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Chlorophyll fluorescence response of wheat to exogenous application of growth regulators under terminal drought stress

Fluorescencja chlorofilu w odpowiedzi pszenicy na egzogenną aplikację regulatorów wzrostu w stresie suszy

ABSTRACT

Drought stress negatively affects plant photosynthesis and disturbs the electron transport activity. Evaluation of the chlorophyll fluorescence parameters might reflect influence of the environmental stress on plants and can be applied as an indicator of the primary photochemistry of photosynthesis. In current study the effect of foliar application of benzylaminopurine (BAP, a synthetic cytokinin) and abscisic acid (ABA) on chlorophyll fluorescence parameters of relatively drought tolerant (Pishtaz) and susceptible (Karaj3) bread wheat genotypes under well watered and terminal water deficit condition have been evaluated. Terminal drought was induced by withholding water at anthesis stage (Zadoks scale 65). Results showed that coefficient of non-photochemical quenching of variable fluorescence (q_N), quantum yield of PS II photochemistry (Φ_{PSII}) and photochemical quenching (qP) were affected by hormone spray treatments. So that evaluation of parameters at 7 day after foliar treatments revealed that ABA significantly increased electron transport rate (ETR) and q_N while considerably decreased Φ_{PSII}, g_s and maximum quantum yield of photosystem II (F_v/F_m). However exogenous application of cytokinin could increase g_s, F_v/F_m and Φ_{PSII} and the highest value of these parameters was recorded in *cytokinin* treated plants of Pishtaze cv. under well watered condition. Nevertheless, evaluation of the parameters in different periods after spraying showed that with approaching the maturity stage some traits like as g_s, Fv/Fm and ETR significantly decreased in both genotypes. Evaluation of g_s and Chlorophyll fluorescence parameters of genotypes between different irrigation levels showed that although cv. Pishtaz showed

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higher performance of PSII under well watered condition, it failed to maintain its superiority under stress condition. This finding suggests that some more responsive parameter like g_s, F_v/F_m and Φ_{PSII} can be considered as reliable indicator for understanding the biochemical and physiological effects of exogenous application of phytohormones under terminal drought stress.

Key words: abscisic acid, cytokinin, photosynthetic capacity, stomatal conductance, terminal water deficit

STRESZCZENIE

Stres suszy wpływa negatywnie na fotosyntezę roślin oraz zakłóca transport elektronów. Ocena parametrów fluorescencji chlorofilu może odzwierciedlać wpływ stresu środowiskowego na rośliny i może być stosowana jako wskaźnik pierwotnych reakcji fotochemicznych fotosyntezy. W prezentowanych badaniach oceniano wpływ dolistnego stosowania benzyloaminopuryny (BAP, syntetyczna cytokinina) i kwasu abscysynowego (ABA) na parametry fluorescencji chlorofilu w stosunkowo odpornych na suszę (Pishtaz) i podatnych (Karaj3) genotypach pszenicy w warunkach dobrego nawodnienia i skrajnego deficytu wody. Krańcową suszę wywoływano w fazie kwitnienia (65 w skali Zadoks). Wykazano, że współczynnik niefotochemicznego wygaszania fluorescencji (qN), wydajność kwantowa reakcji fotochemicznych PS II (ΦPSII) i fotochemiczne wygaszanie (qP) ulegały zmianom po opryskiwaniu liści hormonami. Ocena parametrów po 7 dniach od dolistnego podania hormonu wykazała, że ABA znacząco zwiększa sprawność transportu elektronów (ETR) i qN, znacznie zmniejsza ΦPSII, gs i maksymalną wydajność kwantową fotosystemu II (Fv/Fm). Jednak egzogenne zastosowanie cytokininy może zwiększyć gs, Fv/Fm i ΦPSII, a najwyższą wartość tych parametrów odnotowano w traktowanych cytokininą roślinach odmiany Pishtaze w warunkach dobrego nawodnienia. Ocena parametrów w różnych okresach po oprysku wykazała, że w bardziej dojrzałych roślinach wskaźniki takie jak gs, Fv/Fm i ETR znacznie zmniejszyły się w obu genotypach. Ocena gs i parametrów fluorescencji chlorofilu genotypów w warunkach różnych poziomów nawodnienia wykazała, że chociaż genotypy Pishtaz wykazały wyższą wydajność PSII w stanie dobrego nawodnienia, nie udało się utrzymać przewagi w warunkach stresu. Odkrycie to sugeruje, że niektóre bardziej czułe parametry takie jak gs, Fv/Fm i ΦPSII mogą być wiarygodnym wskaźnikiem dla zrozumienia biochemicznych i fizjologicznych efektów egzogennego stosowania fitohormonów w warunkach krańcowego stresu suszy.

