Effect of a short photoinhibition stress on photosynthesis, chlorophyll *a* fluorescence, and pigment contents of different maize cultivars. Can a rapid and objective stress indicator be found?

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Abstract

The effect of a short cold stress in combination with photoinhibition stress, similar to a low temperature and a high irradiance situation during early morning in the spring time, was examined on four maize cultivars common for Belgium, that differ in early vigour. After 1 h of 2 °C and 500 µmol(photon) m^{-2} s^{-1}, quantum efficiency and maximum photosynthesis rate at saturating irradiance decreased on average by 11 and 8 %, respectively. For one cultivar, Magister, the decrease was the largest: by 23 and 10 %, respectively. For this cultivar it was combined with a decrease of the water vapour conductance after the stress. The decrease of F_v/F_0 due to the cold/light stress was dependent on the cold tolerance (early vigour) of the cultivars. F_v/F_0 changed with –45.5 and –40.2 % for the cultivars Ardiles and Banguy, respectively (cultivars with a less good early vigour) in comparison to –36.3 and –35.9 % for Fjord and Magister, which have a good early vigour. Also the ratio of total chlorophylls/total carotenoids changed in dependence on cold tolerance of the cultivars. For more cold tolerant cultivars, the relative amount of total carotenoids (x+c) was higher, indicating a higher protective state. Both the parameter F_v/F_0 and the ratio of total chlorophylls to total carotenoids can be used to differentiate the cold tolerant cultivars from the cold non-tolerant ones. F_v/F_0 has the advantage because its resolving power is larger and the measurement is less expensive than determination of the pigment ratio.

Additional key words: carotenoids; chilling stress; high irradiance; low temperature; *Zea mays*.

Introduction

Zea mays L. (maize) is a subtropical crop that was introduced in Belgium in the 50's because of its high feeding value for animals and its good storage quality during the winter period. In view of its subtropical origin, maize is sensitive to cold (Miedema 1982, Stamp *et al.* 1983, Haldimann 1998). Since the crop is a C_4-plant it needs much higher temperatures for optimal photosynthesis in comparison to C_3-plants such as wheat. Maize needs relatively high temperatures to germinate, develop, and grow. This is sometimes difficult in the cold and wet conditions of Northern and Western Europe.

In the frame of sustainable agriculture, arable land should be covered during the whole year. Sowing maize as early as possible enables an earlier uptake of nutrients which in turn reduces leaching out. An early sowing can also improve the yield due to a longer growing season and an earlier harvest, allowing an earlier sowing of a green manure crop.

The earlier the sowing starts, the lower are the mean growing temperatures and the more frequent is the combination of a clear morning (high photosynthetically active radiation, PAR) and temperatures (T) near to freezing. Under these conditions photoinhibition of photosynthesis can occur. Photoinhibition is a complex of regulatory processes which introduce inefficiency (Osmond 1994). The same author describes chronic photoinhibition, as slowly reversible, if stress is not maintained. The first reaction is a decrease of quantum efficiency of the irradiance

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Abbreviations: CF – chlorophyll *a* fluorescence; Chl – chlorophyll; DAS – dark adapted state; ETR – apparent electron transport rate of photosystem 2; F_m, maximum CF yield in DAS; F_v, variable CF yield in DAS; F_0, minimum CF yield in DAS; I – irradiance; I_c – compensation irradiance; PAR – photosynthetically active radiation; P_{max} – maximum photosynthetic rate at photon saturation; P_N – net photosynthetic rate; PS, photosystem; R_D, dark respiration rate; T – temperature; x+c, content of total carotenoids; αc – quantum efficiency.
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response curve. Prolonged exposure to high irradiance can also result in an additional decline of the maximum photosynthetic rate (P_{max}). When the chilling/high irradiance stress continues, it can result in chilling injured maize, whose rate of respiration (R_D) and ion leakage increase (Creencia and Bramlage 1971). Further, wilting of leaves may occur in chilled plants, particularly in those not kept at high humidity (Amin 1969, Wright and Simon 1973).

