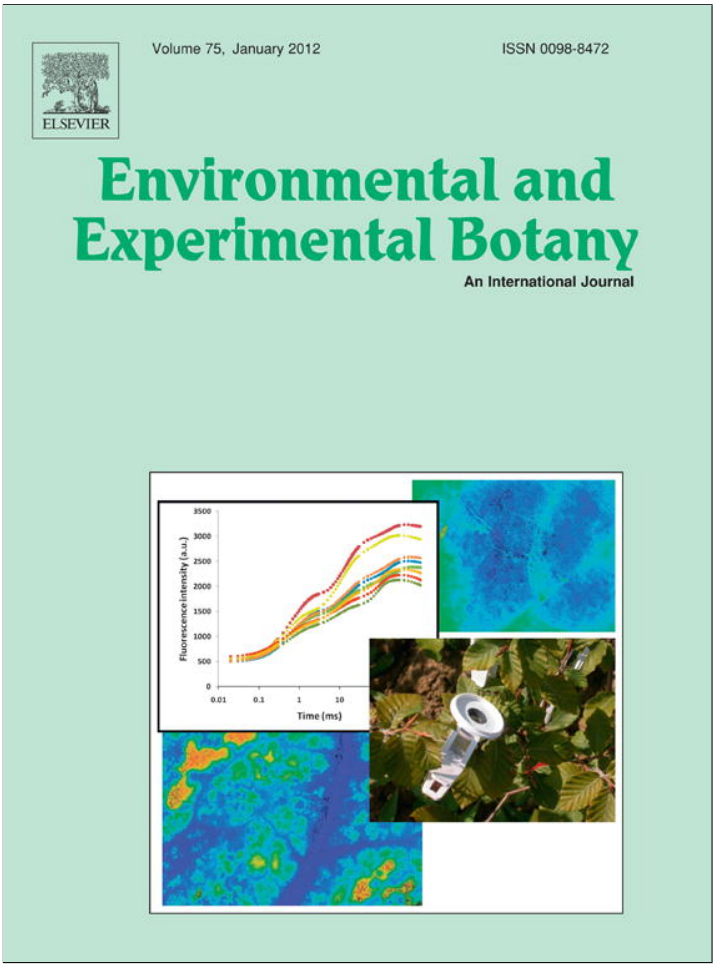

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Screening for drought tolerance in mutant germplasm of sesame (*Sesamum indicum*) probing by chlorophyll *a* fluorescence

Seyni Boureima[a,c,*], Abdallah Oukarroum[b], Macoumba Diouf[a], Ndiaga Cisse[a], Patrick Van Damme[c]

[a] *Centre d'étude régional pour l'amélioration de l'adaptation à la sécheresse, Thiès, Senegal*
[b] *Department of Chemistry and Biochemistry, University of Quebec in Montreal, C.P. 8888, Succ. Centre-Ville, Montreal, Quebec, H3C 3P8 Canada*
[c] *Ghent University, Faculty of Bioscience Engineering, Ghent, Belgium*

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ABSTRACT

Drought is one of the major constraints limiting crop productivity in African Sahel. The aim of this study was to select mutant sesame (*Sesamum indicum* L.) lines with improved levels of drought resistance. Twenty-one M4-M5 sesame lines of unknown drought tolerance, and their three parental sources with well-known and contrasting drought tolerance levels were evaluated at the vegetative stage in a factorial pot experiment, using a completely randomized design with three replicates. After 2 weeks of growth, water was withheld for 16 days as drought stress treatment. Chlorophyll *a* fluorescence data, as well as stomatal conductance and flag leaf temperature were recorded during the stress period. Recorded chlorophyll *a* fluorescence transients were analyzed by the JIP-test to translate stress-induced damage in these transients to changes in biophysical parameters allowing quantification of the energy flow through the photosynthetic apparatus. Large genotypic differences in the extent to which drought stress affected chlorophyll *a* fluorescence transients were observed. Drought stress reduced the performance index and stomatal conductance, and increased flag leaf temperature but had little effect on maximum quantum yield of primary photochemistry. A drought factor index is proposed in this work to screen for improved drought tolerance in twenty-one M4-M5 sesame lines. Mutant lines shi165, lc162, mc112, lc164, icn115, icn141, mt169, dwf172 and cc102 exhibited drought factor index values superior to those of the known drought tolerant cultivars Birkan and 38-1-7. A significant and negative relationship was found between the drought factor index and the leaf temperature index. Finally, we succeeded in obtaining drought tolerant lines with good secondary traits by using mutagenesis and chlorophyll fluorescence technique.

1. Introduction

Cultivated sesame (*Sesamum indicum* L.) is indigenous to Africa (Weiss, 2000), but the crop was initially domesticated on the Indian subcontinent (Bedigian, 2003). The latter author reported that plants in the genus *Sesamum* produce unique antioxidants not found in other edible oils that allow sesame oil to resist oxidative rancidity. These antioxidants contribute to its reputation for yielding high quality oil. The edible part of the crop (seeds) has been used for many centuries as a source of oil, proteins, vitamins and minerals for people as well as in animal feed (Weiss, 2000).

