[Now published in full after the initial publication as an Epub ahead of print on Sept 15, 2020, at www.fluorideresearch.online/epub/files/100.pdf]

COMPARATIVE ANALYSIS OF FLUORIDE INHIBITION OF PHOTOSYNTHESIS IN C_3 (WHEAT) AND C_4 (MAIZE) PLANTS

Bhupendra Singh,[a] Anjana Jajoo[b,*]

Indore, India

ABSTRACT: Effects of fluoride (F) on photosynthetic activity of maize and wheat plants were studied by analyzing chlorophyll *a* (Chl *a*) fluorescence induction kinetics. The results revealed that F affects the overall primary photochemistry by inhibiting the number of active reaction centres (RC) of Photosystem II (PSII). However, the efficiency of each of the active RC is not affected. As compared to wheat (C_3 plant), photosynthesis of maize (C_4 plant) was relatively more inhibited by F toxicity. Results from the present investigation strongly indicate that the most significantly changed Chl *a* fluorescence parameters could be used as an efficient tool for the diagnosis of F toxicity in maize. The present findings may be helpful to select suitable crops in F endemic areas.

Keywords: Chlorophyll *a* fluorescence; Maize; Photosynthesis; Photosystem II; Wheat.

INTRODUCTION

Fluoride (F) exists in soil, air, and water, in varying amounts, naturally and/or due to diverse industrial activities.[1,2] Chronic exposure to F can be toxic and cause varying degrees of pathological changes in humans[3,4] and domestic animals.[5-8] F exposure also induces various pathological changes in plants.[9] However, very limited research work has been conducted so far on F exposure in relation to morphological, biochemical, photochemical, and physiological alterations in plants.[9]

C_4 plants such as maize, sorghum, and sugarcane, have an approximately 50% higher photosynthesis efficiency than C_3 plants such as rice, wheat, and potato.[10] Photosynthesis is a sensitive and vital process which has an important affect on crop yield and is significantly influenced by any kind of environmental stress. Varying concentrations of F cause reduction in chlorophyll content in *Cyamopsis tetragonoloba (*cluster bean),[11] *Oryza sativa* (rice),[12] and *Citrullus lanatus* (watermelon) [13] which ultimately reduce the photosynthetic efficiency under F stress. It has been reported that crops and crop varieties respond differently to increased soil F concentration and accumulate differential F amounts in their vegetative and reproductive parts.[14] The toxicity of F adversely affects germination, growth, mineral nutrition, photosynthesis, breathing, cell enzyme activity, and crop yield.[15] However, the mechanism of F toxicity in plants is still unclear. Moreover, no comparative study has yet been performed on effects of F on C_3 (wheat) and C_4 (maize) plants. Therefore, in the present study, chlorophyll *a* (Chl *a*) fluorescence kinetics were measured to evaluate the efficiency of various components involved in the photosynthesis process. Chl *a* fluorescence analysis provides relevant information about the physiology of plants growing under abiotic stress and is particularly suitable as an indicator of photosystem II (PSII) efficiency.[16]

MATERIAL AND METHODS

Growth conditions: Wheat (*Triticum aestivum*; Purna HI 1544) and maize (*Zea mays*; Ganga safed) cultivars were used as plant material. Five uniform seeds were

[a]School of Life Science, Devi Ahilya University, Indore 452017, India; [b]School of Biotechnology, Devi Ahilya University, Indore 452017, India; *For correspondence: Dr Anjana Jajoo, Professor, School of Biotechnology, Devi Ahilya University, Indore 452017, India; E-mail: anjanajajoo@hotmail.com

[Now published in full after the initial publication as an Epub ahead of print on Sept 15, 2020, at www.fluorideresearch.online/epub/files/100.pdf]

sown in each pot and allowed to germinate in black polyethylene bags having 3:1 ratio of soil and compost. The pots were irrigated with F solutions by using sodium fluoride (NaF) for treatment. Concentration of NaF (400 mg/L) was selected after performing initial preliminary experiment of concentration response (data not shown). Tap water was used for irrigation in control plants. Four pots were kept for each treatment and experiment was done thrice. After 35 days of cultivation various measurements were performed. The experiments were performed in natural conditions at the botanical garden of School of Life Science.

