Variable Chlorophyll$_a$ Fluorescence and its Potential Use in Tree Seedling Production and Forest Regeneration[1]

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Abstract.--An integrating fluorometer for detection of variable chlorophyll$_a$ fluorescence has proven useful in determining the physiological status of conifer seedlings. Information obtained can be used in selecting lifting dates, in evaluating post-storage vigor and in assessing the effectiveness of nursery watering and fertilizer regimes.

INTRODUCTION

Radiation incident on a seedling canopy or leaf can be absorbed, reflected or transmitted (Fig. 1). Of the incident photosynthetically active radiation AR 400 - 700 rim) the leaf absorbs about 90%. The absorbed energy may be used by the photochemical system to fix carbon, be dissipated as heat or emitted as fluorescence. The energy of fluorescence emission *in vivo* represents only about 3 - 5% of the excitation energy. Variable chlorophyll$_a$ fluorescence (Fv) is closely linked to the photochemical activity of the chloroplasts and therefore can be used as a non-destructive probe of the photochemical/photosynthetic processes. These processes are influenced by many factors including the plant's status with respect to water (watering regime), nutrients (fertilizer treatment), temperature (seasonal, diurnal) and light (daylength and irradiance level).

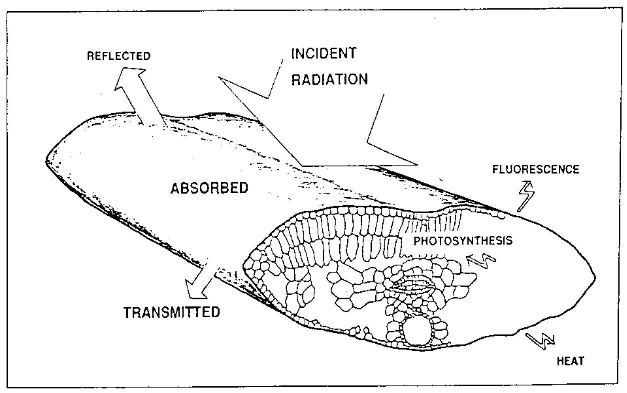

Figure 1.--Schematic drawing of a western hemlock (conifer) needle section. Disposition of the incident light (PAR) is proportional to the size of the arrows. It is this fluorescence that is the basis of Fv measurement. (Redrawn in part from Tucker and Emmingham, 1977).

MATERIALS AND METHODS

Measurement

Seedlings are dark-adapted for 15 to 20 min prior to Fv measurement. This is required to ensure an initial zero photochemical activity and CO_2 fixation state. The shoot of the dark-adapted seedling was then placed in the spherical cuvette of the integrating fluorometer (Fig. 2) (Toivonen and Vidaver, 1984) interfaced to a computer for data acquisition and analysis of Fv. Gas exchange was measured with an **ADC Mk III** infra-red gas analyzer

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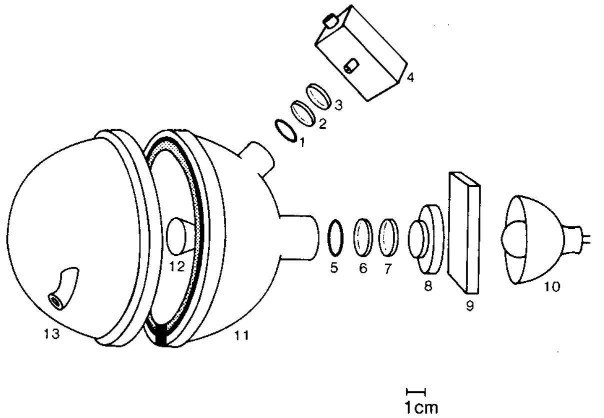

Figure 2.--Exploded view of the integrating fluorometer probe. (1) rubber O ring, (2) CS 7-59 filter, (3) CS 2-64 filter, (4) optical detector assembly, 5) O ring, (6) CS 4-96 filter, (7) CS 3-71 filter, (8) photographic shutter, (9) heat absorbing filter, (10) Sylvania EFP (100 W, 12 V) projector lamp, (11) supporting hemisphere (it supports the light and detector assembly and is fitted to a stand), (12) dispersion cone, (13) detachable hemisphere. (Reproduced from Toivonen and Vidaver, 1984).