Like many other crop species sesame is unfortunately sensitive to drought during its vegetative stage (Boureima et al., 2011). The sensitivity of sesame to drought is reflected in the changes that occur subsequently in its metabolism, growth, development and yield. A great variability in the response to drought stress has been observed between sesame genotypes, with some exhibiting a high degree of tolerance, while others are extremely sensitive (Boureima et al., 2011). As sesame is cultivated in semi-arid regions across the world, production potential is often limited by drought stress.

Increased sesame production under drought can be achieved only by direct breeding or genetic manipulation. However, genetic and breeding improvement efforts in sesame have been limited and the results of such efforts slow to emerge. Ashri (2007) stated that the main reason for this limited success is that sesame is a crop mainly produced in developing countries and usually by smallholders. So far, none of the Consultative Group on International Agricultural Research (CGIAR) centers has been mandated to conduct research on sesame. There is a growing demand for edible oil and biofuel and sesame seeds, which contain up to 50% oil, could become an alternative source of income for small-scale farmers thus helping to alleviate rural poverty. In this respect, three FAO expert consultations (Anon., 1981, 1985; Ashri, 1987) recommended the use of induced mutations to enhance the genetic variability of sesame, selecting characters of economic interest.

* Corresponding author at: BP 3320, Thiès, Senegal. Tel.: +221 70 202 73 18; fax: +221 33 951 49 95.
E-mail address: boureimaseyni@yahoo.fr (S. Boureima).

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Finding an efficient and rapid screening method to select for drought tolerance in a large numbers of sesame genotypes would allow the identification of genotypes best suited for cultivation in semi-arid regions that frequently experience drought.

Chlorophyll *a* fluorescence measurement, a non-intrusive method, is widely used for monitoring and screening plants for environmental stress resistance (Oukarroum et al., 2009, 2007; Strauss et al., 2006). The principle underlying chlorophyll fluorescence analysis is relatively straightforward. Light energy absorbed by chlorophyll pigments in a leaf can: (i) be used to drive photosynthesis (photochemistry), (ii) excess energy can be dissipated as heat or (iii) be re-emitted as light-chlorophyll fluorescence (Maxwell and Johnson, 2000; Govindjee, 1995). These three processes occur in competition, such that any increase in the efficiency of one of them will result in a decrease in the yield of the other two (Strasser et al., 2004).

Fluorescence yield can be quantified by exposing a dark-adapted leaf to light of a defined wavelength and measuring the amount of light re-emitted at longer wavelengths (Maxwell and Johnson, 2000). The illumination of this dark-adapted leaf results in characteristic changes in the intensity of chlorophyll *a* fluorescence, known as the Kautsky effect (Kautsky and Hirsch, 1931).

The Kautsky transient shows a rapid rise completed in less than 1 s, with a subsequent decline towards a steady state (Strauss et al., 2006). The fast rise can be subdivided into main phases (O-J-I-P) that are representative of the three different reduction processes of the electron transport chain (Krause and Weis, 1991; Schansker et al., 2005). The slowest phase of the fluorescence rise, namely from I–P, which occurs at approximately 30–300 ms respectively, was shown to parallel the re-reduction of plastocyanin $(PC)^+$ and $P700^+$ in photosystem (PS) I (Schansker et al., 2003; Schreiber et al., 1989). The J–I phase has been shown to have kinetic properties expected for the reduction/oxidation of the plastoquinone (PQ) pool (Tóth et al., 2007; Schansker et al., 2005; Joliot and Joliot, 2002; Schreiber et al., 1989), and it has been suggested that the O–J rise represents the reduction of the acceptor side of PS II. Strasser and Strasser (1995) have developed the JIP-test, which is used to translate the original fluorescence measurements of these O–J–I–P transients into several phenomenological and biophysical expressions that quantify PSII status (Strasser and Tsimilli-Michael, 2001; Strasser et al., 2000). The JIP-test is highly suited to in vivo investigations of the behaviour of the photosynthetic apparatus, since the shape of the O–J–I–P fluorescence transient is sensitive to stress caused by changes in many environmental conditions (Tsimilli-Michael et al., 1998, 1999; Krüger et al., 1997).

There is a large amount of evidence in the literature (Živčák et al., 2008; Force et al., 2003) demonstrating the advantage of using the JIP-test to evaluate PSII status. More recently, a performance index (PI_{ABS}) has been developed by Strasser et al. (2000). This latter parameter was proven to be more sensitive to environmental stresses than the maximum quantum yield of primary photochemistry (F_V/F_M) (Oukarroum et al., 2007; Strauss et al., 2006). PI_{ABS} is an integrative parameter including three independent parameters: (i) density of fully active reaction centers (RCs); (ii) efficiency of electron movement by trapped excitons into the electron transport chain beyond the primary quinone acceptor of PSII (Q_A); and (iii) the probability that an absorbed photon could be trapped by the RCs.

Drought stress decreased PI_{ABS} (Živčák et al., 2008; Oukarroum et al., 2007) and lower stomatal conductance and CO_2 assimilation rates in plants (Ranjbarfordoei et al., 2000; Janoudi et al., 1993). The decrease in stomatal conductance is attributed to the stomatal closure when plants experience drought. The subsequent reduction of the transpiration rate results in an increase of leaf temperature (Jackson et al., 1981).