In recent years, maize cultivars were often compared when grown between 14 and/or 25 °C (Haldimann *et al.* 1995, Haldimann 1998, 1999, Körnerová and Holá 1999, Fracheboud *et al.* 2000, 2002, Lidon *et al.* 2001, Lee *et al.* 2002). Some researchers changed temperatures and watched the stress effect. The stress temperatures were 10 °C (Haldimann 1997, Janda *et al.* 1997), 5–6 °C (Szalai *et al.* 1997b, Fracheboud *et al.* 1999, Leipner *et al.* 2000) or ≤ 2 °C (Smillie *et al.* 1987, Hetherington and Öquist 1988, Szalai *et al.* 1997a). Much less literature was found where maize was subjected to a temperature of 3 °C or below, even though that are the temperatures to which the plants are exposed in the springtime in the fields in Northern and Western Europe.

The aim of this research was to investigate the effect of a short photoinhibition stress, comparable to the one of a clear morning in spring (high PAR) and temperatures near freezing, on four maize cultivars that differ in early vigour (Van Waes *et al.* 2003), which can be related to a different cold tolerance. We tried also to find a rapid and objective method for differentiating between the cold tolerant and less tolerant cultivars.

Materials and methods

Plants: Four cultivars of *Zea mays* (L.) were chosen, namely Ardiles, Banguy, Fjord, and Magister. All are registered in the Belgian recommended list (Van Waes *et al.* 2003). Ardiles and Banguy have a slow early vigour, showing yellow leaves due to the cold stress in spring in comparison to Fjord and Magister (quick early vigour, green leaves).

Seeds were sown in 12-cm pots containing a standard peat soil. 0.5 kg fertilizer per m^3 of soil (N + P + K + MgO; 14 + 16 + 18 + 2) was added. Plants were grown in a greenhouse during September–October 2003, at the Department of Crop Husbandry and Ecophysiology, Centre of Agricultural Research, Merelbeke. Temperature was set at 20 °C day and night. The greenhouse was ventilated at 22 °C. Plants were irrigated to field capacity when necessary, 1–3 times a week.

Cold stress treatments: Mature leaves (third leaf starting from the bottom of the plant) were placed horizontally on a nylon wired bed in a growth chamber (type *1600US*, *Weiss Technik*, Germany) at a temperature of 2 °C, 70 % relative humidity, and PAR of 500 µmol(photon) m^{-2} s^{-1} during 1 h. Using these environmental settings, no chilling-induced wilting occurred. The temperature and irradiance chosen correspond to situations of the outdoor stress conditions during springtime. Those situations were determined by using the 'Typical Reference Year' for Belgium (Dogniaux *et al.* 1978).

Photosynthesis and chlorophyll (Chl) *a* fluorescence: An open gas-exchange system (*Li-6400*, *LI-COR*, Lincoln, USA) was used in combination with the leaf chamber fluorometer (*Li-6400-40*, *LI-COR*, Lincoln, USA). Irradiance response curves were measured at 20 °C, 400 µmol(CO_2) mol^{-1}, and a vapour pressure deficit between 0.8 and 1.5 kPa. The irradiances used for the measurements were 1 500, 1 000, 500, 250, 100, 50, 0 µmol(photon) m^{-2} s^{-1}. At least 5 leaves were measured per cultivar. The irradiance response parameters were calculated by fitting the curve $P_N = P_{max} [1 - \exp(-\alpha c (I - I_c)/P_{max})]$. Dark respiration rate, R_D was calculated by putting $I = 0$.

Chl *a* fluorescence (CF) was measured simultaneously with the photosynthesis measurements when an overview of the whole photosynthesis process was needed. For separate CF measurements a pulse amplified modulated fluorometer was used (*PAM-2000*, *Walz*, Germany). Before photosynthesis/CF and separate CF measurements, leaves were allowed to dark adapt for 20 min at 20 °C.