Słowa kluczowe: kwas abscysynowy, cytokinina, wydajność fotosyntezy, przewodnictwo szparkowe, krańcowy deficyt wodny

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is the most important staple food for the 35% of the world's population (FAOSTAT, 2012). Like some other crops, wheat is subjected to many environmental stresses, which reduce yield and affect yield stability. Drought is a major abiotic stress that severely affects crop production worldwide. Up to 44% of all the world's cultivated systems are in the drylands and they often have low productivity. In these regions imbalances between water availability and plant demand lead to drought stress, especially at late growth stages. In most semi-arid areas there is an expected precipitation decrease over the next century of 20% or more due to climatic change. Interestingly, only 20% of the world's croplands are irrigated, but they produce 40% of the global harvest meaning that irrigation more than doubles land productivity (13). In Mediterranean areas, wheat and barley are often the only possible rainfed crops that farmers can grow, and is often subjected to water deficit at the end of growth season. Terminal drought occurs in the

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Mediterranean-type climates because they are dependent on rainfall throughout autumn and winter and during the last months of spring, rainfall decreases and evaporation increases when plants enter their key growth stages that affect yield determination.

Photosynthesis is an essential process to maintain crop growth and development, and it is well known that photosynthetic systems in higher plants are most sensitive to drought stress (Falk et al. 1996). Terminal drought can result in significant reduction of leaf photosynthesis before seed growth initiate and finally leading to decreased grain yield (8). Inhibition of photosynthesis under water deficit condition may resulted from alternation in ultrastructure of the organelles and concentration of various pigments and metabolites including enzymes involved in this process as well as stomatal regulation. The stomatal control system plays a critical role for managing water deficit under drought, because it response rapidly to small environmental changes to optimize the exchange of water for carbon (14). In fact stomatal closure is the earliest response to drought and it can be dominant limitation to photosynthesis at mild to moderate drought stress. Although stomatal closure is vital for prevention of desiccation, it reduces the CO_2 acquisition and photosynthetic rate. It has been suggested that stomatal closure under water deficit may occur through two different ways. Hydropassive stomatal control process refers to direct influence of soil water content on stomatal aperture (9). While during the hydroactive closure, stomata as osmotically regulated valves exclusively response to plant hormone levels and leads to movement of ions across the guard cell membranes (7). However it appears that hydroactive and hydropassive stomatal control processes have operated together (14). Furthermore, declines of the photosynthetic rate under drought stress can be through nonstomatal limitations that caused by impairments in photochemical processes (i.e. decrease in NADPH and ATP supply) and/or biochemical reactions, i.e. reduced RuBP regeneration and carboxylation efficiency (6). In this context, the application of stomatal conductance (*g*s) has been proposed as an indicator to assess the difference between stomatal and nonstomatal limitations to photosynthesis under water-limited environments (2). Despite the importance of gs, this information is not sufficient, and supplementary methods are necessary for more accurate and comprehensive evaluating the effects of water stress on photosynthesis. The techniques based on measurement of chlorophyll fluorescence appear to be suitable tool for understanding photosynthetic metabolism and thus identify plant performance under water deficit condition (Li et al., 2006). It has been revealed that some chlorophyll fluorescence parameters, such as the maximum quantum yield of PS II photochemistry (F_v/F_m) and the basal fluorescence (F_0), correlate with drought tolerance (4).

Additionally, it has been suggested that exogenous application of some phytohormones (e.g. abscisic acid and cytokinin) create an ability in plants to adapt to drought stresses by mediating a wide range of adaptive responses (25). Abscisic acid (ABA) can enhance proline biosynthesis and improve the movement of photosynthetic assimilates to the developing seeds. It is known that cytokinin application under abiotic stressful conditions can postpone the leaf senescence directly by scavenging free radicals and increase osmoprotectants content (1; 25). The role of ABA in the regulation of stomatal opening is partly recognized. However, application of ABA to maize resulted in partial protection of the PSII photochemistry against photoinhibition. This was accompanied with higher photochemical and non-photochemical quenching in ABA-treated leaves, considerable increase in the amount of total carotenoids and xanthophylls and activity of xanthophyll cycle (17). Conversely there is only scarce information about cytokinins effects on stomatal function and chlorophyll fluorescence parameters. It seems that cytokinin can affect chlorophyll fluorescence indirectly via non-stomatal effects. It has been revealed that cytokinin has different roles in alleviation of the negative effects of water stress on chlorophyll and carotenoids contents, regulating the photochemical activities of PSI and PSII, adjustment of content and activity of ribulose-1,5-bisphosphate carboxylase or phosphoenolpyruvate carboxylase (16).

