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Characterization of potato genotypes by chlorophyll fluorescence during plant aging in a Mediterranean environment

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Abstract

Field experiments were conducted in Sicily (south Italy) during two seasons to characterize by chlorophyll (Chl) fluorescence four genotypes (Spunta, Sieglinde, Daytona, and Ninfa) of potato (*Solanum tuberosum* L.) for off-season production during plant aging and to analyse the possible relation between Chl parameters and tuber yield. Chl fluorescence parameters [initial fluorescence (F_0), maximum fluorescence (F_m), F_v/F_m, time in which maximal fluorescence occurs (T_{max})] gained from Kautsky kinetics and Chl content were measured weekly, from 5th to 6th leaf appearance to beginning of plant senescence in the first season and to full plant senescence in the second season. F_0 and F_v/F_m were the most reliable Chl fluorescence parameters for the definition of genotypic differences while Chl content and T_{max} were the most reliable Chl parameters to predict plant aging. Tuber yield was highly correlated with Chl content, T_{max}, F_0, and F_m.

Additional key words: leaf senescence; *Solanum tuberosum*; tuber yield.

Introduction

Modifications in the function of the photosynthetic electron-transport system can be assayed in intact leaves by analysis of chlorophyll (Chl) *a* fluorescence (Koch *et al.* 1994). This type of fluorescence reflects changes in the light-dependent process of photosynthesis, such as radiant energy absorption, excitation energy transfer to reaction centres, and electron transport through photosystems 1 and 2 (Krause and Weis 1991).

Chl fluorescence is a non-destructive, rapid indicator of photo-chemical quantum yield and photoinhibition (Papageorgiou 1975, Krause and Weis 1984, 1991, Krause 1988, Roháček 2002). It has been used as an indicator of plant responses to stress, especially air and water pollution (Lichtenthaler 1988), water deficit in potato (Jefferies 1992, Basu *et al.* 1998) and bread wheat (Hassan 2006), high and low-temperature in potato (Kristjansdottir and Merker 1993), sunflower and maize (Yordanov *et al.* 1997), and tomato (Willits and Peet 2001), and salinity in sugar beet, sunflower, and bean (Smillie and Nott 1982). Larcher (1994) suggested that for monitoring heat stress, Chl fluorescence may be a more reliable measurement of photosynthesis than CO_2 exchange, which can be influenced by stomata closure not primarily induced by heat. Chl fluorescence is 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 in sugarcane for photochemical activity of cross-combination (Zhang *et al.* 2000). Furthermore, changes in Chl fluorescence induced by alteration in N application rate have also been studied in sunflower (Ciompi *et al.* 1996), tobacco (Balachandran and Osmond 1994), sorghum (Cechin 1998), maize (Khamis *et al.* 1990), and bean (Lima *et al*. 1999).

Measurements of Chl fluorescence parameters gained from Kautsky kinetics in the field to determine the actual response of the photosynthetic apparatus in different genotypes of crops under natural conditions have been very limited in number (Bilger *et al.* 1995, Earl and Tollenaar 1998).

In Mediterranean countries, potato is a very important crop, occupying an overall area of about one million ha and producing 19 million t of tubers (Frusciante *et al.* 1999). In south Italy, as in other southern coastal areas of the Mediterranean basin, the potato crop is cultivated during the winter-spring cycle (from December to May–June) to obtain off-season production, which has high economic value because it can be sold on foreign markets within the European Union (Foti 1999). The climatic conditions during this time of year—characterized by a

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relatively low temperature, a short photoperiod, and limited solar radiation—have an appreciable effect on plant growth, substantially modifying the morphological and phenological characteristics of the plants, compared to those cultivated in the spring-summer cycle (Mauromicale *et al.* 2003). With the exception of one study that shows interesting results, albeit with respect to one year alone (Mauromicale *et al*. 2006), no systematic study on Chl fluorescence parameters of off-season potato has yet been reported. Such additional information is needed to improve the adaptation of genotypes to off-season production (considering that most cultivars have been developed for northern European conditions). The aim of the present work was to study, under field conditions, in four potato genotypes Chl content and Chl fluorescence parameters gained from Kautsky kinetics in relation to plant age and to detect any relationships between Chl fluorescence parameters and tuber yield.

