CHLOROPHYLL FLUORESCENCE AS AN INDICATOR TO DETECT DIFFERENTIAL TOLERANCE OF SNAPBEAN CULTIVARS IN RESPONSE TO O_3 STRESS

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Abstract: The potential use of chlorophyll fluorescence (CF) induction assay as a tool for screening and characterizing the tolerance of ozone (O_3), contrasting cultivars of snapbeans (*Phaseolus vulgaris* L.), was investigated. A range of CF parameters was examined for snapbeans treated with O_3. Chlorophyll fluorescence parameters such as Fo, Fmax, and Fv, Fv/Fmax were compared in O_3 tolerant and susceptible snapbean cultivars grown under O_3 stress conditions. O_3-stressed leaves showed significantly higher constant-yield (Fo) but greatly reduced variable fluorescence (Fv) and decreased Fv/Fmax ratios. In the O_3-sensitive cultivar snapbean cv BBL-290, O_3 stress resulted in a strong inhibition of the fast and slow fluorescence-induction transients and altered the form of the kinetic curves of CF in leaves. In particular, the fluorescence quenching rate and Fv/Fmax ratios were markedly decreased in O_3-stressed leaves. In contrast, leaves of the O_3-resistant snapbean cv Astro showed only minor changes in CF. The values of the Fv/Fmx ratio decreased in the O_3-sensitive cultivar much more drastically than the O_3-resistant cultivar. Based on CF measurements, it appears that O_3-induced stress blocked photosynthetic electron transport between photosystem (PS) II and PS I. The close agreement between changes in fluorescens and visual symptoms of O_3-induced injury suggest that the CF patterns, the rate of fluorescence-induction transients, and the Fv/Fmax ratio can provide valuable tools to investigate the photosynthetic and metabolic mechanisms affects by O_3-induced stress. Chlorophyll fluorescence analysis could also be a useful technique which could be used by plant breeders to screen large numbers of plant rapidly for air pollution sensitivity.

INTRODUCTION

Chlorophyll fluorescence (CF), a sensitive indicator of photosynthetic light energy conversion, has been an important tool in the characterization of photosynthetic reaction mechanisms (Papageorgious, 1975; Schreiber, 1983; Krause & Weis, 1984; Hetherington and Smillie, 1984; Omasa *et al.*, 1987; Oquist & Wass 1988; Cassells & Hurley, 1990; Carter *et al.*, 1990). Partial reactions of photosynthesis were reflected in parts of the complex fluorescence induction curves displayed during a dark-light transition (Schreiber *et al.*, 1978; Bradbury & Baker, 1983; Lichtenthaler & Rinderle, 1988). Fluorescence provides a nondestructive method for monitoring photosythetic electron transport, any alteration of electron transport at or beyond the primary acceptor (referred as Q) of PS II will increase

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the level of fluorescence (Miles, 1980; Lichtenthaler & Rinderle, 1988). Recently, the method of CF induction has gained wide application in different areas of stress physiology and in developing tests for plant resistance to unfavorable environmental stress factors such as air pollution (Schreiber *et al.*, 1978; Heath *et al.*, 1982; Schneckenburger & Frenz, 1986; Omasa *et al.*, 1987; Schreiber & Bilger. 1987; Schmidt *et al.*, 1990) chilling (Havaux & Lannoye, 1984; MacRae *et al.*, 1986; Hetherington & Oquist, 1988; Neuner & Larcher, 1990; Walker *et al.*, 1990), heat (Seemann *et al.*, 1984; Weis, 1984, Schreiber & Bilger, 1987; McMichael *et al.*, 1989), drought (Havaux & Lannoye, 1985; Conroy *et al.*, 1986; Havaux *et al.*, 1988), water stress (Ben *et al.*, 1987), salt (Smillie & Nott, 1982; Larcher *et al.*, 1990), herbicide (Shaw *et al.*, 1985; Habash *et al.*, 1985; Harris & Camlin, 1988; McMichael *et al.*, 1989), and irradiation (Smillie, 1982; Tevini, 1985; Dijak *et al.*, 1987; Tevini *et al.*, 1988). The application of CF kinetics in the stuey of varietal difference to environmental stress has also been reported (Havaux *et al.*, 1988; McMichael *et al.*, 1989; Neuner & Larcher, 1990; Walker *et al.*, 1990).

Recently, we have also developed a rapid and nodestructive tests for O_3 tolerance based on O_3 stress induced changes in the variable component of CF in intact leaf tissues. Because environmental stress such as air pollutants may participate in oxidant and reductant reactions, they can interfere with electron flow in photosystem (PS) I and II (Arndt, 1974; Chang & Heggestad, 1974; Nieboer *et al.*, 1976; Shimazki *et al.*, 1984). The instrument is designed to provide fast and accurate measurement of CF induction kinetics key parameters such as Fo, Fv, Fmax, Fv/Fmax, t 1/2 in various types of samples. The parameters measured can be used to evaluate the function of photosystem II in photosynthesis (Miles 1980, & 1990).