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Although each individual aspect has been studied to some extent, yet an integrated understanding of phytohormones effects on stomatal controls and chlorophyll fluorescence remains elusive. In this study, we hypothesised that exogenous utilization of abscisic acid and cytokinin may affect the stomatal behaviour and chlorophyll fluorescence parameters in wheat (*Triticum aestivum* L.) cultivars with different drought tolerance grown under terminal drought. In a glasshouse study, we investigated the effect of spraying a synthetic cytokinin and ABA on the chlorophyll fluorescence parameters when the plants were subjected to water deficit condition after anthesis.

MATERIALS AND METHODS

A pot experiment was arranged in factorial based on a randomized complete block design with three replications at greenhouse under well watered and terminal drought stress. The experiment was repeated twice for a total of three replications.

Seeds of wheat (*Triticum aestivum* L. cv. Karaj3 and Pishtaz) cultivars were obtained from Seed and Plant Improvement Institute, Karaj (SPII). Pishtaz (Adlan/Ias58//Alvand) is a relatively tolerant against terminal-drought stress wheat cultivar and Karaj3 ((Drc*Mxp/Son64*Tzpp-Y54) Nai60) is a drought susceptible bread wheat genotype. Five vernalized seedlings were grown in polyvinyl chloride pots, 20 cm in diameter, and filled to a depth of 34 cm with soil containing a mixture of clay, silt and sand in the ratios of 16%, 36% and 48%, respectively, with an electric conductivity of 1.63 $dS.m^{-1}$ and pH 7.2. The concentrations of total N, P, and K were 0.08%, 22.9 mg kg^{-1}, and 181 mg kg^{-1}, respectively.

The plants were grown in glasshouse with natural light and day/night temperatures of 28/19°C. Greenhouse was cooled using fan-pad system. Supplementary light was provided in the greenhouse for 16h per day. The positions of the pots in the glasshouse were changed every 2 days to ensure that all plants experienced the same range of conditions. All of the pots were also watered every two days by hand to maintain the soil water content close to field capacity (FC) through the daily weighting pots until anthesis stage (Zadoks scale 65), when terminal drought was induced by withholding water from half of the pots. FC of the soil was measured at Agriculture College soil laboratory on gravimetric basis (Nachabe, 1998) and 100 and 40% of FC in the soil were considered as well-watered and drought condition, respectively.

When plants were at the initial stage of grain formation (Zadoks scale 71), the pots of each cultivar were randomly divided into 3 groups. First group were sprayed with 6-benzylaminopurine (BAP, a synthetic cytokinin) with concentration of 50×10^{-6} M. The second group were sprayed with 25×10^{-6} M ABA. Both hormones were sprayed continuously for 4 consecutive days at the rate of 50 ml per pot on the leaves and spikes daily for 4 days with 0.5% (v/v) Teepol as surfactant. The plants sprayed with the same volume of 0.5% Teepol solution were taken as a control (as third group). Exogenous application of plant growth regulators were carried out according to method described by Yang et al. (27). Plant growth regulators were obtained from Sigma Chemical Company. Phenological monitoring was made at 3-day intervals and the phenological stage was considered when 50% of the plants had achieved the specific stage.

Chlorophyll fluorescence was measured in fully expanded attached flag leaves (*In vivo* measurements) from all phytohormone treatments and drought levels. During the grain filling period flag leaf (first upper leaf) is the potentially efficient photosynthetic organ in wheat. After 7 and 14 days from the last day of phytohormone spraying (T_1 and T_2, respectively), five flag leaves from both well-watered and drought-stress conditions for each cultivar were chosen to evaluate chlorophyll fluorescence parameters. Fluorescence test performed by the pre-darkening (dark adaptation) of the leaf followed by short exposure to a saturating light intensity. Plants were pre-darkened for 1 hour and Chlorophyll fluorescence was measured using a pulse amplitude modulated (PAM-2000)

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portable fluorometer (Walz, Effeltrich, Germany) connected to a notebook computer. Saturating pulses of white light (duration 700 ms, 2500 μmol m^{-2} s^{-1}) were applied.