Materials and methods

Site, climate, and soil: Field experiments were conducted in two seasons (2001 and 2002) 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 off-season 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, the mean maximum and minimum temperatures and precipitations on the average of 30 years (1959–1988) are given in Table 1. With regard to temperature and rainfall during the trials, in the 2002 season the crop received less rainfall (129 *vs*. 195 mm) and higher maximum temperatures (20.1 *vs*. 18.8 °C) from January through May compared to the 2001 season (Table 1). The soil type is calcixerollic xerochrepts (USDA Soil Taxonomy), moderately deep, with a loam-clay texture.

Table 1. Average maximum and minimum temperature and precipitation for 1959–1988 and during the 2001 and 2002 seasons at the experimental field, south of Siracusa (37°03'N, 15°18'E, 10 m a.s.l.), Italy.

Meteorological variable	Year	Month January	February	March	April	May
Max. air temperature [°C]	1959–1988	15.3	15.6	17.0	19.8	24.7
	2001	16.2	15.4	18.0	20.2	24.3
	2002	16.2	17.3	20.7	21.1	25.3
Min. air temperature [°C]	1959–1988	7.5	8.0	8.9	10.7	14.1
	2001	7.9	8.0	8.3	11.6	15.8
	2002	9.7	7.2	11.0	11.5	15.0
Precipitation [mm]	1959–1988	60	42	44	24	16
	2001	66	35	5	72	17
	2002	62	33	11	6	17

Experimental design and plants: Experiments were arranged in a randomised block design in both seasons with four replications including 4 genotypes (Spunta, Sieglinde, Daytona, and Ninfa) of potato (*Solanum tuberosum* L.). Plot size was 4.2×4.2 m, with 84 plants spaced 0.3 m apart, in rows separated from one another by 0.7 m (equivalent to a plant density of 4.76 plants per m^2).

The two cultivars Spunta and Sieglinde are the most widely cultivated in the Mediterranean region. Spunta is an early ripening ware potato, with long, regular, and very large tubers; plants produce few 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 (Mauromicale *et al.* 2006). Ninfa is also a new Italian cv., of medium to late maturity, with oblong, regular, and very large tubers; stems are fairly tall and erect; marketable tuber yields are superior to those of commercial cvs. frequently cultivated in Southern Italy (Ranalli *et al*. 2005).

Management practices: Planting was done manually on 29 January in 2001 and 22 January in 2002 using whole disease-free tubers. In both trials, phosphorus (100 kg ha^{-1}), potassium (150 kg ha^{-1}), and insecticide (*Chlorpyrifos*, 30 kg ha^{-1}) were applied before planting; nitrogen (in total 100 kg ha^{-1}) was supplied ¼ pre-planting as ammonium sulphate and ¾ two weeks after emergence as ammonium nitrate.

All plants emerged 35 and 40 d after planting in 2001 and 2002, respectively. Drip irrigation was carried out when the accumulated daily evaporation (from an unscreened class A Pan situated about 40 m from the crop) reached 40 mm and supplying 100 % of maximum evapotranspiration. Five and six irrigations were performed, respectively, in the 2001 and 2002 and the total quantity

of irrigation water supplied was 110 and 130 mm, respectively.

The usual crop management was used: herbicide (*Linuron*) post emergence and fungicide (*Kocide*, 1.5 kg m^{-3} of water and *Cimox 25 WP*, 1.0 kg m^{-3} of water).

Chl fluorescence parameters were recorded in the field with a continuous excitation portable fluorometer (*Fi_m 1500*, *Analytical Development Company BioScientific*, Herts, UK). It uses a 650 nm radiation to provide the saturating pulse and detects at wavelengths above 730 nm. F_0 is determined by a system which measures the fluorescence signal 100 000 times per s; an algorithm is then used to determine the best fit through data sets 8–24 which are recorded at the onset of irradiation; this line of best fit is then extrapolated back to T_{zero} (the start of irradiation) to determine F_0.

