Chlorophyll fluorescence and chlorophyll content in field-grown potato as affected by nitrogen supply, genotype, and plant age

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Abstract

Field experiments were conducted in Sicily (south Italy) to assess chlorophyll (Chl) fluorescence parameters in response of potato crop to nitrogen dose, to variation in genotype and in plant age, and to detect relationships between Chl content, fluorescence parameter F_v/F_m, and tuber yield. The experiment included five nitrogen doses (0, 10, 20, 30, and 40 g m^{-2}) and four genotypes (Spunta, Sieglinde, Daytona, and Igea). Chl fluorescence parameters (initial fluorescence, F_0, maximum fluorescence, F_m, variable fluorescence, F_v, F_v/F_m, T_{max} (the time required to reach F_m), and Chl content were measured weekly between the appearance of the fifth and sixth leaves and the onset of plant senescence. A positive linear relationship was established between nitrogen supply and Chl content, F_0, and T_{max}. Nitrogen supply up to 10 g m^{-2} also had a positive effect on F_m and F_v, but above this rate it reduced F_v/F_m. Spunta had the highest Chl content, F_m, F_v, and F_v/F_m, but the lowest F_0, whereas Sieglinde had the lowest Chl content, F_v, F_v/F_m, and T_{max} and the highest F_0. The cvs. Igea and Daytona exhibited intermediate Chl fluorescence parameters. Chl content and T_{max} decreased with increasing plant age, whereas F_0, F_m, and F_v increased until complete canopy development and thereafter declined until crop maturity. Tuber and plant dry matter yield were significantly correlated with Chl content, F_0, and T_{max}. Thus Chl fluorescence and content detect differences in the response of potato to N supply, can discriminate between genotypes, predict plant age, and yield performance under field conditions.

Additional key words: dry matter; seasonal course; *Solanum tuberosum*; tuber yield.

Introduction

Modifications in the function of the photosynthetic electron-transport system can be assayed in intact leaves by the analysis of chlorophyll (Chl) *a* fluorescence, which reflects changes in irradiance-dependent processes of photosynthesis, such as photon absorption, excitation-energy transfer to reaction centres, and electron transport through photosystems PS2 and PS1 (Krause and Weis 1991), and provides qualitative and quantitative information on photosynthetic processes in chloroplasts (Roháček and Barták 1999). Chl fluorescence provides a non-destructive, rapid means of assessing both photo-chemical quantum yield and photoinhibition (Krause 1988, Krause and Weis 1991), and has been widely employed as an indicator of plant response to stresses, especially those imposed by air and water pollution (Lichtenthaler 1988), water deficit (Cornic and Massacci 1996), high and low temperature (Kristjansdottir and Merker 1993, Willits and Peet 2001), and salinity (Smillie and Nott 1982). Larcher (1994) has suggested that it may represent the most reliable way of quantifying photosynthesis in heat-stressed plants. It has been used as a means to screen plants for heat tolerance (Yamada *et al.* 1996, Ranney and Ruter 1997). It is also a useful tool for screening durum and bread wheat for drought tolerance (Flagella *et al.* 1994, 1995), as a selection criterion in durum wheat for high grain yield (Araus *et al.* 1998), and for the measurement of photochemical activity in cross-combination sugarcane (Zhang *et al.* 2000).

In recent years, the use of Chl fluorescence has become commonplace in plant eco-physiology, to the extent that no investigation of the photosynthetic performance of plants growing in field conditions seems complete

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without some fluorescence data (Maxwell and Johnson 2000). Developments in instrumentation and methodology, which have enabled accurate measurements of Chl fluorescence, have led to significant advances of both theoretical and practical benefit to plant and crop physiology and ecology.

Mineral nitrogen is the most important nutrient input in agriculture (Navarro Pedreño *et al.* 1996). Its application has a substantial effect on the leaf area index (LAI) of potatoes causing an increase in both the rate of leaf expansion and the number of emerging leaves (Vos and Biemond 1992, Vos and Van der Putten 1998). The nitrogen effect on LAI directly influences seasonal patterns of photon interception and crop production (Vos 1995, Vos and Van der Putten 1998), and indirectly crop yield, since this is dependent on radiation use efficiency (RUE), the ratio between the amount of radiation intercepted and gain in plant dry mass during a particular time interval (Monteith 1977).

