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Acclimation of chlorophyll fluorescence activities under drought stress in *Avena* species

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Abstract
Acclimation of chlorophyll fluorescence was investigated in seven oat species (*viz., Avena sativa, A. strigosa, A. brevis, A. vaviloviana, A. abyssinica, A. marocana* and *A. sterilis*) under drought stress environment. Significant variation was observed among the different species of oats in relation to various parameters of chlorophyll fluorescence under stress and non stress environment. The level of chlorophyll fluorescence (Fs) and maximal fluorescence (Fms) were significantly decreased in all the species except in *A. sterilis.* The quantum yield and elctron transport rate (ETR) also decreased under stress. The photochemical quenching (qp) was equal under stress and non stress environment. However, drought stress increased non-photochemical quenching of chlorophyll fluorescence (qN). The photochemical efficiency of PSII (Fv/Fm) under stress was less affected at vegetative stage but reduced at flowering stage. Minimum decrease in Fv/Fm was recorded in *A. abyssinica, A. sativa* and *A. sterilis* at vegetative stage and in *A. sterilis* at flowering stage indicating the less effect of stress environment on photochemistry of PS II which intern their drought stress tolerance. As the tolerance of PSII against drought is high, the fluorescence parameter Fv/Fm is useful for selection of genotypes for drought tolerance.

Keywords: *Avena* species, Chlorophyll fluorescence, Stress tolerance, Water deficit.

Introduction

Oat (*Avena sativa* L.) is the sixth most important cereal crop in the world (Oliver *et al.,* 2011). It is well adapted to a wide range of soil types and mostly grown in cool moist climates and they can be sensitive to hot dry weather between head emergence and maturity. In India, oat is mostly used as feeds of animals gown in winter in north western and central India. The crop occupies maximum area in Uttar Pradesh (34%) followed by Punjab (20%) (Pandey and Roy, 2011).

Drought stress is one of the major causes for crop loss worldwide, reducing average yields by more than 50%. (Wang *et al*., 2003). Drought leads to water deficit in plant tissues thus causing a significant reduction in photosynthesis rate. The ability to maintain the photosynthetic machinery functionality under water stress, therefore, is of major importance for drought tolerance. Plants react to water deficit with a rapid closure of stomata to avoid further water loss via transpiration (Cornic, 1994).

In recent years, the technique of chlorophyll fluorescence has become ubiquitous in plant ecophysiology studies. A number of reviews exist that discuss the theoretical background of both measurement and analysis (Horton and Bowyer, 1990; Krause and Weis, 1991; Govindjee, 1995). Fluorescence can provide insights into the ability of plant to tolerate those environmental stress into the extent to which those stresses have damaged the photosynthetic apparatus (Fracheboud *et al.*, 1999; Maxwell and Johnson, 2000). Many previous studies used a sustained decrease in the efficiency of excitation capture of open PSII in dark adapted leaves (Fv/Fm) and yield of energy conversion in light adapted leaves (Photosynthetic yield) as reliable indicator of photo inhibition of plant in response to stress (Seaton and Walker, 1990; Wagner and Dreyer, 1997; Wang and Kellomarl, 1997; Lu and Zhang, 1998). Study conducted by Yin *et al.* (2006) for *Populus Przewalski* on photosynthetic response to drought stress revealed that drought stress not only significantly decreased net P_N, transpiration rate (E), Stomatal conductance (gs), efficiency of photosystem II (Fv/Fm and yield) and increased intrinsic water use efficiency (WUE) under controlled optimal conditions, but also altered the diurnal gas exchange, chlorophyll fluorescence and WUE. Souza *et al.* (2004) evaluated the responses of photosynthesis to water stress in cowpea plants, both in terms of CO_2 assimilation, as measured by leaf gas exchanges, and of the functionality of the photosynthetic apparatus, as assessed by chlorophyll fluorescence measurements.

At an advanced phase of stress, a down regulation of PS II activity was observed along with some impairment of photochemical activity, as revealed by decreases in the maximum quantum yield of PS II (Fv/Fm).

Therefore, a better understanding of the mechanisms that enable plants to adapt to water stress while maintaining growth, development and productivity would help in screening drought resistant lines. Thus, the present investigation was undertaken to evaluate the promising species of oat (*Avena*) under water stress on the basis of chlorophyll fluorescence for the selection of drought tolerant species which can be used in oat improvement programme.