Measurements were made on leaflets of fully expanded leaves of the upper canopy 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 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, seven measurements were taken weekly in the 2001 season from 5^{th}–6^{th} leaf appearance to beginning of plant senescence (from 62 to 110 d after planting – DAP) and nine measurements in the 2002 season from 5^{th}-6^{th} leaf appearance to full plant senescence (from 59 to 122 DAP). In all, 448 measurements in 2001 and 576 in 2002 for each Chl fluorescence parameter were made.

Chl content was measured in the field using a portable Ch 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 measurement: The harvest area consisted of 20 plants from the centre plot. Plants were harvested by hand at 121 (first season) and 134 (second season) DAP, when about 80 % of haulm was dry. Plants were individually separated into above-ground biomass (stems + leaves), roots, and tubers, and weighed. The tubers of each plot were weighed to determine fresh yield. A sample of tubers, above-ground biomass, and roots for each plant was dried (in a 105 °C oven) and weighed.

Statistical analysis: All data were submitted to Bartlett test for the homogeneity of variance and then analysed using ANOVA (Snedecor and Cochran 1989) as a factorial combination of genotypes×measurement date to indicate plant age. Means were compared by LSD test, provided the F test was significant. To define the response of the trend between measurement date and Chl fluorescence parameter and Chl content, analyses of regression (linear and quadratic) were performed. Correlation analyses were made among the Chl fluorescence parameters and between these parameters and yield. *CoStat* version *6.003* (*CoHort Software*) was utilized.

Results

Chl content decreased significantly and linearly with plant ageing in both seasons (Fig. 1). Spunta, Ninfa, and Daytona exhibited higher Chl content (respectively 39.3, 40.0, 38.7 SPAD in the first season and 37.9, 37.8, 36.3 SPAD in the second season) compared to those of Sieglinde (36.7 and 35.4 SPAD in the 2 seasons) (Table 3). Analysis of the variance showed that measurement date as an index of plant age accounted for 76 % of the total variation in 2001 and 90 % in 2002, against only 14 % of the genotype in 2001 and 5 % in 2002 (Table 2).

Initial fluorescence (F_0): Regardless of the measurement date, in both seasons the F_0 value was significantly highest in Sieglinde, intermediate in Daytona and Ninfa, and lowest in Spunta (Table 3). Changes of F_0 during the plant ageing showed a typical bell pattern in both seasons. In the first season, F_0 values increased with time from 62 to 77 DAP and then declined in a linear fashion over the season (Fig. 2). In the second season, F_0 had similar behaviour increasing with time from 59 to 89 DAP and then decreasing over the season (Fig. 2). For this parameter, the cause of variance was the genotype with 49 and 50 % of total variance and measurement date with 44 and 37 % in 2001 and 2002, respectively (Table 2).

Maximum fluorescence (F_m): In both seasons, F_m decreased significantly and linearly from the first to the last measurement date (Fig. 2). As regards the genotype, in both seasons Spunta, Sieglinde, and Ninfa showed the highest values, whereas Daytona the lowest ones (Table 3); differences were more evident in 2002 than in 2001. Variance analysis showed that measurement date accounted for 73 and 49 % and genotype only for 14 and 30 % of the total variation in 2001 and in 2002, respectively (Table 2).

T_{max} in both seasons consistently diminished significantly and linearly from the first measurement date to the last one (Fig. 2). T_{max} in both seasons was smaller in Sieglinde and Daytona compared with the other two genotypes (Table 3), but differences were significant only in 2002. This parameter, similarly to F_m, was affected mainly by measurement date which accounted for 72 % of total variance in 2001 and 41 % in 2002 (Table 2).

Genotype accounted in 2001 for only 11 % and in 2002 for 38 % of total variation.

F_v/F_m: Averaged over the measurement date, the F_v/F_m ratio was highest in Spunta, intermediate in Ninfa and Daytona, and lowest in Sieglinde (Table 3). As regards the plant age, F_v/F_m in both seasons showed a tendency to increase from the first to the last measurement; the differences were more evident in the first season than in the second one (Fig. 1). The cause of variance in 2001 was genotype for 38 % and measurement date for 52 % of total variation; on the contrary, in 2002 the genotype proved to be the main cause for 54 % of total variation, and the measurement date for only 19 % (Table 2).