An increased N supply stimulates the photosynthetic capacity of leaves *via* increases in the contents of stromal and thylakoid proteins in leaves (Evans and Terashima 1988, Evans 1989). In *Phaseolus* and spinach, N deficiency reduces both ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) activity and whole-chain electron transport to roughly the same extent (Caemmerer and Farquhar 1981, Evans and Terashima 1987). These two processes are closely linked to one another, but some reports suggest that the balance between RuBPCO activity and electron transport is unaffected by N supply (Evans and Terashima 1988, Makino *et al.* 1992).

The ability of plants to assimilate CO_2 is, in part, a function of the rate of electron transfer (Strong *et al.* 2000), and therefore the Chl fluorescence of a leaf can be used as an indicator of photosynthetic activity (Schreiber *et al.* 1995). Good correlations have been established between Chl fluorescence and root growth, gas exchange, electrolyte leakage, visible leaf damage, and leaf water potential (Larcher 1994).

The potato is a very important crop in Mediterranean countries, occupying an overall area of about 1 Mha, with an annual production of 19 Mt of tubers (Frusciante *et al.* 1999). In southern Italy, as in other southern coastal areas of the Mediterranean basin, the potato crop is produced during the winter-spring cycle (from November–December to May–June) to obtain off-season harvest. No systematic study of the eco-physiological response to N supply has yet been carried out in the Mediterranean climate and the abnormal seasonal cycle. This information is relevant for genotype adaptation, and may also help to generate savings in N input, which would both lessen production costs and mitigate against excessive leaching to groundwater.

The objectives of the present research programme were to study, *via* Chl fluorescence, the response of a potato crop to variation in fertiliser N rate, genotype, and plant age, and to detect relationships, if any, between Chl fluorescence parameters and yield, in order to explore the possibility of applying this technique as a predictive tool in field studies.

Materials and methods

Site, climate, and soil: Field experiment was conducted along the coastal plain area south of Siracusa (37°03'N, 15°18'E, 10 m a.s.l.), which is a typical area for early potato cultivation in south Italy. The climate in this area is semi-arid-Mediterranean, with mild winters and often rainless springs. Frost is virtually absent (2 events in 30 years). During the potato crop season for early production (December–May), the mean day temperature and mean minimum night temperature of 30 years (1959–1988) are, respectively, 16.8 and 8.7 °C in December, 16.2 and 9.0 °C in January, 15.3 and 7.5 °C in February, 17.0 and 8.0 °C in March, 19.8 and 8.9 °C in April, 24.0 and 13.4 °C in May. Rainfall over the same period averages about 234 mm.

The soil type is calcixerollic xerochrepts (USDA Soil Taxonomy), moderately deep, with a loam-clay texture. At the start of the experiments, the soil characteristics analysed in our laboratory were as follows: sand (38 %), silt (28 %), clay (34 %), limestone (1 %), pH (8.0), organic matter (1.5 %), total N (1 200 mg kg^{-1}), P (10 mg kg^{-1}), K (108 mg kg^{-1}). All soil analyses were performed according to procedures approved by Italian Soc. Soil Sci. (Violante 2000).

Experimental design and plants: The experiment was arranged in a randomised split-plot design with four replications including 5 nitrogen rates (0, 10, 20, 30, and 40 g m^{-2}) as main plots and 4 genotypes of potato (*Solanum tuberosum* L.), *e.g.* Spunta, Sieglinde, Daytona, and Igea as sub-plots. Sub-plots' size was 4.2×4.2 m, with 84 plants. Nitrogen was supplied ¼ pre-planting as ammonium sulphate and ¾ two weeks after emergence as ammonium nitrate.