Measurement of fluorescence induction kinetics: The Chl *a* fluorescence induction kinetics was measured at room temperature using a Plant Efficiency Analyzer (PEA, Hansatech, King's Lynn, Northfolk, England). Control leaves exhibited a polyphasic rise called O–J–I–P Chl *a* fluorescence transient; the O to J phase (ends at ~2 ms), the J to I phase (ends at ~30 ms), and I to P phase (ends at ~500 ms). The JIP test was named after the basic steps in the fluorescence transient, when plotted on a logarithmic time scale.[17] Plants were dark adapted for 15 min before measurements. Parameters (O-J-I-P-parameters) were calculated based on induction curves of fast chlorophyll fluorescence. The energy pipeline model was prepared using the Biolyzer HP 3 software (the Bioenergetics Laboratory's chlorophyll fluorescence analysis program, University of Geneva, Switzerland). [15]

PI was calculated as shown in Equation 1:

$$PI_{ABS} = \frac{RC}{ABS} \times \frac{\phi Po}{1 - \phi Po} \times \frac{\psi o}{1 - \psi o} \quad \text{.....Equation 1}$$

Where:

PI = performance index
RC = reaction centre
ABS = absorption
ϕPo = exciton trapped per photon absorbed
ψo = the probability that an electron can move further than Q_A[18]

Total chlorophyll content measurement: Measurement of total chlorophyll content (in SPAD unit) was done using at Leaf SPAD chlorophyll meter.[19]

Statistical analysis: Data was analyzed by using Graphpad Prism 5.01 software, Inc. La Jolla, CA, USA. Results were analyzed using an unpaired two-sided t-test. Significance was determined at $p<0.05$ and statistical analyses were performed using Microsoft Excel 2007. All the assays were carried out in replicates (three to four sets of each analysis).

Descriptions of the various terms used in the fluorescence induction curves are shown in Table 1.

[Now published in full after the initial publication as an Epub ahead of print on Sept 15, 2020, at www.fluorideresearch.online/epub/files/100.pdf]

Table 1. Descriptions of the various terms related to the fluorescence induction curves

Parameter	Description
Fv/Fm	Maximum quantum efficiency of PSH photochemistry
Fv/Fo	Water-splitting complex of PSH
Fo	Minimal fluorescence
Fm	Maximal fluorescence
DF	Driving force which quantifies the potential of plant photosynthesis
Vj	Relative variable fluorescence
1 – Vj	Efficiency of trapped electron by which it can move ahead of Q_A^- (Primary plastoquinone) is equal to (1 –Vj)
RC/ABS	Density RCs per chlorophyll
Area	Reflection of the size of the plastoquinone pool

RESULTS

In the present study, we have observed that the chlorophyll concentration was found to be reduced in both plants treated with F. However, it was relatively more prominent in maize as compared to wheat plant (Figure 1).

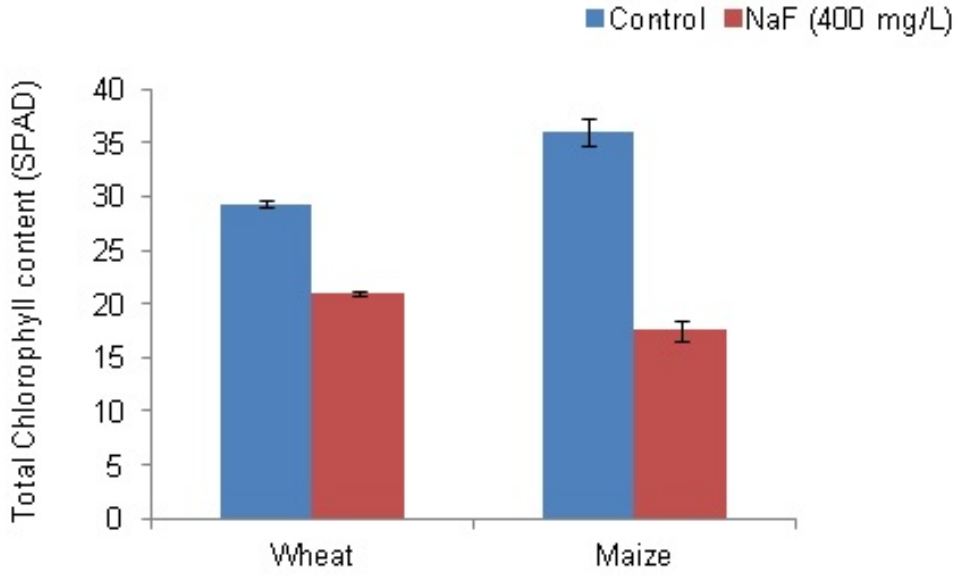


Figure 1. Total chlorophyll content of wheat and maize in control and fluoride treated plants.

The Chl *a* fluorescence induction curves of maize and wheat plants after F treatment can be seen in Figures 2A and 2B.

