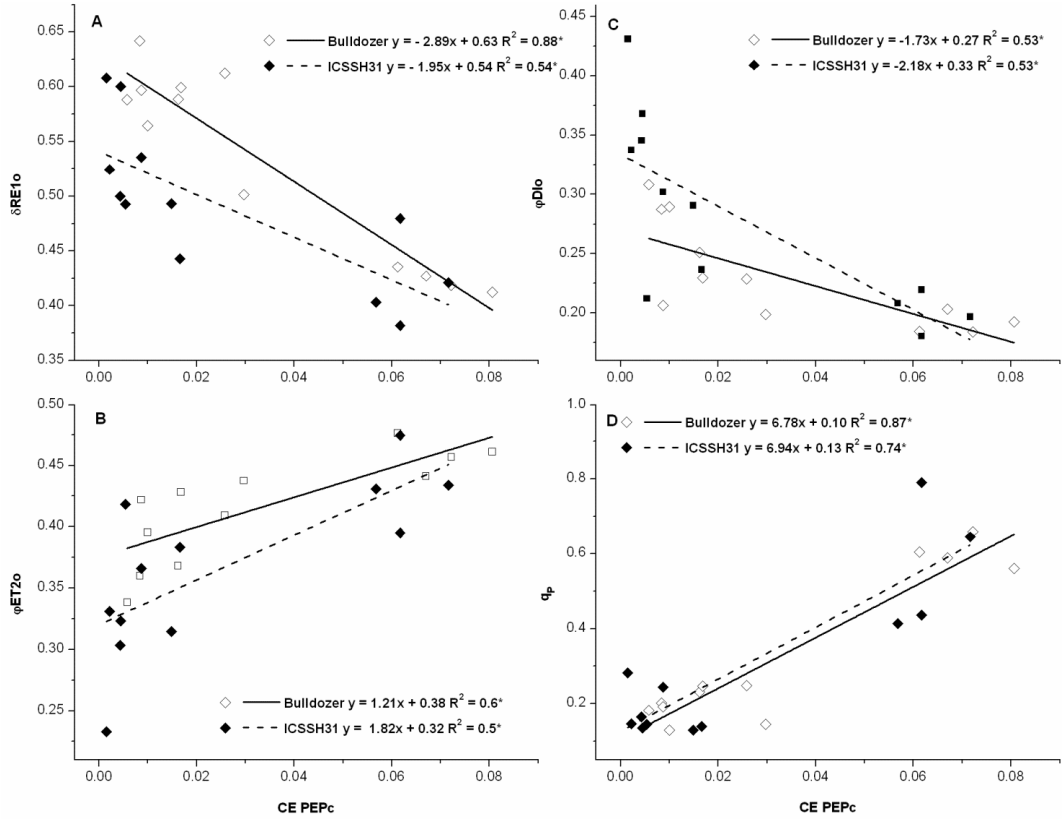

Fig. 3.9 Relationship among phosphoenolpyruvate carboxilase effieicny (CE PEPc) and: the efficiency of the reduced intersystem electron acceptor to the PSI end acceptor ($\delta RE1_o$; panel A), the quantum yield of the electron transport flux from Q_A to Q_B ($\varphi ET2_o$; panel B), the quantum yield for energy dissipation (φDIo; panel C) and the no photochemical quenching (q_P).

Concluding remarks

Photosynthetic response to progressive drought stress

Young sweet sorghum plants showed a higher capacity to maintain the PSII electron transport functional activity under progressive drought than mature plants. Such capacity was attributed to the efficient down-regulation mechanism ofPSII electron trasport, mainly by increased light energy dissipation (DI_o/CS_m) and the closure of active reaction centers (RC/CS).

Even though at young developmental stages the earliest indication of drought was given by the stomatal conductance (g_s) it did not provoke any symptoms of photoinhibition. It was speculated that the premature chlorophyll degradation induced by drought at mature stageswas a decisive factor reducing their drought tolerance. Besides that, the capacity to release the excessive energy (DI_o/CS_m) seems to become ineffective when plants enters to the blooming stage.

Some evidence of a possible role of soluble sugars as photo-protective compounds against the oxidative effects of drought indicated that at young stages the predominant sugars playing such a role were glucose and sucrose, while at booting stage only fructose seems to act as a photo-protective compound. Moreover, it is suggested that the soluble sugars beside their well-known functions such as osmoregulation, they could participate in the complex system that controls ROS production/reduction. Since this statement was based on the linear relationship found between sugars accumulation and the energy light dissipation and closure of active reaction centers, caution should be taken as further photochemical and biophysical analysis would be needed.

In addition to that it was found that the acceptor side of PSI was more sensitive to drought than the donor side of PSII. What is more, such sensitivity was closely related also to the plant age, suggesting that in booting plants the blockage of transfer of electrons to PSI is the main cause for photoinhibition.

Photosynthetic and biochemical response of sweet sorghum hybrids to cold stress

Under field conditions the cold effects of early sowing induced biochemical and physiological disruptions in all the hybrids studied. Seedling cold acclimation resulted in the loss of chlorophyll pigments and/or the inhibition of its re-synthesis, which was linked to low total nitrogen content. Moreover, the low N content in the leaves reduced significantly the PSII electron transport activity thus the CO_2 assimilation capacity as indicated by the reduced biomass accumulation up to the 5^{th} leave stage. Even though all the hybrid seedlings showed a similar degree of such metabolic limitations, Bulldozer was able to revert more efficiently the early season cold effects. Taking as reference this results we should highlight the high end of season performance of Bulldozer when sown under cold conditions. So the high biomass production potential of Bulldozer makes it valid candidate to extend sweet sorghum production areas to temperate zones. On the other hand, ICSSH31 hybrids was one of the hybrids that could not significantly increase its end of season biomass production when sown early in the season, suggesting that could not effectively revert cold effects at the seedling stage.

The photosynthetic and biochemical adaptation mechanisms of the most contrasting hybrids found in the field study (Bulldozer and ICSSH31) were analyzed in detail under controlled environmental conditions. Even though neither direct nor modulated chlorophyll *a* fluorescence revealed significant differences between hybrids, it was confirm that the hybrid ICSSH31 was more sensitive to cold than Bulldozer. Such sensitivity was evidenced by an anticipated accumulation of zeaxanthin, larger lutein loss, and an over expression of Rubisco during the re-warming period. Furthermore, after 48 hours of exposure to chilling temperatures ICSSH31 underwent a degradation of chlorophyll a and b, while in Bulldozer did not show signs of chlorophyll loss. Therefore the poor performance of the ICSSH31 after four days of chilling could be related to the great metabolic cost to acclimate its photosynthetic apparatus by re-

synthesis pigments (chlorophyll a, b, lutein and beta-carotene), increment of Rubisco content, and anticipated engagement of zeaxanthin in the thermal energy dissipation mechanisms. Even though the results obtained from this study (controlled environmental conations) cannot be extrapolated to field situations, our study may contribute to improve the understanding of the physiological processes that govern the tolerance/sensitivity of different origin/genetic background sweet sorghum hybrids under sub optimal growing conditions.

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