Spunta and Sieglinde are the most widely grown cultivars in the Mediterranean region. Spunta is a ware potato, of early maturity with long, regular, and very large tubers; plants produce few, but erect and vigorous, stems. Sieglinde is a firm flesh early cultivar with oblong, regular, and moderate-sized tubers; plants produce numerous stems which are of medium height, semi-erect, and moderately vigorous. Daytona is a new Italian cultivar, of late maturity with short, oval, and regular tubers. Stems are of medium size. Igea is also a new Italian cultivar, of medium to late maturity, with oblong, regular, and very large tubers. Stems are fairly tall and erect.

Management practices: Planting was done manually on 22 January using whole disease-free tubers. Phosphorus

(10 g m^{-2}), potassium (15 g m^{-2}), and chlorpyrifos (3 g m^{-2}) were applied before planting. All plants emerged. Drip irrigation was carried out when the accumulated daily evaporation reached 40 mm and supplying 100 % of maximum evapotranspiration. The usual crop management was used: post emergence with linuron and pest control when needed.

Chl fluorescence parameters were recorded in the field with a portable fluorescence induction monitor (*Fi_m 1500*, *Alma Group Company*, Hoddesdon, Herts, England). Measurements were made on the terminal of the youngest fully expanded leaf (usually the third or fourth leaf from the apex) after a 20 min dark adaptation period between 11:00 and 13:00 (local solar time). Dark adaptation time was the time needed to obtain a steady value of F_v/F_m.

Leaf clips were applied to fully sun exposed leaflets of 4 potato plants randomly sampled in the centre of each sub-plot. Measurements were made with saturation irradiance up to 3 000 µmol m^{-2} s^{-1}. In order to study the influence of plant age on Chl fluorescence, nine measurements were taken weekly between 5^{th}–6^{th} leaf appearance and beginning of plant senescence. In all, 2 880 measurements for each Chl fluorescence parameter were made.

Chl content was measured in the field using a portable Chl meter (*SPAD 502*, *Minolta Camera*, Osaka, Japan). Triplicate readings were taken at each leaf of the four plants previously marked for Chl fluorescence measurements.

Yield: The harvest area consisted of 20 plants from the centre of each sub-plot. Plants were harvested by hand on 22 May, when about 70 % of haulm was dry and mass of tubers and epigeal biomass was determined. A sample of tubers and epigeal biomass for each plant was dried in a thermo-ventilated oven at 105 °C for 72 h and weighed.

Statistical analysis: All data were submitted to Bartlett test for the homogeneity of variance and then were analysed using analysis of variance (ANOVA) as a factorial combination of nitrogen dose×genotype×measurement date as indicator of plant age. Means were compared by *LSD* test, provided the *F* test was significant. Polynomial effects up to second degree were made where appropriate to define the response of trend (linear or quadratic) between treatments and Chl fluorescence parameters or Chl content.

Results

Chl content: Analysis of the variance showed that N dose and measurement date as index of plant age accounted for 45 and 40 %, respectively, of the total variation, against only 10 % of the genotype (Table 1). The Chl content increased linearly and significantly with increasing nitrogen rates, but decreased significantly with plant ageing (Table 2). Spunta exhibited a higher Chl content (on average, 41.4 SPAD) compared to those of Daytona (38.2 SPAD), Igea (38.4 SPAD), and Sieglinde (36.5 SPAD).

Table 1. Mean square as absolute value and percentage of total (in brackets) of main effects resulting from analysis of variance of studied chlorophyll (Chl) parameters in field-grown potato. df = degree of freedom, **significant at $p \leq 0.01$.

Parameter	Source of variation (main effects)		
	N dose (df 4)	Genotype (df 3)	Measurement date (df 8)
Chl content	2584** (45 %)	590** (10 %)	2315** (40 %)
F_0	1041270** (16 %)	3394922** (51 %)	1540067** (23 %)
F_m	5293696** (37 %)	3277704** (23 %)	4227232** (29 %)
F_v	2601632** (19 %)	7481120** (54 %)	1427066$_{**}$ (10 %)
F_v/F_m	0.0581** (10 %)	0.3584** (65 %)	0.0462** (8 %)
T_{max}	17790** (8 %)	18755** (8 %)	166144** (72 %)

Initial fluorescence (F_0) represents the basal emission of Chl fluorescence when redox components of photosystems are fully oxidised. This requires appropriate dark adaptation. For this parameter, the main cause of variance proved to be the genotype with 51 % of total variance (Table 1). Regardless of the other studied factors, the F_0 value was significantly highest in Sieglinde, intermediate in Daytona and Igea, and lowest in Spunta (Table 2). F_0 values increased significantly and linearly as the amount of N applied increased, but was unvaried from 30 to 40 g m^{-2} (Table 2).