[Now published in full after the initial publication as an Epub ahead of print on Sept 15, 2020, at www.fluorideresearch.online/epub/files/100.pdf]

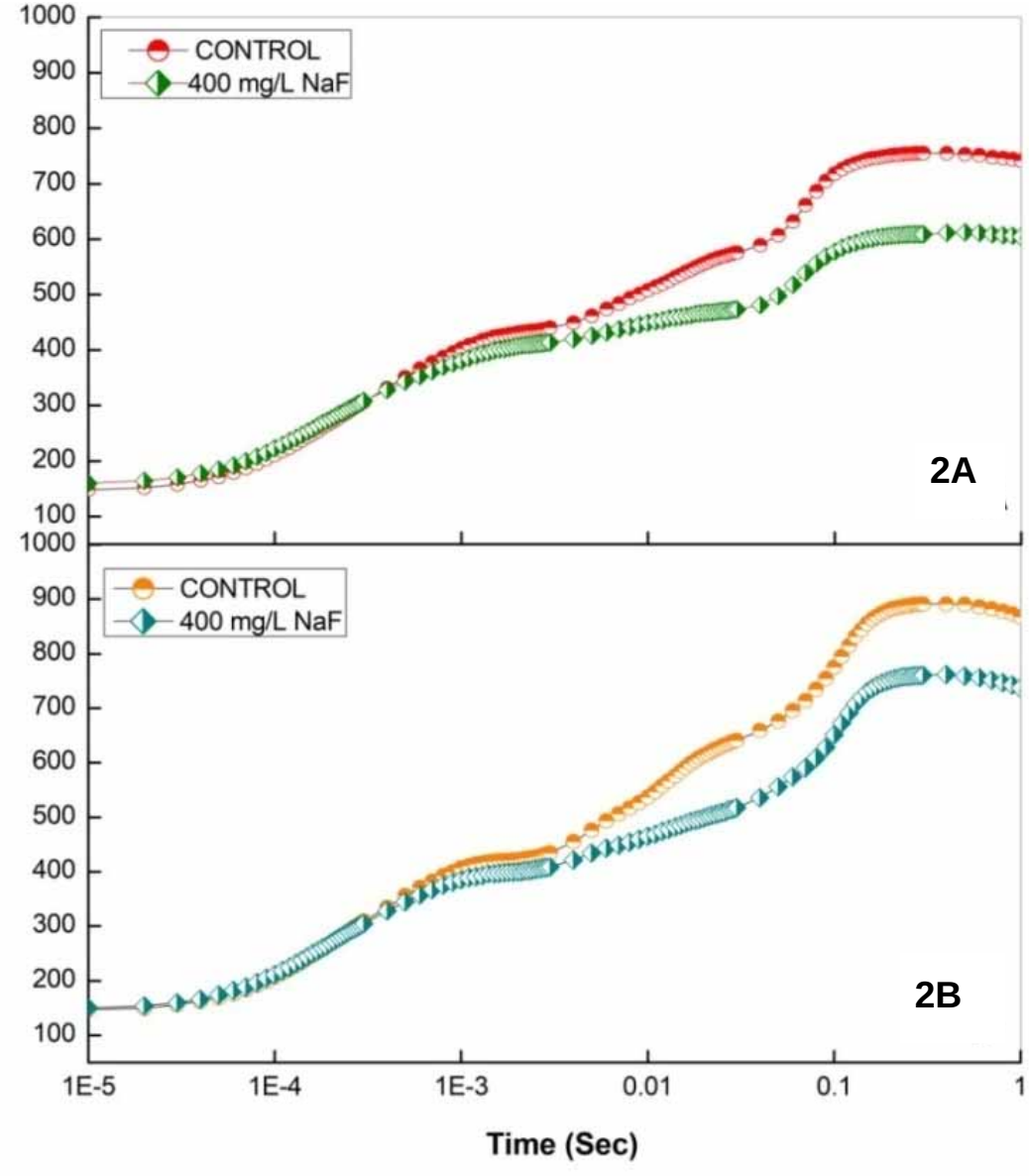


Figure 2. The OJIP Chl *a* fluorescence transient curve (log time scale) in (2A) maize and (2B) wheat leaves in control and fluoride treated plants.

The plants exhibit a polyphasic rise called O-J-I-P fluorescence transient. The shape of the O-J-I-P fluorescence rise has been related to a major change in the photosynthetic electron transport.[20] The intensity of fluorescence in the O-J-I-P transient decreased with F treatment. Various parameters were calculated from these curves (Tables 2A and 2B). The maximum quantum yield of PSII was measured by taking ratio of variable fluorescence to maximal fluorescence (Fv/Fm). After F treatment, value of Fv/Fm in maize plant decreased by 8% and in wheat plants by 4%. The value of ratio of variable fluorescence to initial fluorescence (Fv/Fo) which deals with status of water splitting complex of PSII decreased by 33.6% in maize

[Now published in full after the initial publication as an Epub ahead of print on Sept 15, 2020, at www.fluorideresearch.online/epub/files/100.pdf]

plant and by 21.4% in wheat after treatment with F. Density of active RCs per chlorophyll (RC/ABS) decreased by 25.4% in maize plant and by 16.7 in wheat plant when treated with 400 mg/L of F. Changes in the value of 1-V_j were observed more in maize (14.5%) as compared to wheat (6%) with F treatment. Present study revealed that F treatment caused about 57.5% reduction in Performance Index (PI) on absorption basis in maize and 38.2% reduction in wheat plant.