The objective of this work is to evaluate the use of the CF induction technique to investigate the feasibility of characterizing CF transients for susceptible and resistant cultivars of crop plants to O_3 stress.

MATERIALS AND METHODS

Plant Materials

Two cultivars of snapbean (*Phaseolus vulgaris* L.) shown previously to differ in their response to O_3 stress (Heggestad *et al.*, 1980; Lee *et al.*, 1984) were used in this study. The O_3-sensitive (O_3-S) cultivar Bush Blue Lake-290 (BBL-290), and O_3-resistant (O_3-R) Astro snapbean were used. Plants were grown from seed and germinated in 15-cm diameter clay pots containing a sand: soil (1:3) potting mixture. The beans were thinned to 1 plant per pot after germination and grown in charcoal-filtered air greenhouse equipped with thermostat and humidity controls (Lee & Bennett, 1982). Plants were fertilized weekly with 100 ml 1% solution of Peters 20-20-20 fertilizer solution (R.B. Peters Co., Inc., Allentown, PA, USA) containing essential micronutrient.

Samples for CF analysis were obtained from fully-expanded mature first trifoliate and young third trifoliate leaves 21 to 28 days after planting. The experimental design was a randomized block containing two cultivars and six replications. The experiments were repeated three times.

Environmental Conditions

Greenhouse environmental conditions during the plant growth period were as follows: temperature, day (17 to 30 °C)/night (15 to 25°C); relative humidity (RH), 55 to 98%; photosynthetically active radiation (PAR) level of 1,500 to 2,400 μmol m^{-2} sec^{-1} at midday.

Ozone Fumigation

Ozone fumigations were conducted in Controlled Environments, Inc., Model PGW 36 walk-in type growth chambers (Conviron Controlled Environ., Pembina, N. D., USA, with 3.2 m^2 of floor space). Temperature, PAR, and RH condition were 25±1°C, 350 μmol m^{-2} sec^{-1} and 70 ± 5%, respectively. Test plants were equilibrated in a fumigation chamber and a non-fumigation control chamber for 1 to 2 h before exposure to O_3. Ozone was generated by passing research grade (99.99%) O_2 through a high voltage electric discharge ozonizer. The O_3 concentration was monitored with a chemiluminescent O_3 analyzer (Model 8002, Bendix Corp., Ronceverte, WV, USA). The unit was calibrated with a Dasibi Model 1003 PC O_3 calibrator/monitor (Dasibi Environ. Corp., Glendale, CA, USA). The plants were fumigated for 5 h at a concentration 499 μg m^{-3} (0.25 ppm.) O_3 or 599 μg m^{-3} (0.30 ppm.) for 3 h. Six replicates of each cultivar were sampled from the O_3 and control chambers after 0, 1, 2, 3, or 4 h of fumigation and the leaves immediately removed for CF analysis. After O_3 exposure, plants from each cultivar were returned to the greenhouse and O_3 injury levels were evaluated after 48 h exposure. A rating of 0 to 10 was given for the first terminal trifoliate leaf, where 0 indicated no damage and 10 indicated 100% necrosis.

Statistical Analysis

Analysis of variance (ANOVA) was performed on chlorophyll florescence parameters data and O_3 injury score data to determine significant differences, followed by a Duncan's Multiple Range Test.

Fluorometer Equipment and Chlorophyll Fluorescence

For all fluorescence induction kinetics measurements, a filtered fluorometer was constructed and as illustrated in Fig. 1, which was modified from the method in Method of Enzymology by Miles (1980). A low voltage (6 V) car battery was used as a power supply for an actinic lamp (miniature lamp, GE 1493) housed in a Bausch and Lomb microscope illuminator producing 530 μmol m^{-2} s^{-1} light intensity. The light was passed through a shutter, a set of blue (Corning filter 5030, Corning Lab Sci. Co., Oneonta, NY, USA) and blue-green (Corning filter 4303) cut-off filters and conducted to the leaf segment. Fluorescence was recorded at 45° to the leaf surface by a red-sensitive photodiode (HUV-4000 B, EG and G Company, Silver Spring, MD. USA) through a 683 nm red interference filter (Corning filter 2030). The electrical analogue signals from the photodiode were recorded on a Series 2090 digital storage oscilloscope (Nicolet Co., Madison WI, USA) or accessed by a microcomputer system via an analogue/digital interface.