(IRGA). Dry wt. was determined by oven drying samples at 90° C for 24 h. Unless otherwise stated all Fv and gas exchange measurements were carried out at 22 - 25° C.

Normalization

Once data are collected, the fluorescence (Fv) transients need to be compared. The Fv transients (curves) are normalized to compensate for differences in chlorophyll content (ie. plant size) (Fig. 3). The normalization formula uses a value for instantaneous fluorescence (F_o) (see Papageorgiou, 1975 for a review of variable chlorophyll fluorescence).

The formula is:

$$Fv = \frac{F_{\vartheta} - F_o}{F_o}$$

where Fv is normalized variable fluorescence at time t,
F_{ϑ} is non-normalized fluorescence at time t,
and Fo is O-level fluorescence

Averaging

To evaluate a seedling population or seedlot, a normalized Fv transient or induction curve for each of several seedlings is used (Fig. 4). Each curve represents more than 1000 data points obtained at a predetermined frequency over a fixed time period. Averaging is done by summing the values at each sampling point of the normalized curves, then dividing by the number of replicates. Data given in results represent the averaged response of 3 - 5 seedlings.

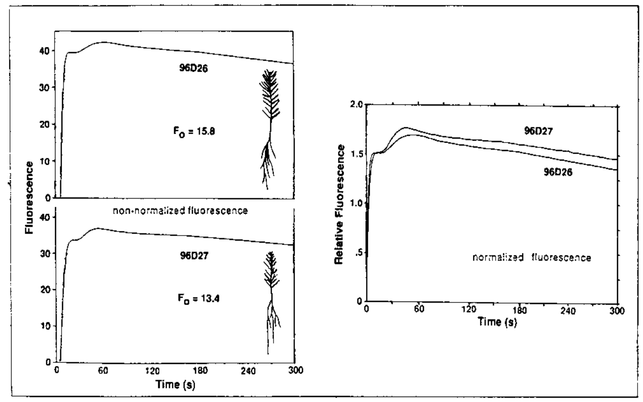

Figure 3.--The normalization operation largely compensates for differences in amplitude of individual White spruce seedling Fv curves. Non-normalized F_{ϑ} curves (left) of seedling 961326 and 961327 are from the same seedlot (8981).Their normalized Fv transients are compared in the right.

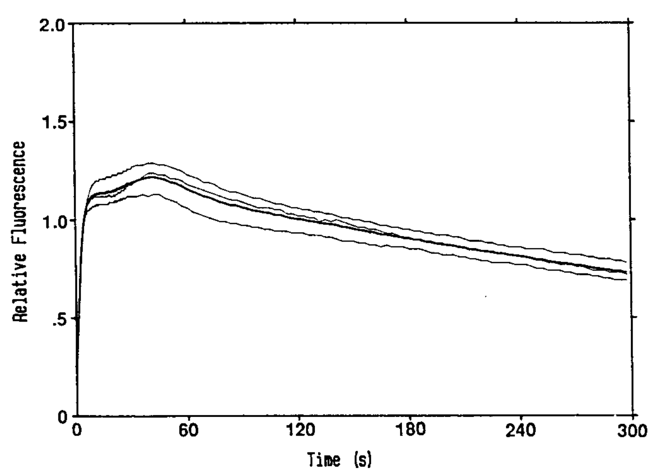

Figure 4.--Normalized Fv responses of individual White spruce seedlings (seedlot 8981) are shown as fine lines. The averaged Fv response of the three seedlings is shown as the broad line.

RESULTS

Photochemical Inactivation

Fv curves shown in Fig. 5 show a sequential decline in Fv amplitude for 1-0 White spruce container-grown stock. This decrease in fluorescence is accompanied by reductions in the rate of apparent photosynthesis (APS). The fluorescence decline represents a crease in the rate of the primary photosynthetic process of water splitting (Toivonen and Vidaver, 1988), thus reducing the potential for photodamage in high ambient light under cold temperatures (Peeler and Naylor, 1988) when biochemical CO_2 assimilation is inhibited. Inactivation of photosynthetic activity (shutdown) is considered by us to be an indicator of the winter hardening-off process.