Table 2A. Effect of fluoride on Chl *a* fluorescence parameters in maize plants. (Values are mean±SD)

Treatment	Parameter				
	Fv/Fm	RC/ABS	Fv/Fo	(1-Vj)	$PI_{(abs)}$
Control	0.820±0.006 (100%)	0.742±0.04 (100%)	4.551±0.20 (100%)	0.523±0.02 (100%)	15.73±0.23 (100%)
400 mg/L NaF	0.752±0.004* (91.7%)	0.554±0.03* (74.6%)	3.026±0.06* (66.4%)	0.446±0.03* (85.5%)	6.70±0.12* (42.5%)

* Compared to the control group: p<0.0001

Table 2B. Effect of fluoride on Chl a fluorescence parameters in wheat plants. (Values are mean±SD)

Treatment	Parameter				
	Fv/Fm	RC/ABS	Fv/Fo	(1-Vj)	$PI_{(abs)}$
Control	0.850±0.001 (100%)	0.918±0.05 (100%)	5.669±0.04 (100%)	0.618±0.01 (100%)	28.99±0.26 (100%)
400 mg/L NaF	0.817±0.001* (96.0%)	0.765±0.01* (83.3%)	4.458±0.03* (78.6%)	0.581±0.004* (94.0%)	17.92±0.04* (61.8%)

*Compared to the control group: p<0.0001

An analysis of Chl *a* fluorescence parameters can be done by making leaf model using biolyzer HP3 software (Figure 3).The electron transport in PSII cross-section (ETo/CSm), the absorption flux per cross-section (ABS/CSm), and the trapped energy flux per PSII cross-section (TRo/CSm) were decreased with F treatment in both plants, but more remarkably in maize. However, dissipation per cross-section (DIo/CSm) increased with F treatment more in maize as compared to wheat. In addition, the density of active RCs, as indicated by the number of open circles, was also reduced by F treatment in both plants while an increase was observed in the

[Now published in full after the initial publication as an Epub ahead of print on Sept 15, 2020, at www.fluorideresearch.online/epub/files/100.pdf]

number of dark circles which represent the inactive PSII centres. On F treatment, there was an increase in the number of inactive PSII centres more prominently in maize.