Materials and Methods

***Plant material and growth condition*:** Seed of seven oat species *viz*., *Avena strigosa* Schreb. (IG 03-543), *Avena brevis* Roth. (IG 03-471), *Avena vaviloviana* (Malzev) Mordv. (IG 03-548), Avena *abyssinica* Hochst. (IG 03-456), *Avena sativa* L. (JHO 822), *Avena marocana* G. (IG 03-486) and *Avena sterilis* L. (IG 03-529-1) were sown in porcelain pots (30 x 33 cm) containing garden soil at pot culture experimental site of IGFRI, Jhansi, India (25°27'N, 78°35'E, 275 m a.s.l.) during November to April, 2006-07. The soil was clay loam in texture, neutral in reaction (pH 6.57). The available nitrogen, phosphorus and potassium were 23.62 g m^{-2}, 1.36 g(P) m^{-2} and 27.92 $g(K_2O)m^{-2}$ respectively. The mean maximum and minimum temperature were 29.48 °C and 11.85 °C. The photo synthetically active-radiation (PAR) ranged from 1000 to 1580 µ mol m^{-2} s^{-1} during the growth period. After uniform germination three plants in each pot were maintained. After 15 days of germination one set of pot was kept under stress and another set of pots was watered regularly. The water stress was created by withholding of irrigation at vegetative and flowering stage of crop growth. After extreme stress, when wilting of leaves started, plants were re-watered (with irrigation at 100% field capacity). The crop was grown as per recommended agronomical practices.

***Fluorescence measurements*:** Chlorophyll fluorescence was measured by using Pulse Modulated, Field Portable Chlorophyll Fluorometer (OS5-FL, Optisciences, USA). The light and dark adapted parameters such as F_0, Fs, F_m, Fms, Fv, Ft and Foq were measured on attached dark adapted leaf and also in another second leaf lightened by constant actinic light. The fluorescence parameters determined on both light and dark adapted leaves, the following parameters were calculated: the maximal photochemical efficiency of PSII photochemistry (Fv/Fm), the photochemical quenching coefficient (qp=Fms-Fs/Fms-Fo), non-photochemical quenching coefficient (qN=Fm-Fms/Fm-Fo), the efficiency of excitation capture by open PSII centres (Fv/Fo), quantum yield of PSII (Y=Fms-Fs/Fms) and electron transport rate represents the apparent photosynthetic electron transport rate which calculated as (ETR=Y x PAR x 0.5 x 0.84). Photosynthetically active radiation **(**PAR) corresponds to the flux density of incident Photosynthetic Photon Flux Density (PPFD) [µmol quanta $m^{-2}s^{-1}$], transport of one electron requires absorption of two quanta, as two photo systems are involved (factor 0.5). It is assumed that 84% of the incident quanta are absorbed by the leaf (factor 0.84).

Results and Discussion

The chlorophyll fluorescence parameters like fluorescence under steady condition (F_s), maximum fluorescence under steady condition (F_{ms}), yield of quantum efficiency (Yield), electron transport rate (ETR), photochemical quenching (qP), photochemical efficiency of PSII (F_v/F_m) and the efficiency of excitation capture by open PSII centres (F_o/F_v) were significantly decreased under drought stress at vegetative and flowering stages of the crop growth in all *Avena* species. The Fs decreased by 11 to 52% and 1 to 31% under drought stress over the non stress at vegetative and flowering stages respectively (Table 1). The maximal fluorescence under steady state condition (Fms) also decreased 27 to 66% at vegetative stage and 21 to 36% at flowering stage in water stressed leaves over well watered leaves (Table 1). Minimum decrease was observed in *A. sterilis* as compared to other species tested. The yield of quantum efficiency (yield) significantly reduced in all the species as water stress was imposed at both the stage of crop growth (Fig. 1a). The decrease in yield ranged from 11.41% (*A. abyssinica*) to (77.65%) *A. sativa.* Under stress environment the electron transfer rate (ETR) also decreased, however the rate of decrease was pronounced in some species of *Avena*. Minimum decrease was observed in *A. sativa* (17.54%) followed by *A. brevis* (19.88%) and *A. sterilis* (20%) at vegetative stage (Fig.1b), however at flowering stage minimum decrease in ETR was observed in *A. brevis* (21.92%).

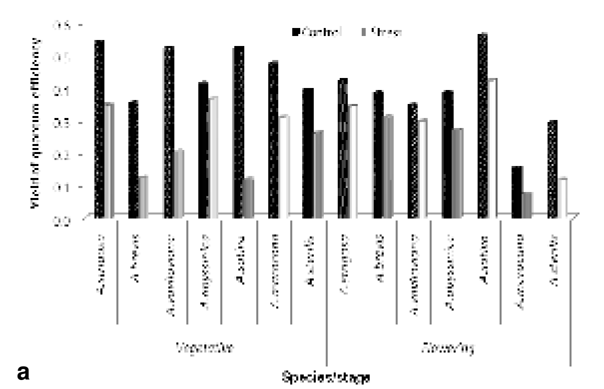
The photochemical efficiency of PS II (F_v/F_m) was less affected under water stressed environment at vegetative stage but reduced significantly at flowering stage of crop growth (Fig. 2a). Minimum decrease in F_v/F_m values under stress environment were observed in *A. abyssinica*

Table 1. Fluorescence (Fs) and maximal fluorescence (Fms) under steady state condition in *Avena* species at two growth stages as influenced by moisture stress.

