1 **Differential heat sensitivity index in barley cultivars (*Hordeum vulgare* L.)**
2 **monitored by chlorophyll *a* fluorescence OKJIP**

3

4 Abdallah Oukarroum ^{a,b *}, Saïd El Madidi ^c, Reto J. Strasser ^b

5

6 ^a Present address: Department of Chemistry and Biochemistry, University of Quebec in
7 Montreal, C.P. 8888, Succ. Centre-Ville, Montreal, Quebec, H3C 3P8 Canada.

8 ^b University of Geneva, Bioenergetics and Microbiology laboratory, CH-1254 Jussy-
9 Geneva, Switzerland.

10 ^c University Ibn Zohr, Faculty of Sciences, Department of Biology, BP/S-80 000 Agadir,
11 Morocco

12

13 * Corresponding author: Dr. A. Oukarroum

14 Email address: Oukarroum.abdallah@uqam.ca

15 Tel.: +1 514 987 3000

16 Fax: +1 514 987 4054

17

18

19

20

21

22

23

1 **Abstract**

2

3 The objective of this study was to differentiate the heat tolerance in ten varieties of
4 barley (*Hordeum vulgare* L.) originating from Morocco. Five modern varieties and five
5 landraces (local varieties) collected at five different geographical localities in the south
6 of Morocco were investigated in the present study. After two weeks of growth, detached
7 leaves were short term exposure to various temperatures (25, 30, 35, 40, and 45 °C) for
8 10 min in the dark. Two chlorophyll *a* fluorescence parameters derived from chlorophyll
9 a fluorescence transient (OKJIP) (performance index (PI_{ABS}) and relative variable
10 fluorescence at the K-step (V_K)) were analysed. Heat treatment had a significant effect
11 on the PI_{ABS} and V_K at 45 °C treatment and the analysis of variance for PI_{ABS} and V_K is
12 highly significant between all varieties. The slope of the relationship between logPI_{ABS}
13 and V_K named heat sensitivity index (HSI) was used to evaluate the thermotolerance of
14 photosystem II (PSII) between the studied barley varieties. According to this approach,
15 barley varieties were screened and ranked for improving heat tolerance. HSI was found
16 to be a new indicator with regard to distinguishing heat tolerance of different barley
17 cultivars.

18

19 *Keywords:* JIP-test; barley varieties; heat sensitivity index

20

21

22

23

1 **Introduction**

2

3 Barley plants (*Hordeum vulgare* L.) grown in a wide geographic range under different
4 geographical conditions and are subjected during their development to various
5 environmental stresses such as drought stress and high temperature. Heat stress due to
6 high ambient temperatures is one of the major abiotic stresses that limit plants
7 productivity (Hall, 2001; Allakhverdiev et al., 2008; Shao et al., 2015). Indeed, heat
8 stress disturbs the normal cellular homeostasis and then affects plants growth and
9 development (Wahid et al., 2007). Beside metabolic processes occurring in plants,
10 photosynthesis is considered as a sensitive metabolic process in photosynthetic
11 organisms to high temperature stress and is often inhibited before impairment of other
12 cell functions (Berry and Björkman, 1980). Furthermore, photosystem II (PSII) is
13 considered to be the most thermo-sensitive components of photosynthetic apparatus
14 (Baker, 1991). Bilger et al. (1987) reported that Calvin-Benson cycle activity was
15 sensitive to rapid heat-stress treatments. Further, rubisco (ribulose 1,5-bisphosphate car-
16 boxylase/oxygenase) activase in the Calvin cycle is also one of the most thermosensitive
17 compounds associated with the photosynthetic apparatus (Eckhardt et al., 1997; Law
18 and Crafts-Brandner ,1999; Crafts-Brandner et al., 2000). Furthermore, high
19 temperatures -in the range 35-45°C- induced a drastic inhibition of carbon-dioxide
20 fixation and photophosphorylation capability (Berry and Björkman 1980). However,
21 photosystem I (PSI) has been reported to be heat resistant comparatively to PSII
22 (Havaux, 1992; Yan et al., 2013).

1 It is known that responses of plants to high temperature are species dependent and it is
2 primordial to understand how these plant species cope with heat stress (Oukarroum et
3 al., 2012; Wang et al., 2014). It has been reported for instance that photosynthesis
4 studied at level of PSII in wheat plants are more sensitive to heat stress than the rice
5 plants, which is a species of the hot regions and it is adapted to higher temperatures (Al-
6 Khatib and Paulsen, 1999). Moreover, alterations of photosynthesis activity under heat
7 stress was suggested to be a good indicators of thermotolerance of the plants (Wahid et
8 al., 2007).

9 It has been well documented that the short-term exposure of plants to high temperatures
10 provokes changes of the structure and function of the photosynthetic apparatus (Yaman
11 et al., 1998; Yan et al., 2011, 2013a). Early studies in respect to heat-induced damage of
12 PSII found that heat treatment leads to various damages, such as inhibition of the
13 donation of electrons by OEC (Guissé et al., 1995); inhibition of the electron flow from
14 Q_A to Q_B (Cao and Govindjee, 1990) and inhibition of the charge-separation activity of
15 the PSII reaction center (Allakhverdiev et al., 1996).

16 Study of the energetic behaviour of photosynthetic organism was highly investigated by
17 Chlorophyll (Chl) *a* fluorescence measurements (Strasser et al., 2004; Stirbet and
18 Govindjee, 2011). Chl *a* fluorescence method was widely applied to probe the stress-
19 induced damage in photosynthetic apparatus and for monitoring and screening plant for
20 environmental stress tolerance (Oukarroum et al., 2007; Oukarroum et al., 2009;
21 Goltsev et al., 2012; Yan et al., 2013b; Pollastrini et al., 2014; Chen et al., 2015;
22 Salvatori et al., 2015). In fact, Chl *a* fluorescence kinetics provides detailed information
23 on the structure and functions of PSII (Strasser et al., 1995; 2004).