Pigment contents: The contents of Chl *a* and *b*, and total carotenoids and xanthophylls (x+c) were determined by extraction with N,N-dimethylformamide and spectrophotometric measurements using a *Cary 50 conc* UV-VIS spectrophotometer (*Varian*, Australia) and equations of Wellburn (1994).

Statistical analysis: The effect of different parameters was analyzed *via* ANOVA by using a statistical package *SPSS 11.5* (*SPSS*, USA).

Results

Leaves of different cultivars grown and measured at 20 °C (controls, Fig. 1*A*) showed no clear difference between the irradiance response curves of photosynthetic CO_2 fixation. Due to the cold stress (Fig. 1*B*), the average response curve for Magister decreased in comparison to the others and the standard errors for all cultivars increased. Irradiance response parameters were calculated from the model and from the rate of dark respiration (R_D). Since a significant difference was found between the modelled and measured R_D, although the r^2 of the fit was always more than 0.97, the measured R_D was always used in the comparisons (Table 1). From the statistical analysis (Table 2) we found out that for the photosynthetic parameters only P_{max} and αc were significantly affected by the cold stress. P_{max} and αc decreased on average for the 4 cultivars by –11 and –8 %, respectively; for the cv. Magister by –23 and –10 %, respectively (Table 1). No significantly different reaction was found related to the early vigour of the cultivars.

No significant differences were found for the irradiance response curve parameters between the cultivars when no stress was applied. When stress was applied, P_{max} was significantly lower for Magister than for Fjord [15.70 *versus* 22.33 μmol(CO_2) m^{-2} s^{-1}] (Table 1).

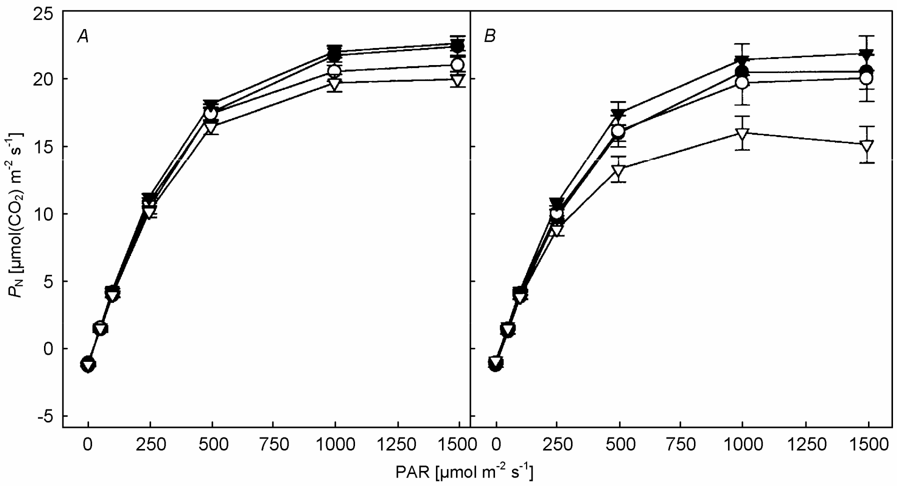

Fig. 1. Irradiance saturation curves of net photosynthetic rate, P_N [μmol(CO_2) m^{-2} s^{-1}] measured at 20 °C with cvs. Adriles (●), Banguy (○), Fjord (▼), and Magister (∇). Means ± S.E. of at least 5 replicates. *A*: control; *B*: cold stress of 2 °C combined with 500 μmol(photon) m^{-2} s^{-1}.

Table 1. Parameters of the irradiance response curve of photosynthetic CO_2 fixation ($n \geq 5$), contents and ratios of pigments ($n = 20$), and parameters of chlorophyll *a* fluorescence ($n = 20$) for controls and cold stressed maize cultivars Ardiles, Banguy, Fjord, and Magister measured at 20 °C. P_{max} [μmol(CO_2) m^{-2} s^{-1}], α_c [μmol(CO_2) $μmol^{-1}$ (photon), I_c [μmol(photon) m^{-2} s^{-1}], R_D [μmol(CO_2) m^{-2} s^{-1}], and pigment contents [mg m^{-2}(leaf area)].