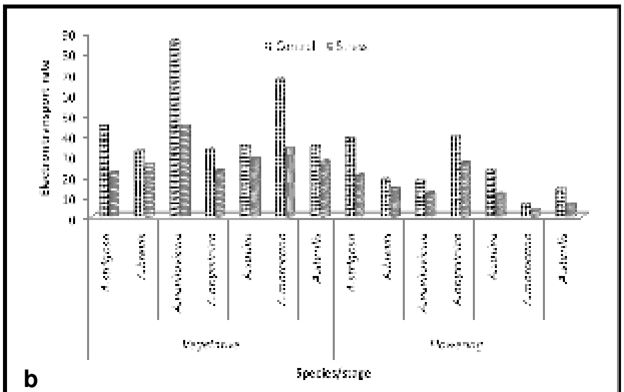
Species	FS (arbitrary *units)*				Fms (arbitrary *units*)			
	Vegetative		Flowering		Vegetative		Flowering	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
A. strigosa	392	188	220	181	852	288	380	265
A. brevis	260	136	147	108	401	155	283	206
A.vaviloviana	454	367	167	123	948	460	245	198
A. abyssnica	348	224	358	261	592	353	581	369
A. sativa	275	182	186	163	572	206	423	321
A. marocana	268	190	167	116	510	275	197	125
A. sterils	155	138	200	198	227	165	282	224
S (CD: P<0.05)	5.55		5.65		3.67		3.63	
T (CD: P<0.05)	2.96		3.02		1.96		1.94	
SxT (CD: P<0.05)	7.85		7.99		5.18		5.13	

(S-Species; T-Treatment; SxT-Species treatment)

(1.9%), followed by *Avena sativa* (3.9%), *A. sterilis* (4%), and *A. brevis* (7.5%) at vegetative stage and in *A. sterilis* (25.5%) at flowering stage. The F_v/F_0 which indicates efficiency of excitation energy capture by open PSII reaction centres was increased in *A. sterilis* and *A. sativa* under stress at vegetative stage while decreased significantly at flowering stage (Fig. 2b).

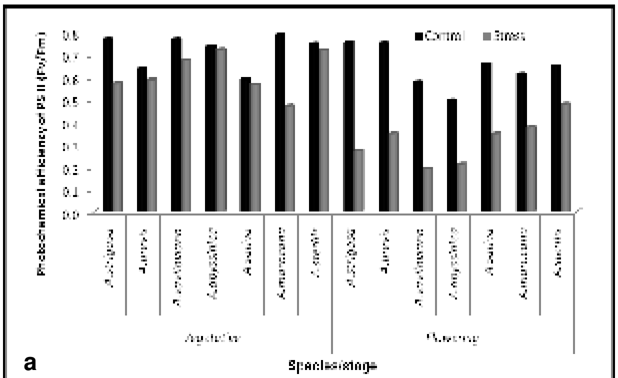


a

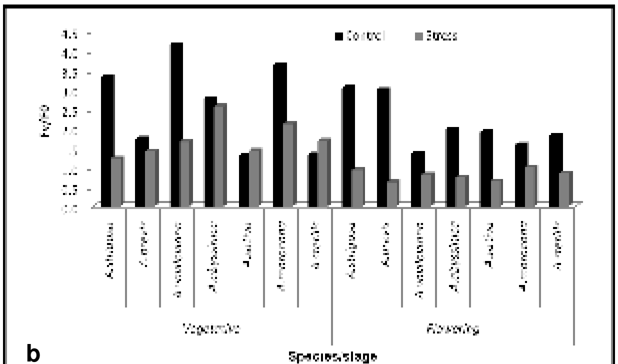


b

Fig 1. Yield of quantum efficiency (yield) (a) and electron transport rate (ETR) (b) as influenced by soil moisture stress in *Avena* species.



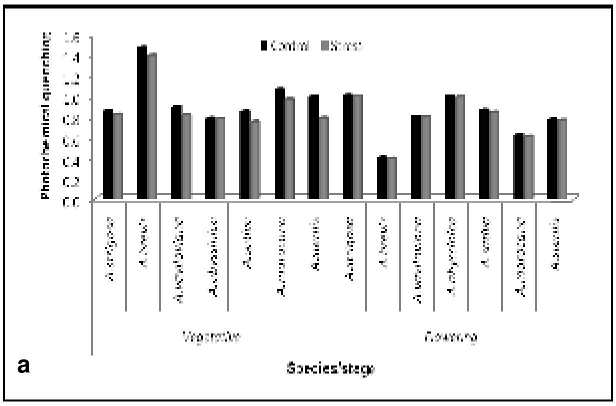
a



b

Fig 2. Photochemical efficiency of PSII (Fv/Fm) (a) and Fv/F0 (b) as influenced by soil moisture stress in *Avena* species.