1 Chl *a* fluorescence OKJIP is emitted by photosynthetic organisms on a dark-to-light
2 transition and is characterized by a polyphasic fluorescence increase. The fluorescence
3 intensity increases from a minimum fluorescence intensity (F_0) to a maximum
4 fluorescence intensity (F_M) and with two intermediate steps named as J (F_J) and I (F_I).
5 This fluorescence transient was divided into three phases (O–J, J–I and I–P) and these
6 phases reflect three different reduction processes of the electron transport chain and K
7 step (at about 200–400 μ s) can often be visualised under heat treatment or by subtraction
8 of differences between fluorescence transients (Strasser et al., 2004; Oukarroum et al.,
9 2007).
10 Furthermore, analysis of fluorescence parameters derived from Chl *a* fluorescence by
11 JIP-test model provide information about specific fluxes, phenomenological fluxes and
12 vitality indices (Strasser 1986, Strasser et al., 2004). This model has been proposed for
13 the analysis of the polyphasic rise OJIP measured during the first second of illumination
14 and has been shown to be a valuable tool in plant vitality monitoring. JIP-test uses the
15 theory of energy fluxes in biomembranes to calculate several phenomenological and
16 biophysical expressions for a given physiological state. In addition, JIP-test model was
17 also involved to introduce new parameters for monitoring dark chilling tolerance in
18 soybean genotypes (Strauss et al., 2006); ranking drought tolerance of barley varieties
19 (Oukarroum et al., 2007) and sesame lines (*Sesamum indicum* L.) (Boureima et al.,
20 2012).
21 It is accepted that temperatures will increase significantly in future (Weltzin et al.,
22 2003). However, it is necessary to search for tolerant plant varieties able to adapt in a
23 changing temperature environment. Recently, JIP-test model was used to drive a new

1 fluorescence parameter named heat sensitivity index (HSI) (Chen et al., 2016). This
2 parameter was introduced as an indicator of plant heat sensitivity. HSI was performed to
3 classify and characterise heat tolerance in four *Ageratina adenophora* populations and it
4 corresponds to the absolute value of the slope (K) of the relationship between two Chl *a*
5 fluorescence parameters: the logarithm of performance index and the relative variable
6 fluorescence at the K-step.

7 In the present work, we investigate the heat tolerance in ten Moroccan barley varieties
8 (five modern varieties and five landraces). Analysis tolerance of these different varieties
9 to heat stress could be encouraging to select varieties able to adapt environmental
10 conditions in hostile areas such as in arid and semi-arid regions. Our hypothesis is that
11 the tolerance of the modern and landrace barley varieties to heat treatment can be
12 elucidated at PSII level by studying the behaviour and change in chlorophyll
13 fluorescence parameters. According to the approach proposed by Chen et al., (2016) and
14 the calculated HSI, heat tolerance response of these ten barley varieties was analysed.

15

16 **Material and methods**

17 **Plant material**

18 The following modern barley varieties were used: Arig 8 (Ar), Igrane (Igr), Lannaceur
19 (La), Massine (Ma) and Rabat 071 (Ra) obtained from the National Institute of
20 Agricultural Research (INRA) of Morocco and five landraces (Aït Baha (Ab), Ighrem
21 (Ig), Immouzzet (Im), Tarodant (Ta) and Tizinit (Tz)) collected by the Provincial
22 Direction of Agriculture (DPA) originating from five localities in the south of Morocco
23 (Table 1).

1 **Plant growth conditions**

2 Grains were sterilised by 10 min treatment with a 5% calcium hypochlorite solution and
3 then rinsed several times with distilled water. The seeds were allowed to germinate for
4 48 h on a sheet of Watman filter paper placed in Petri dishes. The germinated seeds
5 were transplanted into plastic pots (4 l) containing commercial peat soil with 3 plants
6 per pot and in 5 replicates. In the growth chamber experiment, the plants were grown in
7 a controlled environment with day/night air temperature of 25/18 °C, under long-day
8 conditions (16 h light, 8 h dark) and by giving additional light if needed (OSRAM HQIT
9 400W lamps were used). The experimental design was hierarchical in a completely
10 randomised design.

11

12 **Chlorophyll (Chl) *a* fluorescence transient and heat treatment**

13 All Chl *a* fluorescence measurements were conducted on detached third leave on each
14 plant after two weeks of growth, at room temperature with a portable fluorimeter (Plant
15 Efficiency Analyser, built by Hansatech Instruments Ltd. King's Lynn Norfolk, UK)
16 with high time resolution (10 µs). The plants were kept in dark for one hour before the
17 measurements were started. Detached leaves were heated at various temperatures (25,
18 30, 35, 40, and 45 °C) for 10 min in the dark, and kept for 5 min at room temperature
19 before Chl *a* fluorescence measurements. For each variety, 15 measurements were
20 made. The measurements consisted of a single strong 1 s light pulse (3500 µmol photons
21 m⁻² s⁻¹, an excitation intensity sufficient to ensure closure of all PSII reaction centers)
22 provided by an array of six light-emitting diodes (peak 650 nm). The Chl *a* fluorescence

1 emission induced by the strong light pulses was measured and recorded between 10 μ s
2 to 1 s by the instrument.
3 All leaves exhibit a polyphasic Chl *a* fluorescence rise OJIP during the first second of
4 illumination after dark adaptation (see treatment at 25 °C in Fig. 1 for a typical
5 fluorescence transient). The fluorescence intensity increases quickly from a minimum
6 fluorescence intensity (F_o) to a maximum fluorescence intensity (F_M). The OJIP
7 transient can also be divided to two rise phases: the photochemical phase O-J and the
8 thermal phase J-I-P (Strasser et al., 1995). In the single turnover range F_o (measured at
9 50 μ s) to F_J (measured at 2 ms), the bands K (at about 200 to 400 μ s) is visualised at 45
10 °C.