Changes of F_0 during the plant ageing showed a typical bell pattern; F_0 values increased with time from 21 March (plant stage 5^{th}–6^{th} leaf development) to 15 April (complete canopy development) and then declined in a linear fashion over the season (Table 2).

Maximum fluorescence (F_m) is obtained at the fully saturating irradiance for the plant when the electron acceptor Q_A is fully reduced. The analysis of variance showed that N dose, genotype, and plant age accounted for 37, 29, and 23 %, respectively, of the total variation (Table 1).

Table 2. Chlorophyll (Chl) content and Chl fluorescence parameters of field grown potato as affected by nitrogen dose, genotype, and measurement date as indicator of plant age. *Different letters*, within each factor (N dose, genotype, or measurement date) and parameter, indicate significant differences for $p \leq 0.05$. L = linear; Q = quadratic; $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$. Relationship tested by regression analysis, between N dose or measurement date and responses of each character.

	Treatment	Chl content (SPAD)	F_0 [relative]	F_m [relative]	F_v [relative]	F_v/F_m	T_{max} [ms]
N dose [g m^{-2}]	0	32.4 e	896 d	2857 c	2006 c	0.691 a	175 c
	10	36.8 d	951 c	3291 ab	2378 a	0.709 a	189 b
	20	40.1 c	1038 b	3231 b	2210 b	0.664 b	196 ab
	30	41.4 b	1098 a	3335 a	2253 b	0.669 b	197 ab
	40	43.2 a	1073 ab	3250 ab	2182 b	0.663 b	205 a
	L	**	*	NS	NS	NS	NS
	Q	NS	NS	NS	NS	NS	NS
Genotype	Spunta	41.4 a	849 c	3337 a	2479 a	0.739 a	197 a
	Sieglinde	36.5 c	1184 a	3189 b	2074 c	0.632 c	178 b
	Daytona	38.2 bc	991 b	3013 c	2028 c	0.666 b	193 a
	Igea	38.4 b	1023 b	3232 b	2242 b	0.680 b	202 a
Measurement date [days from 1st March]	21	44.5 ab	857 e	3021 d	2147 cde	0.699 ab	262 a
	27	45.7 a	899 de	3240 c	2345 ab	0.711 a	259 a
	31	43.5 b	1107 b	3405 b	2277 bc	0.674 b	222 b
	37	40.6 c	1190 a	3626 a	2453 a	0.672 b	192 c
	46	39.1 d	1233 a	3274 c	2109 de	0.626 c	182 c
	51	37.4 e	1003 e	3239 c	2236 bcd	0.687 ab	162 d
	58	35.9 f	1019 c	3047 d	2153 cde	0.672 b	165 d
	66	32.0 g	923 d	2968 d	2090 de	0.678 ab	142 e
	76	30.5 h	871 de	2914 d	2041 e	0.691 ab	146 e
	L	***	NS	NS	NS	NS	***
	Q	NS	*	NS	NS	*	*

N supply caused a significant increase in F_m. Regardless of other factors and averaged over the N amounts, the increase of F_m was about 13 % with respect to unfertilised plants (Table 2). As regards the genotype, Spunta showed the highest values, Sieglinde and Igea the intermediate, and Daytona the lowest ones. The pattern of changes in F_m during the plant cycle was similar to that of F_0 (Table 2).

Variable fluorescence (F_v): Obtained by subtracting F_0 from F_m, this reflects the reduction at a given time of the primary electron acceptor, which, in the oxidised state, quenches fluorescence (Jefferies 1992). As for F_0, the main cause of variance of F_v was the genotype with 54 % of total variation. Averaged over the N dose and measurement date, F_v was greatest in Spunta, intermediate in Igea, and lowest in Sieglinde and Daytona (Table 2). Nitrogen supply significantly increased F_v, especially at 10 g m^{-2} (Table 2). As regards the plant age, the F_v was highest in early and intermediate growth stages and lowest at senescence stage (Table 2).

