

## Principal component analysis of chlorophyll fluorescence transients for tolerance to drought stress in coconut seedlings

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### ABSTRACT

Study was conducted in coconut for understanding the importance of chlorophyll fluorescence (Chl. fluorescence) transients in seedling selection for drought tolerance. The observations on Chl. fluorescence transients along with leaf water potential ( $\psi_{leaf}$ ) were carried out in selected hybrid combinations of Dwarf x Tall during irrigated and water with-held conditions. The data from the whole experiment as well as transformed values were subjected to principal component analysis (PCA). The results clearly indicated significant differences between seedling combinations and between the two periods. The interaction between the combinations and treatment also was found to be significant. The ranking of the combinations based on Chl. fluorescence transients indicated the combinations with LCT as the pollen parent showed better adaptation to water stress conditions followed by Fiji Tall combinations. The PCA considering the Chl. fluorescence transients as well as  $\psi_{leaf}$  indicated relative dispersion of the genotypes which imply a high level of genetic diversity for water-stress tolerance in the combinations examined. Further, the observations clearly revealed that performance of seedlings, under stress cannot be predicted from performance in non-stress condition. Based on principal component loadings it can be suggested that Chl. fluorescence transients viz.,  $F_0$  and  $t_{1/2}$  and  $\psi_{leaf}$  are important traits which can be used to differentiate and screen the coconut seedlings that can adapt to water stress condition.

**Key words:** Coconut, chlorophyll fluorescence, principal component analysis, leaf water potential, water stress.

### INTRODUCTION

Coconut is a perennial plantation tree crop of the tropics. As it is grown mainly under the rainfed conditions, water stress situations prevailing during the summer months (March-May) affect the growth and productivity (Rajagopal *et al.*, 15). Impact of water stress on the physiology and biochemistry of this crop has been well established (Kasturi Bai *et al.*, 7; Chempakam *et al.*, 3) and breeding strategies for drought tolerance were formulated (Rajgopal *et al.*, 17). The nature of gene action governing some of these drought sensitive traits also have been understood which can be exploited in selective breeding for drought tolerance (Rajagopal *et al.*, 18). Chlorophyll fluorescence (Chl. fluorescence) of photosynthetic systems has become one of the most powerful techniques for quantifying environmental effects on the chloroplast thylakoid membrane system (Krause and Weis, 9; Govindjee, 4) and has been widely used in various fields of plant physiological studies (Lichtenthaler, 11; Srivastava *et al.*, 19). In an earlier study, Kasturi Bai *et al.* (8) reported changes in the photochemical efficiency of PS II with the reduction in leaf water potential in coconut seedlings. They observed blockage of electron flow on the water side

of the PS II and damage to the photochemical apparatus during water stress. Since all the Chl. fluorescence transients are inter-related and highly dependent on micro-climate variables and soil water status, it is not easy to select the transients which contribute more towards better adaptation of the seedlings to drought situations under nursery level. Hence, in this paper, an assessment of the Chl. fluorescence emission of coconut seedlings was made to compare the performance under irrigated and water withheld condition and to identify the seedling combinations with contrasting response to water stress. The observation will help in the identification of the seedling combination which can adapt to water stress conditions.

### MATERIALS AND METHODS

Seedling combinations in which three dwarf coconut cultivars viz., Chowghat Dwarf Green (CGD), Malayan Yellow Dwarf (MYD) and Gangabondam Green Dwarf (GBGD) each crossed with six tall cultivars viz., West Coast Tall (WCT), Laccadive Ordinary Tall (LCT), Federated Malay States Tall (FMST), Fiji Tall (FJT), Java Giant Tall (JVT) and Philippines Ordinary Tall (PHOT) were selected for the observations. All the talls selected were reported to be relatively tolerant to drought situations while

the dwarfs were relatively susceptible to drought (Rajagopal *et al.*, 16). These seedlings (18 months of age) were growing in red sandy loam soil in CRD in the Institute farm (12° 30' N, 70° 00' E and 11m MSL). Irrigation was provided to the seedlings continuously till field capacity of soil (non-stress) and stress was imposed by withholding irrigation for twenty days.