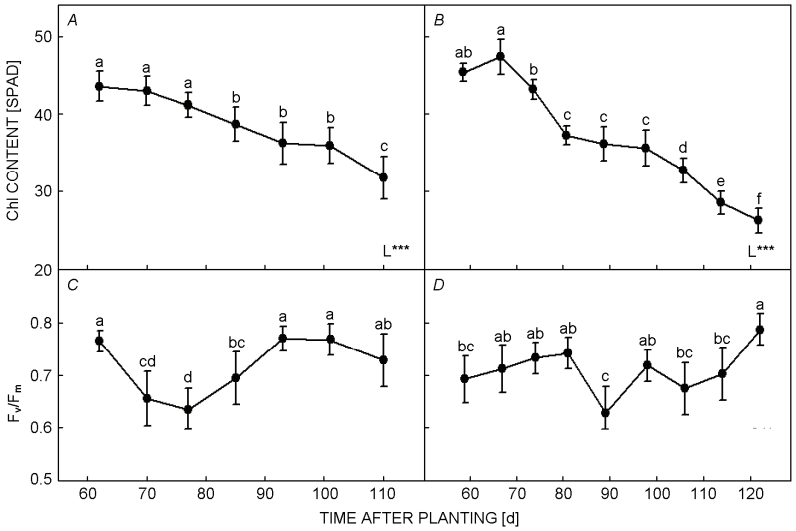

Fig. 1. Chlorophyll (Chl) content and F_v/F_m ratio of field-grown potato in 2001 season (*A, C*) and in 2002 season (*B, D*) as affected by measurement date as indicator of plant age. Means±SD of 16 values. *Different letters* within each parameter and season indicate significant differences for $p \leq 0.05$. L = linear, Q = quadratic, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$; relationship tested by regression analysis.

Table 2. Mean square as absolute value and percentage of total (*in brackets*) of main effects resulting from variance analysis of studied chlorophyll (Chl) parameters in field-grown potato in seasons 2001 and 2002. df = degree of freedom, NS = not significant; $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$.

Parameter	Source of variation (main effects)			
	Genotype (df 3) 2001	Measurement date (df 6)	Genotype (df 3) 2002	Measurement date (df 8)
Chl content	83^{**} (14 %)	437^{***} (76 %)	52^{**} (5 %)	854^{***} (90 %)
F_0	480000^{***} (49 %)	434000^{***} (44 %)	734300^{***} (50 %)	553500^{***} (37 %)
F_m	288000^{*} (14 %)	1537000^{***} (73 %)	431000^{*} (30 %)	697120^{***} (49 %)
T_{max}	6400 NS (11 %)	40490^{***} (72 %)	33900^{***} (38 %)	32580^{***} (41 %)
F_v/F_m	0.055^{***} (38 %)	0.076^{***} (52 %)	0.091^{***} (54 %)	0.033^{***} (19 %)

Table 3. Chlorophyll (Chl) content and Chl fluorescence parameters of field-grown potato as affected by genotype in the years 2001 and 2002. Means ± SD of 28 (2001) or 36 (2002) values. *Different letters* within each parameter and season indicate significant differences for $p \leq 0.05$.

Genotype	2001					2002				
	Chl [SPAD]	F_0 [relative]	F_m [relative]	T_{ma} [ms]	F_v/F_m	Chl [SPAD]	F_0 [relative]	F_m [relative]	T_{max} [ms]	F_v/F_m
Spunta	39.3±4.7a	575±138c	2547±295ab	215±34a	0.770±0.070a	37.9±7.9a	786±159c	3367±284a	205±41a	0.760±0.060a
Sieglinde	36.7±4.5b	830±184a	2660±253a	190±46a	0.691±0.09b	35.4±7.1b	1132±232a	3305±262a	162±41b	0.641±0.100c
Daytona	38.7±7.0a	741±100b	2458±332b	208±57a	0.701±0.090b	36.3±7.4ab	924±178b	3133±226b	166±37b	0.703±0.080b
Ninfa	40.0±3.5a	725±160b	2554±222ab	220±55a	0.705±0.090b	37.8±8.4a	917±148b	3361±150a	225±78a	0.728±0.070ab