11

12 **Heat sensitive index (HSI)**

13 Several parameters can be derived from the polyphasic Chl *a* fluorescence OJIP rise that
14 provide information about photosynthetic fluxes. In this study and according to Chen et
15 al., (2016), HSI corresponds to the absolute value of the slope (K) of the relationship
16 between the logarithm of performance index (PI_{ABS}) and the relative variable
17 fluorescence at the K-step (V_K); these two parameters were calculated at various
18 temperature treatments (25, 30, 35, 40, and 45 °C). Indeed, to investigate the sensitivity
19 to heat treatment between plant varieties and developed an effective model; it is
20 necessary to choose different high temperature treatments that include mild (<40 °C),
21 moderate (40–44 °C) and strong (>44 °C) heat treatment.

22

23

1 **The performance index (PI_{ABS})**

2 The performance index (PI_{ABS}) was one of the chlorophyll fluorescence parameters that
3 provide useful and quantitative information about the physiological state of plants and
4 their vitality. The PI_{ABS} has been created out of three independent expressions: the
5 concentration of reaction centers per chlorophyll $\gamma_o/(1-\gamma_o)$, an expression related to
6 primary photochemistry $\phi_{Po}/(1-\phi_{Po})$ and an expression related to electron transport
7 $\psi_o/(1-\psi_o)$.

$$8 \quad PI_{ABS} = [\gamma_o/(1-\gamma_o)] \cdot [\phi_{Po}/(1-\phi_{Po})] \cdot [\psi_o/(1-\psi_o)]$$

9 ϕ_{Po} ($= 1-(F_o/F_M) = F_V/F_M$) corresponds to the efficiency by which an absorbed photon
10 will be trapped by PSII reaction centers. The symbol γ represents the ratio of reaction
11 centre chlorophylls and the total chlorophyll of PSII. The expression $\gamma_o/(1-\gamma_o)$ is
12 proportional to the parameters estimated by JIP-test as equal to the ratio of reaction
13 centers and the absorbance (RC/ABS). Therefore:

$$14 \quad \gamma_o/(1-\gamma_o) \sim Chl_{RC}/Chl_{antenna}$$

$$15 \quad RC/ABS = [(F_{2ms}-F_{50\mu s})/4 \cdot (F_{300\mu s}-F_{50\mu s})] \cdot F_V/F_M$$

16 ψ_o ($=1-V_J$) is the fraction of electrons transported beyond Q_A^- per exciton trapped by the
17 reaction centers (RC) of PSII. It is the probability that the energy of a trapped exciton is
18 used for electron transport beyond Q_A .

19

20 **Relative variable fluorescence (V_t) at the K-step (V_k)**

21 V_t is defined as $(F_t - F_o)/(F_M - F_o)$ and this expression can be taken as a measure of the
22 fraction of the primary quinone electron acceptor of PSII in its reduced state [Q_A^-/Q_A]

1 (total)] (Strasser et al., 1995). Then, the expression of the relative variable fluorescence at
2 the K-step is:

3
$$V_K = (F_K/F_o)/(F_M/F_o)$$

4

5 **Statistical analysis**

6 The statistical analysis was conducted with the software package SPSS for Windows
7 (version 13). The data were subjected to an analysis of variance (ANOVA). Means and
8 standard deviations were determined for each variety.

9

10 **Results**

11

12 Heat treatment up to 40 °C showed in barley leaves and following a dark-to-light
13 transition a polyphasic OJIP rise (O–J, J–I and I–P phases) (example in Fig. 1). This
14 OJIP rise reveals three different reduction processes of the electron transport chain
15 (Schansker et al., 2003; Stirbet and Govindjee, 2012). O–J rise represents the reduction
16 of the acceptor side of PSII. The J–I phase has been shown to have kinetic properties
17 expected for the reduction/oxidation of the plastoquinone (PQ) pool (Schansker et al.,
18 2005; Schreiber et al., 1989). The I–P phase was shown to be parallel the re-reduction of
19 plastocyanin (PC)⁺ and P700⁺ in photosystem I (PS I) (Schansker et al., 2003; Schreiber
20 et al., 1989). On the other hand, heat treatment at 45 °C provoked a decrease in
21 fluorescence intensity of the OJIP rise and a change in its shape compared to the 25 °C
22 treatment. A decrease in F_M and increase in F_o (data not shown) and an additional K-
23 band at 0.3 ms were observed in the OJIP transients of all barley varieties treated at 45

1 °C. This K-band appeared dominant with a concomitant J-I step disappearing in the
2 fluorescence rise. We noted here that all studied barley varieties showed the same
3 pattern response to heat treatment.

4

5 **Performance index (PI_{ABS})**

6 The effect of heat treatments on changes that occurred in the PI_{ABS} in fully expanded
7 leaves is shown in Fig. 2. The analysis of variance for PI_{ABS} showed the significance of
8 the differences between varieties responses (Table 2). Following heat stress, PI_{ABS} was
9 decreased in all varieties. At 45 °C treatment, a significant decrease of PI_{ABS} parameter
10 was much more pronounced and the value decreased by more than 50 % compared with
11 control value in all varieties. The significant decrease of PI_{ABS} at 45 °C treatment was
12 observed in Immouzzar variety; it decreased by 82 % compared to control (25 °C).

13

14 **Relative variable fluorescence at the K-step (V_K)**

15 A visual K-band on OJIP transient was exhibited at 45 °C in all barley varieties. The
16 appearance of K-band has been associated with a dissociation of the oxygen-evolving-
17 complex. High temperature led to an increase in V_K value in all plants (Fig. 2). The
18 higher value of V_K was reached at 45 °C heat treatment in all barley varieties. Table 2
19 showed that the analysis of variance for V_K is highly significant between all varieties.
20 The significant increase of V_K at 45 °C treatment was observed in Immouzzar variety
21 and it increased by 81 % compared to control (25 °C).