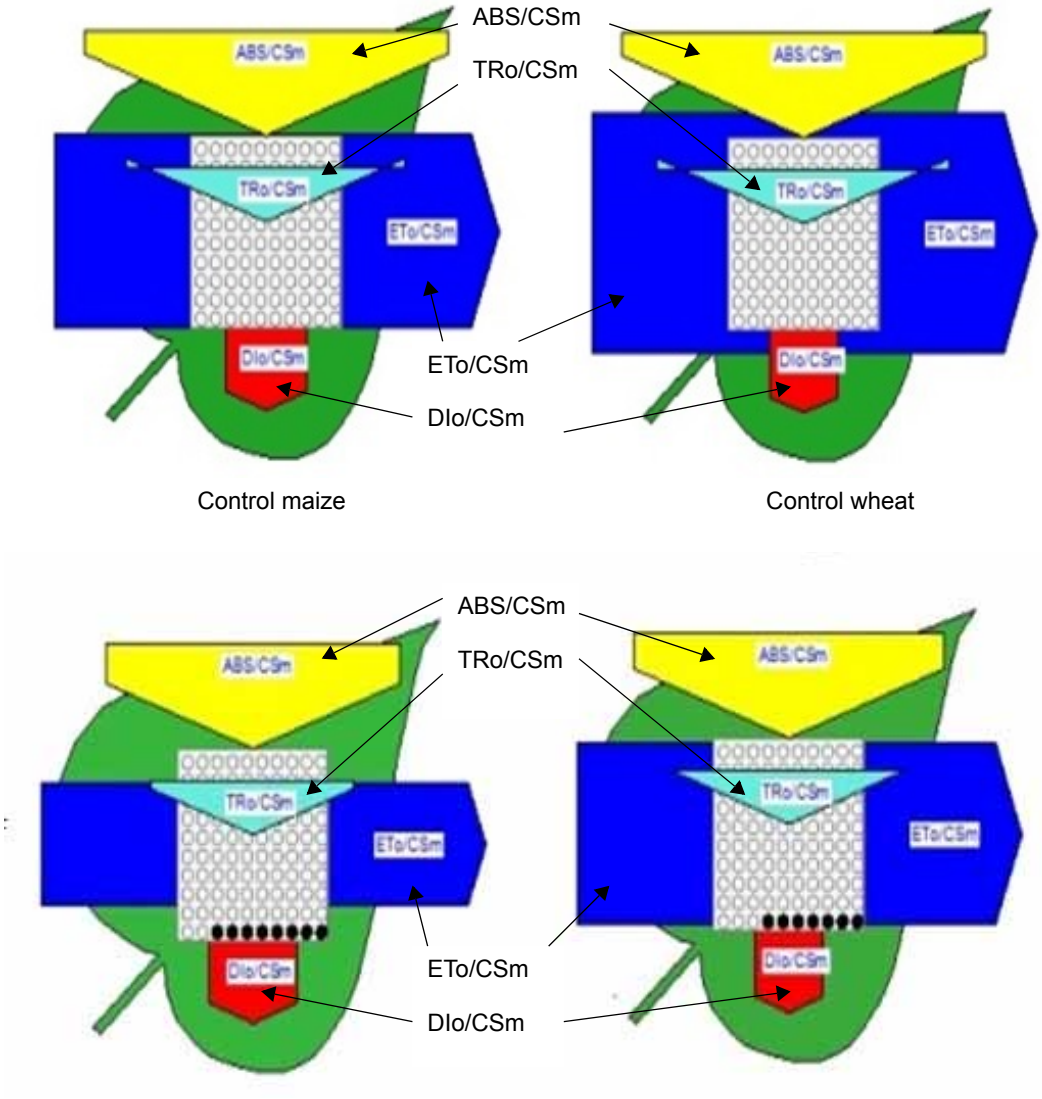
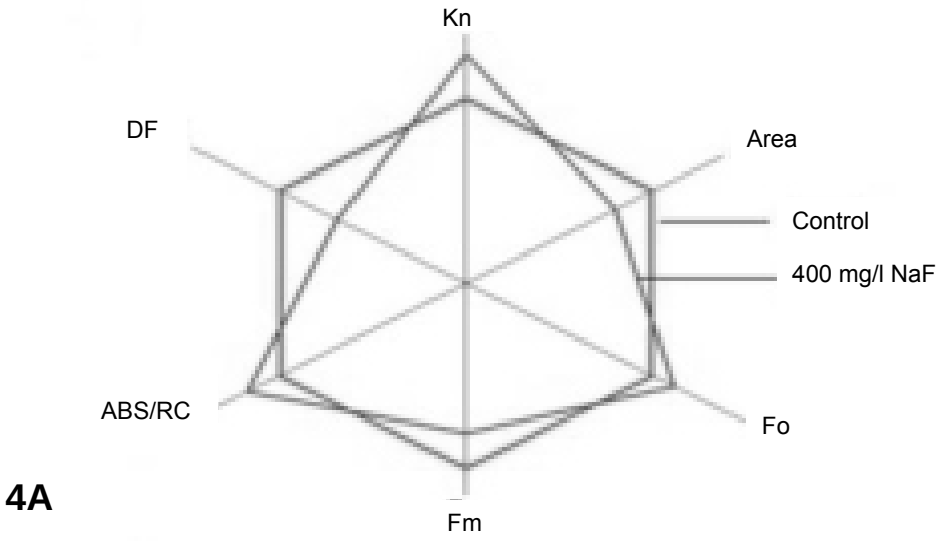


Figure 3. Leaf pipeline model calculated per cross section in control and fluoride-treated maize and wheat plants. Width of the corresponding arrow denotes activity of that parameter. Empty and filled black circles indicate active and non-active reaction centres of PS-II, respectively. ABS/CSm=absorption flux per cross-section; TRo/CSm=trapped energy flux per PSII cross-section; ETo/CSm=electron transport in PSII cross section; and DI/CSm=dissipation per cross-section.