In this research, seeds of three sesame cultivars (32-15, Birkan and 38-1-7) were irradiated with gamma rays and mutants induced from these cultivars were evaluated for drought tolerance along with their parental sources. Measurements of chlorophyll *a* fluorescence, stomatal conductance and leaf temperature were carried out. The hypothesis was that the PI_{ABS} and the drought factor index (DFI), a parameter calculated from PI_{ABS}, would be sensitive enough to evaluate the response of these induced sesame mutants to drought stress and to rank them according to drought stress tolerance.

2. Material and methods

2.1. Plant material development

The material of the present study consisted of 24 sesame lines. These lines included 21 gamma-ray-induced mutants and their three respective parental sources with known drought resistance: 32-15 (drought sensitive); 38-1-7 (moderately tolerant); and Birkan (tolerant). Mutants were induced in 2008 and confirmed in 2009 (Boureima et al., 2009; Diouf et al., 2010) at the experimental station of the "Centre National de Recherche Agronomique (CNRA)", Bambey, Senegal (latitude 14°42′ North and longitude 16°28′ West).

Two irradiation doses (300 and 400 Gy) were used for the three parents. Irradiated seeds (50 g/dose) were planted in the field to raise a first generation of plant (M1). Seeds were harvested from these M1 plants and M1 plant progenies were grown in 30 m rows, 60 cm apart with plants at 20 cm within rows to produce a second generation (M2). After every tenth row, one parent control row (untreated) was sown. Potential mutants selected in M2 were grown as M3 generation to confirm true breeding behaviour during the 2009 rainy season in 2 m long rows, 60 cm apart. Eventually 21 mutant lines that out-yield their parental sources and/or with various agronomic characteristics (e.g. white seed colour which is highly valued on the world market, non-shattering mutants, early flowering mutants, and mutants with basal branches or with more capsules per node, hairiness which may be associated with drought tolerance) were selected for drought tolerance screening.

2.2. Plant growth conditions

Seeds were allowed to germinate for 48 h on a piece of Whatman filter placed in petri dishes prior to be transplanted in a glasshouse to ensure homogeneous emergence. Emerged seedlings were then transplanted in 10 L plastic pots previously filled with sandy soil taken from the experimental station of CNRA Bambey. Pots were watered to maximum water holding capacity a day prior to the transplantation of the seedlings. Plants were grown in a glasshouse at the "Centre d'Etude Régional pour l'Amélioration de l'Adaptation à la Sécheresse (CERAAS)", Thiès, Senegal (latitude 14°81′ North and longitude 16°28′ West) with a day/night air temperature regime of 35/25 °C, under short-day conditions (12 h natural light).

The experiment was conducted in a completely randomized design with three replicates. After 2 weeks of growth, plants were thinned to 2 plants per pot and the drought stress treatment was initiated. Water was withheld for 16 days. In this period of water withholding, the control plants continued to be well-watered (maximum water holding capacity) every 48 h.

2.3. Chlorophyll a fluorescence measurements

Chlorophyll *a* fluorescence (Chl *a*) measurements were taken from the third fully developed leaf from the apex of the plant, using a HandyPEA (Plant Efficiency Analyser, Hansatech Ltd., UK)

fluorimeter according to Oukarroum et al. (2007). Selected leaves were dark-adapted for at least 30 min prior to measurements. Immediately after the dark-adaptation period, a 4 mm diameter circular portion of the leaves was illuminated homogeneously with three light emitting diodes (LED) providing a pulse of saturating light intensity of 3000 $\mu mol\, m^{-2}\, s^{-1}$. Chlorophyll *a* fluorescence was recorded digitally by the HandyPEA between 10 μs and 1 s. All leaves exhibit a polyphasic Chl *a* fluorescence rise during the first second of illumination after the dark adaptation. The different steps of the polyphasic fluorescence transient were labelled in alphabetical order from the slower to the faster part of the transient. The most marked step at 2 ms is called the J-step. The fluorescence rise up to the J-step provides information about single turnover events of the primary reactions of photochemistry, mainly Q_A reduction (Oukarroum et al., 2007). During the time interval from 2 to ~200 ms multiple charge separations occur and the redox components of the electron transport chain became reduced. The different phases of this process show up in the fluorescence rise as the steps J, I and P. The step with the highest fluorescence intensity was called P (peak).

2.4. The JIP-test

Biolyzer software (Maldonado-Rodriguez, 2000) was used to load full fluorescence transients. Data collected were used to calculate JIP parameters according to the equations of the JIP-test (Tsimilli-Michael and Strasser, 2001): maximal fluorescence intensity (F_M); fluorescence intensity at 50 μs (considered as F_o); fluorescence intensity at 300 μs ($F_{300\,\mu s}$) required for calculation of the initial slope (M_o) of the relative variable fluorescence (V) kinetics; and the fluorescence intensity at 2 ms (the J-step) denoted as F_J. The JIP-test was used to quantify the amount of energy that flowed through PSII (Strauss et al., 2006).

The parameters measured initially (onset of fluorescence induction) were: (i) the specific energy fluxes (per reaction center) for absorption (ABS/RC), trapping (TR_o/RC), dissipation at the level of the antenna chlorophylls (DI_o/RC) and electron transport (ET_o/RC); (ii) the flux ratios related to photosynthetic yields, e.g. the maximum quantum yield of primary photochemistry ($\varphi_{Po} = TR_o/ABS = F_V/F_M$), the efficiency ($\psi_o = ET_o/TR_o$) with which a trapped exciton can move an electron into the electron transport chain further than Q_A^-, the quantum yield of electron transport ($\varphi_{Eo} = ET_o/ABS = \varphi_{Po}\psi_o$); (iii) the phenomenological energy fluxes (per excited cross-section, CS) for absorption (ABS/CS), trapping (TR_o/CS), dissipation (DI_o/CS) and electron transport (ET_o/CS). The fraction of active PSII reaction centers per excited cross-section (RC/CS) was also calculated.