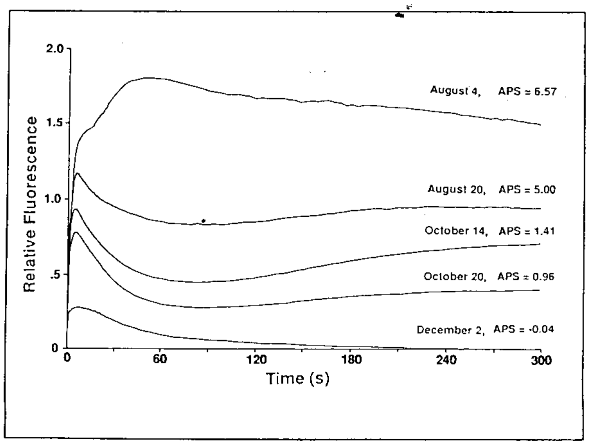

Figure 5.--Sequential inactivation of photosynthetic activity in White spruce seedlings (8981). The Fv decline with the approach of Fall reflects the inactivation of photochemical water splitting (shutdown). The decline in Fv appears to be mainly daylength dependent. These seedlings experienced no frost during the inactivation period. APS is expressed as mg CO_2 g dry wt^{-1} h^{-1}.

From this series of curves, we suggest that these seedlings could have been lifted for cold dark storage by Oct. 20. The -18 C cold hardiness tests (British Columbia Ministry of Forests (BCMOF)), (Simpson, 1985) performed immediately after Fv established that the seedlings had also achieved cold-hardiness at that time. The barely positive APS rates determined at 25° C would have been negative at ambient temperatures. The nursery operational lifting date was Dec. 2 for the same stock.

Provenance Differences in Shutdown Time

Differences were detected using Fv in the level of photochemical inactivation in two provenances of White spruce. In Fig. 6, the upper curve shows that more southern provenance seedlings (seedlot #8534, ca. 54° 50'N, Fort St. James, B.C.) had not reached the same level of inactivation by Nov. 17 as the northern provenance (#8981, ca. 57° 50' N, Fort Nelson, B.C.). The earlier progression toward shutdown in the northern provenance may indicate an adaptation to the earlier onset of winter conditions which would be experienced by these seedlings.

Recovery From Cold Dark Storage

Root growth capacity (RGC) assessment of seedling vigor following removal from cold dark storage usually takes one to three weeks (Burdett, 1979). Seedlings in which Fv had returned to near pre-shutdown levels within 48 h after removal from storage (Fig. 7, Table 1) also had high RGC scores. Seedlings exhibiting little recovery of Fv after 48 h had poor RGC's (Table 1 .

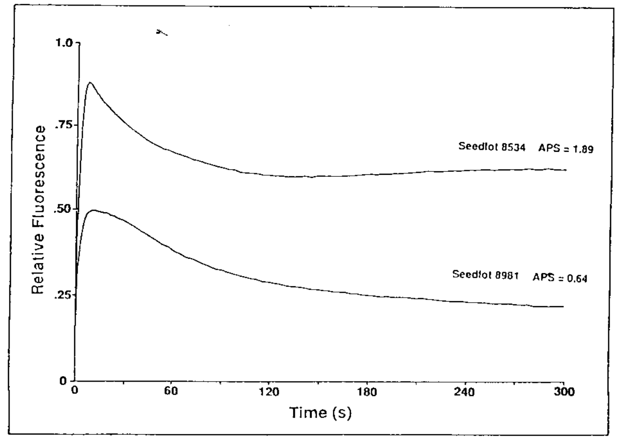

Figure 6.-- Provenance differences in the photosynthetic inactivation of White spruce seedlings. Other data as in Fig. 5.

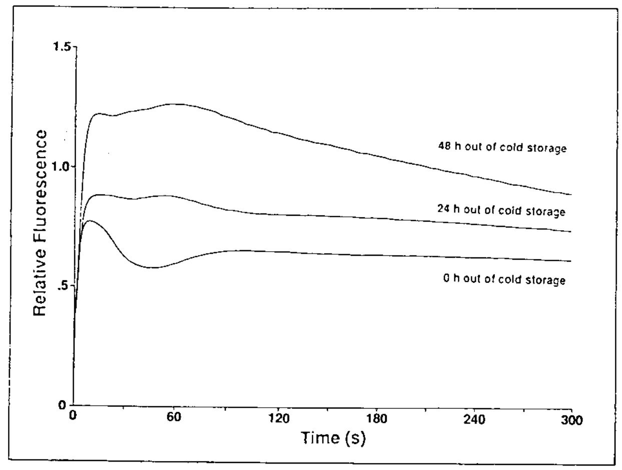

Figure 7.--Recovery of Fv on removal from cold dark storage in White spruce seedlings (seedlot 4073). On removal from storage the seedlings were repotted, watered and equilibrated to room temperature. Note that within 48 h, Fv had largely recovered to levels expected from fully active seedlings (top curve in Fig. 5).