Chlorophyll fluorescence transients were measured in dark-adapted leaf of coconut seedlings with Morgan, CF-1000, Chlorophyll fluorescence meter, USA using a black light - weight clamp for dark adaptation as described earlier (Kasturi Bai *et al.*, 8). Leaflets were dark adapted for 30 minutes before measurements. The Chl. fluorescence transients over five seconds were measured after an active pulse of 680  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The inbuilt microprocessor of the instrument calculates all the main fast Chl. fluorescence parameters such as initial fluorescence ( $F_0$ ), maximum fluorescence ( $F_m$ ) after a flash of actinic radiation and the variable fluorescence ( $F_v = F_m - F_0$ ), photochemical efficiency ( $F_v/F_m$ ) and  $t_{1/2}$  i.e., the half rise time from  $F_0$  to  $F_m$ . In addition to the above parameters, there is some quantification of the slow fluorescence transients such as  $F_t$  (terminal fluorescence) and  $F_q$  (fluorescence quenching i.e.,  $F_m - F_t$ ). Fluorescence derived ratio such as  $F_m/F_0$  (Questa *et al.*, 10) was calculated from these measurements.  $\psi_{leaf}$  was measured with Scholanders' Pressure Chamber as modified for coconut (Rajagopal *et al.*, 13).

The observations on  $\psi_{leaf}$  as well as Chl. fluorescence transients were carried out during the summer months of March to April during both the periods. Four measurements were made from six replicate seedlings in each combination on the third leaf from the spindle and mean of these readings were subjected to analysis of variance to test for significant differences between the treatments (Panse and Sukhatme, 12). The mean trait values obtained from the whole experiment, i.e. data from irrigated (NS) and stress treatment (S), hereafter referred to as NS & S set were used for principal component analysis (PCA) to partition plant performance. A second data set relating to stress performance was created by calculating  $(S-NS)/NS \times 100$  (transformed data set) for individual traits. Principal components were then derived from these data sets to separate the seedling combinations in different groups taking into account all evaluated parameters and to identify stress-tolerant combination, i.e. combinations that were least affected by the water stress treatment. Other multivariate analysis such as cluster and discriminate analysis also was carried out (Adams and Wiersma, 1).

## RESULTS AND DISCUSSION

The study was conducted for understanding the importance of Chl. fluorescence transients in seedling selection for drought tolerance. During the experimental period (March to April) solar radiation ranged between 273.6 and 351  $\text{Wm}^{-2}$ , maximum air temperature between 36 and 37°C and VPD between 3 and 3.6 k Pa. The soil moisture content, which was 10% during non-stress period, got reduced to 4.2% (with a soil water deficit of 102 mm). This reduction (58%) in soil moisture during stress can be attributed to the higher evaporation demand prevailing during the period with high pan evaporation rate (5.5 mm day<sup>-1</sup>).

The results clearly indicated significant differences in  $\psi_{leaf}$  and Chl. fluorescence transients between the seedling combinations as well as between the two treatments (Table 1). The interaction between the variety as well as the treatments also was found to be significant. As compared to irrigated seedlings significant changes in the values of traits have been observed in the stressed seedlings (Table 2). Based on the degree and relevance of the traits for stress adaptations the combinations were ranked on a scale of 1 (least affected) to 16 (severely affected) (Table 2). Interestingly all the combinations with LCT as the pollen parent were within rank 5, indicating comparatively better adaptation to the stress condition. Similarly, all the combinations with FJT as the pollen parent were found within 6 to 10 rank. Among the combinations MYD x FMS, GBGD x JVT and CGD x JVT were comparatively less adaptive to the stress condition.