The nomenclature for identification of fluorescence transient in the time course of fluorescence induction has been adapted in this paper (Krause & Weis, 1984). Chlorophyll fluorescence values were record directly with a storage digital oscilloscope, and the fluorescence signal and digital read out were obtained the

inital fluorescence (Fo), the maximum peak height (Fmax), variable fluorescence (Fv); FV is equal to Fmax-Fo, and the time or velocity required for fluorescence to rise to (P), designated as (RIS), and the time or speed required for the fluorescence to decline from (P) to the semi-steady state (S), designated as (DES), the cursor paddles were used to move the displayed wave form on screen to take the fluorescence transient data when expansion was applied. Fluorescence intensity spectra of each cultivar were obtained by screening the fluorescence emission at a 5 msec speed.

For CF measurement, a section of fully-expanded first trifoliate leaf, about 1.5 cm × 2.5 cm long, avoiding the midrib, was cut from each plant and placed on a moist paper towel, and immediately covered with plastic film to prevent water loss (Hetherington & Smillie, 1984). Samples were stored in a dark box or a laboratory bench drawer at least 10 to 15 min for dark adaptation before exposure to actinic light for measuring the CF. The leaf section was placed in the sensing probe dark compartment box with the abaxial surface down on an exposure glass plate, which was a part of fluorometer instrument (Fig. 1). After 2 to 3 min dark adaptation, the light switch was turned on to measure the kinetics of fluorescence emission at 683 nm. Changes in initial and variable fluorescence and the rate of rise in PS II and subsequently decline of CF are compared with assessments of relative O_3 tolerance made from growth chamber observations.

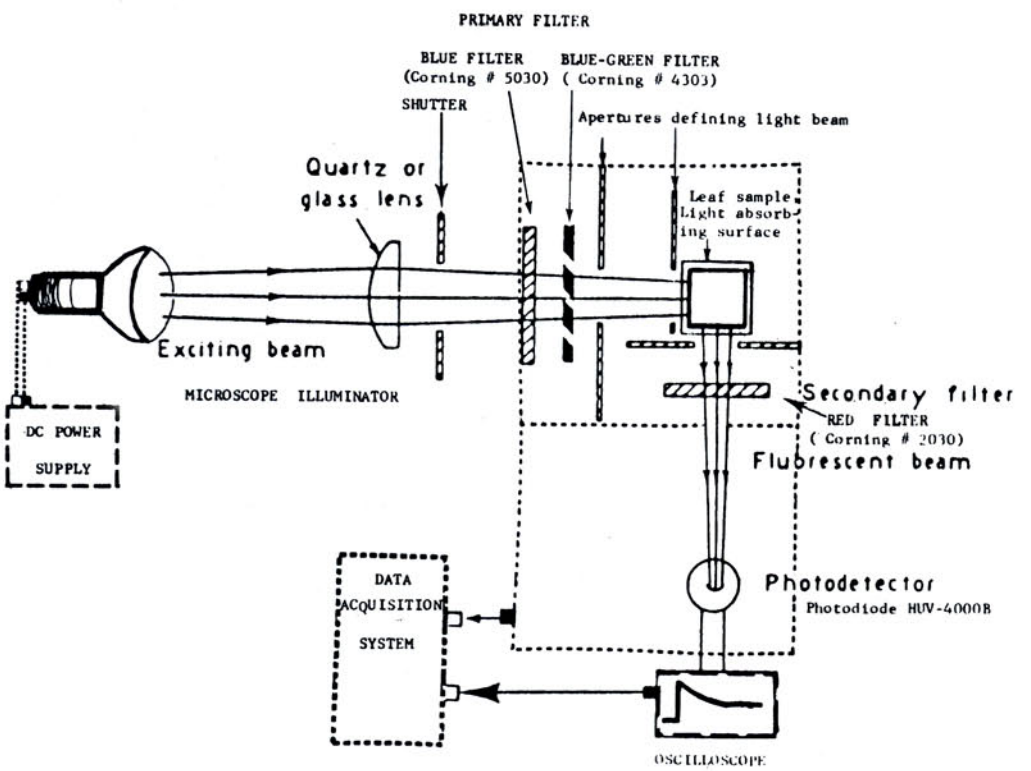

Fig. 1. Schematic illustration of fluorometric system assembly used for chlorophyll fluorescence detection.

RESULTS AND DISCUSSION

Plant Response to O_3 Stress

A wide range of sensitivity in plants, both within and between species, is evident from the literature in regard to photochemical oxidants (Heggestad *et al.*, 1980; Heagle, 1989). Air-pollutant-induced visible injury and symptoms may become evident on the leaves or stems with severe damage. Decreased photosynthetic rates may be recognized only at harvest or when ameliorative measurements become fatal. Furthermore, genetic variability for tolerance in crops has been minimally studied, nor has any suitable methods or technology been available to select for pollutant-tolerance (Reinert *et al.*, 1982). New methods should be developed to monitor or screen O_3 stress in physiological studies. Detection of adverse effects of O_3 on field-grown crops that reduced yield without producing visible symptoms should also be developed.