Table 1.-- Maximum Fv values 48 h following removal from cold dark storage and corresponding RGC scores for White spruce seedlings. (Values are means ± standard errors.)

Seedlot	n=	Fv(max)	RGC
4073	5	1.28 ± 0.16	4.88 ± 0.15
8503	6	0.37 ± 0.40	0.62 ± 0.5
8782	6	0.53 ± 0.01	1.06 ± 0.44
8533	6	0.63 ± 0.2	1.13 ± 0.02

This correlation is consistent with the findings of Van den Driessche (1987) which indicated that new root growth is dependent on current photosynthetic activity.

Natural Water Stress and Recovery

From June 1 to July 15, 1987 there were large ambient temperature and relative humidity fluctuations at the growing sites (Fig. 8). This resulted in increased water stress in White spruce (seedlot #8534) seedlings on a 48 h watering cycle as indicated by both fluorescence and CO_2 exchange data (Fig. 9). When temperatures markedry declined at the beginning of July, both CO_2 exchange and Fv recovered.

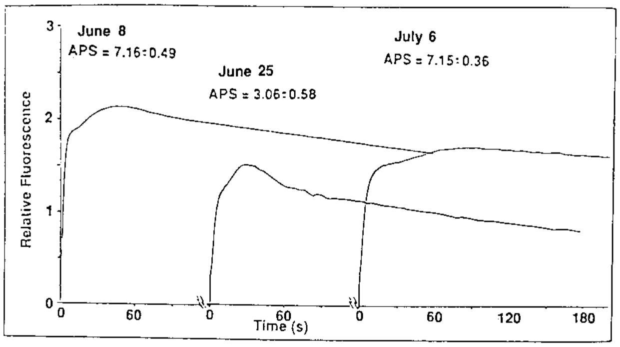

Figure 8.--Changes in Fv and APS of white spruce seedlings (seedlot 8534) under water stress occurring during the second year of the production cycle. Both June 8 and July 6 represent periods of cooler temperatures and therefore lowe water stress potential. APS (mean ± standard error) given as CO_2 g dry wt^{-1} h^{-1}.

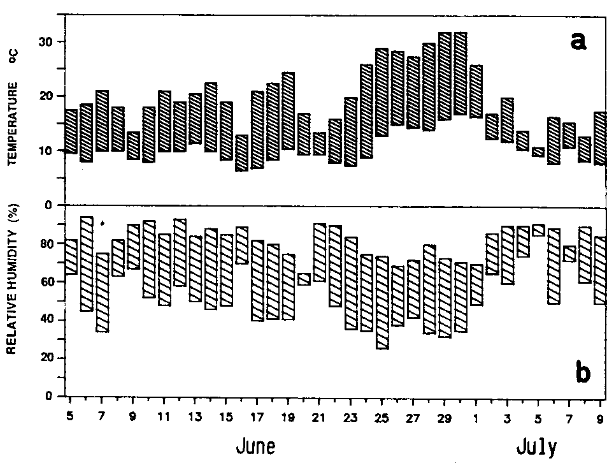

Figure 9.--Temperature range and humidity range data for the period June S to July 9, 1987 from a weather station at SFU. Fv and APS measurements were done on June 8 and 25, and on July 6, 1987.

Induced Water Stress and Recovery

Withholding water for various intervals induced symptoms of water stress in 2-0 seedlings of seedlot 8981. On rewatering, recovery (see Figs. 10 -11) depended on the fluctuations in temperature and humidity (Fig 12).

Seedlings last watered on July 14 exhibited mild water stress on July 16 (Fig. 10). Following watering (immediately after Fv assessment on July 16), the seedlings showed some recovery over the next day. High evaporative demand during July 17 (Fig. 12) resulted in marked water stress as indicated by reduced Fv on July 18 (Fig. 11). Following rewatering on July 18, together with lower temperatures, higher relative humidities and therefore lower evaporative demand; the seedlings recovered overnight as indicated by the July 19 Fv curve.

