



Chlorophyll Fluorescence parameters, SPAD chlorophyll and yield in *Brassica* cultivars

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Abstract

In order to evaluate chlorophyll fluorescence fluctuations in different cultivars of *Brassica*, a factorial experiment with randomized complete block design was laid during two winter seasons 2011-12 and 2012-13. Cultivars differed significantly for SPAD chlorophyll values, different components of chlorophyll fluorescence and seed yield. Environment had a profound effect on all the parameters studied except Fv/Fm and Fv/Fo. However, positive relation existed between SY and Fv/Fm (0.268), Fv/Fo (0.389), ETR (0.0118) and SPAD (0.636**). RLC1 (*B. juncea*) and Hyola PAC401 (*B. napus*, hybrid) out yielded the other cultivars indicating cultivars with less disruption in photochemical efficiency of PSII, higher electron transport rate and SPAD chlorophyll greenness were higher yielders.

Keywords: Brassicas, electron transport rate, fluorescence, photochemical efficiency

Introduction

Variations in environment limit plant photosynthesis which is associated with malfunction of biochemical reactions. The photosystem II is highly sensitive to environmental limiting factors, PSII reaction centre and its chemical reactions are adversely affected by changing climatic conditions. There are many constraints during seed filling which generally affects the seed yield. Photo inhibition may occur during this phase due to interaction between drought stress, high temperature and radiation levels. Using the single factor analysis, it is not appropriate to distinguish between effects of each variable separately. Under high radiation, moisture stress enhances inhibition of electron transport while variable fluorescence (Fv), initiative fluorescence (Fo) and quantum yield (Fv/Fm) are reduced. Further, non-photochemical quenching increases under stress leading to decline in photochemical quenching (Lu *et al.*, 2002). Chlorophyll fluorescence measurement is a non-destructive non-time consuming and relatively simple technique for studying the equilibrium between metabolic and energy evolving processes, that may be affected by both temperature and drought stresses (Paknejad *et al.*, 2007, Sharma *et al.*, 2011, 2014).

Under field conditions fluorescence measurement denotes actual response of photosynthetic system which is more restricted under natural conditions (Bigler *et al.*, 1995). However, plants normally face maximum stress during seed filling, where environmental factors can adversely affect leaf photosynthesis which in turn limits the supply of photo assimilates to the desirable sinks/seeds (Flagella *et al.*, 1994). The prime objective of the study was evaluation of chlorophyll fluorescence in various *Brassica* cultivars under field conditions and relation between seed yield with different components of chlorophyll fluorescence.

Materials and Methods

The experiment was conducted at the experimental farm of oilseeds section in the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana (Punjab) during winter (*rabi*) season 2011-12 and 2012-13 under irrigated conditions. The material for the present study consisted of eight popular varieties of *Brassic*as i.e. PBR210, PBR91 and RLC1 (*B. juncea*), GSL1, GSC6, GSC5 and Hyola PAC401 (*B. napus*) and PC5 (*B. carinata*). During both the years, crop was sown in the first week of November and the trails were laid out in

randomized block design with 3 replications. Each variety consisted of 5 rows of 3m row length. Row to row and plant to plant distance was 30 x10 cm for *B.juncea* (Indian mustard) and *B.carinata* (Ethiopian mustard) while 45x10 cm for *B. napus* (Gobhi Sarson). All the recommended agronomic and protection practices were followed to raise a healthy crop. Three plants per replication were randomly tagged and the 3rd and 4th fully developed leaf on the main shoot were selected to record chlorofluorescence and SPAD chlorophyll readings.

Chlorophyll fluorescence was determined with Os30p model by Opti Sciences after the leaves were dark adapted with dark adapting clips as described by Schreiber *et al.* (1986). The initial fluorescence (Fo), maximal fluorescence (Fm) were analyzed and quantum efficiency of open PSII centers-quantum yield (Fv/Fm) was automatically calculated by the instrument. The leaf surfaces were previously adapted to the dark for 15 minutes so that all the centers of PSII were in open stage (all the primary acceptors oxidized) and the energy dissipation through heat was minimal. The Fo was obtained with low intensity light (less than $0.1 \mu\text{molm}^{-2}\text{s}^{-1}$) not to induce any effect in the fluorescence variable. The Fm was obtained by continuous light excitation (at $2500 \mu\text{molm}^{-2}\text{s}^{-1}$) provided by an array of LEDs focused on the leaf surface to provide homogenous irradiation over a 4mm (0.16in) diameter leaf surface. The fluorescence variable (Fv) was calculated from the difference between Fm and Fo. The electron transport rate was calculated according the formula given by Jin *et al.* (2011). Chlorophyll content in the designated leaves of intact plant was measured with Minolta SPAD502 at 90DAS taking care the midrib should not come under the sample area/sensor of the instrument. The mean of 10 readings was recorded as SPAD values. The character means for each replication were subjected to analysis of variance (ANOVA) for the factorial randomized complete block design (CPCSI 2008) developed at PAU. The correlation coefficients among different characters were computed using the same software.