The data (NS & S set) consisting of the nine traits and the 18 combinations were subjected to  $D^2$  analysis. Hierarchical classification was done based on  $D^2$  values. A total of five groups could be obtained of which one was forming an independent (GBGD x LCT) group (Table 3). Through the PCA it was seen that 75% of the total variation was explained by the first three principal components with first contributing 46% (Table 5, NS & S set). Based on the coefficients in the eigen vectors it was seen that the characters  $F_0$ ,  $F_t$  and  $\psi_{leaf}$  with high coefficients in the first vector are important discriminating characters. The character  $F_m/F_0$  appearing with high coefficient in the 2<sup>nd</sup> principal component and the character  $t_{1/2}$  appearing with high coefficient in the 3<sup>rd</sup> principal component are also important characters in discrimination.

The mean values worked out group wise indicated (Table 3) that the hybrid GBGD x LCT which stands as an independent group is characterized by lowest value of  $F_0$ , highest value of  $F_t$  and highest value of  $\psi_{leaf}$  among the groups implying normal functioning of

**Table 1.** Chlorophyll fluorescence transients (relative fluorescence units) and leaf water potential ( $\psi_{leaf}$  -bars) in coconut seedling combinations (mean for non-stress and stress condition).

Combination	F <sub>0</sub>	Fm	Fv	Fv/Fm	Ft	Fq	t <sub>1/2</sub>	Fm/F <sub>0</sub>	$\psi_{leaf}$
MYD x WCT	228	858	630	0.69	486	373	128	3.96	-9.75
MYD x LCT	202	906	707	0.79	541	365	157	4.50	-9.13
MY D x FJT	208	834	627	0.73	435	399	135	4.01	-8.75
MYD x JVT	234	921	687	0.74	436	485	124	3.95	-7.20
M YD x FMS	296	793	497	0.54	411	382	152	2.98	-11.57
MYD x PHOT	243	976	733	0.74	460	516	135	4.07	-9.87
CGD x WCT	551	1186	637	0.51	752	434	114	2.59	-13.10
CGD x LCT	302	1151	850	0.75	653	498	110	3.89	-7.55
CGD x FJT	302	954	635	0.63	543	430	103	3.46	-10.25
CGD x JVT	376	967	575	0.54	424	543	116	3.20	-12.07
CGD x FMS	354	1144	790	0.68	660	538	123	3.58	-8.72
CGD x PHOT	300	1202	881	0.73	711	491	113	4.07	-6.48
GBGD x WCT	366	932	566	0.58	593	339	96	3.25	-14.12
GBGD x LCT	193	1031	838	0.81	720	311	119	5.49	-7.18
GBGD x FJT	219	939	720	0.76	533	405	113	4.32	-8.10
GBGD x JVT	285	909	624	0.63	662	247	131	3.71	-13.18
GBGD x FMS	429	1034	605	0.59	737	296	105	3.01	-14.30
GBGD x PHOT	557	1264	709	0.58	790	489	94	3.12	-14.67
Probability (P < 0.05)	97.9**	233.8**	203.2**	22.7**	161.9**	152.4**	40.7**	5.47**	2.84**
Treatment	32.1**	70.4**	57.4**	0.032**	50.9**	NS	7.0**	0.24**	0.61**
Interaction	136.3**	298.8*	243.6**	0.136**	216.2**	NS	29.8**	1.02**	2.61**

Probabilities are indicated by symbols: \* 5 %, \*\* 1%, ns (non-significant).

**Table 2.** Ranking of coconut seedlings based on changes (% +/-) in Chl. fluorescence transients and  $\psi_{leaf}$  during stress period as compared to non-stress period.