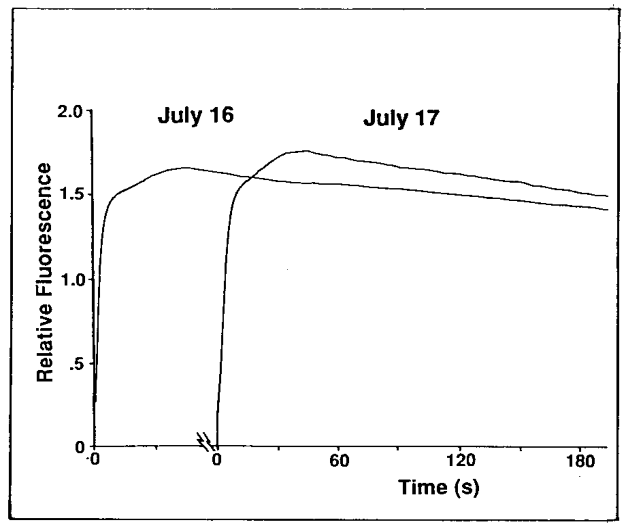

Figure 10.--Response of white spruce (Seedlot 8981) to 48 hours without water on July 16 and the recovery 18 hours after rewatering on July 17. This period of time was characterized as having a low evaporative potential.

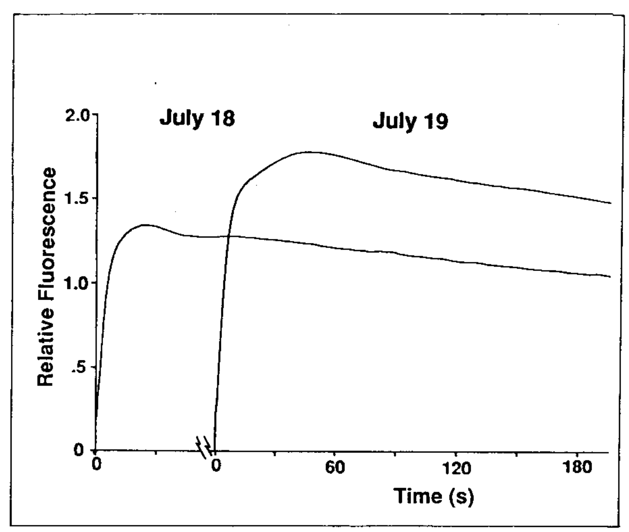

Figure 11.--Response of white spruce (seedlot 8981) to 48 hours without watering on July 18 and the recovery 18 hours after rewatering on July 19. The day prior to July 18 had relatively high evaporative potential.

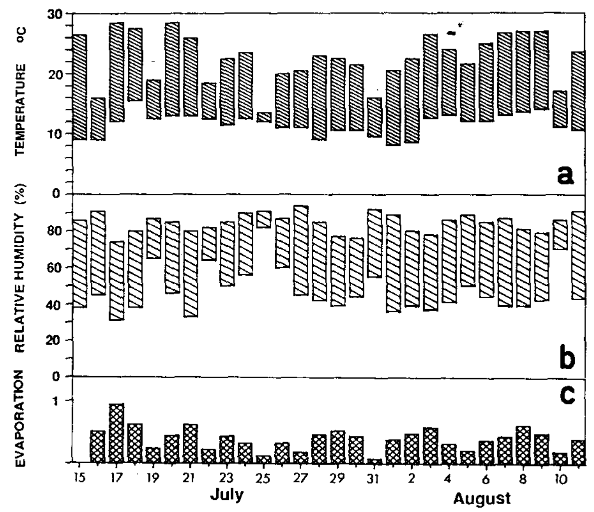

Figure 12.--Temperature range, humidity range, and relative evaporation data for the period of July 15 to August 10, 1987 from a weather station at SFU. Evaporation was measured with a Piche evaporimeter and the units are relative.