Results and Discussions

Chlorophyll fluorescence

Initiative fluorescence (Fo) values were lower in the first year ranging from 62.1 to 75.2 in *B.juncea* and 88.8 to 97.0 in *B.napus* and 76.5 in *B.carinata*. Due to erratic rains and comparatively high temperature fluorescence values were higher in all the cultivars during second crop season (Table 1 and 2). Fovaried from 92.3 (RLC1) to 192.8 (PBR91), 102.6 (GSL1) to 120.3 (GSC5). Over the years, GSC5 possessed highest Fo followed by GSL1. Fo values are related to chlorophyll fluorescence of PSI receptors and considering significant Fo differences between the cultivars, it seems the receptors chlorophylls had variable efficiency. As SPAD values decreased in the 2nd year (data not included) it should be partly responsible for photo inhibition. Chloroplasts needs N to generate chlorophyll through proteins, chlorophyll production rates became slower and as a result leaves will become more susceptible to photo inhibition.

Maximal fluorescence was 355 (RLC1), 413.9 (Hyola) and 298.7 (PC5) during the 1st year while Fm was highest in the cultivars PBR210 (445.6), GSC5 (554) and PC5 (455.3). Environment registered significant impact on Fm and Fo over the years. Genotypic mean over the years showed maximum Fm in RLC1 (361.7), GSC5 (477.8) and PC5 (377). In 2nd year variable fluorescence (Fv) values were higher indicating Q in reduced state while lower Fv values during 1st year indicated Q in oxidized state which further implies disruption in the normal electron transfer in photolysis at PSII where as a negligible effect on electron after first electron receptor Q is evident thereafter (Table3).

Fv/Fm ratio was highest in RLC1 trailed by PBR91. Photochemical efficiency of GSC6 and GSC5 in *B. napus* and PBR91 and RLC1 in *B. juncea* was comparable at 100DAS. Hyola PAC401 had higher Fv/Fm values (0.757) followed by GSC6 (0.748) and GSC5 (0.743). During field studies in 2012-13, photochemical efficiency of PSII declined as denoted by lower Fv/Fm values. Damage to PSII over the years was less in GSC6 and GSL1 as the photochemical efficiencies were reduced to lesser

Table 1. Weekly mean meteorological data recorded during the crop season 20011-12 at PAU Ludhiana

No.	Standard Meteorological Week (SMW)	Temperature (°C)			Relative humidity (%)			Rainfall (mm)	No. of Rainy Days	Total evaporation (mm)	Sun-shine (hrs)
		Max.	Min.	Mean	M*	E*	Mean				
46	Nov. 12-18	28.3	12.4	20.4	95	38	66	0	0	17.1	8.1
47	Nov. 19-25	27.1	12.4	19.8	98	55	76	0	0	12.1	0.9
48	Nov. 26-Dec 2	24.6	9.8	17.2	97	46	71	0	0	11.3	4.6
49	Dec. 3-9	25.4	12.2	18.8	93	50	71	11.4	2	13.6	6.4
50	Dec. 10-16	20.9	6.0	13.5	99	49	74	0	0	7.7	7.6
51	Dec. 17-23	19.4	4.3	11.9	99	47	73	0	0	9.2	6.2
52	Dec. 24-31	20.1	3.1	11.6	99	46	73	0	0	8.6	6.5
1	Jan. 1-7	17.1	7.3	12.2	97	72	84	12.6	2	8.8	2.8
2	Jan. 8-14	16.6	3.0	9.8	99	56	77	2.0	0	1.3	8.0
3	Jan. 15-21	14.3	7.7	11.0	94	83	88	38.0	2	1.0	2.0
4	Jan. 22-28	18.4	5.4	11.9	93	46	70	0	0	1.5	8.6
5	Jan. 29- Feb 4	21.7	6.1	13.9	96	45	70	0	0	1.9	6.3
6	Feb. 5- 11	17.8	4.2	11.0	94	46	70	0	0	14.5	7.6
7	Feb. 12-18	19.7	6.7	13.2	89	49	69	1.2	0	16.6	5.8
8	Feb. 19-25	20.9	8.1	14.5	89	52	70	0	0	16.2	6.5
9	Feb. 26- Mar. 4	24.1	8.4	16.3	90	41	65	0	0	32.1	8.9
10	March 4-11	24.5	10.1	17.3	90	41	65	0	0	24.5	8.0
11	March 12-18	26.4	10.8	18.6	89	39	64	0	0	27.4	8.8
12	March 19-25	27.9	12.6	20.3	84	31	57	0	0	39.5	6.7
13	Mar. 26-Apr. 1	31.8	15.3	23.6	84	34	59	0	0	34.8	7.6
14	April 1-8	35.1	18.6	26.9	76	31	54	0	0	39.8	9.3