F_v/F_m: As proposed by Butler and Kitajima (1975) and Kitajima and Butler (1975), this is a useful ratio which has been shown to be proportional to the quantum yield of photosystem 2 (PS2) photochemistry and exhibits a high degree of correlation with the quantum yield of net photosynthesis. The main cause of variance proved to be the genotype with 66 % of total variation, followed by the N dose (12 %) and plant age (8 %). Averaged over the other factors, the F_v/F_m was greatest in Spunta (0.74) and least in Sieglinde (0.63) (Table 2). The rate of the three highest N doses significantly reduced the F_v/F_m ratio in comparison to lowest dose and control without N supplied (Table 2). Small and often insignificant differences were found during the crop cycle as shown in Table 2.

T_{max}: The time at which the maximal fluorescence is a measure of the pool size of electron acceptors on the reducing side of PS2. This parameter was, unlike F_v/F_m, mainly affected by measurement data which accounted for 72 % of total variance against only 8 % accounted for by both genotype and N dose. T_{max} consistently declined with plant age, passing from about 260 ms of the young plants to about 140 ms of the old plants (Table 2). T_{max} linearly increased ($p \leq 0.05$) with increasing N supply and was smaller in Sieglinde compared with the other genotypes (Table 2).

Correlation among parameters was positive for T_{max} and F_m ($p \leq 0.001$), F_v ($p \leq 0.01$), F_0 ($p \leq 0.05$), and Chl content. The F_v/F_m ratio was obviously correlated negatively with F_0 and positively with F_v and resulted uncorrelated with F_m. A very high, positive correlation was also found between F_0 and F_m, and between F_m and F_v (Table 3). Fresh tuber yield was positively and significantly

Table 3. Correlation coefficients (r_{xy}) and significance of correlations between chlorophyll (Chl) content and Chl fluorescence parameters and among Chl fluorescence parameters in field grown potato. $^{*}p\leq0.05$; $^{**}p\leq0.01$; $^{***}p\leq0.001$.

	Chl content	F_0	F_m	F_v	F_v/F_m	T_{max}
Chl content	–	0.3381*	0.6211***	0.444**	–0.0842	0.8275***
F_0	0.3381*	–	0.5793***	0.093	–0.7194***	0.0388
F_m	0.6211***	0.5793***	–	0.813***	–0.0300	0.3401*
F_v	0.4440**	0.0933	0.8131***	–	0.4801***	0.3172*
F_v/F_m	–0.0842	–0.7194***	–0.0300	0.480***	–	–0.1525
T_{max}	0.8275***	0.0388	0.3401	0.317*	0.1525	–

Table 4. Correlation coefficients (r_{xy}) and significance of correlations between tuber yield and chlorophyll (Chl) parameters and between plant dry matter yield and Chl parameters in field-grown potato. $^{*}p\leq0.05$; $^{**}p\leq0.01$; $^{***}p\leq0.001$.

	Chl content	F_0	F_m	F_v	F_v/F_m	T_{max}
Plant dry matter yield	0.969**	0.948*	0.938*	0.609	–0.430	0.948*
Tuber yield	0.992***	0.978**	0.833	0.494	–0.510	0.976**

correlated with Chl content, F_0, and T_{max}. These significant and positive correlations were also observed with regard to total dry matter yield, which, in addition, also showed a positive association with F_m (Table 4).