Three parameters of fluorescence including Fv/Fm (maximum photosystem II quantum yield of dark-adapted samples), Y (quantum yield) and qP (photochemical quenching) were calculated online by PAM fluorometry and the saturation pulse method. The ratio between variable and maximal fluorescence (F_v/F_m) was measured in dark-adapted leaves. The ratio of variable to maximum fluorescence (F_v/F_m) derived from the measurement was used as maximum photochemical efficiency of photosystem II (PS II). The quantum yield of electron transport through PS II (Y) was calculated according to Genty et al. (15). Electron Transport Rate (ETR) that was used as a function of the quantum yield and illumination, was calculated as formula described by Brestic and Zivcak (6). Coefficient of non-photochemical quenching of variable fluorescence (q_N) was estimated as the following formula: $[(F_m-F_{m'})/ (Fm-F_{0'})]$, where $F_{m'}$ is maximum fluorescence from light-adapted leaf and $F_{0'}$ is minimal fluorescence from light-adapted leaf. Quantum yield (efficiency) of PS II photochemistry (Φ_{PSII}) was calculated as the following formula: $[(F_{m'} - F_{s'})/ F_{m'}]$ where $F_{s'}$ is steady-state fluorescence at determined light level. Leaf stomatal conductance (g_s) was measured using a portable porometer (Delta-T AP4, England). Ten intact flag leaves were selected for the measurements. Principal component analysis and graphical display were performed using Statgraphics and SAS 9.1 software. The data was analyzed statistically using Fisher's analysis of variance technique and then Duncan multiple range tests ($p < 0.05$) were performed using the SAS statistical analysis package.

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) is shown in Table 1. ANOVA is a collection of statistical models used in order to analyze the differences among variables treatments and is useful in comparing variables for statistical significance. Analysis of variance showed that F_v/F_m significantly was affected by the measurement stage, phytohormone spraying, irrigation level and cultivars (Table 1). Terminal drought stress significantly reduced the Fv/Fm. The highest value was recorded in cv. Pishtaz under well watered condition. Foliar application of the ABA reduced Fv/Fm over control. However, mean comparisons revealed that the highest F_v/F_m parameter was related to first measurement stage (7 days after the last spray) in cv. Pishtaz sprayed by cytokinin under well watered condition, whereas the lowest value was recorded in ABA treated plants of cv. Karaj3 under moderate drought at T_1 stage. However, concise comparison of the Fv/Fm with other chlorophyll fluorescence parameters showed that maximum quantum yield of Photosystem II is much more responsive to treatments. Although exogenous application of cytokinin increased Fv/Fm parameter in both genotypes, this increase was significantly higher in drought tolerant cultivar (cv. Pishtaz). However, Vlčková et al. (26) reported that cytokinin application under light condition initially induced assimilate production and accumulation in the detached leaves; however, this accumulation led paradoxically to the deterioration of photosynthetic function via feedback inhibition in later stages of artificial senescence.

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Table 1. Analysis of variance (ANOVA) chlorophyll parameters of bread wheat cultivars in greenhouse condition.

SOV	DF	F_v/F_m	Y	ETR	q_p	Φ_{PSII}	q_N	g_s
Replication	2	0.0004 ^{ns}	0.0119 ^{ns}	37.965 ^{ns}	0.0003 ^{ns}	0.0018 ^{ns}	0.0085 ^{ns}	0.856 ^{ns}
(A)cultivars	1	0.0141 **	0.0100 ^{ns}	31.008 ^{ns}	0.0020 *	0.0070 ^{ns}	0.1688 *	0.436 ^{ns}
(B) irrigation	1	0.0916 **	0.0059 ^{ns}	1.727 ^{ns}	0.0016 *	0.0191 **	0.0172 ^{ns}	20726.480**
Phytohormon (C)	2	0.0637 **	0.0366 ^{ns}	169.663 **	0.0026 **	0.0291 **	0.0958 *	16914.892 **
measurement stage (D)	1	0.0030 *	0.0886 *	444.765 **	0.0005 ^{ns}	0.0001 ^{ns}	0.0148 ^{ns}	646.801 **
A*B	1	0.0281 **	0.0005 ^{ns}	13.825 ^{ns}	0.0000 ^{ns}	0.0244 **	0.0071 ^{ns}	66.894 ^{ns}
A*C	2	0.0155 **	0.0081 ^{ns}	111.218 **	0.0001 ^{ns}	0.0065 *	0.0195 ^{ns}	156.642 **
A*D	1	0.0046 *	0.0087 ^{ns}	1350.700**	0.0030 **	0.0010 ^{ns}	0.0256 ^{ns}	1067.220 **
B*C	2	0.0002 ^{ns}	0.0058 ^{ns}	66.722 *	0.0022 **	0.0009 ^{ns}	0.0405 ^{ns}	1083.324 **
B*D	1	0.0137 **	0.0079 ^{ns}	6.814 ^{ns}	0.0007 ^{ns}	0.0003 ^{ns}	0.00002 ^{ns}	16.056 ^{ns}
C*D	2	0.0177 **	0.0269 ^{ns}	112.565 **	0.0031 **	0.0220 **	0.0528 ^{ns}	262.203 **
A*B*C	2	0.0032 *	0.0681 *	11.307 ^{ns}	0.0013 *	0.0117 **	0.0591 ^{ns}	2.871 ^{ns}
A*B*D	1	0.0135 **	0.0036 ^{ns}	0.288 ^{ns}	0.0011 ^{ns}	0.0086 *	0.0041 ^{ns}	85.805 ^{ns}
A*C*D	2	0.0479 **	0.0030 ^{ns}	115.858 **	0.0006 ^{ns}	0.0059 ^{ns}	0.0706 ^{ns}	253.455 **
B*C*D	2	0.0168 **	0.0080 ^{ns}	7.544 ^{ns}	0.0002 ^{ns}	0.0115 **	0.0293 ^{ns}	99.430*
A*B*C*D	2	0.0079 **	0.0491 ^{ns}	24. 213 ^{ns}	0.0006 ^{ns}	0.0167 **	0.0514 ^{ns}	299.778 **
Error	46	0.0006	0.0145	20.014	0.0004	0.0020	0.0249	27.861