Fresh tuber yield and dry above-ground biomass: In both seasons, tuber yield was significantly higher in Spunta, Ninfa, and Daytona, without differences among these, than in Sieglinde (Fig. 3). Above-ground biomass was higher in Daytona and Ninfa than Spunta; Sieglinde showed the lowest value (Fig. 3).

Correlation among Chl parameters: Positive correlations of Chl content with F_m and T_{max} ($p \leq 0.001$) were found. The F_v/F_m ratio was correlated ($p \leq 0.001$)

negatively with F_0 and positively with F_m, while it proved uncorrelated with Chl content and T_{max}. A high positive correlation was also found between F_0 and F_m ($p \leq 0.001$) (Table 4).

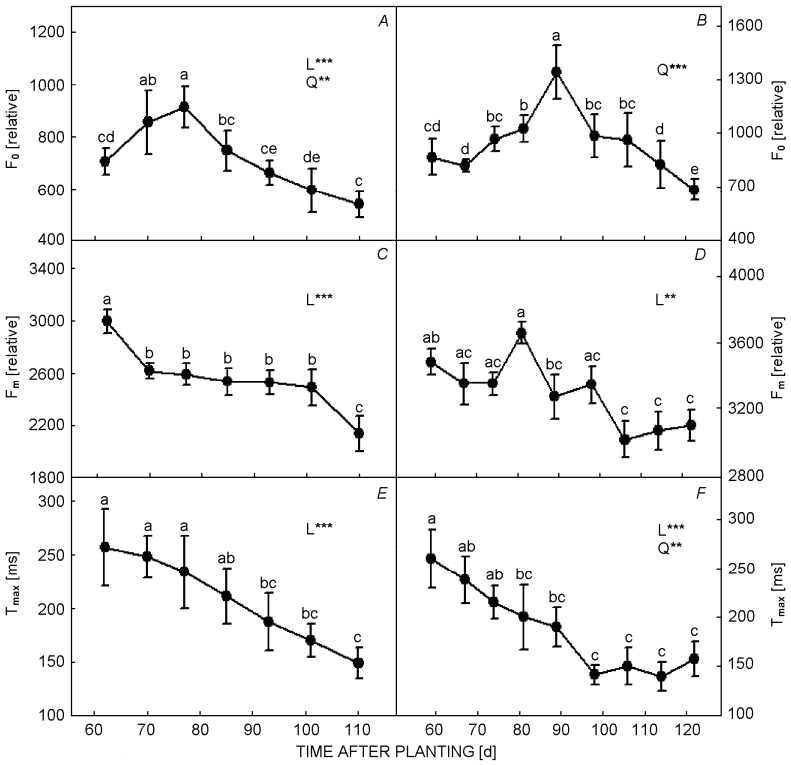

Fig. 2. F_0, F_m, and T_{max} of field-grown potato in 2001 season (*A, C, E*) and in 2002 season (*B, D, F*) as affected by measurement date as indicator of plant age. Means±SD of 16 values. *Different letters* within each parameter and season indicate significant differences for $p \leq 0.05$. L = linear, Q = quadratic, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$; relationship tested by regression analysis.

Table 4. Correlation coefficient (r_{xy}) and significance of correlations referred to all data of the two seasons between chlorophyll (Chl) content and Chl fluorescence parameters and among Chl fluorescence parameters in field-grown potato. Correlation coefficients between tuber yield and Chl parameters in field-grown potato are also included. $^{**}p \leq 0.01$; $^{***}p \leq 0.001$.