Laboratory experiments on differential difference in snapbean cultivar in response to O_3 were performed on plants grown in greenhouse and moved to controlled environmental growth chambers. Fig. 2 shows the differential injury on two snapbean cultivars, the O_3-S cv BBL-290 and O_3-R cv Astrov. Visible

Fig. 2. Photograph of 3-week old snapbean (*P. vulgaris* L.) plant exposed to 0.25 ppm O_3 for 3 h, showing comparative leaf injury in the O_3-sensitive cultivar BBL-290 (Left), and in the O_3 resistance cultivar Astro (Right). Photo was taken 48 h after exposure. BBL-290 leaves showed severe flecking or bronzing injury on the upper surface of the leaves, while Astro leaves showed only a very few spots of bronzing injury.

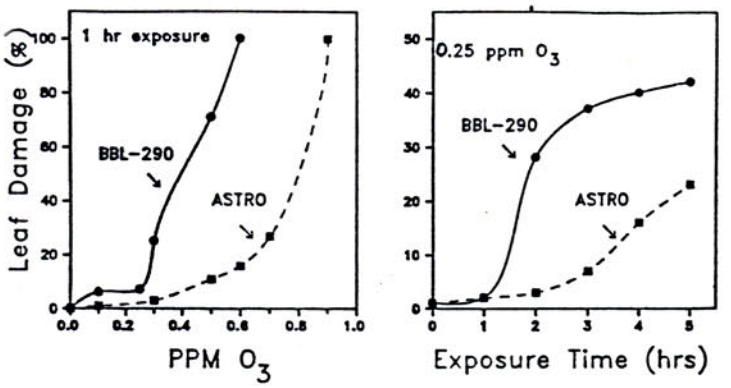

Fig. 3. Dose-response curves showing cultivar differences in O_3 sensitivity. Right: leaf damage at 0.25 ppm O_3 after 1 to 5 h of exposure. Left: leaf damage as a function of O_3 concentration. Plants were rated for foliar O_3 injury at 48 h after fumigation. The first trifoliate leaves were assessed for the percentage of the surface showing injury.

injury increased in the order: first trifoliate leaves (expanded leaves)>2nd trifoliate leaves (50 to 70% expanded leaves)>3rd trifoliate leaves (young leaves with less than 40% expansion). Ozone injury was expressed by chlorosis, stippling, and bifacial necrosis. The O_3-S snapbean cultivar began to display signs of injury in the fumigation chamber after 1.5 to 2 h O_3 exposure. However, the O_3-R cultivar exposed under identical conditions exhibited little visible injury.

Figure 3 shows graphically the comparative dose-response curvese for O_3 injury in the two cultivars as a function of O_3 concentration and duration of O_3 exposure. As seen in this figure, BBL-290 exhibited more foliar injury at a lower O_3 concentration and exhibited more foliar injury than the highest O_3 level of Astro. Visible injury less than 20% in Astro when plants were exposed to 0.7 ppm O_3 for 1 h, or 0.25 ppm O_3 for 4 h. In contrast, BBL-290, showed 50% injury after 1 h at 0.4 ppm O_3; 100% injury after 1 h at 0.6 ppm O_3; and 30% O_3 injury after 2 h at 0.25 ppm O_3 fumigation.

Characterization of O_3 Susceptibility with Chlorophyll Fluorescence

Typical changes in the intensity of leaf fluorescence from chlorophyll *a* in bean plant tissues, during constant illumination after a period of dark adaptation are shown in Figure 4. Upon illumination of a nonstressed bean leaf, fluorescence rose very rapidly to an initial point (O), or Fo (less than 1 *ns*), and then a small rise from (O) to a point (I) followed by a dip (D) in fluorescence. This O-I-D change occurred in 10-to 50-*ms* range, and is due to the primary electron acceptor of PS II being reduced and then then oxidized by intersystem electron carriers (Miles, 1980 & 1990). From the point of (I), the fluorescence would rise more slowly to a maximum level (P) or Fmax. Under these conditions, (P) or (Fmax) was reached after 1 to 3 sec. This slow rise of fluorescence from Fo to Fmax signal is associated with PS II activity and intersystem electron transport (Krause & Weis, 1984). A block or defect in PS II could affect the rate and pattern of the induction curve. After the (P) has been reached, the fluorescence slowly declines and oxidants generated by electron flow mediated by PS I and by the reduction of CO_2 exert an effect. In the bean leaf a semi-steady state (S)