22

23

1 **Relationship between $\log PI_{ABS}$ and V_K**

2 In order to determine HSI in our studied barley varieties, the logarithm of PI_{ABS}
3 ($\log PI_{abs}$) is calculated and correlated to V_K . $\log PI_{ABS}$ is defined as the total driving
4 force for photosynthesis (Strasser et al., 2004). Here, we observed a high correlation
5 between $\log PI_{ABS}$ and V_K (see example in Fig. 4). Indeed, $\log PI_{ABS}$ values decreased
6 linearly as the K-step level (V_K) increased from 25 to 45 °C. This linear relationship
7 indicates that the most important consequence of the PSII damage is the loss of OEC
8 centers function.

9

10 **Heat sensitive index**

11 According to Chen et al. (2016), the absolute value of the slope (K) of the relationship
12 between $\log PI_{abs}$ and V_K quantifies plant tolerance to high temperature and in other
13 words, the lower HSI means a stronger tolerance to heat treatment. The slope of the
14 straight line was obtained from the corresponding equation from the plot of $\log PI_{ABS}$
15 versus V_K (see Fig. 4). Table 3 presents all slop values obtained from the relationship
16 between $\log PI_{ABS}$ and V_K in all studied barley varieties. The HSI varied between 3.39
17 (Ighrem variety) and 6.48 (Igrane variety).

18

19 **Discussion**

20

21 Photosynthetic apparatus and mainly oxygen evolving complex of PSII has been
22 identified as the most heat sensitive component of the photosynthetic transfer chain
23 (Berry and Björkman, 1980; Havaux and Tardy, 1996; Oukarroum et al., 2013). The

1 decrease in F_M and increase in F_o (level of fluorescence when all the primary quinone
2 acceptors Q_A are in the oxidized state) were observed in the OJIP at 45 °C transients.
3 These change revealed an inhibition of electron transport from OEC toward PQ pool
4 (Havaux, 1993; Yamane et al., 2000). It has been reported that the increase of F_o has
5 been attributed to physical separation of the PSII from associated pigment antennae
6 (Guissé et al., 1995). A well-known consequence of high temperature in plant is the
7 destruction of OEC and this effect was revealed on the OJIP transient by a dominant K-
8 band (at about 200 to 400 μ s). This K-band consists of a fast rise with a maximum at
9 300 μ s followed by a reduction in fluorescence intensity (Fig. 1). The fluorescence
10 transient at K-peak is the result only of one stable charge separation that lets to Q_A^-
11 accumulation and the absence of electron donors would lead to the accumulation of
12 $P680^+$ (Tóth et al., 2007). On the other hand, the dip after the K-peak is related the
13 reopening of PSII RCs by electron transfer from Q_A^- to Q_B (Strasser, 1997). Assessing
14 the response of the different varieties with respect to V_K showed that the significant
15 effect on OEC was observed at 45 °C in all varieties. The consequence of destruction of
16 OEC is an inhibition of the photosynthetic electron transport chain. It is worth to note
17 that the appearance of K-band is considered as a major change in OJIP rise kinetics
18 under heat treatment. Further, K-peak value has been predicted as a heat indicator with
19 respect to plant response to high temperature (Srivastava et al., 1995; Lu and Zhang,
20 2009; Oukarroum et al., 2013). In this context, V_K would contribute to an understanding
21 of the physiological basis of heat tolerance variation between barley varieties.
22 Beside an increase in K value in heated detached leaves, our data showing a significant
23 decrease of PI_{ABS} . This decrease indicated a down-regulation of the photochemical

1 activity. PI_{ABS} is a multi-parametric expression and was highly used to identify plant
 2 vitality exposed to environmental stress and it takes into consideration the three main
 3 functional steps: the density of active reaction centers per chlorophyll, the ratio of the
 4 de-excitation rate constants for photochemical and non-photochemical events and the
 5 efficiency of the conversion of excitation energy to electron transport toward the
 6 plastoquinone pool (Strasser et al., 2004). Several papers have pointed out that PI_{ABS} is a
 7 very sensitive indicator of the physiological status of plants subjected to environmental
 8 stress. Indeed, PI_{ABS} to screen drought tolerance (Oukarroum et al., 2007; Boureima et
 9 al., 2012); chilling tolerance (Strauss et al., 2006); ozone elevation (Bussotti et al.,
 10 2007); nutrient deficiency (Kalaji et al., 2014) and chilling (Van Heerden and Krüger,
 11 2002).

12 Barley is a typical crop of marginal areas in Morocco and represents about 42 % of the
 13 cereal acreage (Forster et al., 2004). Based on change in PI_{ABS} , barley varieties has been
 14 screening and ranking forward their degree of tolerance to a specific environmental
 15 stress. Recently Chen et al., (2016) established a new parameter called heat sensitive
 16 index (HSI); this new parameter discriminate heat tolerance in four croftonweed
 17 populations and was calculated as the absolute value of the slope (K) of the relationship
 18 between $\log PI_{ABS}$ and V_K . In previous studies, some approaches to determine the heat-
 19 tolerance of leaves have been published based on chlorophyll fluorescence transient.
 20 The F_o -level of a sample is measured when at the same time the temperature of the
 21 sample is increased (Schreiber and Berry (1977). The F_o -rise in this fluorescence-
 22 temperature dependence corresponds to a shift in the Q_A/Q_B^- equilibrium towards Q_A^-
 23 (Ducruet 1999) and it was related to an effect of heat stress on the acceptor side of PSII.