*M = Morning, E = Evening

extent in these varieties. Weather conditions of a year significantly influenced photosynthetic activity of *Brassicac*s. In the dry year of 1st crop season Fv/Fm were higher than in the wet year of 2012-13. These observations are in accordance with the findings on chlorophyll fluorescence in various plants (barley, oats, wheat, rice, sorghum) in relation to environmental stresses (Sayed, 2003, Yamane *et al.*, 2008, Jia *et al.*, 2008 and Hura *et al.*, 2009). Fo had significant differences implying differences in antenna chlorophyll efficiency (Anonymous1993b, Yang *et al.* 1996) between cultivars. Significant variations of Fm, Fv and Fv/Fm ratio over the years in different cultivars revealed differences of quantum yield. It may therefore be concluded that these cultivars had different quantum yield and observed yield differences can be attributed to capacity of electron acceptors which varied in relation to

chlorophyll content. Further the role of environment on these traits is also significant. Photochemical efficiency of PSII (Fv/Fm) varies between 0.75 to 0.85 in non-stresses /healthy plants and a close correlation exists with net photosynthesis and quantum yield in intact leaves (Vazan, 2002). The extreme photo synthetically photon flux density (PPFD) is an important factor affecting quenching in Fv/Fm ratio under normal conditions (Lu *et al.*, 2002). Decline in slope of Fv/Fm is a valuable criterion for evaluation of photoinhibition in plants subjected to environmental fluctuations. However, it is desirable to know if Fv/Fm quenching had arisen from an increase in Fo or variations in other components. For example when all reaction centers are open and photochemical quenching is at minimum, any increase in Fo indicates increase of fluorescence and destruction of PSII reaction centre or disruption in electron

Table 2. Weekly mean meteorological data recorded during the crop season 2012-13 at PAU Ludhiana

No.	Standard Meteorological Week (SMW)	Temperature (°C)			Relative humidity (%)			Rainfall (mm)	Total evaporation	Sunshine (hrs)
		Max.	Min.	Mean	M*	E*	Mean			
45	Nov 5-11	28.7	12.4	20.5	91	39	65	0.0	13.8	5.5
46	Nov 12-18	26.2	10.5	18.3	95	45	70	0.0	10.7	7.7
47	Nov 19-25	25.8	9.0	17.1	89	36	63	0.0	14.0	6.9
48	Nov 26-Dec 2	23.7	7.5	15.6	89	39	64	0.0	15.9	8.1
49	Dec 3-9	24.1	5.9	15.0	91	37	64	0.0	14.0	8.1
50	Dec 10-16	20.1	9.7	14.9	91	65	78	17.4 (2)	9.7	4.4
51	Dec 17-23	19.4	6.9	13.1	93	59	76	0.0	12.4	7.6
52	Dec 24-31	13.8	7.3	10.6	92	74	83	0.0	8.8	4.9
1	Jan 1-7	10.5	4.7	7.6	90	75	82	0.0	6.0	0.2
2	Jan 8-14	18.6	4.7	11.6	92	51	72	0.0	9.7	6.9
3	Jan 15-21	17.9	6.7	12.3	96	67	81	8.2 (1)	10.1	5.1
4	Jan 22-28	19.5	4.2	11.9	97	49	73	0.0	13.5	8.4
5	Jan 29-Feb 4	20.9	8.1	14.5	98	61	79	22.6 (2)	12.3	3.3
6	Feb 5-11	19.8	7.4	13.6	99	66	83	1.0	15.0	8.7
7	Feb 12-18	20.4	9.5	14.9	97	65	81	33.4 (2)	13.3	6.2
8	Feb 19-25	20.0	11.1	15.6	99	73	86	33.4 (3)	12.7	5.3
9	Feb 26-Mar 4	23.9	9.7	16.8	98	56	77	4.0 (1)	18.3	10.0
10	March 4-11	28.7	12.9	20.8	94	50	72	0.0	24.6	9.4
11	March 12-18	27.2	12.8	20.0	95	51	73	5.4 (1)	24.9	9.4
12	March 19-25	28.8	14.6	21.7	95	48	71	29.0 (1)	30.3	9.6
13	Mar 26-Apr 1	28.0	15.2	21.6	90	47	69	1.2	26.4	8.0
14	April 2-8	32.0	15.3	23.6	81	25	53	0.0	45.5	11.1