Strasser et al. (2000) introduced a multi-parametric expression, the so-called performance index (PI_{ABS}):

$$PI_{ABS} = \left(\frac{\gamma}{1-\gamma}\right)\left(\frac{\varphi_{Po}}{1-\varphi_{Po}}\right)\left(\frac{\psi_o}{1-\psi_o}\right)$$

γ represents the ratio of chlorophylls reaction centre (Chl_{RC}) for the total amount of chlorophyll ($Chl_{RC+antenna}$) of PSII during the 50 μs to 1 s time range. The ratio of reaction centre and the absorbance (RC/ABS) was expressed as $[\gamma/1-\gamma]$.

Therefore, $RC/ABS = [(F_{2\,ms} - F_{50\,\mu s})/4(F_{300\,\mu s} - F_{50\,\mu s})]\ (F_V/F_M)$. The factor 4 is used to express the initial fluorescence rise per 1 ms. The contribution of the light reactions to primary photochemistry was estimated using the following equation:

$$\left[\frac{\varphi_{Po}}{1-\varphi_{Po}}\right] = \frac{TR_o}{DI_o} = \frac{k_P}{k_N} = \frac{F_V}{F_o}$$

The contribution of the dark reactions was estimated by:

$$\left[\frac{\psi_o}{1-\psi_o}\right] = \frac{ET_o}{TR_o - ET_o} = \frac{F_M - F_{2\,ms}}{F_{2\,ms} - F_{50\,\mu s}}$$

2.5. Drought factor index (DFI)

In this experiment, the drought factor index (DFI) was a measurement of the decrease of the Performance Index (PI_{ABS}) induced by the 16 days drought period. This approach has been successfully used by Strauss et al. (2006) to rank a number of soybean [*Glycine max* (L.) Merr.] genotypes for dark chilling tolerance, and by Oukarroum et al. (2007) to probe the responses of barley cultivars (*Hordeum vulgare* L.) to drought stress.

DFI was defined as:

$$DFI = \log A + 2\log B$$

where A was the relative PI_{ABS} measured at the 8th day of drought stress induction, and B the relative PI_{ABS} measured on the 16th day of drought stress.

The relative PI_{ABS} for each period of drought stress was calculated as $PI_{ABS\ unwatered}/PI_{ABS\ control}$. It was postulated that drought-tolerant genotypes could tolerate drought stress for longer periods of time than the drought-sensitive ones. Strauss et al. (2006), working on chill stress in soybean, stated that the reduction in PI_{ABS} in any given genotype after the treatment period B could be more significant (by a factor of 2) than a reduction in PI_{ABS} during the period A. As a consequence, drought-sensitive genotypes that exhibited the largest reduction in the PI_{ABS} during the latter stages of drought stress also had the lowest (most negative) DFI values.

2.6. Measurements of stomatal conductance and leaf temperature

Stomatal conductance was measured using a leaf porometer (SC-1 porometer, Decagon) on the third fully expanded leaves from the apex of the plant, between 10 and 12 a.m. Measurements of flag leaf temperature (FLT) were carried out on the third expanded pair of leaves of each genotype using a handheld infrared thermometer (Quicktemp 860-T2, Testo, Germany) according to Garrity and O'Toole (1995)'s method. Briefly, a 45° angle was created between the leaf surfaces and the horizon for each temperature measurements. The distance between the leaf and the infrared thermometer was 10 cm. Measurements were taken at solar noon (±1 h) to minimize solar angle interactions with the viewing direction. Flag leaf temperature were measured every 4th day.

A flag leaf temperature index (FLTI) was calculated as follows:

$FLTI = [(FLT_o - FLT_1)/FLT_o]$, where FLT_1 and FLT_o represent leaf temperature measured under optimal and drought stress conditions, respectively.

2.7. Statistical analyses

All statistical analyses were performed using R software (v.2.9.0). Normal distribution of data was determined using the Shapiro–Wilk W-test. ANOVA results were considered significant at $P < 0.05$ and means comparisons were done using Tukey HSD test.

3. Results

Fig. 1 illustrates the maximum quantum yield of primary photochemistry for selected genotypes after 16 days of drought stress treatment. F_V/F_M was not affected until 14 days of water withholding (Table 1). At the end of the drought stress period (16 days),

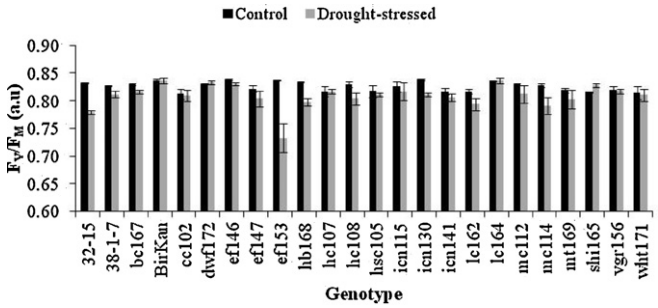

Fig. 1. Quantum yield of primary photochemistry of 24 sesame genotypes 16 days after withholding water. u.a, arbitrary unit.

there were evidence of significant genotypic variations for drought tolerance. There was also a significant genotypes × treatment interaction (Table 1). For most genotypes, the decrease in F_V/F_M was not significantly different between treatments except for mutant lines ef153, 32-15, mc114, lc162, hb168, mt169 and cultivar 32-15 in which the decrease in F_V/F_M was greater in the drought treated genotypes (Fig. 1). Mutant ef153 exhibited the highest decrease of F_V/F_M (13%) compared to the control. From these data, it is clear that the latter parameter was least affected by moderate drought stress.