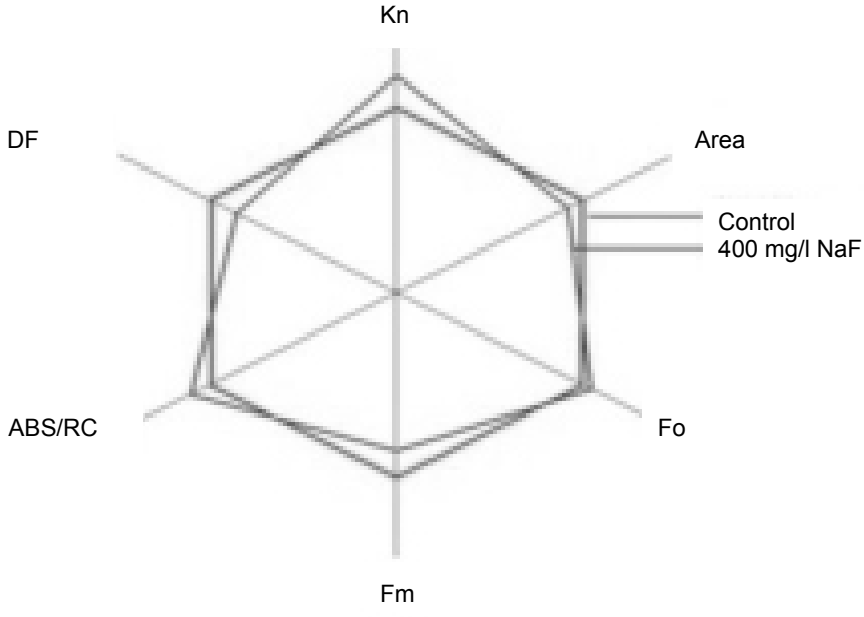
Spider plot of chosen calculated Chl *a* fluorescence parameters of maize and wheat plants was prepared (Figures 4A and 4B). Initial fluorescence (Fo) increased by F treatment by 12% in maize and 4.5% in wheat. Maximal fluorescence (Fm) which indicates that PSII donor side damage declined with F treatment by 19% in maize and 14.6% in wheat. Area (the area over the fluorescence curve between Fo and Fm) decreased in maize and wheat by 20% and 8%, respectively, after F treatment. The driving force (DF)[18] which quantifies the potential of plant photosynthesis decreased

[Now published in full after the initial publication as an Epub ahead of print on Sept 15, 2020, at www.fluorideresearch.online/epub/files/100.pdf]

with F treatment in both plants. Non-photochemical de-excitation rate constant (Kn) increased by 23.3% in maize and by 7% in wheat after treatment of F. With F treatment, ABS/RC ratio, which is dependent on the ratio of active to nonactive reaction centers (RCs), increased more in maize than in wheat, indicating a decrease in active RCs antenna size. Area, DF, Kn, and Fm were the most affected parameters in both plants but more prominently in maize as clearly evident from spider plot.



4A



4B

Figures 4A and 4B. Radar plot of chosen calculated chlorophyll a fluorescence parameters quantifying the activity of PSII in control and fluoride treated (4A) maize and (4B) wheat. Kn=non-photochemical de-excitation rate constant; DF=driving force; ABS/RC=absorption per reaction centre; Fm=maximal fluorescence intensity; Fo=minimal fluorescence intensity; Area=reflection of the size of the plastoquinone pool.

DISCUSSION

Measurement of total chlorophyll content is an indicator of the photosynthetic capacity of a plant. F may reduce chlorophyll by entering the chloroplasts in the form of F ions, which can bind to the central complexed Mg^{2+} in the porphyrin ring, thereby undermining the chlorophyll molecules and resulting in decreased chlorophyll content.[21] Other possible causes for the decreasing of the pigment content may be break down of chlorophyll, inhibition of chlorophyll biosynthesis,[12] stress-induced increase in the activity of the chlorophyll degrading enzyme chlorophyllase,[22] and F-induced reduction in Fe^{+2} which is essential for chlorophyll biosynthesis (Figure 1).[23] The effect of F on the shape of Chl *a* fluorescence transient is very clear (Figure 2). A decrease in F_v / F_m ratio suggested that F decreased the quantum efficiency of PSII photochemistry either by causing a decrease in the rate of primary charge separation or by disconnection of some minor antenna from PSII.[24,25] F caused decrease in active PSII RCs followed by decrease in size of the chlorophyll antenna serving each RC and the reaction center density.[27,28] In the present study, changes in the value of 1–Vj were observed more in F treated maize as compared to wheat plant. This result suggested that F affects re-oxidation capacity of Q_A^- and electron transport at the acceptor side of PSII.[29] The PI combines three independent functional steps of photosynthesis, the density of RCs in the chlorophyll bed, excitation energy trapping, and conversion of excitation energy to electron transport, into a single multi-parametric expression.[30, 31] This is a function of ψ, ϕPo, and RC/ABS.[32] The PI decreased due to reduced vitality with F treatment leading to a decrease in ϕPo. The ϕPo is a parameter that expresses maximum efficiency of PSII, which is controlled by the primary photochemistry of PSII, non-radiative loss of excited states in light-harvesting antennae, and excited states quenched by oxidised PQ (Plastoquinone) molecules from the PQ pool.

The leaf model of phenomenological energy fluxes per cross-section was made to visualize the derived parameters from the Chl *a* fluorescence induction curve (Figure 3). This model gives information about the efficiency of flow of energy from antennae to the electron transport chain components through the cross-section of PSII. [33] The ETo/CSm, ABS/CSm, and TRo/CSm decreased with F treatment which is more remarkable in maize. ETo/CSm decreased due to lower energy absorption by antenna pigments (ABS/CSm), lower energy trapping by RCs (TRo/CSm), and higher energy loss as heat (DIo/CSm). The decrease in TRo/CSm is mainly due to the decrease in the density of the active RCs.This is an indication that the major down regulation of PSII is accomplished by the inactivation of the RCs. However, DIo/CSm increased which indicates an increase in the dissipation of absorbed energy in non-photochemical form.