	Control Ardiles	Banguy	Fjord	Magister	Cold stress Ardiles	Banguy	Fjord	Magister
P_{max}	23.08	21.41	23.12	20.45	21.53	20.23	22.33	15.70
α_c	0.0634	0.0686	0.0691	0.0641	0.0604	0.0623	0.0655	0.0574
I_c	24.0	23.7	23.7	23.7	29.1	23.2	23.7	22.1
R_D	-1.06	-1.28	-1.25	-1.18	-1.38	-1.20	-1.24	-1.06
Chl *a*	305.7	274.1	317.5	335.7	322.7	277.4	322.6	310.9
Chl *b*	62.6	71.3	62.9	67.6	78.0	60.8	62.3	62.6
x+c	64.9	59.1	62.2	66.2	68.4	56.0	63.0	61.2
a/b	4.91	3.87	5.05	4.97	4.16	4.54	5.18	4.99
(*a*+*b*)/(x+c)	5.66	5.84	6.12	6.09	5.85	6.00	6.11	6.10
F_0	0.140	0.134	0.136	0.151	0.155	0.147	0.142	0.149
F_m	0.729	0.698	0.688	0.722	0.511	0.517	0.511	0.508
F_v	0.589	0.564	0.552	0.571	0.356	0.370	0.368	0.359
F_v/F_m	0.808	0.808	0.803	0.791	0.697	0.715	0.721	0.707
F_0/F_m	0.192	0.192	0.197	0.210	0.304	0.285	0.279	0.293
F_v/F_0	4.215	4.197	4.070	3.773	2.298	2.508	2.595	2.419

As concerns the Chl *a* fluorescence parameters (Table 2), F_0 was not significantly affected by the cold stress in contrast to the other fluorescence parameters related to DAS. F_0 and F_v/F_0 were significantly related to the cold tolerance of the cultivars. For neither of the parameters a significant interaction was found between cold stress and cold tolerance (early vigour). This means that more cold tolerant cultivars showed a similar reaction to cold stress as the less cold tolerant cultivars. For F_v/F_0 we found a significance level for the interaction that was the lowest of all: 0.088 (which is close to 0.050). Therefore, a new experiment was done with more leaves to find out if the reaction of the plants to this parameter holds. If so, we could have a fast tool to separate more cold tolerant plants from the others. According to Babani and Lichtenthaler (1996) and Roháček (2002) this parameter reflects changes in the maximum ratio of quantum yields of photochemical and concurrent non-photochemical processes in photosystem 2 (PS2) related to DAS and is a very sensitive indicator of the maximum efficiency of photochemical processes in this photosystem and/or the potential photosynthetic activity. The sensitivity of F_v/F_0 in comparison to F_v/F_m and F_0/F_m is also proven here.

Table 2. Significance levels for the different parameters: P_{max} [μmol(CO_2) m^{-2} s^{-1}], αc [μmol(CO_2) $μmol^{-1}$(photon), I_c [μmol (photon) m^{-2} s^{-1}], R_D [μmol(CO_2) m^{-2} s^{-1}] of the irradiance response curves ($n \geq 5$), chlorophyll and carotenoid contents (n = 20), and CF parameters F_0, F_m, F_v/F_m, F_0/F_m, and F_v/F_0 (n = 5 or 20) of the control and stress treatments for four maize cultivars. For abbreviations see the text.