	Chl content	F_0	F_m	T_{max}	F_v/F_m
Chl content	–	0.224^{**}	0.369^{***}	0.399^{***}	0.045
F_0	0.224^{**}	–	0.235^{***}	–0.051	-0.683^{***}
F_m	0.369^{***}	0.235^{***}	–	–0.061	0.294^{***}
T_{max}	0.399^{***}	–0.051	–0.061	–	0.038
F_v/F_m	0.045	-0.683^{***}	0.294^{***}	0.038	–
Tuber yield	0.601^{***}	-0.823^{***}	-0.783^{***}	0.570^{**}	0.202

Correlation between Chl parameters and tuber yield: Tuber yield was significantly and positively ($p \leq 0.001$) correlated with Chl content and T_{max} and negatively ($p < 0.001$) with F_0 and F_m (Table 4).

Discussion

Under the particular conditions in which the experiment was conducted, all Chl fluorescence parameters were significantly affected by the two factors under study, namely genotype and plant age. The main examples of variation in the experiment were F_0 and F_v/F_m among genotypes, and Chl content, F_m, and T_{max} in relation to plant age.

F_0 represents the basal emission of Chl fluorescence when redox components of photosystems are fully oxidised. F_v/F_m as proposed by Butler and Kitajima (1975) is a useful ratio which is proportional to the quantum yield of photosystem 2 (PS2) photochemistry and exhibits a high correlation with the quantum yield of net photosynthesis. F_m is obtained at the fully saturated irradiance for the plant when the electron acceptor Q_A is fully reduced. T_{max} is the time at which the maximal fluorescence occurs as a measure of the pool size of electron acceptors on the reducing side of PS2.

F_v/F_m and F_0 are the most reliable Chl fluorescence parameters for the definition of genotypic differences,

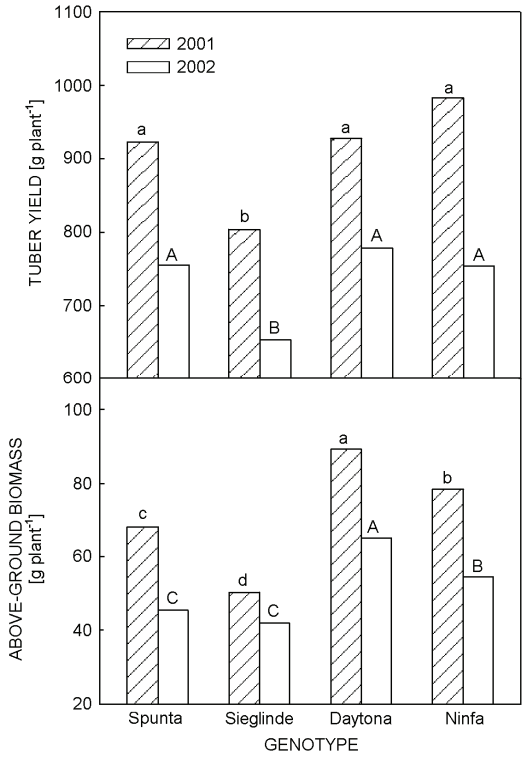

Fig. 3. Tuber yields and dry above-ground biomass of field-grown potato in seasons 2001 and 2002 in relation to genotypes. *Different letters* within each season indicate significant differences for $p \leq 0.05$.

and could represent a practical means to discriminate between genotypes under field conditions. In both seasons, with more or less significant differences, Spunta showed the highest values of F_v/F_m ratio but the lowest F_0. On the contrary, Sieglinde showed the lowest values of F_v/F_m ratio and the highest ones of F_0. Ninfa and Daytona exhibited an intermediate behaviour. On the basis of these indices, Spunta seems to have higher efficiency of photochemical processes in PS2, photon absorption capacity of all Chls, and quantum yield of net photosynthesis than Sieglinde. High capacity and efficiency may be associated with high yield. Indeed, both in previous (Mauromicale and Ierna 1997, Ierna and Mauromicale 2006) and present research, Spunta gives higher tuber yield than Sieglinde.