Combination	F <sub>0</sub>	Fm	Fv	Fv/Fm	Ft	Fq	t <sub>1/2</sub>	Fm/F <sub>0</sub>	$\psi_{leaf}$	Rank
MYD x WCT	17.59	-28.43	-40.65	-23.51	-38.92	-13.61	7.74	-35.36	-49.85	06
MYD x LCT	1.57	-6.31	-32.52	-2.78	-43.49	8.71	5.72	-27.21	-29.24	03
MYD x FJT	-13.12	-34.84	-41.33	-10.32	-58.78	3.34	19.77	-24.61	-29.41	05
MYD x JVT	7.17	-10.47	-15.80	-5.99	-22.47	1.81	2.50	-16.51	-0.051	02
MYD x FMS	48.12	-52.43	-81.07	-61.68	-62.19	-39.83	5.26	-68.31	-109.99	15
MYD x PHOT	7.42	-25.52	-34.41	-13.70	-53.58	10.91	-23.98	-31.36	-97.54	09
CGD x WCT	119.95	-18.05	-67.48	-60.09	5.02	-47.72	-27.11	-62.41	-223.99	12
CGD x LCT	-6.84	-7.34	-7.38	0.358	-6.05	-5.01	-20.24	6.72	-53.55	01
CGD x FJT	44.73	-32.88	-52.09	-33.38	-37.19	-26.49	-22.01	-53.33	-113.68	10
CGD x JVT	114.1	-26.14	-62.12	-55.23	-42.63	-9.42	-21.97	-64.15	-178.75	14
CGD x FMS	20.04	-27.44	-42.37	-22.33	-46.09	-7.16	-3.51	-27.78	-61.36	08
CGD x PHOT	5.98	-9.29	-9.53	-0.097	-24.68	20.83	0.06	-13.09	-34.12	03
GBGD x WCT	176.36	-4.64	-51.07	-53.22	-5.86	-0.41	-75.38	-66.10	-144.60	13
GBGD x LCT	-13.98	-17.07	-17.71	-0.88	-28.59	19.60	-16.45	-4.16	-33.39	04
GBGD x FJT	9.47	-25.10	-33.49	-11.14	-42.52	5.41	-21.66	-31.62	-31.43	07
GBGD x JVT	70.75	-47.10	-72.43	-46.61	-55.97	-17.20	-3.66	-66.64	-129.45	16
GBGD x FMS	164.43	3.05	-45.44	-46.27	7.22	-6.73	-40.46	-60.96	-132.56	09
GBGD x PHOT	262.61	26.96	-37.95	-51.71	31.84	12.27	-52.17	-65.31	-118.12	11

**Table 3.** Group means for clustering of the coconut seedlings under non-stress and stress conditions (NS & S data set).

Traits	N = 1	N = 4	N = 2	N = 3	N = 8
	GBGD x LCT	GBGD x WCT, GBGD x FMS, GBG D x PHOT, CGD x WCT	MGDx LCT, GBGD x JVT	CGD x LCT, CGD x PHOT, CGD x FMS	MYD x WCT, MYD x FJT, MYD x JVT, GBGD x Fiji, MYD x PHOT, CGD x FJT, CGD x JVT, MYD x FMS,
F0	192.833	475.667	243.250	318.278	263.271
Fm	1030.500	1103.708	907.000	1165.278	905.208
Fv	837.833	629.000	665.250	840.333	637.896
Fv/Fm	0.814	0.567	0.708	0.718	0.671
Ft	720.167	718.000	601.083	674.333	465.979
Fq	310.500	389.417	306.083	509.167	441.625
t $\frac{1}{2}$	118.667	102.042	144.000	115.222	125.625
Fm/F0	5.489	2.993	4.109	3.848	3.744
$\psi_{leaf}$	-7.183	-14.046	-11.158	-7.583	-9.694

the PS II. The second group consisting of four hybrids is characterized by highest value of F0 and lowest value of  $\psi_{leaf}$  among the groups. This situation implies that the seedlings are under stress. The third group consisting of two hybrids was characterized by highest value of t $\frac{1}{2}$  indicating active PSII centers. The 4<sup>th</sup> group consisting of three hybrids was characterized by highest values of Fm and Fq. The 5<sup>th</sup> group consisting of eight hybrids was characterized by lowest values of Fm and Ft among the different groups. The situations in the last two groups also imply the seedling combinations are under the influence of water stress.