*M = Morning *E = Evening * Figure in parentheses are the number of rainy days

Source: School of Climate Change and Agricultural meteorology, PAU, Ludhiana

Table 3. Initial fluorescence (Fo), maximal fluorescence (Fm) and maximum quantum yield (Fv/Fm) in *Brassica* cultivars

Varieties	100 DAS								
	Fo			Fm			Fv/Fm		
	2011-12	2012-13	Mean	2011-12	2012-13	Mean	2011-12	2012-13	Mean
PBR 210	75.2	97.0	86.1	283.7	445.7	364.7	0.731	0.710	0.721
PBR 91	62.1	102.8	82.5	273.5	415.8	344.7	0.758	0.694	0.726
RLC 1	70.0	92.3	81.1	355.0	378.4	366.7	0.759	0.678	0.719
GSL 1	95.3	102.6	98.9	359.0	469.3	414.2	0.720	0.727	0.724
GSC 6	89.6	106.2	97.9	364.7	540.2	452.4	0.748	0.740	0.744
GSC 5	97.0	120.3	108.6	401.7	554.0	477.8	0.743	0.711	0.727
Hyola PAC 401	88.8	104.4	96.6	413.9	491.8	452.8	0.757	0.739	0.748
PC 5	76.5	109.6	93.0	298.7	455.3	377.0	0.736	0.698	0.717
Mean	81.8	104.38		343.8	468.8		0.744	0.712	

cp (=0.05) G=7.62, Y=1.11, G x Y=21.5, G=41.1, Y=2.72, G x Y=NS, G= 0.019, Y= NS, G x Y=NS

transport for excitation of reaction centers, but Fm quenching may result from non-photochemical quenching. These changes are associated with photo inhibition. Simulating field conditions in a controlled environment is very difficult, where as

field is a task (Moffat *et al.*, 1990).

Efficiency of the water splitting complex on the donor side of PSII (inferred by Fv/Fo) is most sensitive component of photosynthetic electron

Table 4. Variable fluorescence (Fv), activity of water splitting complex (Fv/Fo) and electron transport rates at 100 DAS in *Brassica* cultivars.

Varieties	Fv			Fv/Fo			ETR		
	2011-12	2012-13	Mean	2011-12	2012-13	Mean	2011-12	2012-13	Mean
PBR210	208.6	317.3	263.0	2.7	2.6	2.7	23.8	18.2	21.0
PBR91	211.4	280.0	245.7	3.3	2.1	2.7	24.3	20.2	22.3
RLC1	285.0	256.1	270.6	4.0	2.2	3.1	25.0	15.7	20.4
GSL1	263.7	335.0	299.3	2.8	2.3	2.6	23.5	21.5	22.5
GSC6	275.1	400.7	337.9	3.0	3.0	3.0	22.5	15.5	19.0
GSC5	304.7	395.7	350.2	3.0	3.0	3.0	19.0	22.4	20.7
Hyola PAC401	325.0	353.9	339.5	3.6	2.7	3.2	26.6	19.8	23.2
PC5	222.2	309.6	265.9	2.9	2.3	2.6	23.7	18.0	20.9
Mean	262.0	331.0		3.16	2.5		23.5	18.9	

cp(=0.05)G=46.0 Y=2.14 G xY=NSG=0.317 Y=NS G xY=NSG=1.54 Y=0.48 G xY=4.37

transport chain. Fv/Fo was higher and varied significantly within the cultivars (Table 4). However a decline was observed during the 2nd crop season. The decline in Fv/Fo was considerable in PBR 91, RLC 1, GSL 1, Hyola PAC 401 and PC5 which could be due to inhibition of osmotic ally driven uptake of water and also due to impairment of electron transport. GSC 6 and GSC 5 had similar efficiencies over the two crop seasons followed by comparable water splitting efficiency in PBR210 inferring no inhibition in water uptake. Overall, mean of the genotypes indicated similarities of Fv/Fo in PBR 210 and PBR 91 (2.7), GSL1 and PC 5 (2.6)