The relative values of selected expressions can be plotted in the form of spider plot. This plot provides a direct visualisation of the behaviour of a sample and thus facilitates comparison of control and treated samples (Figure 4). Fo is an indicator for irreversible damage in PSII, associated with light harvesting complex II (LHCII) dissociation and blocking of electron transfer on the reducing side of PSII.[34] Area is proportional to the pool size of the electron acceptor Q_A on the reducing side of PSII and also secondary plastoquinone (Q_B), PQ, and Photosystem I (PSI) acceptors.[18] The DF which quantifies the potential of plant photosynthesis decreased in maize and

wheat plants with F treatment.[32] The fluorescence parameter Kn that is equivalent to non-photochemical de-excitation constant for photochemistry, is a qualitative indicator of rate constant and was found to decrease after F treatment. ABS/RC is affected by the active/inactive RC ratio and more inactive centres were observed in maize after F treatment. Fluoride treatment disturbs photochemistry of PSII resulting in more inactive centres. Photosystem I (PS I) is more tolerant as compared to PSII.[35] This study will contribute to understanding of basic photosynthetic mechanisms affected by F in crop plants and will also be helpful in the selection of suitable crops for F endemic areas.

ACKNOWLEDGMENT

The first author is grateful to the University Grant Commission, New Delhi, India, for financial assistance (F1-17.1/2016-17/RGNF-2015-17-SC-MAD-9395).

REFERENCES

1 Choubisa SL. Fluoride distribution in drinking groundwater in Rajasthan, India. Curr Sci 2018;114(9):1851-7.
2 Choubisa SL, Choubisa D. Status of industrial fluoride pollution and its diverse adverse health effects in man and domestic animals in India. Environ Sci Pollut Res 2016;23(8):7244-54.
3 Choubisa SL, Choubisa DK, Joshi SC, Choubisa L. Fluorosis in some tribal villages of Dungarpur district of Rajasthan, India. Fluoride 1997;30(4):223-8.
4 Choubisa SL. Endemic fluorosis in southern Rajasthan (India). Fluoride 2001;34(1):61-70.
5 Choubisa SL. Some observations on endemic fluorosis in domestic animals of southern Rajasthan (India). Vet Res Commun 1999;23(7):457-65.
6 Choubisa SL. Osteo-dental fluorosis in horses and donkeys of Rajasthan, India. Fluoride 2010;43(1):5-10.
7 Choubisa SL. Fluorosis in dromedary camels of Rajasthan, India. Fluoride 2010;43(3):194-9.
8 Choubisa SL. Fluoride toxicosis in immature herbivorous domestic animals living in low fluoride water endemic areas of Rajasthan, India: an observational survey. Fluoride 2013;46(1):19-24.
9 Baunthiyal M, Ranghar S. Physiological and biochemical responses of plants under fluoride stress: an overview. Fluoride 2014;47(4):287-93.
10 Kajala K, Covshoff S, Karki S, Woodfield H, Tolley BJ, Dionora MJ, et al. Strategies for engineering a two-celled C_4 photosynthetic pathway into rice. J Exp Bot 2011;62(9):3001-10.
11 Sabal D, Khan TI, Saxena R. Effect of sodium fluoride on cluster bean (*Cyamopsis tetragonoloba*) seed germination and seedling growth. Fluoride 2006;39(3):228-30.
12 Gupta S, Banerjee S, Mondal S. Phytotoxicity of fluoride in the germination of paddy (*Oryza sativa*) and its effects on the physiology and biochemistry of germinating seedlings. Fluoride 2009;42(2):142-6.
13 Ram A, Verma P, Gadi BR. Effect of fluoride and salicylic acid on seedling growth and biochemical parameters of watermelon *(Citrullus lanatus)*. Fluoride 2014;47(1):49-55.
14 Jothimani P, Pandian BJ. Assessing the fluoride contamination in the groundwater of Tiruppur district, Tamil Nadu. Madras Agric J 2017;104(7-9):235-41.
15 Panda D. Fluoride toxicity stress: physiological and biochemical consequences on plants. Int J Bio-res Env Agril Sci 2015;1:70-84.
16 Kalaji HM, Schansker G, Ladle RJ, Goltsev V, Bosa K, Allakhverdiev SI, et al. Frequently asked questions about *in vivo* chlorophyll fluorescence: practical issues. Photosynth Res 2014;122(2):121-58.
17 Force L, Critchley C, van Rensen JJ. New fluorescence parameters for monitoring photosynthesis in plants. Photosynth Res 2003;78(1):17-33.