1 then F_o rise is only an indirect measure for the loss of the manganese cluster. Another
2 approach uses F_K/F_J (or (V_K/V_J)) parameter for monitoring heat stress. However this
3 parameter monitors a limitation of electron donation on the donor side of PS II and the
4 lowering of the J-step observed after the K-step represents a quantitative measure for the
5 inactivation of the PS II donor side. However, the J-step is not well defined in
6 physiological terms. Furthermore the use only one parameter likes F_K/F_J (or (V_K/V_J))
7 cannot distinguish well the tolerance variation between our barley varieties (Oukarroum
8 et al., 2009). Here, the interest point is that HSI indicates the limitation of electron
9 donation on the donor side of PS II provoked by heat treatment (V_K) and PI_{ABS} which
10 was described as the result of three main functional steps of the photosynthetic activity
11 of PSII (Strasser et al., 2004). In fact, PI_{ABS} is a highly sensitive parameter to any stress
12 and cannot differential well one stress treatment from other stresses and V_K increased
13 when F_o goes up when heat treatment temperatures excess 40 °C. Then, the use of these
14 parameters (PI_{ABS} and V_K) together gives us a suitable model to investigate the
15 sensitivity of heat response is different barley varieties (see also Chen et al., 2016).
16 Here, we used this parameter to rank ten barley varieties in respect to their HSI (Table
17 3). The principle of this model suggests that the smaller HSI value is correlated to high
18 heat tolerance. Interestingly, Table 3 showed that the first three barley varieties heat
19 tolerant are landraces. However, we note that in contrast, three modern barley varieties
20 are heat sensitive. It is necessary to note that Zeven (1998) defined a landrace as an
21 autochthonous variety with a high capacity to tolerate biotic and abiotic stress. Here
22 Ighrem variety (an autochthonouse variety) presents the smaller HSI value, and then this
23 variety is predicted to be tolerant to high temperature. On the other hand, in our study

1 the most susceptible variety (higher HSI value) was Igrane. In this regard, our data
2 herein shows that heat tolerance vary between the studied barley varieties. This means
3 that there is higher heat response variability within barley varieties (Table 2). In a
4 previous study, Brestic et al. (2012) reported that the genotype comparison between
5 thirty winter wheat (*Triticum aestivum* L.) differed either in their initial PSII
6 thermostability or in acclimation capacity and this difference was associated to the
7 origin of the genotypes. However, a correlation between PSII thermostability of these
8 thirty winter wheat (*Triticum aestivum* L.) grown in field and agronomical parameters
9 did not analysed. Also, it has been reported that altitude and local topography exert a
10 significant influence on plant heat tolerance (Iba, 2002). Our data also corroborated the
11 findings of Chen et al. (2006) that heat tolerance depends on the geographical growth of
12 plants. We note here that the landraces were collected from a semi-arid area.

13 In summary we have shown that the measurement of OKJIP fluorescence transients and
14 change in fluorescence parameters using the JIP-test (e.g. PI_{ABS} and V_K) can be used to
15 introduce the new HSI as tools for screening varieties able to cope with heat treatment.
16 Landraces seem to have efficient mechanism tolerance to cope with high temperature
17 like Ighrem variety. It might be that landraces have been developed an acclimated
18 mechanism against sub-optimal thermal stress. El Madidi et al. (2005) reported that
19 there is a need to identify large number of Moroccan landraces with a high degree of
20 tolerance for use in breeding programmes for disadvantaged areas, where drought and
21 heat stresses preclude an increase in yields. Indeed, Mediterranean environment appears
22 more affected by climate change than most of other climatic zones and mainly by an
23 increase of air temperature (Bussotti et al., 2014). The detection of heat tolerance *in vivo*

1 in early plant developmental stage with bioenergetic behaviour is the main goal of this
2 study. Preventing crop yield decrease in large arid and semi-arid agricultural areas
3 where high temperature stress can occur, such as in Morocco, was cardinal. However, it
4 is in our interest to understand this inter-genotypical heat tolerance when plants were
5 confronted to simultaneous environmental stresses.

6

7 **Acknowledgements**

8 This work was supported by the Swiss National Science Foundation, (ISCB Project
9 PS4).

10

11 **References**

12

13 Allakhverdiev, S. I., Y. M. Feyziev, A. Ahmed, H. Hayashi, J.A. Aliev, V.V. Klimov,
14 N. Murata, R. Carpentier, 1996. Stabilization of oxygen evolution and primary electron
15 transport reactions in photosystem II against heat stress with glycinebetaine and sucrose.
16 J. Photochem. Photobiol. B 34, 149–157.

17

18 Allakhverdiev, S. I., V. D. Kreslavski, V. V. Klimov, D. A. Los, R. Carpentier, P.
19 Mohanty, 2008. Heat stress: an overview of molecular responses in photosynthesis.
20 Photosynth. Res. 98, 541–550.

21

22 Al-Khatib, K., G. M. Paulsen, 1999. High-temperature effects on photosynthetic
23 processes in temperate and tropical cereals. Crop Sci. 39, 119–125.

1 Baker, N. R., 1991. A possible role for photosystem II in environmental perturbation of
2 photosynthesis. *Physiol. Plant.* 81, 563–570.
3

4 Berry, J. A., O. Björkman, 1980. Photosynthetic response and adaptation to temperature
5 in higher plants. *Annu. Rev. Plant Physiol.* 31, 491–543.
6

7 Bilger W., U. Schreiber, O. L. Lange, 1987. Chlorophyll fluorescence as an indicator of
8 heat induced limitation of photosynthesis in *Arbutus unedo* L. In JD Tenhunen, FM
9 Catarino, OL Lange, eds, *Plant Responses to Stress*. Springer, Berlin, pp 391–399
10

11 Boureima, S., A. Oukarroum, M. Diouf, N. Cisse, P. Van Damme. 2012. Screening for
12 drought tolerance in mutant germplasm of sesame (*Sesamum indicum*) probing by
13 chlorophyll a fluorescence. *Environ. Exp. Bot.* 81, 37–43.
14