and GSC 6 and GSC 5 (3.0). The higher values in RLC1 (3.1) and Hyola (3.2) indicated negligible effect of environment on osmotic ally uptake of water. Significant differences in ETR occurred over the years but Hyola PAC 401 had statistically higher ETR as compared to other two *B. napus* cultivars (GSC 6 and GSC 5). Electron transport rates were reduced in 2nd year. Mean reduction in ETR was 23%. Higher electron transport was recorded in Hyola PAC 401 over the years while this trait was comparable in RLC 1, GSC 5 and PC 5. Genotypes differed significantly for various chlorofluorescence characteristics and varied significantly over the years

Table 5. Coefficient of correlation between different components of chlorofluorescence in *Brassica* genotypes

Characters	Fo	Fm	Fv/Fm	Fv	Fv/Fo	ETR	SPAD	SY
Fo	1							
Fm	0.904**	1						
Fv/Fm	0.386	0.671**	1					
Fv	0.849**	0.993**	0.726**	1				
Fv/Fo	0.158	0.538*	0.638**	0.619**	1			
ETR	-0.049	-0.112	0.091	-0.122	-0.131	1		
SPAD	0.833**	0.895**	0.652**	0.89**	0.366	0.103	1	
SY	-0.677**	-0.829**	0.261	-0.851**	0.389	0.012	0.636**	1

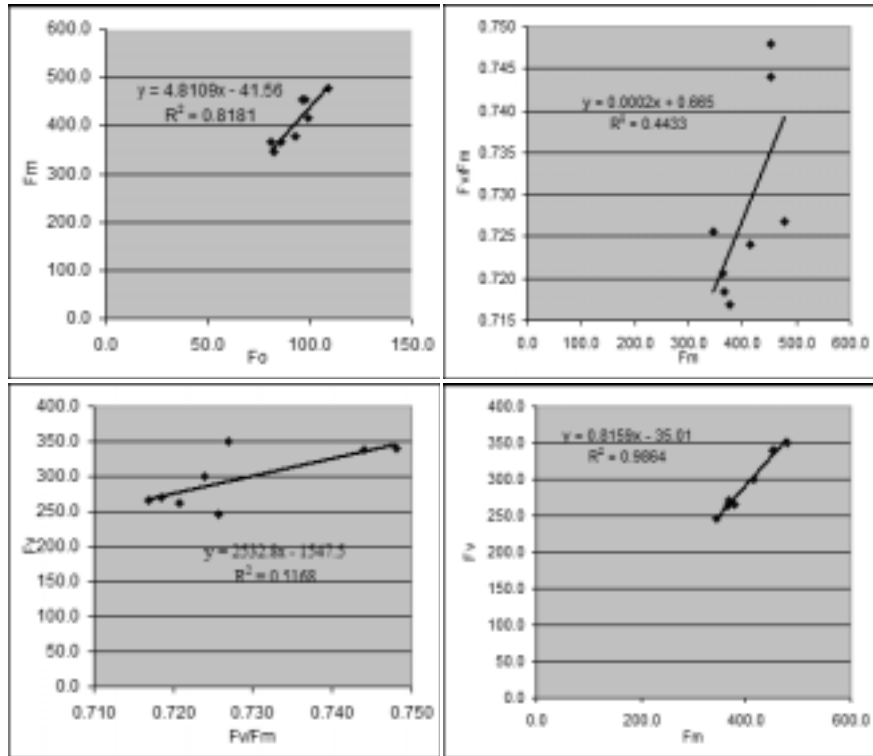


Fig 1: Relationship between different components of chlorofluorescence in *Brassicas*