The reason for doing an ANOVA is to see if there is any difference between groups on some variable. SOV – source of variance, Df – degrees of freedom, Fv/Fm – maximum quantum yield of Photosystem II, ETR – electron transport rate, g_s – stomatal conductance (mmol m^{-2} s^{-1}), Y – quantum yield of electron transport through PS II, qP – photochemical quenching, q_N – coefficient of non-photochemical quenching of variable fluorescence, Φ_{PSII} – quantum yield of PS II photochemistry. Ns – non-significant, * – $p<0.05$, ** – $p<0.01$.

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Drought stress leads to a considerable decrease in net photosynthetic rate, due to stomatal closure, which contains the diffusion of CO_2 into the leaf or non-stomatal factors, such as inhibition of Rubisco or ATP synthesis (20). This condition may result in photoinhibition which is due to an imbalance between the rate of photodamage to PSII (D_1 protein) and the rate of the repair of damaged parts (22). However, under severe drought stress, the capacity for repair of damaged parts becomes suboptimal and an irreversible inhibition of PSII can be detected through chlorophyll fluorescence. Therefore it seems that F_v/F_m parameter could be considered as a useful parameter to evaluate the extent of photoinhibition of photosynthesis (21).

The lowest F_v/F_m parameter in plants treated with ABA can be attributed to effects of these hormones on stomata closure. It seems that there is positive correlation between actual quantum yield of PSII electron transport and stomatal conductance (18). Variance analysis of g_s parameter revealed that the irrigation levels, phytohormone spraying and measurement stage was significant. Moreover, the interaction effect of cultivar× irrigation× phytohormone× measurement stage was significant at $p<0.01$ level (Table 1). The highest g_s parameter was recorded in cytokinin treated plants of Pishtaz cultivar under well watered condition during first measurement stage (Table 2, 3, 4). However, mean comparison of g_s parameter between the different irrigation levels showed that terminal drought stress could decrease this parameter up to 48%. Comparison of g_s parameter between phytohormones indicated that exogenous application of cytokinin could increase this parameter up to 69% over to control. On the contrary, spraying of ABA could result in 38% reduction of g_s when compared with control plant. Stomatal conductance plays an imperative role in the plant-atmosphere water exchange and significantly correlates with photosynthetic capacity. Stomatal closure is the earliest response to drought and the dominant limitation to photosynthesis at mild to moderate drought. g_s has been introduced as a key parameter to assess limitations to photosynthesis and growth potential in barley genotypes (18). Furthermore, when the relationships of g_s with yield components and agronomic traits have been evaluated, there has been found a positive significant correlation between g_s at early milky maturity period and grain numbers per spike (3). However, in parallel to stomatal limitation, advanced down-regulation Rubisco activity leads to decreased ribulose-1, 5-bisphosphate (RuBP) regeneration, which becomes the dominant limitation (non-stomata) at severe drought, and thereby significantly reduces CO_2 assimilation and consequently leads to an increase in chlorophyll fluorescence (5). This trend was confirmed by principle component analysis (PCA) analysis (Figure 1). In the present study, the PCA described a suitable amount of the total variation. The correlation coefficient between any two traits is approximated by the cosine of the angle between their vectors. In Figure 1, the most

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Table 2. Changes in chlorophyll parameters of bread wheat cultivars under different irrigation levels which were measured after 7 and 14 d of phytohormone spraying (T_1 and T_2).