Factor	Cold stress	Cold tolerance	Interaction
P_{max}	0.027	0.181	0.366
Ac	0.011	0.970	0.750
I_c	0.628	0.134	0.178
R_D	0.429	0.281	0.244
F_0 (n = 5)	0.345	0.036	0.118
F_m	0.001	0.448	0.585
F_v	0.001	0.241	0.394
F_v/F_m	0.001	0.104	0.295
F_0/F_m	0.001	0.104	0.295
F_v/F_0	0.001	0.025	0.088
F_0 (n = 20)	0.001	0.899	0.013
F_m	<0.001	0.523	0.837
F_v	<0.001	0.404	0.340
F_v/F_m	<0.001	0.433	<0.001
F_0/F_m	<0.001	0.447	<0.001
F_v/F_0	<0.001	0.001	<0.001
Chl *a*	0.391	<0.001	0.160
Chl *b*	0.605	0.003	0.107
x+c	0.869	0.088	0.532
a/b	0.746	<0.001	0.594
(*a*+*b*)/(x+c)	<0.001	<0.001	0.001

In the second experiment, CF parameters related to DAS were measured on 20 leaves. This number was chosen based on the detected variance of the previous experiment and to be able to detect 5 % difference at a 95 % significance level between the cultivars. ANOVA of the results (Table 2) showed that all CF parameters were significantly affected by cold stress: F_0 and F_0/F_m increased but F_m, F_v, F_v/F_m, and F_v/F_0 decreased (Table 1). As concerns the cold tolerance, a significant difference was found for one parameter, F_v/F_0. Furthermore, we found for that parameter a significant interaction meaning that the cold tolerant and the non-cold tolerant cultivars reacted different to the cold stress. For Ardiles and Banguy an average decrease of the parameter was –45.5 and –40.2 %, respectively, while for Fjord and Magister it was –36.3 and –35.9 %, respectively (Fig. 2).

Table 3. Comparison between the F-levels of ANOVA of F_v/F_0 and (*a*+*b*)/(x+c) of the control and stress treatments for four maize cultivars (n = 20).

Factor	Cold stress	Cold tolerance	Interaction
F_v/F_0	3795.96	9.76	60.33
(*a*+*b*)/(x+c)	12.66	96.18	11.28

In the same experiment, we found values [mg m^{-2}] 308.3±3.3 for Chl *a*, 66.0±0.7 for Chl *b*, and 62.6±0.6 for x+c. These contents or the ratio of Chl *a/b* were not significantly affected by cold stress, but the ratio (*a*+*b*)/(x+c) was. For this ratio, we detected also a significant difference in cold tolerance and a significant interaction, meaning that the cold tolerant and the cold non-tolerant cultivars reacted differently to the cold stress. This effect was comparable to the statistical results found for F_v/F_0. For cvs. Ardiles and Banguy an average increase of the parameter was +3.28 and +3.31 %, respectively, but for Fjord and Magister it was –0.10 and +0.26 %, respectively.

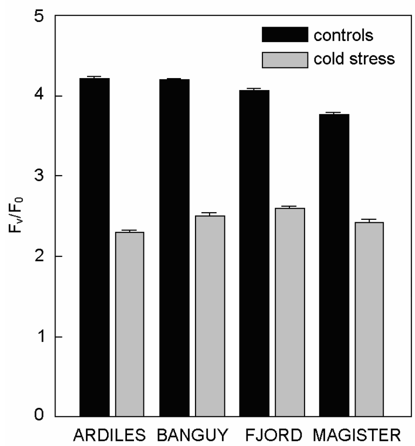

Fig. 2. Values of the chlorophyll fluorescence ratio F_v/F_0 (means ± standard errors) before and after the cold stress for the four cultivars of maize (n = 20).

The F-level of ANOVA of the two interesting parameters for cold tolerant and non-cold tolerant cultivars (interaction term) was the largest for F_v/F_0 (Table 3). This parameters enabled the easiest detection of differences. Furthermore, the cost for determining F_v/F_0 is much cheaper and less time consuming than determination of the ratio (*a*+*b*)/(x+c).