15 Bussotti, F., R. J. Strasser, M. Schaub, 2007. Photosynthetic behavior of woody species
16 under high ozone exposure probed with the JIP-test: a review. *Environ. Pollut.* 147,
17 430–437.
18

19 Bussotti, F., F. Ferrini, M. Pollastrini, and A. Fini, 2014. The challenge of
20 Mediterranean sclerophyllous vegetation under climate change: From acclimation to
21 adaptation. *Environ. Exp. Bot.* 103, 80–98.
22

1 Brestic, M., M. Zivcak, H. M. Kalaji, R. Carpentier, S. I. Allakhverdiev, 2012.
2 Photosystem II thermostability in situ: Environmentally induced acclimation and
3 genotype-specific reactions in *Triticum aestivum* L. *Plant Physiol. Biochem.* 57, 93–
4 105.
5
6 Cao, J., Govindjee, 1990. Chlorophyll a fluorescence transient as an indicator of active
7 and inactive Photosystem II in thylakoid membranes. *Biochim. Biophys. Acta.* 1015,
8 180–188.
9
10 Chen, S. G., Y. Kang, M. Zhang, X. X. Wang, R. J. Strasser, B. Zhou, and S. Qiang,
11 2015. Differential sensitivity to the potential bioherbicide tenuazonic acid probed by the
12 JIP-test based on fast chlorophyll fluorescence kinetics. *Environ. Exp. Bot.* 112, 1–15
13
14 Chen, S., J. Yang, M. Zhang, R. J. Strasser, S. Qiang, 2016. Classification and
15 characteristics of heat tolerance in *Ageratina adenophora* populations using fast
16 chlorophyll a fluorescence rise O-J-I-P. *Environ. Exp. Bot.* 122, 126–140.
17
18 Crafts-Brandner, S.J., M.E. Salvucci, 2000. Rubisco activase constrains the photosyn-
19 thetic potential of leaves at high temperature and CO₂, *Proc. Natl. Acad. Sci. U.S. A.*
20 97, 13430–13435.
21

- 1 Ducruet, J.M. 1999. Relation between the heat-induced increase of F_o fluorescence and a
2 shift in the electronic equilibrium at the acceptor side of photosystem 2. *Photosynthetica*
3 37, 335–338.
- 4
- 5 Eckhardt, N.A., A.R. Portis, 1997. Heat denaturation profiles of ribulose-1,5-
6 biphosphate carboxylase/oxygenase (Rubisco) and Rubisco activase and the inability of
7 Rubisco activase to restore activity of heat-denaturated Rubisco, *Plant Physiol.* 113,
8 243–248.
- 9
- 10 El Madidi, S., Z. Diani, and F. Bani Aameur, 2005. Variation of agro-morphological
11 characters in Moroccan barley landraces under near optimal and drought conditions.
12 *Genet. Res. Crop. Evol.* 52, 831–838.
- 13
- 14 Forster, B.P., R. P. Ellis, J. Moir, V. Talamè, M. C. Sanguineti, R. Tuberosa, D. This, B.
15 Teulat-Merah, I. Ahmed, S. A. E. Mariy, H. Bahri, M. El-Ouahabi, N. Zoumarou-Wallis
16 M. EL-Fellah, M. Ben Salem, 2004. Genotype and phenotype associations with drought
17 tolerance in barley tested in North Africa. *Ann. Appl. Biol.* 144, 157–168.
- 18
- 19 Guissé, B., A. Srivastava, R. J. Strasser, 1995. The polyphasic rise of the chlorophyll *a*
20 fluorescence (OKJIP) in heat-stressed leaves. *Archs. Sci. Genève* 48, 147–160.
- 21
- 22 Goltsev V., I. Zaharieva, P. Chernev, M. Kouzmanova, H. M. Kalaji, I. Yordanov, V.
Krasteva, V. Alexandrov, D. Stefanov, S. I. Allakhverdiev, R.J. Strasser, 2012. Drought-

1 induced modifications of photosynthetic electron transport in intact leaves: analysis and
2 use of neural networks as a tool for a rapid non-invasive estimation *Biochim. Biophys.*
3 *Acta* 1817, 1490–1498.

4

5 Hall, A.E., 2001. *Crop Responses to Environment*. CRCPress LLC, Boca Raton,
6 Florida.

7

8 Havaux, M., 1992. Stress tolerance of photosystem II in vivo antagonistic effects of
9 water, heat, and photoinhibition stresses. *Plant Physiol.* 100, 424–432

10

11 Havaux, M., 1993. Rapid photosynthetic adaptation to heat stress triggered in potato
12 leaves by moderately elevated temperatures. *Plant Cell Environ.* 16, 461–467.

13

14 Havaux, M., F.Tardy, 1996. Temperature-dependent adjustment of the thermal stability
15 of photosystem II in vivo: possible involvement of xanthophyll-cycle pigments. *Planta*
16 198, 324–333.

17

18 Iba, K., 2002. Acclimative response to temperature stress in higher plants: Approches of
19 gene engineering for temperature tolerance. *Annu. Rev. Plant Biol.* 2002. 53:225–45.

20

21 Kalaji, H.M., A. Oukarroum, V. Alexandrov, M. Kouzmanova, M. Brestic, M. Zivcak,
22 I.A. Samborska, M.D. Cetner, S.I. Allakhverdiev, and V. Goltsev, 2014. Identification

1 of nutrient deficiency in maize and tomato plants by in vivo chlorophyll a fluorescence
2 measurements. Plant Physiol. Biochem. 81, 16–25.
3

4 Law R. D., S. J. Crafts-Brandner, 1999. Inhibition and Acclimation of Photosynthesis to
5 Heat Stress Is Closely Correlated with Activation of Ribulose-1,5-Bisphosphate
6 Carboxylase/Oxygenase. Plant Physiol, 120, 173–181
7

8 Lu, C., J. Zhang, 1999. Effect of water stress on photosystem II photochemistry and its
9 thermostability in wheat plants. J. Exp. Bot. 336, 1199–1206.
10

11 Oukarroum, A., S. El Madidi, G. Schansker, R. J. Strasser, 2007. Probing the responses
12 of barley cultivars (*Hordeum vulgare* L.) by chlorophyll *a* fluorescence OLKJIP under
13 drought stress and re-watering. Environ. Exp. Bot. 60, 438–446.