Cultivar	Irrigation	Measurement stage	F_v/F_m	Y	ETR	q_p	Φ_{PSII}	q_N	g_s
Pishtaz	W	T_1	0.777 a	0.585 b	21.011 a	0.977 ab	0.780 a	0.052 a	78.444 a
	W	T_2	0.751 b	0.712 a	6.889 b	0.946 b	0.744 ab	0.002 a	61.622 b
	S	T_1	0.611 c	0.607 ab	21.089 a	0.974 ab	0.685 b	0.119 a	43.311 c
	S	T_2	0.695 b	0.664 ab	7.944 b	0.970 ab	0.701 ab	0.037 a	32.744 d
Karaj3	W	T_1	0.698 b	0.602 ab	12.039 b	0.969 ab	0.694 b	0.146 a	70.333 a
	W	T_2	0.695 b	0.658 ab	14.989 ab	0.978 ab	0.717 ab	0.141 a	73.278 a
	S	T_1	0.666 b	0.586 b	10.111 b	0.978 ab	0.716 ab	0.143 a	35.711 cd
	S	T_2	0.663 b	0.628 ab	14.544 ab	0.985 a	0.703 ab	0.166 a	36.178 cd

Values are given as means of three replicates. Figures not sharing the same letters in the same column differ significantly at $p< 0.05$. W; well watered, S; terminal drought stress. Different letters between the rows indicate statistically significant differences. Fv/Fm – maximum quantum yield of Photosystem II, ETR – electron transport rate, g_s – stomatal conductance (mmol m^{-2} s^{-1}), Y – quantum yield of electron transport through PS II, qP – photochemical quenching, q_N – coefficient of non-photochemical quenching of variable fluorescence, Φ_{PSII} – quantum yield of PS II photochemistry.

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Table 3. Effect of different phytohormone spraying on chlorophyll parameters of bread wheat cultivars at different time intervals after treatment.

Cultivar	Phytohormone	Measurement stage	F_v/F_m	Y	ETR	q_p	Φ_{PSII}	q_N	g_s
Pishtaz	control	T_1	0.695 bc	0.674 ab	20.367 b	0.968 abc	0.702 b	0.027 a	52.033 de
	control	T_2	0.708 bc	0.676 ab	6.250 d	0.972 abc	0.711 b	0.054 a	40.550 f
	CK	T_1	0.841 a	0.559 b	13.000 bcd	0.972 abc	0.839 a	0.032 a	90.833 a
	CK	T_2	0.734 b	0.686 ab	8.017 cd	0.936 c	0.730 b	0.002 a	79.667 bc
	ABA	T_1	0.547 e	0.555 b	29.783 a	0.987 ab	0.655 b	0.199 a	39.767 f
	ABA	T_2	0.727 b	0.702 a	7.983 cd	0.965 abc	0.726 b	0.002 a	21.333 h
Karaj3	control	T_1	0.693 bc	0.678 ab	14.783 bc	0.967 abc	0.702 b	0.012 a	56.783 d
	control	T_2	0.696 bc	0.663 ab	15.833 bc	0.989 ab	0.720 b	0.137 a	45.967 ef
	CK	T_1	0.688 bc	0.569 b	8.333 cd	0.980 ab	0.742 b	0.218 a	73.083 c
	CK	T_2	0.716 b	0.676 ab	13.167 bcd	0.955 bc	0.713 b	0.037 a	87.167 ab
	ABA	T_1	0.667 c	0.537 b	10.108 cd	0.974 abc	0.671 b	0.204 a	29.200 gh
	ABA	T_2	0.626 d	0.589 b	15.300 bc	1.000 a	0.698 b	0.288 a	31.050 g

Values are given as means of three replicates. Figures not sharing the same letters in the same column differ significantly at $p< 0.05$. CK – Cytokinin (benzylaminopurine*), ABA* – Abscisic acid, Fv/Fm – maximum quantum yield of Photosystem II, ETR – electron transport rate, g_s – stomatal conductance (mmol m^{-2} s^{-1}), Y – quantum yield of electron transport through PS II, qP – photochemical quenching, q_N – coefficient of non-photochemical quenching of variable fluorescence, Φ_{PSII} – quantum yield of PS II photochemistry. Pishtaz as relatively drought tolerant and Karaj3 as susceptible bread wheat genotypes were selected.

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Table 4. Response of chlorophyll parameters of bread wheat to different phytohormone spraying under different soil moisture regimes levels which were measured after 7 and 14 d of spraying (T_1 and T_2).