Discussion

The possible photoinhibition is subdivided into two main classes: dynamic and chronic (Osmond 1994). In our case, where plants/leaves were subjected to high PAR and temperatures near to freezing, we deal with chronic photoinhibition. During chronic photoinhibition, the irradiance response curve of photosynthesis is displaced downwards. The first indication to be expected is the decrease of αc. When the stress is more severe or prolonged also a decrease of P_{max} is to be expected. Indeed we found that those parameters were affected by the applied cold stress. The decrease was slightly more significant for αc. For cv. Magister we found that the decrease of P_{max} due to the stress was remarkably higher than for the other cultivars. As a consequence of the stress and in comparison to the other cultivars, we also found a relatively larger decrease of the stomatal conductance at high PAR for Magister (data not shown). This indicates that in Magister the stomata were more closed, which consequently interfered with the supply of CO_2 for photosynthesis. The effect found for P_{max} of cv. Magister was also combined with a lowered electron transport rate at high PAR after cold stress. In the first experiment, F_0 was not affected by the cold stress, but F_m was. F_0 can increase due to stress, indicating damage to PS2 (Demmig and Björkman 1987). This was not due to the applied 1 h cold/light stress. F_m decreased on average by 29 % due to the applied stress. F_v/F_m, F_0/F_m, and F_v/F_0 were all affected by the cold stress, even though the F-value of the ANOVA test was the largest for F_v/F_0. F_v/F_0 has a high power of discernment (Babani and Lichtenthaler 1996, Roháček 2002). For F_v/F_0 the differences due to cold stress and cold tolerance (early vigour) of the cultivars were detected. Cold tolerant cultivars should react to cold stress in a different way than cold non-tolerant cultivars. Therefore, the second experiment was done with an increased sample size to detect the 0.05 significance level (Snedecor and Cochran 1980). In the second experiment all CF parameters were affected by cold stress: also F_0 was affected and increased for all cultivars but cv. Magister. If the changes were calculated per cultivar, we found that F_0 changed for Ardiles, Banguy, Fjord, and Magister by +10.7, +11.0, +4.9, and –1.8 %, respectively. This was in accordance with the significant interaction found for F_0. Indeed, Ardiles and Banguy, the less cold tolerant cultivars (lowest early vigour), showed a larger, although not significant, increase in F_0 due to stress than Fjord and Magister (quicker early vigour). F_m and F_v decreased significantly for all cultivars with an average of –27.9 and –36.1 %, respectively. Only for the parameter F_v/F_0, both factors (cold stress and cold tolerance) and their interaction were significant. This indicates that the parameter is affected by the stress and there is a difference in cold tolerance. Furthermore, the reaction of cold tolerant cultivars in comparison to cold non-tolerant ones is different. The results of the second experiment were also in accordance with the first experiment using a smaller sample size. As such, this was a non-destructive parameter that can be used to determine objectively the cold tolerance. Nevertheless, the changes of the measured CF parameters give an indication of photoinhibition. A more explicit indication is given by the analysis of the Chl *a* fluorescence relaxation kinetics in the dark (Lichtenthaler and Burkart 1999).

The ratio $(a+b)/(x+c)$ increased significantly for the cold non-tolerant cultivars in comparison with the cold tolerant ones. Already during the 1-h stress, relatively less total carotenoids were found in the cold non-tolerant cultivars. This finding is in accordance with the fact that carotenoids protect the photosynthetic apparatus against damage from photo-oxidation (Siefermann-Harms 1987, Young 1991). Higher $(x+c)/(a+b)$ ratios were also found in maize grown at 14 °C in comparison to 24 °C (Haldimann *et al.* 1995), indicating that leaves attempt to protect themselves against damage from excessive energy by increasing the relative amount of carotenoids. Nevertheless, the change of F_v/F_0 is far more useful for testing different plants/cultivars, since the costs of measuring are much lower and the resolving power is larger.

In conclusion, we applied a photoinhibition stress comparable to a morning stress in springtime, but the direct effect on P_N was small with the exception of the cv. Magister. On the other hand, effects on CF parameters and the ratio of $(a+b)/(x+c)$ can be used to judge cold tolerance on an objective basis.

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