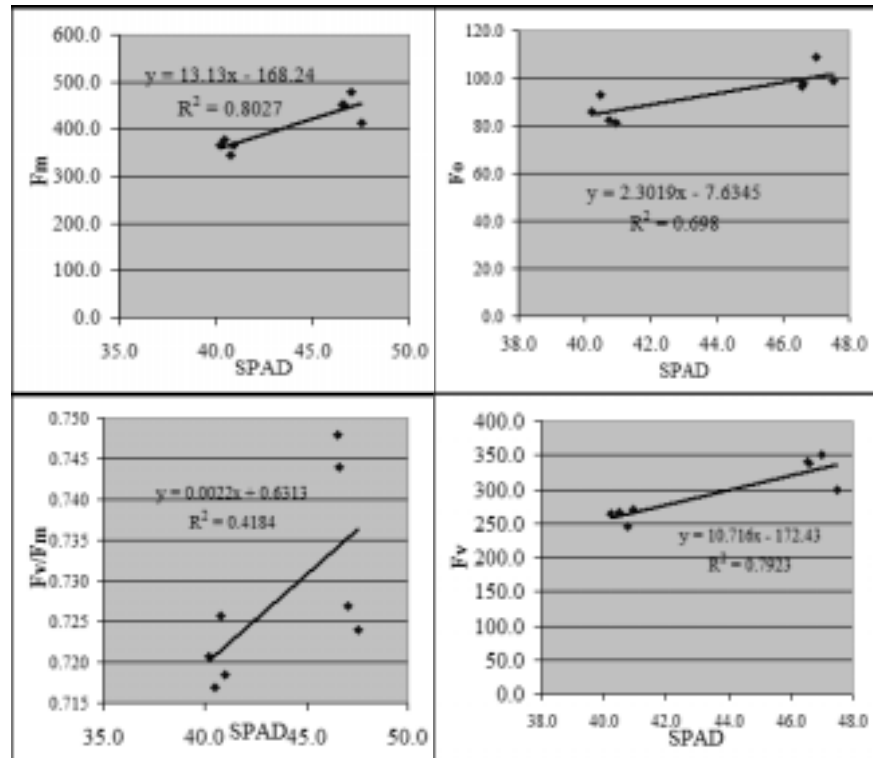


Fig2: Relationship between SPAD values and chlorofluorescence in *Brassicas*.

of study (except for Fv/Fm and Fv/Fo). Interaction (G x Y) was significant only for electron transport rates. Mean increase in the cultivars was recorded for Fo (27.6 %), Fm (26.6%) and Fv (22.8 %) over the 1st year of study. Mean photochemical efficiency of PSII in the cultivars declined by 4.43% (Fv/Fm), 20.8% (Fv/Fo) and 23.0% (ETR) during 2012-13 as compared to 1st year of study revealing less damage than the normal range (0.75-0.82), efficient osmotically driven water uptake and electron transport rate. Linear regression between different components of chlorofluorescence (Fig 1) and SPAD chlorophyll values (Fig 2) indicated strong relationship between them. It is possible to use fluorescence parameters for evaluating yield of *Brassica* cultivars under field, because the present results showed regular variations in the fluorescence parameters.

Correlations studies: Maximal fluorescence (Fm) showed positive association with Fo (0.904**). Fv/Fm and Fm were positively associated (0.671**). Variable fluorescence (Fv) exhibited positive association with Fo (0.849**), Fm (0.995**) and Fv/Fm (0.726**). Positive relation was also evident between Fv/Fo and Fm (0.538*) and also Fv/Fo and Fv/Fm (0.638**). SPAD chlorophyll value was positively correlated with the Fo (0.835**), Fm (0.895**), Fv/Fm (0.632**) and Fv (0.890** Table 5). Leaf chlorophyll content is often well correlated with leaf photosynthetic rates (Seeman *et al.*, 1987). Correlation between SPAD and Fv/Fm values as means of PSII efficiency are limited (Percival *et al.*, 2008). A negative association existed between SY and Fo (-0.677**), Fm (-0.829**), Fv (-0.851**), implying increase in these parameters of fluorescence declined the yield. However positive relation was observed between SY and Fv/Fm (0.268), Fv/Fo (0.389) and ETR (0.012). Genotypes like RLC1 and Hyola PAC401 showed less disruption in photochemical efficiency of PSII and electron transport rate and were higher yielders. These are the two sensitive parameters prone to environmental stresses. SPAD values were also positively correlated with SY (0.636**). Except for Fo, there was a high correlation between grain yield and other fluorescence parameters in wheat (Paknejad *et al.*, 2007). However, in study

of Arous *et al.* (1998) Fo with Fv and Fm had the strongest correlation with grain yield, while a weak correlation for Fv/Fm. This is contrary to the present findings and those of Arous *et al.* (1998) in field conditions. Moffat *et al.* (1990) reported a negative correlation between grain yield and Fv in wheat plants. Similar results were also observed in the present investigation. Present study revealed that fast fluorescence parameters could be used as valuable measures to determine the impact of environment on variation in productivity under field conditions. Fv/Fm, Fv/Fo and ETR appeared more suitable criteria amongst the studied parameters and non-significant interactions between genotypes and year showed similar response during the experimental period.

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