[Now published in full after the initial publication as an Epub ahead of print on Sept 15, 2020, at www.fluorideresearch.online/epub/files/100.pdf]

18 Strasser RJ, Tsimilli-Michael M, Srivastava A. Analysis of the chlorophyll *a* fluorescence transient. In: Papageorgiou G, Govindjee, editors. Advances in photosynthesis and respiration: chlorophyll fluorescence a signature of photosynthesis. Netherlands: Kluwer Academic Publishers; 2004. pp. 321-62.

19 Mathur S, Sharma MP, Jajoo A. Improved photosynthetic efficacy of maize (*Zea mays*) plants with arbuscular mycorrhizal fungi (AMF) under high temperature stress. J Photochem Photobiol B: Biol 2018;180:149-54.

20 Joly D, Carpentier R. Sigmoidal reduction kinetics of the photosystem II acceptor side in intact photosynthetic materials during fluorescence induction. Photochem Photobiol Sci 2009;8(2):167-73.

21 Weinstein LH, Davison AW. Fluorides in the environment: effects on plants and animals. Wallingford, Oxon, UK: CABI Publishing, CAB International; 2004.

22 Noreen Z, Ashraf M. Changes in antioxidant enzymes and some let metabolites in some genetically diverse of radish (*Raphanus sativus* L.). Environ Exp Bot 2009;67(2):395-402.

23 Elloumi N, Abdallah FB, Mezghani I, Rhouma A, Boukhris M. Effect of fluoride on almond seedling in culture solution. Fluoride 2005;38(3):193-8.

24 Kalaji HM, Bosa K, Kościelniak J, Żuk-Gołaszewska K. Effects of salt stress on photosystem II efficiency and CO_2 assimilation of two Syrian barley landraces. Environ Exp Bot 2011;73:64-72.

25 Tomar RS, Jajoo A. Fluoranthene, a polycyclic aromatic hydrocarbon, inhibits light as well as dark reactions of photosynthesis in wheat (*Triticum aestivum*). Ecotoxicol Environ Saf 2014;109:110-15.

26 Zhang L, Li Q, Ma L, Ruan J. Characterization of fluoride uptake by roots of tea plants (*Camellia sinensis* (L.) O. Kuntze). Plant soil 2013;366(1-2):659-69.

27 Mathur S, Kalaji HM, Jajoo A. Investigation of deleterious effects of chromium phytotoxicity and photosynthesis in wheat plant. Photosynthetica 2016;54(2):185-92.

28 Christen D, Schönmann S, Jermini M, Strasser RJ, Défago G. Characterization and early detection of grapevine *(Vitis vinifera)* stress responses to esca disease by in situ chlorophyll fluorescence and comparison with drought stress. Environ Exp Bot 2007;60(3):504-14.

29 Tomar RS, Jajoo A. A quick investigation of the detrimental effects of environmental pollutant polycyclic aromatic hydrocarbon fluoranthene on the photosynthetic efficiency of wheat (*Triticum aestivum*). Ecotoxicology 2013;22(8):1313-18.

30 Strasser RJ, Srivastava A, Tsimilli-Michael M. Screening the vitality and photosynthetic activity of plants by fluorescence transient. In: Behl RK, Punia MS, Lather BPS, editors. Crop improvement for food security. Hisar India: SSARM;1999. pp.126.

31 Tsimilli-Michael M, Eggenberg P, Biro B, Köves-Pechy K, Vörös I, Strasser RJ. Synergistic and antagonistic effects of arbuscular mycorrhizal fungi and Azospirillum and Rhizobium nitrogen-fixers on the photosynthetic activity of alfalfa, probed by the polyphasic chlorophyll *a* fluorescence transient OJIP. Appl Soil Ecol 2000;15(2):169-82.

32 Srivastava A, Strasser RJ, Govindjee. Greening of peas: parallel measurements of 77 K emission spectra, OJIP chlorophyll *a* fluorescence transient, period four oscillation of the initial fluorescence level, delayed light emission, and P700. Photosynthetica 1999;37(3):365-92.

33 Li X, Cai J, Liu F, Dai T, Cao W, Jiang D. Cold priming drives the sub-cellular antioxidant systems to protect photosynthetic electron transport against subsequent low temperature stress in winter wheat. Plant Physiol Biochem 2014;82:34-43.

34 Costa ES, Bressan-Smith R, Oliveira JG, Campostrini E, Pimentel C. Photochemical efficiency in bean plants (*Phaseolus vulgaris L. and Vigna unguiculata L. Walp*) during recovery from high temperature stress. Braz J Plant Physiol 2002;14(2):105-10.

35 Singh-Rawal P, Jajoo A, Bharti S. Fluoride affects distribution of absorbed excitation energy more in favour of photosystem I. Biol plantarum 2010;54(3):556-60.