The relative deviation of PI_{ABS} values (ΔPI_{ABS}) for each genotype in percent of control is shown in Fig. 2. From this figure, we can define two markedly contrasting groups of genotypes: one group with ΔPI_{ABS} value <0, e.g. genotypes that perform well in drought rather than optimal conditions, and a second group of genotypes having a ΔPI_{ABS} value > 0. The first group included mutants shi165, mc112, lc164, lc162, icn141 and icn115. The second group can arbitrarily be divided into 3 sub-groups. A particularly drought-sensitive group having ΔPI_{ABS} value ≥50% and comprising genotypes 32-15, ef153 and hb168; a second subgroup of tolerant genotypes with a small relative deviation of PI_{ABS} and including mutants mt169, icn130, ef146, dwf172, cc102 and Birkan. Genotypes wht171, vgr156, mc114, bc167 and 38-1-7 formed an intermediate sub-group. Significant differences were revealed between genotypes using PI_{ABS} by ANOVA analyses (Table 1).

The effect of drought stress on PI_{ABS} and its three main components is shown in Fig. 3 for two performing genotypes (lc162 and BK) and two drought-sensitive genotypes (32-15 and wht171). Decrease in PI_{ABS} due to drought stress, was mainly attributable to a decrease in electron transport component activity as shown by the large decrease in the parameter $\psi_o/(1-\psi_o)$ in the drought stress-sensitive genotypes 32-15 and wht171 (Fig. 3). In this experiment, the density of active photosystems (RC/ABS) was also affected by drought stress. In contrast, the efficiency of primary photochemistry or trapping, represented by the parameter $\varphi_{Po}/(1-\varphi_{Po})$ was less altered by drought stress.

The drought stress-tolerant genotype Birkan had a drought factor index (DFI) of −0.06 compared to −1.08 for the drought-sensitive genotype 32-15 (Table 2). Nine genotypes had higher DFI

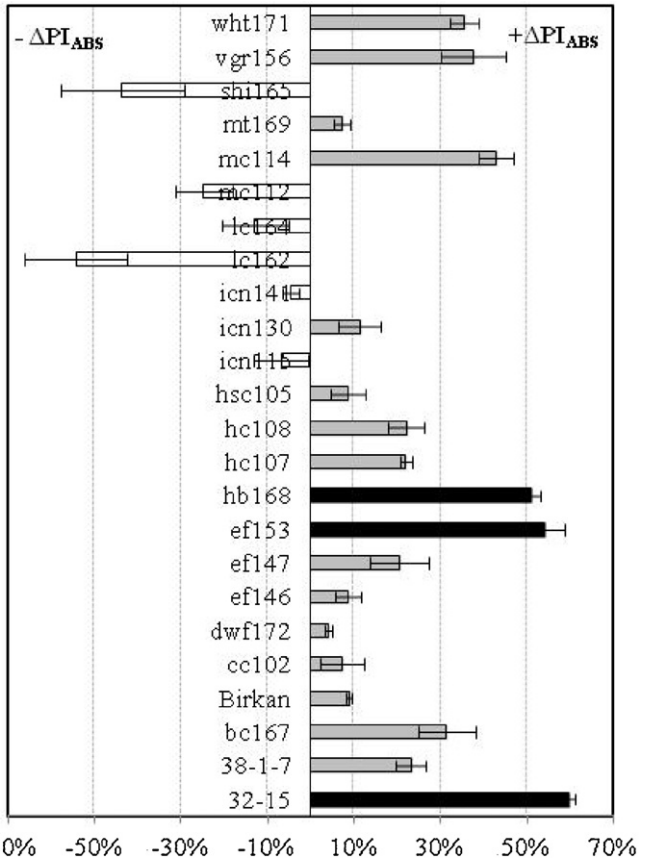

Fig. 2. Relative deviation of PI_{ABS} in percent of control of each sesame genotype tested for drought resistance; $\Delta PI_{ABS\ rel} = [(PI_{ABS\ control} - PI_{ABS\ stress})/PI_{ABS\ control}] \times 100$. Uncolored bars evidence highly drought-resistance genotypes with $\Delta PI_{ABS} < 0$; black bars show highly drought-sensitive genotypes with $\Delta PI_{ABS} \geq 50\%$; whereas grey bars represent intermediates. Bars represent standard error of mean.