Further the transformed data set was subjected to D $\frac{1}{2}$  analysis and PCA to identify stress tolerant combinations. A total of six groups were obtained of which one was forming an independent (CGD x WCT) group (Table 4). Through the PCA it was seen that 82% of the total variation was explained by the first three principal components with first contributing 58% (Table 5, transformed data set). Based on the coefficients in the eigen vectors it was seen that the characters F0, Fv, Ft, t $\frac{1}{2}$  and  $\psi_{leaf}$  with high coefficients in the first vector are important discriminating characters. The character Fq appearing with high coefficient in the 2<sup>nd</sup> and 3<sup>rd</sup> are also important characters in discrimination. The mean values worked out group wise indicated (Table 4) that the hybrid CGD x WCT which stands as an independent group (group IV) is characterized by lowest values of Fv, Fv/Fm, Fq and very low  $\psi_{leaf}$  among the group means. The 1<sup>st</sup> group consisting of four hybrids is characterized by lowest value of Fm, Ft and 2<sup>nd</sup> lowest value of Fv and Fm/F0 among the groups. The second group consisting of five hybrids was characterized by highest value of t $\frac{1}{2}$  and highest value for  $\psi_{leaf}$ . The third group consisting of three hybrids has only medium values for most of the characters. The 4<sup>th</sup> group consisting of

two hybrids had lowest values for F0 and highest values for Fv/ Fm and Fm/ F0 among the group means. The 5<sup>th</sup> group consisting of three hybrids was characterized by highest values for F0, Fm and Ft and lowest values for t $\frac{1}{2}$  and Fm/ F0. To summarize group wise mean in the transformed data set clearly indicate that among the seedling combinations, MYD x JVT, MYD x FJT, MYD x WCT, CGD x FMS, CGD x PHOT (2<sup>nd</sup> group) CGD x LCT and GBGD x LCT (4<sup>th</sup> group) are relatively tolerant to water stress than the other combinations.

The observations clearly demonstrated differences among seedling combination in Chl. fluorescence transients and adaptation to reduced water availability conditions. The PCA considering the Chl. fluorescence transients as well as  $\psi_{leaf}$  indicated relative dispersion of the genotypes which imply a high level of genetic diversity for water stress tolerance in the combinations examined. For example. Tables 3 and 4 clearly indicate differential response of the seedling during the two periods. The eight combinations which were in one group (group V- Table 3) got segregated into different groups (Table 4). Among the combination MYD x JVT and MYD x WCT showed relative tolerance to water stress with high values for t $\frac{1}{2}$  and  $\psi_{leaf}$  while the rest of the combinations did not show any response as revealed by Chl. fluorescence values. However the combinations CGD x WCT, GBGD x WCT, GBGD x PHOT as well as GBGD x FMS (group II) with high F0 values and low  $\psi_{leaf}$  imply positive response to water stress (Table 3) showed the same response during stress period also (Table 4). The combination GBGD x LCT with low F0 and high  $\psi_{leaf}$  values could adapt to the stress condition. On the other hand CGD x LCT showed high adaptation during stress condition, although the response was not good as revealed by the grouping based on non - stress and stress mean

(Table 3). This implies that performance under stress cannot be predicted from performance in non-stress or from the mean trait values obtained from the whole experiment. F0 values have been used to rank relative temperature resistance in poplar (Haveux *et al.*, 5).