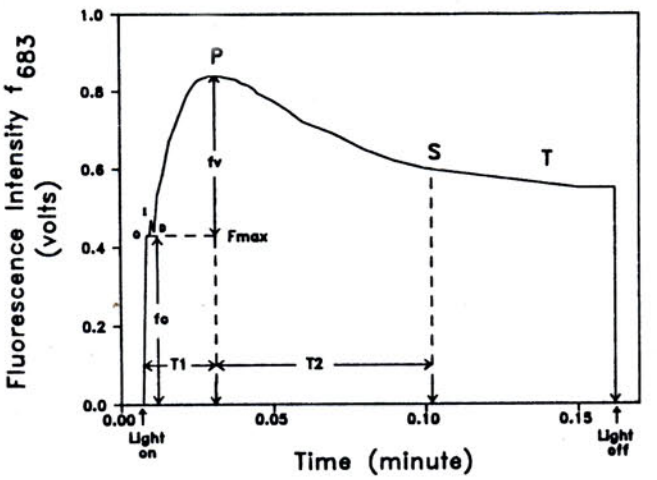

Fig. 4. Typical changes in the intensity of chlorophyll fluorescence from chl *a* in a dark adapted leaf of *P. vulgaris* at room temperature. The change in Fv is correlated with the reduction of the electron acceptor (Q) in the reaction center of PS II.

fluorescence level was reached after about 7 to 9 sec of illumination. For a slow fluorescence-induction transient, a steady-state fluorescence level was reached at about 60 to 90 sec.

Fluorescence-Induction Response in Leaves of Different Stage of Development Within a Cultivar

The susceptibility of leaf tissue to injury by O_3 was dependent upon leaf age and the stage of development (Fig. 2). The older leaves that were 70 to 90% of their full size (i.e., the first trifoliate leaves) were most sensitive to O_3 exposure; the younger leaves that were ranging from 40 to 70% of their full size (i.e., third trifoliate leaves), were much less sensitivity to O_3.

Analyses of CF responses were made for leaves of O_3-S BBL-290 snapbean under O_3 and nonstress condition (Table 1). The CF induction data indicated that the Fo, Fmax, and FV were generally higher in the third trifoliate leaves (i.e., younger leaves) than the first trifoliate leaves (i.e., older leaves). The O_3-S leaves (i.e., the first trifoliate) had Fv/Fmax ratios more than 6% higher than

Table 1. Relative fluorescence of BBL-290 snapbeans leaves of different ages exposed to 0.25 ppm O_3 for 3 h

Treatment	Trifoliate	Fo	Fmax	Fv*	Ratio**
		($mV\ s^{-1}$)			
Control	first	200 c +	740 b	540 a	0.73 a
	third	260 b	830 a	570 a	0.69 ab
O_3-stressed	first	380 a	720 b	340 c	0.47 c
	third	350 a	860 a	510 b	0.59 b

* Fv=Fmax−Fo ** Ratio=Fv/Fmax

+ Each value is the mean of 6 replicates. Numbers in a column followed by the same letter are not significantly differently different at the 5% level of probability.

the O_3-R leaves (i.e., the third trifoliate). The first trifoliate leaves had significantly lower Fo values than the third trifoliate leaves. The ratio of variable fluorescence to maximal fluorescence (Fv/Fmax), calculated from the CF curves, was lowest in the O_3-S leaves following O_3 exposure. Values in insensitive leaves under O_3-stress remained fairly constant. The younger trifoliate leaves were at the stage of most active growth (40 to 60% expand stage) and their fluorescence-induction curve differed clearly from that older leaves. Fluorescence data were obtained for two types of the same physiological stage of bean leaves suggest a smaller amount of light-harvesting matrices in relation to the photosynthetic reaction centers in O_3-S leaves.

Fluorescence-Induction Response to O_3 in Leaves of Different Cultivars

In order to determine the biochemical and physiological bases for O_3-induced stress and to develop an understanding of the nature of plant tolerance to O_3 we examined the CF in relation to cultivar sensitivity of snapbean plants, which have been shown previously to differ in their response to O_3 within the species (Lee & Bennett, 1982, Lee *et al.*, 1984) to evaluate for susceptibility to O_3 stress and gain insight into the primary processes in photosythesis. Typical examples of CF induction curves for bean leaves affected by O_3 exposure are shown in Figure 5, showing a clean pattern of Fo change. Increasing the duration of O_3 exposure caused the stationary level (Fo) to rise. Thus, an increase in either O_3 concentration or duration of O_3 exposure (Fig 5 & 6) may lead to an increase of Fo and a decrease in Fv/Fmax. After a high O_3 concentration or long duration of O_3 exposure, there was virtually a total loss of variable fluorescence. In addition to obtaining a decrease in the level of variable CF in intact leaf tissues treated with O_3, there was an increase in slowing the rate of CF to the (P) level in comparison with the control plant, which indicates O_3 suppression of activity of the donor part of PS II.