Although Daytona and Ninfa showed intermediate values of Chl parameters, they did not produce yields significantly different from Spunta. This could be explained by the fact that the two Italian genotypes have a greater canopy development than Spunta and Sieglinde, as higher values of dry above-ground biomass show, which allowed it to achieve a higher "whole photosynthetic capacity". In addition, Daytona and Ninfa probably have, compared to Spunta and Sieglinde, a more efficient mechanism of exporting photosynthates from leaves to storage tubers. This is explained by the fact that, unlike Spunta and Sieglinde which are of foreign origin, Daytona and Ninfa were developed for off-season production and evaluated for years in the same Sicilian environment in which the trials were performed and thus show better adaptation to off-season production.

Tuber yield was strongly and positively correlated with Chl content and T_{max}, whereas negatively with F_0 and F_m, but was uncorrelated with F_v/F_m in this study. This concurs with previous results obtained in off-season potatoes by Mauromicale *et al.* (2006).

The differing responses of the genotypes in terms of Chl content and fluorescence and its relationships with yield, suggest the possibility of using Chl fluoremetry in potato breeding programs.

With increasing plant age, Chl content, F_m, and T_{max} linearly decreased, F_0 exhibited a typical bell trend, increasing from young plants to complete canopy development and declining thereafter, while F_v/F_m showed a tendency to increase from first measurements to the last ones. Nonetheless, T_{max} and Chl content proved to be the most reliable parameters to monitor plant age. Effect of plant aging on decrease of Chl content was more evident in the second season than in the first one, probably because in the second season measurements of Chl fluorescence were carried out until the end of the cycle when active leaves were old. The effect of plant age on Chl parameters may also be added to the effect of daily maximum temperature increase: from about 18 °C at start to 26 °C at end of experiment in the first season and from about 18 °C at start to 28°C at end of experiment in the second one. In fact, high temperature (35 °C) accelerates the thylakoid component breakdown in wheat, leading to a decrease in PS2 activity, a similar effect to normal senescence patterns (Harding *et al.* 1990).

Contrary to what several authors have noted, namely that F_v/F_m decreases with leaf ageing (Hong *et al.* 1999, Šesták 1996, Behera and Choudhury 1997, Haimeirong and Kubota 2003), in this research the F_v/F_m ratio tended to augment during the canopy development and plant senescence, but only small and often insignificant differences were found.

In conclusion, results of this study confirmed that Chl fluorescence parameters can be used under field conditions to discriminate between genotypes and predict plant age and yield of field-grown potatoes.

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Gamaleĭ, Y.V.: **Transportnaya Sistema Sosudnykh Rastenii.** [Transport System of Vascular Plants.] – Publishing House of Saint-Peterburg State University, Saint-Peterburg 2004. ISBN 5-288-03343-9. 422 pp., 28.57 roubles.

The book summarizes recent knowledge on transport systems in plants from the level of membrane transport to the long-distance xylem and floem transport. Emphasis is given to the anatomy and function of leaf conductivity elements, especially to minor leaf terminal veins. However, separate extensive chapters deal also with mechanisms of intra- and inter-cellular transport of water and solutes, with ontogeny of conducting elements, evolution of xylem and floem elements in dicots, the role of transport system in regulation of plant growth, and development and effects of environmental factors (irradiance, temperature, air humidity, water stress, nutrition, salinity, atmospheric pressure) on development of transport systems. One of nine chapters summarizes experimental practices used in water and assimilate transport studies, mainly techniques of flux manipulation. The concluding section represents comparative study of transport systems in dicots of different biomes.

The book brings interesting and inspiring concepts. Some of them might be new for scientists oriented mostly on English-written literature. Fusion of categories denoting heterobaric or homobaric leaves with symplastic or apoplastic floem-loading leaves, is an example of such concept. Almost one thousand papers, chapters in books, and monographs are referenced through the book. Most of them (about one third) originate from the nineties but also those of interesting historical value and more recent ones from 2000-2002 are included. Unfortunately, papers of at least several renown scientists working in the field of stem and leaf hydraulics for more than ten years are missing (for example N.M. Holbrook or J.S. Sperry).

The book is completed by an explanatory index of fundamental terms and a list of abbreviations. It makes the book useful for students specialized in plant transport systems. With a rather broad field of study, the book is on the edge of a specialized scientific monograph and a textbook.

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