values than that of Birkan (drought-tolerant). On the other hand, two genotypes had DFI values lower than that of genotype 32-15. Ten genotypes had intermediate DFI values. The 21 mutants were classified in four groups according to their DFI values. The first group (highly tolerant) includes mutants shi165, lc162, mc112, lc164, icn115, icn141, mt169, dwf172 and cc102, having DFI values superior to that of the drought-tolerant Birkan (DFI = −0.06); the second group (tolerant) concerned those mutants having DFI values comprised between the one of Birkan and the considered moderately drought-tolerant 38-1-7 (−0.39 < mutant DFI value ≤ −0.06). This group included genotypes ef146, icn130, hsc105 and hc107. The third group had mutants with DFI values comprised between 38-1-7 and the putative susceptible parent's DFI value (32-15) and was considered as moderately tolerant (−1.08 < mutant DFI value ≤ −0.39). This third group included mutants 38-1-7, bc167, hc108, vgr156, ef147, wht171 and hb168 whereas the fourth group which is the group of putative susceptible mutants had DFI values

Table 1
Analysis of variance for performance index, F_V/F_M, stomatal conductance and leaf temperature 16 days after withholding water: *F* values and significance levels of each variable.

Source of variance	Performance index	F_V/F_M	Stomatal conductance	Leaf temperature
Genotype (G)	1.69^{**}	1.73^{**}	1.11^{NS}	0.17^{NS}
Treatment (T)	3.21^{**}	24.06^{***}	340.31^{***}	176.60^{***}
G × T	1.02^{NS}	1.91^{**}	1.07^{NS}	0.16^{NS}

NS, not significant.
** Significance level, $P < 0.01$.
*** Significance level, $P < 0.001$.

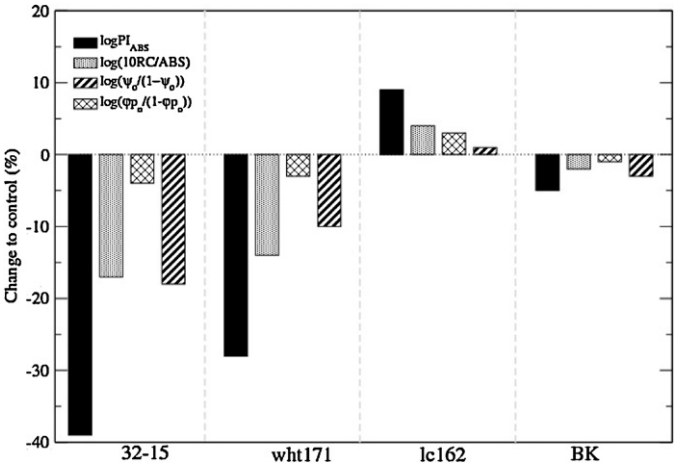

Fig. 3. Effect of drought stress on the driving force (DF = $\log PI_{ABS}$) of photosynthesis and its three partial components log(10RC/ABS), $\log(\varphi_{Po}/(1-\varphi_{Po}))$ and $\log(\psi_o/(1-\psi_o))$ of four representative sesame genotypes chosen on the basis of their different levels of drought tolerance. Horizontal bars represent the response in drought-stressed plants relative to that of well-watered plants 16 days after drought stress treatment initiation.

≤DFI value of the putative susceptible parent 32-15 (DFI = −1.08). This last group was formed by the mutants mc114 and ef153.

A positive relationship ($r^2 = 0.90$) was shown between genotype's DFI values and the relative driving force of $PI_{ABS} = \log(PI_{ABS\ stress}/PI_{ABS\ control}$; Fig. 4). Genotypes having the highest DFI values also exhibited the smallest reduction in relative PI_{ABS} during the drought stress period and the genotypes having the lowest DFI values (most negative) exhibited the largest reduction in relative PI_{ABS}.

Overall mean of stomatal conductance drops from 525.52 mmol m^{-2} s^{-1} six days after water withholding to 48.5 mmol m^{-2} s^{-1} on the 16th day while the watered plants maintained a high level of stomatal conductance ranging between 558.63 mmol m^{-2} s^{-1} and 625 mmol m^{-2} s^{-1} (Fig. 5A). There were evidences of a genotype effect on stomatal conductance with genotype icn130 and Birkan having the highest stomatal conductance. The lowest conductance was recorded for genotype wht171 (Fig. 6A).

Flag leaf temperature in the control plants reached a maximum value of 30 °C, falling to 29 °C by the end of the stress period (Fig. 5B). In contrast, a maximum of 36 °C was recorded in the drought treatment by the end of the stress period. The Temperature difference between stressed and unstressed treatments was 7 °C, 16 days after starting to withhold water but no genotypic difference was recorded. The highest flag leaf temperature indexes were recorded on the most drought-sensitive genotypes 32-15, ef153 and wht171 (0.26, 0.23, 0.23, respectively) (Fig. 6B) whereas the lowest values were recorded in lines mc112, shi165, vgr156 and 38-1-7 (0.03, 0.11, 0.10 and 0.11, respectively).

Table 2
Drought factor index (DFI) values for 21 induced mutant sesame lines and the three parental sources (in bold) following an exposure period of 16 days to drought stress.

Genotype number	Genotype name	Drought factor index (DFI)
1	shi165	0.48
2	lc162	0.46
3	mc112	0.33
4	lc164	0.22
5	icn115	0.08
6	icn141	0.01
7	mt169	−0.02
8	dwf172	−0.04
9	cc102	−0.05
Parent (DT)	**Birkan**	−0.06
10	ef146	−0.10
11	icn130	−0.24
12	hsc105	−0.24
13	hc107	−0.26
Parent (MT)	**38-1-7**	−0.39
14	bc167	−0.43
15	hc108	−0.54
16	vgr156	−0.56
17	ef147	−0.62
18	wht171	−0.74
19	hb168	−0.92
Parent (DS)	**32-15**	−1.08
20	mc114	−1.26
21	ef153	−1.51

DT, drought-tolerant; MT, moderately drought-tolerant; DS, drought-sensitive.