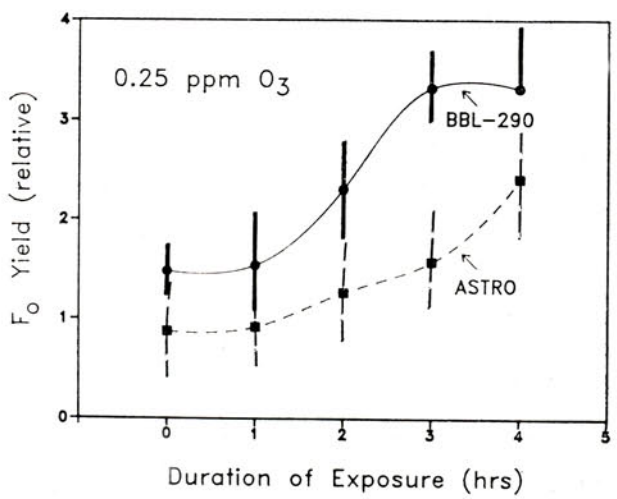

Fig. 5. Chlorophyll fluorescence induction curves of fully expanded first trifoliate leaves of snapbean plants exposed to 0.25 ppm O_3 in relation to Fo yield after 0 to 4 h of exposure. Each point represents the mean of six replicates with standard of the mean bars.

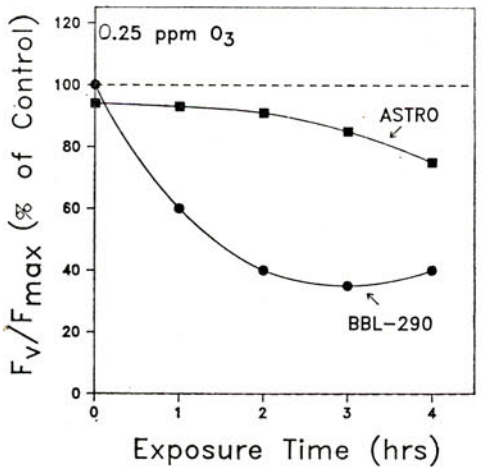

Fig. 6. Influence of exposure time at 0.25 ppm O_3 on Fv/Fmax ratios in Astro and BBL-290 snapbean.

The O_3-exposure damages the donor part of PS II, as is indicated by increase in the level of Fo. The Fo is known to be affected by environmental stress that causes structural alteration at the PS II pigment level (Krause & Weis, 1984). Ozone damage of PS II is characterized by a drastic increase in Fo level and decreased in Fv/Fmax ratio (Table 2). Under no O_3 stress, BBL-290 leaves did not differ from the Astro leaves with respect to the Fv, although the Fo was significantly lower in BBL-290 (O_3-S) than the Astro (O_3-R). This indicates the efficiency of photochemistry in PS II was the same in these two cultivars before O_3 stress. However, under O_3 stress, the lower ratio of Fv to Fmax in BBL-290 leaves indicated that the primary photochemistry of PS II was impaired (Miranda *et al.*, 1981). The O_3-R cultivar after O_3 stress had a Fv/Fmax ratio about 12% greater than the O_3-S cultivar, indicating a greater photochemical efficiency for cultivar Astro. The ratio of Fv/Fmax showed slightly higher values in trifoliate leaves of O_3-S cv BBL-290 than comparable leaves in O_3-R cv Astro. Ozone stress resulted in lower ratio of Fv/Fmax and a change of CF transients in O_3-S and O_3-R cultivars (Table 2). These change might be associated with the degree of reduction of Q with the slowing of electron outflow from PS II to PS I as a result of prolonging O_3 exposure.

Table 2. Relative leaf fluorescence of snapbeans cultivars exposed to 0.25 ppm O_3 for 3 h

Treatment	Cultivar	Fo	Fmax	Fv*	Ratio**
		($mV\ s^{-1}$)			
Control	BBL-290	370 d +	1430 b	1060 a	0.74 a
	ASTRO	485 c	1615 a	1130 a	0.70 ab
O_3-stressed	BBL-290	610 a	1020 b	410 c	0.40 c
	ASTRO	510 b	1390 b	880 b	0.63 b

* Fv=Fmax−Fo ** Ratio=Fv/Fmax

\+ Each value is the mean of 6 replicates. Numbers in a column followed by the same letter are not significantly different at the 5% level of probability.