Irrigation level	Phytohormone	Measurement stage	F_v/F_m	Y	ETR	q_p	Φ_{PSII}	q_N	g_s
Well watered	control	T_1	0.720 bc	0.688 ab	18.817 ab	0.957 bc	0.708 b	0.012 a	78.333 b
	control	T_2	0.745 b	0.709 a	11.233 bcd	0.965 abc	0.743 b	0.072 a	63.333 c
	CK	T_1	0.846 a	0.579 b	8.617 d	0.988 ab	0.840 a	0.029 a	99.667 a
	CK	T_2	0.721 bc	0.679 ab	9.200 cd	0.945 c	0.716 b	0.002 a	104.833 a
	ABA	T_1	0.647 e	0.514 b	22.142 a	0.975 abc	0.663 b	0.257 a	45.167 d
	ABA	T_2	0.703 bcd	0.668 ab	12.383 bcd	0.975 abc	0.732 b	0.142 a	34.183 e
Drought stress	control	T_1	0.668 de	0.664 ab	16.333 abcd	0.979 abc	0.697 b	0.027 a	30.483 ef
	control	T_2	0.659 e	0.630 ab	10.850 bcd	0.996 a	0.687 b	0.119 a	23.183 fg
	CK	T_1	0.682 cde	0.549 b	12.717 bcd	0.964 abc	0.741 b	0.220 a	64.250 c
	CK	T_2	0.729 b	0.684 ab	11.983 bcd	0.946 c	0.726 b	0.037 a	62.000 c
	ABA	T_1	0.567 f	0.578 b	17.750 abc	0.985 ab	0.664 b	0.147 a	23.800fg
	ABA	T_2	0.650 e	0.623 ab	10.900 bcd	0.990 ab	0.692 b	0.149 a	18.200 g

Figures not sharing the same letters in the same column differ significantly at $p< 0.05$. Fv/Fm – maximum quantum yield of Photosystem II, ETR – electron transport rate, g_s – stomatal conductance (mmol m^{-2} s^{-1}), Y – quantum yield of electron transport through PS II, qP – photochemical quenching, q_N – coefficient of non-photochemical quenching of variable fluorescence, Φ_{PSII} – quantum yield of PS II photochemistry.

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prominent relations are: a strong positive association among g_s, F_v/F_m, and Φ_{PSII} as indicated by the small obtuse angles between their vectors (r=cos 0=+1). However, there was a negative correlation between g_s and non-photochemical quenching of variable fluorescence (q_N) (Figure 1) as indicated by the near perpendicular vectors (r=cos180=-1). It was also observed between g_s and ETR. However, PCA revealed that there was not correlation between g_s and qP as indicated by the near perpendicular vectors (r=cos90=0). Variance analysis of ETR parameter showed that exogenous application of phytohormone and measurement stage could significantly affect this parameter ($P<0.01$). In susceptible cultivar ETR parameter significantly was reduced by approaching to maturity stage, while in tolerant cultivar a reversed trend was recorded. In addition three way interaction of cutivar× phytohormone× measurement stage was statistically significant. The highest ETR parameter was recorded in ABA treated plant of Pishtaz cultivar during the first measurement stage (Table 3). Our result indicated that terminal drought stress could not notably affect ETR parameter. This corroborates the result of Dani et al. (10), who reported that ETR parameter was not affected by drought (50% FC) in Eucalypts seedling. The insensitivity of ETR to drought in both genotypes in this study suggests that the photosystems and the electron transport chain are not susceptible to terminal drought stress. A negative correlation was observed between ETR, g_s, F_v/F_m and Φ_{PSII} (Figure 1).

Photochemical quenching refers to a status in which excited chlorophylls pass their energy to another chlorophyll molecules and then excitation is gradually passed to the photochemical reaction centers (photosystem I and photosystem II) where energy is used in photosynthesis. Investigation of qP showed that effect of three way interaction of cutivar× irrigation× phytohormone was statistically significant ($p<0.05$). The highest value of qP was recorded in control plant of cv. Karaj3 under drought stress condition. However, q_N refers to condition in which the excited state can return to the ground state by emitting the energy as heat. Variance analysis q_N indicated that the main effect of cultivar and phytohormone was significant at $p<0.05$ level. Mean comparison of the cultivars showed that non-photochemical quenching of relatively drought tolerant (Pishtaz) was about one-third of susceptible cultivar (Karaj3). On the other hand, the foliar application ABA could significantly increase q_N when ccompared with control. This increase can be attributed to stimulatory effect of this hormone in stomatal closure and this matter is partly confirmed by negative correlation between q_N and g_s (Figure 1). However previous studies showed that plants exhibit lower photochemical quenching and potential photochemical yield under drought stress (6; 19). The first quinone acceptor (Q^-_A) reoxidized to Q_A is reduced, which results in reduced electron transfer rate and increased photochemical quenching for heat dissipation.