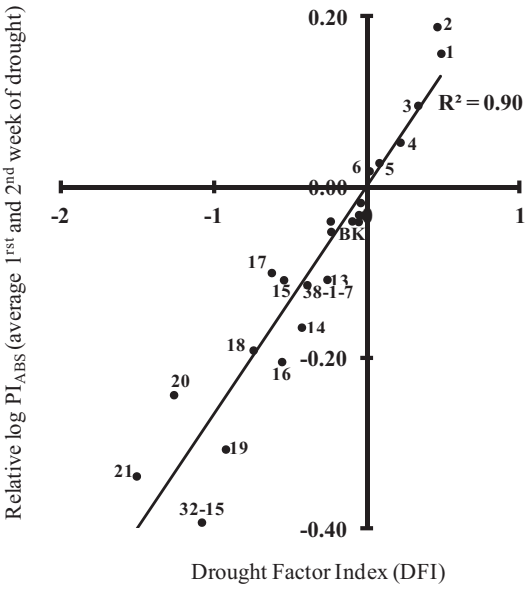

Fig. 4. Relationship between drought factor index (DFI) and relative $\log PI_{ABS} = [(\log PI_{ABS\ stress}/\log PI_{ABS\ control})]$ during 16 days of drought stress. The position of each genotype was identified by a number according to the results shown in Table 2. In contrast, for the three parental sources, their position was identified with their names; BK was used for Birkan.

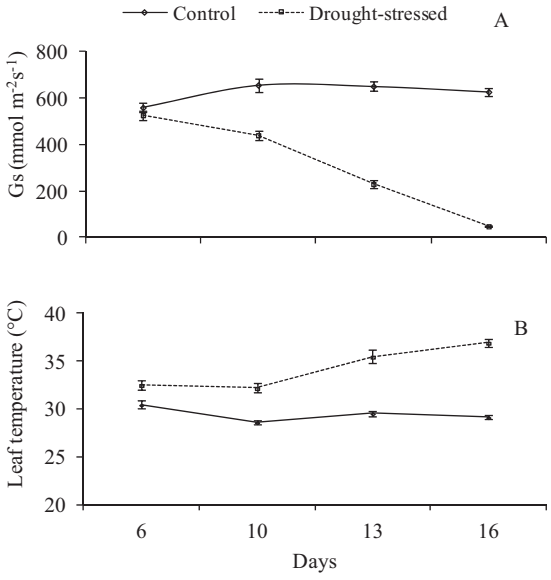

Fig. 5. Drought stress effect on stomatal conductance of 24 sesame genotypes under consideration (A); average leaf temperature at noon (B). Means are calculated across all genotypes tested. Bars represent standard error of mean.

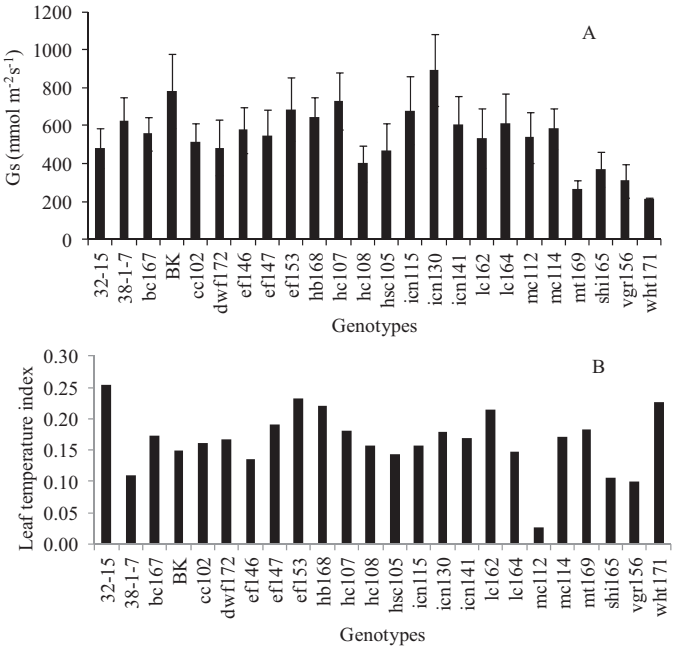

Fig. 6. Genotypic responses after 10 days of drought stress for stomatal conductance (Gs) (A) and leaf temperature index of each genotype (B). Bars represent standard error of mean.

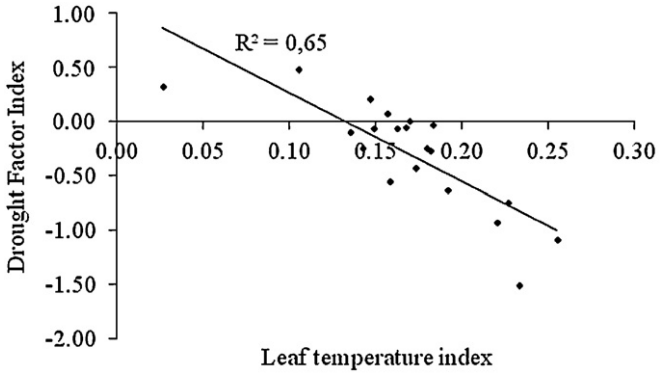

Fig. 7. Relationship between the drought factor index (DFI) and the leaf temperature index of sesame genotypes under consideration.