These effects of O_3 exposure on vaiable CF, Fo, and Fv/Fmax ratio indicate dual action of this stress factor on PS II activity. Thus, suppression of Fv/Fmax at various O_3 exposure times or concentrations can be used as rapid, diagnostic criteria of their physiological status. The quenching of the CF sector from OPST (Fig. 4) and its rates decreased with an increase in O_3 exposure. Under acute O_3 exposure for short duration, the O_3 decrease of CF in O_3-S BBL-290 snapbean was no longer evident at 0.30 ppm O_3 at 3 h of exposure, whereas in O_3-R Astro, a typical picture of fluorescence-induction transients still could be observed even at 0,40 ppm O_3.

According to the CF kinetics date presented here, plants grown under pollutant stress have a partial inactivation of PS II, thus confirming an observation that was reported earlier by Chang and Heggestad (1974). At higher dose of O_3 fumigation and or after 4 to 5 h of chronic O_3 exposure, the O_3-S cultivars experience an irreversible inactivation of PS II reaction centers, since the yield of CF in intact leaves exposed to the O_3 is significantly lower than the control even after several days of recovery under charcoal filtered air greenhouse. Increasing the duration of O_3 caused the stationary level of CF to rise (Fig 5). This could be associated with changes in the degree of Q reduction and with a slowing of electron outflow PS II to PS I as a result of prolonging O_3 exposure.

The curves of CF induction transients in both Fo and Fv/Fmax ratio shown in Fig 6 differed markedly in O_3-R and O_3-S plants in response to O_3 stress. The variable CF of leaves gradually decreases after several hours of O_3 exposure. Loss of Fv as increasing exposure time was considerably sooner on O_3-S plants than O_3-R ones, indicating possible significant differences in activity of their

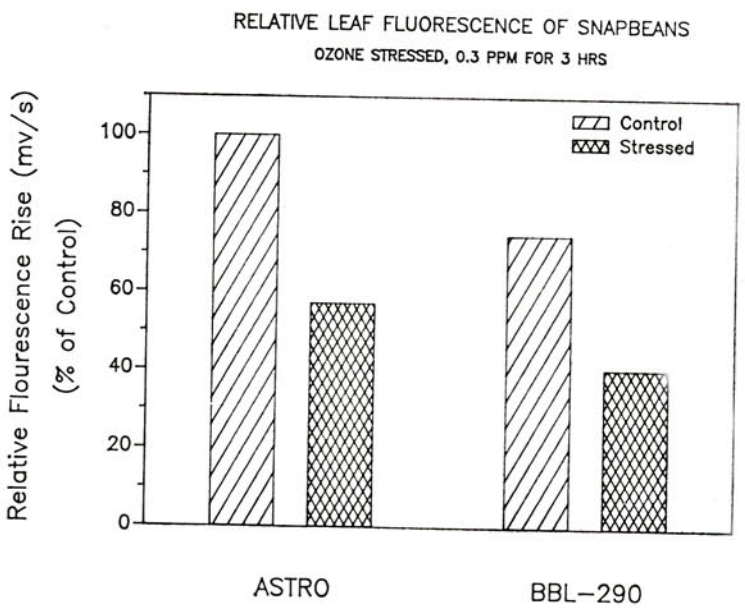

Fig. 7. Rate of fluorescence rise in fully expanded first trifoliate leaves of two cultivars of snapbeans exposed to 0.3 ppm O_3 for 3 h, showing the rate of fluorescence rise (RIS) for (O) to (P) as indicated in Fig. 4. Relative fluorescence units are in mV s^{-1}. Each value is the mean obtained from samples of six different leaves.

photosynthetic apparatus upon the light illumination. Furthermore, the rate of CF rise (RIS) from (O) to (P) and subsequent decline from (P) to (S) in O_3-R Astro was quite different from O_3-S BBL-290 (Fig 7). The rate of CF yield between (O) and (P) (i.e., the value of RIS), the Astro about 11% faster compared to the BBL-290, and the fluorescence decay or decline (DEC) was also about 20% faster in Astro leaf (Fig. 8). Fluorescence increased considerably faster in O_3-R cultivars (Fig. 7), which indicate that more efficient electron outflow from PS II to PS I in comparison with the O_3-S cultivars.