Phosphorus Nutrition

In this trial, phosphorus was applied as 20-20-20 (Green Valley) at varying frequencies during the growing season to 1-0 Douglas-fir seedlings. The frequency of P application was: every 2 wks, 4 wks, 6 wks or no application at all. All other nutrients were applied at regular 2 week cycles. The shape of the Douglas-fir Fv induction curve can be seen to be different from that of White spruce (Fig. 13). This difference is likely due to higher water-splitting activity in Douglas-fir compared to White spruce. The difference appears to be reflected in higher Douglas-fir APS rate (Fig. 13) which is typical for the

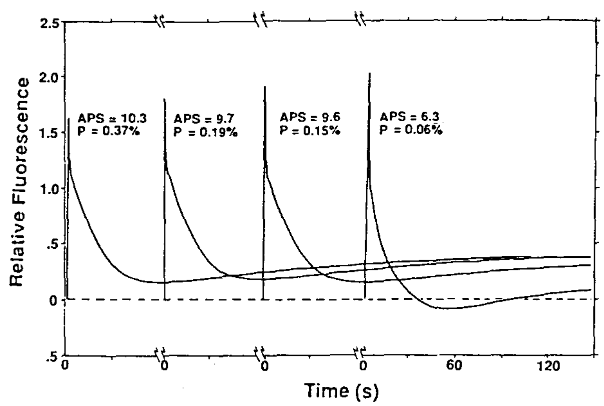

Figure 13.--Changes in Fv, APS, and phosphorus content of Douglas-fir (seedlot 1293) when P was applied (from left to right) every 2 weeks, 4 weeks, 6 weeks, or not at all during the growing season of 1987. APS units are mg CO_2 g dry wt^{-1} h^{-1}, phosphorus (P) content as F, total dry weight.

seedling during the growing season. An initial effect of lowering P was observed with the treatment resulting in 0.19% P content; the initial Fv transient was slightly higher than for the control treatment while APS did not decrease significantly. At the lowest P level (right-hand curve), the initial Fv transient was still higher and APS decreased to 60% of the initial value, and the decline from the initial peak was faster. The effect of these P levels on photochemical water splitting could be associated with some decrease in the activity of the dark reactions of CO_2 assimilation.

DISCUSSION

Data from Fv assessment provides information about the physiological status of conifer seedlings during the nursery production cycle.

In White spruce seedlings inactivation of photochemical water splitting (shutdown) occurs primarily in response to daylength. The onset of shutdown appears to be influenced by the latitude of seedlot provenance; shutdown occurs earlier in the fall in the northern than the more southern provenance. As shutdown is presumably related to the winter-hardening process and may be indicative of the extent of hardening, Fv assessment provides information of potential advantage in the selection of pre-storage lifting dates. Any delay in lifting could result in seedlings being lifted well after the optimal date and unnecessary losses in seedling nutrient reserves could occur. Fv assessment indicates physiological reactivation in the event of a warming trend after the chilling requirement has been filled (see the bottom curve in Fig. 5. and the top curve in Fig. 7). Reactivated seedlings would likely undergo nutrient losses and possibly physical damage while in cold dark storage.

The use of Fv to assess recovery from cold dark storage by monitoring the reactivation of photochemical water splitting could provide a good indication of seedling quality. Recovery of Fv appears to be related to root function. In experiments not shown here (Vidaver, et al. 1988), it was observed that watered and repotted seedlings showing little or no recovery, recovered more rapidly and to a greater extent when shoots were detached and the stem was placed directly in water.

Symptoms of water stress observed with Fv assessment during periods of high evaporative demand could likewise be indicative of the failure of the seedling root system to provide sufficent shoot moisture. Episodes of partial inactivation due to water stress may result in seedling set back or failure to reach BCMOF morphological growth standards during the growing season. Operational application of Fv could alert growers to the need for more effective watering regimes or the production of more efficient root systems.

The data from the P experiments suggest that potential utilization of nutrient stress to regulate morphological development could be monitored by Fv. In withholding P, APS was suppressed which if sustained would obviously restrict seedling growth. In these experiments restoring P, rapidly resulted in the full recovery of Fv (data not show.

SUMMARY

These preliminary results of the application of Fv assessment to the physiological status of conifer seedlings suggests that operational application of the fluorometer system to seedling production could provide substantial benefits to the nursery industry.

1] Fv measurement can provide a simple, rapid, reliable and non-destructive method of evaluating seedling physiological status during the nursery production cycle.

2] Fv measurement provides information which can be used in determining lifting dates, the effects of water stress and nutrient regimes, and for assessing post-storage vigor.

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