14 Oukarroum, A., G. Schansker, R. J. Strasser, 2009. Drought stress effects on
15 photosystem I content and photosystem II thermotolerance analyzed using Chl *a*
16 fluorescence kinetics in barley varieties differing in their drought tolerance. Physiol.
17 Plant 137, 188–199.
18

19 Oukarroum, A., R. J. Strasser, G. Schansker, 2012. Heat stress and the photosynthetic
20 electron transport chain of the lichen *Parmelina tiliacea* (Hoffm.) Ach. in the dry and the
21 wet state: differences and similarities with the heat stress response of higher plants.
22 Photosynth. Res. 111, 303–314.

1 Oukarroum, A., V. Goltsev, R. J. Strasser, 2013. Temperature Effects on Pea Plants
2 Probed by Simultaneous Measurements of the Kinetics of Prompt Fluorescence,
3 Delayed Fluorescence and Modulated 820 nm Reflection. PLoS ONE 8(3): e59433.
4 doi:10.1371/journal.pone.0059433
5
6 Pollastrini M., R. Desotgiu, F. Camin, L. Ziller, G. Gerosa, R. Marzuoli, F. Bussotti,
7 2014. Severe drought events increase the sensitivity to ozone on poplar clones. Environ.
8 Exp. Bot. 100, 94–104.
9
10 Salvatori, E., L. Fusaro, R. J. Strasser, F. Bussotti, F. Manes, 2015. Effects of acute O₃
11 stress on PSII and PS I photochemistry of sensitive and resistant snap bean genotypes
12 (*Phaseolus vulgaris* L.), probed by prompt chlorophyll "a" fluorescence and 820 nm
13 modulated reflectance. Plant Physiol. Biochem. 97, 368–377.
14
15 Schreiber, U., C. Neubauer, C. Klughammer, 1989. Devices and methods for room-
16 temperature fluorescence analysis. Philos Trans R Soc Lond B 323, 241-251.
17
18 Schansker, G., A. Srivastava, Govindjee, R. J. Strasser, 2003. Characterization of the
19 820-nm transmission signal paralleling the chlorophyll *a* fluorescence rise (OJIP) in pea
20 leaves. Funct. Plant Biol. 30, 785–796.
21

1 Schansker, G., S. Z. Tóth, R. J. Strasser, 2005. Methylviologen and
2 dibromothymoquinone treatments of pea leaves reveal the role of photosystem I in the
3 Chl *a* fluorescence rise OJIP. Biochim. Biophys. Acta 1706, 250–261.
4

5 Schreiber, U., J. A. Berry, 1977. Heat-induced changes of chlorophyll fluorescence in
6 intact leaves correlated with damage of the photosynthetic apparatus. Planta 136, 233–
7 238.
8

9 Shao, H, H. Wang, X. Tang, 2015. NAC transcription factors in plant multiple abiotic
10 stress responses: progress and prospects. Front. Plant Sci. 6:902. doi:
11 10.3389/fpls.2015.00902.
12

13 Strauss, A.J., G. H. J. Krüger, R. J. Strasser, P. D. R. Van Heerden, 2006. Ranking of
14 dark chilling tolerance in soybean genotypes probed by the chlorophyll *a* fluorescence
15 transient O-J-I-P. Environ. Exp. Bot. 56, 147–157.
16

17 Strasser, B.J., 1997. Donor side capacity of Photosystem II probed by chlorophyll *a*
18 fluorescence transients. Photosynth Res 52, 147–155.
19

20 Strasser, R.J., 1986. Mono-bi-tri and polypartite models in photosynthesis. Photosynth. Res.
21 10, 255–276.
22

23 Strasser, R.J., A. Srivastava, Govindjee, 1995. Polyphasic chlorophyll *a* fluorescence
24 transient in plants and cyanobacteria. Photochem. Photobiol. 61, 32–42.

1 Strasser, R.J., A. Srivastava, M. Tsimilli-Michael, 2004. Analysis of the chlorophyll *a*
2 fluorescence transient. In: Papageorgiou, G., Govindjee, (Eds), Advances in
3 Photosynthesis and Respiration, Vol. 19. Chlorophyll Fluorescence a Signature of
4 Photosynthesis. Kluwer Academic Publishers, the Netherlands, pp. 321-362.

5

6 Srivastava, A., R. J. Strasser, 1995. How do land plants respond to stress temperature
7 and stress light? Stress and stress management of land plants during a regular day.
8 Archs. Sci. Genève 48, 135–146.

9

10 Stirbet, A., Govindjee, 2011. On the relation between the Kautsky effect (chlorophyll *a*
11 fluorescence induction) and Photosystem II: Basics and applications of the OJIP
12 fluorescence transient. J. Photochem. Photobiol. B 104, 236–257.

13

14 Stirbet, A., Govindjee, 2012. Chlorophyll *a* fluorescence induction: A personal
15 perspective of the thermal phase, the J-I-P rise. Photosynth. Res. 113, 15–61.

16

17 Tóth, S.Z., G. Schansker, G. Garab, R. J. Strasser, 2007. Photosynthetic electron
18 transport activity in heat-treated barley leaves: The role of internal alternative electron
19 donors to photosystem II. Biochim. Biophys. Acta 1767, 295–305.

20

21 Van Heerden, P.D.R., G. H. J. Krüger, 2002. Separately and simultaneously induced
22 dark chilling and drought stress effects on photosynthesis, proline accumulation and
23 antioxidant metabolism in soybean. J. Plant Physiol. 159, 1077–1086.