Imposition of water stress had no significant effect on photochemical quenching (qP) of the *Avena* species (Fig. 3a). On average qP ranges from 0.984 in control to 0.901 in stressed environment at vegetative stage whereas at flowering stage the mean value rages from 0.781 in control to 0.772 in water stressed plants (Fig. 3a). The non-photochemical quenching (qN) increased significantly under water stress (Fig. 3b). Maximum increase in qN under stress environment was recorded in *A. sterilis* as the stress imposed at vegetative and flowering stage of crop growth.



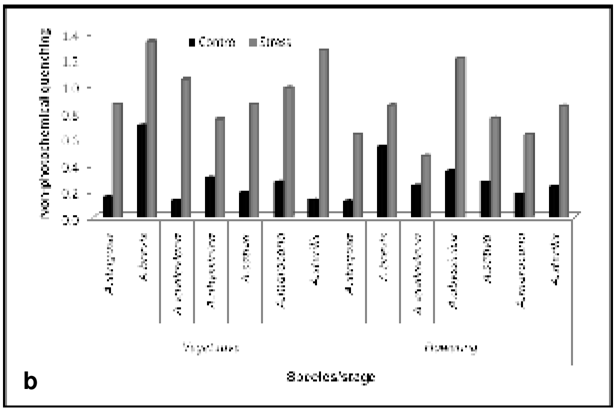


Fig 3. Photochemical quenching (qP) (a) and non-photochemical quenching (qN) (b) as influenced by soil moisture stress in *Avena* species.

In our experiment under stress condition ETR, yield, F_v/F_m and F_v/F_0 reduced significantly in all the species of *Avena*, but the reduction rates varied among species. The species *A. sterilis* showed the less reduction of these parameters under drought stress, that suggests the highest tolerance to drought stress. Decline in F_v/F_m ratio might be due to photoinhibition causing damage to PS II (Cao and Govindjee, 1990) combined with increase in energy dissipation in the chlorophyll pigment, antena system (Adams and Adams, 1996), the process aften observed in the plants under water stress environment. Reddy (2007) further reiterated that decreased value of F_v/F_m could also be due to the partial breakdown of the photosynthetic apparatus under water stress. Since water stress did not affect qP, the reduction in yield and ETR was mainly due to the reduction in F_v/F_0. We observed that reduction of qP was very less under stress whereas qN significantly increased which may be due to increase in dissipation of some exitation energy (Schindler and Lichtenthaler, 1996; Brestic *et al.,* 1995). Similar result were reported by Campos (1998) in *Vigna glabrescens* Flagella *et al.* (1994) and Lu and Zhang (1999) in wheat. The non-photochemical quenching of water stressed plants was significantly higher than that of well watered plants in all the species of *Avena*. An increase in qN induced by water stress was also reported in wheat (Lu and Zhang 1999). The increase in non-photochemical quenching of variable fluorescence was due to an increased rate constant of thermal dissipation of excitation energy and this increase represents a mechanism to down regulate photosynthetic electron transport and match utilization of NADPH and ATP under reduced photosynthesis (Lu and Zhang, 1999; Subrahmanyam and Rathore, 2000). The increase in nonphotochemical quenching could be connected against the damages in reaction centres of PS II, as it was shown by Golding and Johnson (2003).

The fluorescence measurements performed in our experiments revealed tolerant species similarly as it was found in wheat (Araus *et al.,* 1998; Lu and Zhang, 1998 &1999), tall fescue (Huang *et al.,* 1998). The drought is still seen as potent to cause over-reduction of the electron transport chain. However, plants may prevent this through the down regulation of the quantum efficiency of PSII electron transport (Cornic, 1994; Osmond, 1994; Lu and Zhang, 1998; Golding and Johnson, 2003).

Conclusion

The results revealed that genetic variability exists in *Avena* species with repect to drought tolerance using measurements of chlorophyll fluorescence. The species that better tolerate their tissue dehydration can be distinguished from sensitive ones based on the efficiency of electron transport in PS II. Mminimum decrease in Fv/Fm was recorded in *A. sterilis* indicating its drought stress tolerance. As the tolerance of PSII against drought is high, the fluorescence parameter Fv/Fm is useful for selection of genotypes for drought tolerance.

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