In short, our data indicate that in O_3-S cultivars (snapbean cv. BBL-290), O_3 stress resulted in a strong inhibition of the fast and slow fluorescence induction transients and altered the form of the kinetic curves of CF in leaves. In particular, the fluorescence quenching rate and Fv/Fmax ratio were markedly decreased in O_3-stressed leaves. In contrast, leaves of O_3-R cultivars (snapbean cv. Astro) showed only minor changes in CF. The values of the ratio Fv/Fmax decreased in O_3-S cultivar much more drastically than O_3-R ones. Ozone-induced stress blocked photosynthetic electron transport PS II and PS I. The results suggested that CF patterns, the rate of fluorescence induction transients, and the Fv/Fmax ratio could provide a valuable tool to investigate the photosynthetic and metabolic networks affected by O_3-induced stress to biochemical processes. The development of new tools for detecting stress and determining the causes would enhance the ability to researchers to assess the impacts of environmental stress and would play a particularly important role in early detection of ecosystem change due to stress.

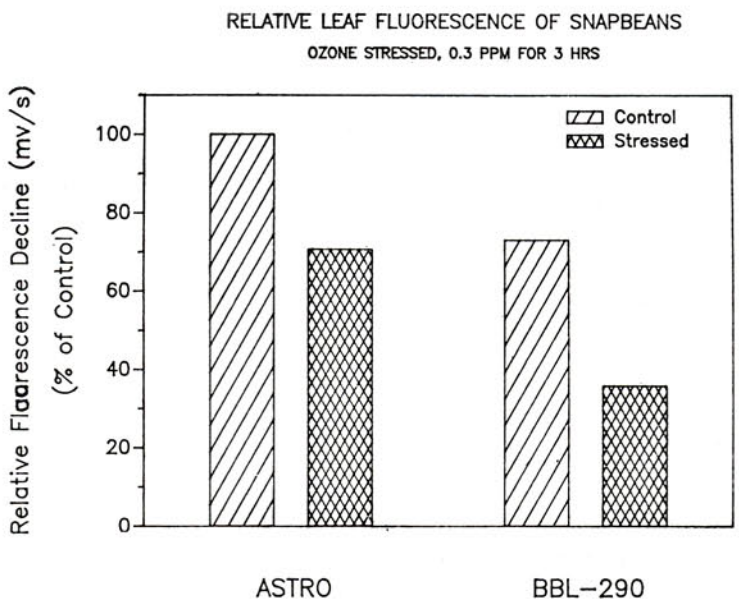

Fig. 8. Rates of fluorescence decay in fully expanded first trifoliate leaves of two cultivars of snapbean plants exposed to 0.3 ppm O_3 for 3 h, showing a decline from Fmax to a semi-steady state (S) level as shown in Fig. 4. Relative fluorescence units are in negative mV s^{-1} to show the rate of decline. Each value is the mean obtained from samples of six different leaves.

CONCLUSION

This paper provides appropriate background to this topic and presents a case study in air pollution research and applications relating to these devices. Particular emphasis was given to the determination of differences in O_3 susceptibility of snapbean cultivars by means of *in vivo* chlorophyll fluorescence measurements.

In order to determine the biochemical and physiological bases for differences in O_3 tolerance of cultivars, we report here the use of chlorophyll fluorescence (CF) to determine susceptibility to O_3 stress and gain insight into the primary processes in photosynthesis.

The yield of CF from leaves of the O_3-R cultivar remained unaffected by O_3 treatment, except when exposed for prolonged duration or high concentrations of O_3. In contrast, the O_3-S cultivar responded to the O_3 stress more drastically than the O_3-R cultivar in regard to CF transients. Thus, the fluorescence-induction kinetics; especially the suppression of Fv/Fmax at various exposure times or concentrations of O_3, can be used to detect the differential sensitivity of snapbean to O_3 stress. These rapid, nondestructive methods could be applied to air pollution field research to study the photosynthetic and metabolic mechanisms affected by air pollutant-induced stress. It could also be used as a rapid, diagnostic criteria of their physiological status.

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葉綠素螢光可作為測試 Snapbean 對臭氧忍受程度的指示劑

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摘 要

利用葉綠素產生螢光的快慢及其强度變化情形，可篩選及檢定 Snapbean 對臭氧是否具有忍耐作用。測試葉綠素螢光强度項目有 Fo, Fv 及 Fv/Fmax 比例。在臭氧逆境下，不抗臭氧的 Snapbean 品系 BBL 對螢光產生及其强度有很大的起伏變化，尤其是螢光消失速率及 Fv/Fmax 比例均顯著減少。但是具抗臭氧的品系 Astro 則變化較小。由螢光的測定，可知臭氧爲害作物係臭氧中斷光體系Ⅱ及光體系Ⅰ之間的電子傳遞所引起的結果。由於臭氧所引起的外表損害程度與其葉綠素螢光的變化大小有一致性，所以由螢光變化之測定，可研究作物在臭氧逆境情況下其光合作用機制及新陳代謝機制受害程度，同時也可供植物育種者作爲大規模並且快速檢測作物對空氣污染物之感受程度，作爲篩選品種之用。