1 Wahid, A., S. Gelani, M. Ashraf, M. R. Foolad, 2007. Heat tolerance in plants. An
2 overview. *Environ. Exp. Bot.* 61, 199–223.
3

4 Wang, D., J. Fan, S. A. Heckathorn. 2014. Acclimation of photosynthetic tolerance to
5 acute heat stress at elevated CO₂ and N. *Plant Sci.* 226, 162–171.
6

7 Weltzin, J.F., M. E. Loik, S. Schwinning, D. G. Williams, P. Fay, B. Haddad, J. Harte,
8 T. E. Huxman, A. K. Knapp, G. Lin, W. T. Pockman, M. R. Shaw, E. Small, M. D.
9 Smith, S. D. Smith, D. T. Tissue, J. C. Zak, 2003. Assessing the response of terrestrial
10 ecosystems to potential changes in precipitation. *Bioscience* 53, 941–952.
11

12 Yamane, Y., Y. Kashino, H. Koike, K. Satoh, 1998. Effects of high temperatures on the
13 photosynthetic systems in spinach: Oxygen-evolving activities, fluorescence
14 characteristics and the denaturation process. *Photosynth. Res.* 57, 51–59.
15

16 Yamane, Y., T. Shikanai, H. Koike, K. Satoh, 2000. Reduction of QA in the dark:
17 another cause of fluorescence F_o increases by high temperatures in higher plants.
18 *Photosynth. Res.* 63, 23–34.
19

20 Yan, K., P. Chen, H. Shao, L. Zhang, G. Xu, 2011. Effects of Short-Term High
21 Temperature on Photosynthesis and Photosystem II Performance in Sorghum. *J. Agr.*
22 *Crop Sci.* 197, 400–408.
23

- 1 Yan, K., P. Chen, H. Shao, C. Shao, S. Zhao, M. Brestic, 2013. Dissection of
- 2 Photosynthetic Electron Transport Process in Sweet Sorghum under Heat Stress. PLoS
- 3 ONE 8(5): e62100. doi:10.1371/journal.pone.0062100
- 4
- 5 Yan, K., P. Chen, H. Shao, S. Zhao, 2013a. Characterization of photosynthetic electron
- 6 transport chain in bioenergy crop Jerusalem artichoke (*Helianthus tuberosus* L.) under
- 7 heat stress for sustainable cultivation. Ind. Crop. and Prod. 50, 809–815.
- 8
- 9 Yan, K., H. Shao, C. Shao, P. Chen, S. Zhao, M. Brestic, X. Chen, 2013b. Physiological
- 10 adaptive mechanisms of plants grown in saline soil and implications for sustainable
- 11 saline agriculture in coastal zone. Acta Physiol Plant (2013) 35:2867–2878
- 12
- 13 Zeven, A.C., 1998. Landraces: A review of definitions and classifications. Euphytica
- 14 104, 127–139.
- 15

16 **Figure captions**

17

18 **Figure 1.** A typical Chlorophyll *a* polyphasic fluorescence rise OJIP, exhibited by

19 heated detached barley plants. The transients are plotted on a logarithmic time scale

20 from 50 μ s to 1 s. The marks refer to the selected time points used by the JIP-test for the

21 calculation of structural and functional parameters. The signals are: the fluorescence

22 intensity F_0 (at 50 μ s); the fluorescence intensity F_K (at 300 μ s); the fluorescence

23 intensities F_J (at 2 ms) and F_I (at 30 ms); the maximal fluorescence intensity, $F_P = F_M$.

1 **Figure 2.** Performance index (PI_{ABS}) of the studied Moroccan barley varieties after heat
2 treatment. Each value represents the mean $\pm$ SD of five independent experiments with
3 about 15 repetitions.

4

5 **Figure 3.** Relative variable fluorescence at the K-step (V_k) of the studied Moroccan
6 barley varieties after heat treatment. Each value represents the mean $\pm$ SD of five
7 independent experiments with about 15 repetitions.

8

9 **Figure 4.** Example of a relationship between and the driving force of performance
10 index ($\text{Log}PI_{ABS}$) and relative variable fluorescence at the K-step (V_k) in Ighrem and
11 Igrane varieties heat-treated. The linear corresponding equation was obtained. Each
12 value is the average of five independent experiments with about 15 repetitions.

13

14

Table 1 The geographical of five sites where the landraces were collected.

Landrace	Altitude	Latitude	Longitude
Aït Baha (Ab)	550 m	30°05 N	9°33 W
Ighrem (Ig)	1800 m	30°06 N	8°27 W
Immouzzar (Im)	1200 m	30°04 N	9°29 W
Tarodant (Ta)	235 m	30°28 N	8°52 W
Tizinit (Tz)	244 m	29°41 N	9°43 W

Table 2 Analysis of variance for performance index (PI) and the relative variable fluorescence at the K-step (V_K): F values and significance levels of each variable.

Source of variation	DF	PI	V_K
Variety (V)	9	9.64**	4.62**
Treatment (T)	4	438.17**	592.91**
Interaction (V x T)	36	3.37**	5.149**

** ; test significatif à 5%

DF; degree of freedom

Table 3 Heat sensitive Index (HSI) calculated as the absolute value of the slope (K) of the relationship between $\log PI_{abs}$ and V_K in five modern barley varieties obtained from the National Institute of Agricultural Research (INRA) of Morocco and five collected by the Provincial Direction of Agriculture (PDA) originating from five localities in the south of Morocco.

Varieties	Origin	HSI
Ig	Landrace	3.39
Im	Landrace	3.40
Tz	Landrace	3.73
La	INRA	3.79
Ar	INRA	4.55
Ab	Landrace	4.55
Ta	Landrace	4.98
Ma	INRA	5.06
Ra	INRA	5.06
Igr	INRA	6.48

Figure 1

Figure 2

Figure 3

Figure 4