There were a significant negative correlation between the drought factor index and the leaf temperature index (Fig. 7).

4. Discussion

In the present study, we investigated drought resistance in 21 induced mutant lines of sesame at the vegetative stage. Maximum quantum yield of photochemistry (F_V/F_M), performance index (PI_{ABS}), stomatal conductance and leaf temperature were the main parameters used for this preliminary screening.

PI_{ABS} was found to be very sensitive to drought and has been used by many researchers to discriminate between cultivars for their responses to numerous abiotic stresses including drought stress (Oukarroum et al., 2007; Živčák et al., 2008), chilling (Strauss et al., 2006) and relative health of urban trees (Hermans et al., 2003). Data suggest that the decrease in PI_{ABS} observed in stressed plants was mainly due to a decrease in intersystem electron transport flux. This also suggests that drought stress could possibly alter sesame plants vitality by inducing loss of electron transport capacity. This assumption was supported by the linear correlation observed between the relative driving force ($DF = \log PI_{ABS\ stress}/\log PI_{ABS\ control}$) for photosynthesis and the relative electron transport activity. Oukarroum et al. (2007) also showed a similar linear correlation in a drought study on barley cultivars (*H. vulgare* L.). Such correlation was also confirmed by other authors (Hermans et al., 2003; Strauss et al., 2006).

In this experiment the active PSII reaction centers per absorbance (RC/ABS) were also altered by the drought stress. This alteration could probably be attributed to a degradation of chlorophyll by an early leaf senescence induced by the drought stress (Taulavuori et al., 2010).

The drought factor index (DFI) was introduced to rank the 21 induced mutant lines as well as the three reference parental sources with known drought tolerance: 32-15 (drought-sensitive), Birkan (drought-tolerant) and 38-1-7 (moderately drought-tolerant). A linear positive correlation was found between DFI and relative $\log PI_{ABS}$ (Fig. 4).

Data showed limited differences in maximum quantum yield (F_V/F_M) between genotypes at 16 days after withholding water. This was consistent with the previous findings of Oukarroum et al. (2007) suggesting that drought stress had little effect on maximum quantum yield of primary photochemistry φ_{Po} ($=F_V/F_M$) in barley cultivars (*H. vulgare* L.).

Rosales-Serna et al. (2000) suggested that multiples indices should be used when making selections for drought stress. In this study, the screening for drought resistance was complemented with stomatal conductance and flag leaf temperature measurements. Stomatal conductance was highly reduced in drought-stressed plants. Data showed that stomatal limitation was one of the main factors explaining the decrease in PI_{ABS} in the drought-sensitive line wht171. Medrano et al. (2002) reported that chlorophyll fluorescence showed a high dependency on stomatal conductance. Several other photosynthetic parameters such as electron transport rate, pre-dawn F_V/F_M, net photosynthesis and internal CO_2 concentration were shown to be more dependent on stomatal conductance (Flexas et al., 1998; Escalona et al., 1999). It has been suggested that stomatal conductance could be used as a physiological index to select for high yield potential in sesame varieties in dry and hot areas (Hall and Yermanos, 1975). The latter authors also stated that dehiscent cultivars generally presented the highest stomatal conductance values compared to closed capsules lines. This is in contrast with our own results because line cc102, an induced closed capsule mutant exhibited higher stomatal conductance than some dehiscent lines.

It has been shown that water deficit stress could cause partial stomatal closure in leaves and subsequently increased their temperature (Jackson et al., 1981). Munns et al. (2010) also suggested that leaf temperature is an indicator of stomatal conductance. Our data were in accordance with the founding of those authors as stomatal conductance was lower and leaf temperature greater in the drought-stressed plants when compared to the control plants.

Low values of leaf temperature index in lines mc112, shi165, vgr156 and 38-1-7 suggested a greater ability to extract water from the soil and to maintain higher relative water content and greater transpiration rate under moisture stress conditions. Differences in canopy temperature among crop cultivars are known to be related to drought avoidance characteristics (Garrity and O'Toole, 1995). The latter authors reported that leaves of cultivars with a history of outstanding vegetative stage drought stress remained coolest under water deficit conditions.

5. Conclusion

Based on drought factor index values, we have identified 9 mutant sesame lines with higher drought tolerance than the parent lines, 4 mutants with similar tolerance and 4 other mutants

were identified to be more drought-sensitive than the drought sensitive parent line "32-15". When considering the three parameters together (DFI, leaf temperature and stomatal conductance), mutant lines shi165, mc112, lc164, icn115, icn141, icn130, dwf172, cc102 and ef146 proved to be outstanding in both dry and wet conditions. Finally, we could evidence large genotypic variation in the drought tolerance of 24 senegalese sesame genotypes through the measurement of O–J–I–P fluorescence transients. In this study, the PI_{ABS} was found to be a very sensitive indicator of drought tolerance in sesame and was used to determine the drought factor index (DFI) which made it possible to evaluate the drought tolerance of these 24 sesame genotypes.

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