Discussion

Under the specific conditions in which the experiment was conducted, all Chl fluorescence parameters were significantly affected by the three factors under study, namely N dose, genotype, and plant age. They reliably detected differences in leaf photosynthesis, specifically in PS2 photochemistry under photoinhibitory field conditions. The main examples of variation in the experiment were (*1*) Chl content and F_m in response to nitrogen rate, (*2*) F_0, F_v, and F_v/F_m between genotypes, and (*3*) T_{max} in relation to measurement date. In our hands, F_m and F_v proved to be highly sensitive indicators for plant N deficiency (as measured in contrasts between fertilized and non-fertilized materials), but were unreliable for plant N content at N fertilization rates above 10 g m^{-2}. Chl content, F_0, and T_{max} instead proved to be more suitable as predictors for N status, since they were linearly correlated with N fertilization rate. The reduction of F_v/F_m at N input above 10 g m^{-2} suggests a negative effect of high N fertilization, but it was not responsive to increases in N rate in the range of 20 to 40 g m^{-2}.

The changes in Chl fluorescence induced by alteration in N application rate may be due to a direct response in Chl content. Ciompi *et al.* (1996) have shown that N deficiency in sunflower increased F_m, did not affect F_0, and did not change F_v/F_m without suffering a reduction in PS2 efficiency. The changes in F_m in plants grown under N deficiency had no physiological significance, since the Chl content fell by about 50 %. Similar responses have been found in tobacco (Balachandron and Osmond 1994) and sorghum (Cechin 1998). In contrast, a decrease in F_v/F_m was reported in maize grown in a sub-optimal regime (Khamis *et al.* 1990) as well as in common bean grown under low N supply, as a consequence of an increase in F_0 and a decrease in F_m (Lima *et al.* 1999). This variation in N-dependent F_v/F_m behaviour probably reflects specific responses to environmental conditions and N dose, and also suggests divergent genetic mechanisms for the efficient recycling of N within different plant species.

F_v/F_m, F_v, and F_0 proved to be the most reliable Chl fluorescence parameters for the definition of genotypic differences, and could represent a practical means to discriminate between genotypes under field conditions. Spunta showed the highest values of Chl content, F_m, F_v, and F_v/F_m, but the lowest F_0. In contrast, Sieglinde showed the lowest Chl content, F_v/F_m, and T_{max} and the highest F_0. Igea and Daytona exhibited intermediate values of both Chl fluorescence parameters and plant growth and development. High F_0 along with low F_m indicate that the photon absorption capacity of all Chls, including the reaction centres, is low (Flagella *et al.* 1994). Spunta is well adapted to the Mediterranean climate, where it gives high tuber yield, while Sieglinde, under the same conditions, usually develops only limited biomass and delivers low tuber yield (Mauromicale and Ierna 1997, Ierna and Mauromicale 2005). On the basis of these physiological and yield parameters, it is reasonable to propose a genuine association between Chl content and the adaptability of a genotype to its growing environment, and its tuber yield. We found that tuber yield was strongly and positively correlated with Chl content, F_0, and T_{max}, but was uncorrelated with fluorescence yield and F_v/F_m. Similar associations have been noted by Araus *et al.* (1998), who suggested that Chl parameters, specifi-

cally F_0, F_m, and $t_{1/2}$ (half-time of the increase from F_0 to F_m) measured in field grown durum wheat may be as efficient for selection purposes as the direct evaluation of yield.

With increasing plant age, Chl content and F_{max} decreased linearly and consistently, F_v/F_m was constant, whereas F_0, F_m, and F_v exhibited a typical bell-shaped curve, increasing up to complete canopy development and declining thereafter. The effect of plant age on Chl fluorescence parameters may be confounded with an effect of daily maximum temperature, which varied from about 18 °C at the start of the experiment to 26 °C at its end. High temperatures (up to 35 °C) accelerate the breakdown of the thylakoid component in wheat, leading to a decrease in PS2 activity, an effect reminiscent of normal senescence patterns (Harding *et al.* 1990). Nevertheless, T_{max} and Chl content proved to be the most reliable parameters to assess plant age.

In conclusion, the results of the present study indicate that Chl fluorescence can be used under field conditions to detect differences in the response of a potato crop to nitrogen supply, genotype, and plant age. The relationships between Chl fluorescence and crop yield and crop growth support the notion that certain Chl fluorescence parameters can be used to predict the yield performance of field-grown potatoes. To this end, further investigations will be necessary to clarify the role of Chl fluorescence on photoassimilate distribution in the plant, given that source-sink relationships are such a major determinant of tuber yield.

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