Fv/ Fm has been valued as the most important Chl. fluorescence transient for understanding the photosystem II efficiency (Krause and Weis, 9; Govindjee, 4). It is interesting to note here that Fv/ Fm did not contribute in the discrimination of the combinations as revealed in the main PCA. Similar type of response has been reported by Hofmann *et al.* (6) in *Trifolium repens*. However it cannot be ruled out that the two seedling combinations GBGD x LCT and CGD x LCT which maintained low F0 and high  $\psi_{leaf}$  during stress also showed higher Fv/Fm and Fm/F0 values. The combinations MYD x FJT, MYD x WCT,

MYD x JVT, CGD x FMS and CGD x PHOT (Table 4) with high  $t\frac{1}{2}$  and  $\psi_{leaf}$  were found to be within 10th rank (Table 2). This corroborates with the reports of Bjorkman, (2) and Krause and Weis (9) that  $t\frac{1}{2}$ , which indicate the relative number of active PS II centre is an important trait for stress adaptation. In coconut a rapid method for drought tolerance screening based on  $\psi_{leaf}$  has already been developed by Rajagopal *et al.* (14). To conclude based on the coefficients in the eigen vectors the observations thus suggest that the Chl. fluorescence transients *viz.*, F0 and  $t\frac{1}{2}$  and  $\psi_{leaf}$  with high principal component loadings, 0.4844, 0.4167 and 0.4702 respectively (Table 5, transformed data set, PC1) are important traits which can be used to differentiate and screen the coconut seedlings that can adapt to water stress conditions.

**Table 4.** Group means for clustering of the coconut seedlings for percent change in the parameters under stress condition over non-stress condition (transformed data set).

	N=4	N=5	N=3	N=2	N=3	N=1
	MYD x FMS, CGD x JVT, CGD x FJT, GB x JVT	MYD x JVT, MYD x FJT, MYD x WCT, CGD x FMS, CGD x PHOT	MYD x LCT, MYD x PHOT, GBGD x FJT	CGD x LCT, CGD x LCT, GBGD x FMS	GBGD x WCT, GBGD x PHOT,	CGD x WCT
F0	69.423	7.530	6.142	-10.409	201.132	119.947
Fm	-39.638	-22.094	-25.642	-12.203	8.455	-18.047
Fv	-66.928	-29.938	-33.474	12.546	-44.817	-67.479
Fv/Fm	-49.224	-12.451	-9.209	-0.259	-50.734	-60.089
Ft	-49.493	-38.187	-46.531	-17.320	11.065	5.017
Fq	-23.245	1.042	8.343	7.293	1.711	-47.722
$t\frac{1}{2}$	-10.594	5.313	-13.308	-18.355	-56.003	-27.112
$\psi_{leaf}$	-63.105	-23.469	-30.062	1.2839	-64.1209	-62.407
	-132.968	-34.958	-52.736	-43.472	-131.761	-223.988

**Table 5.** First three PCs (PC1, PC2 & PC3) for Chl. fluorescence transients and  $\psi_{leaf}$  in coconut seedlings.

Trait	NS & S Mean			Transformed data set		
	PC1	PC2	PC3	PC1	PC2	PC3
F0	-0.4892	0.3313	0.3956	0.4844	0.0038	-0.2739
Fm	0.2389	-0.1320	-0.3166	-0.2288	0.3439	0.0931
Fv	0.0781	-0.2152	-0.0412	-0.3714	0.2635	-0.0644
Fv/Fm	0.1017	-0.2042	0.04912	-0.0228	0.3156	0.0847
Ft	-0.3998	-0.5784	0.43767	0.3684	0.0283	0.3678
Fq	0.2085	0.1459	-0.0329	-0.1478	0.4318	0.6283
$t\frac{1}{2}$	0.1246	-0.2848	-0.5152	-0.4167	-0.7089	0.3650
Fm/F0	-0.0685	-0.5875	-0.0856	-0.1493	-0.0486	-0.1051
$\psi_{leaf}$	0.6809	-0.5182	0.52312	0.4702	-0.1433	0.4803
% variance	45.96	17.67	11.7	57.92	13.25	11.2
Cumulative %	45.96	63.63	75.33	57.92	71.17	82.37

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