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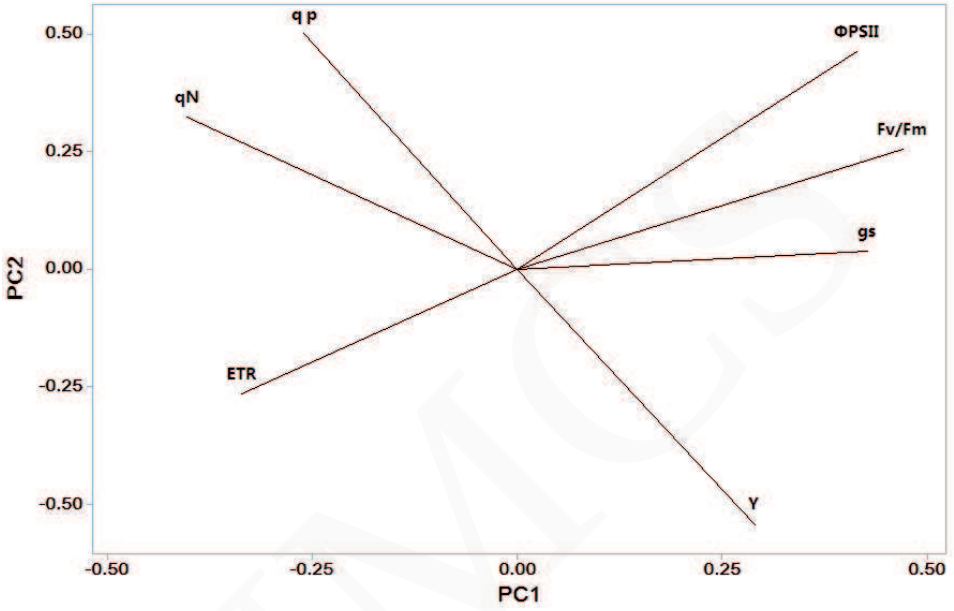


Figure 1. Plot of the first two PCAs showing relation among various chlorophyll fluorescence parameters and stomatal conductance of two bread wheat cultivar. The number of the samples were equals for all investigated traits. Fv/Fm – maximum quantum yield of Photosystem II, ETR – electron transport rate, g_s – stomatal conductance (mmol m^{-2} s^{-1}), Y – quantum yield of electron transport through PS II, qP – photochemical quenching, q_N – coefficient of non-photochemical quenching of variable fluorescence, Φ_{PSII} – quantum yield of PS II photochemistry.

Result revealed that Φ_{PSII} considerably reduced by terminal drought stress and exogenous application of ABA (Table 3, 4). The highest Φ_{PSII} parameter was recorded in cytokinin treated plants of cv. Pishtaze under well watered condition. Mean comparison of ΦPSII between the measurement stages showed that with approaching the maturity stage this parameter slightly increased. However, the findings of the current study do not support the results of Prokopová et al. (24) who reported that exogenous cytokinin application led to reductions in photosynthetic pigment contents and F_v/F_m, inhibition of electron transport and increased q_N.

Variance analysis of Y parameter showed that the effect of measurement stage and interaction effect of cultivar× irrigation× phytohormone were significant at $p<0.05$ level (Table 1). Terminal drought stress slightly reduced this parameter. The highest Y parameter was recorded in intact drought tolerant cultivar (not sprayed) under well watered condition and at second measurement stage. Actually this parameter refers to quantum yield of electron transport from Q_A to Q_B in PS II. According to our results, it seems that this part of the electron transport chain is

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not much sensitive to the investigated treatments. However, drought stress could negatively affect the efficiency of electron transfer and subsequently PSII inactivation reduces electron transport and CO_2 assimilation.The measurement of quantum efficiency, F_v/F_m, provides clear data on the effect of various environmental and biotic effects on the performance of photosynthesis in plants through the effect on photosystem II. Our result revealed that chlorophyll fluorescence analysis should carry out at a time frame because most of the parameters greatly changed over time.

CONCLUSIONS

Present results indicate that between the evaluated parameters Fv/Fm, ETR and Φ_{PSII} considerably responded to irrigation levels and measurement stages. In the current study the tolerant genotype showed significantly higher values of Fv/Fm, g_s and Φ_{PSII} parameters under drought stress. These parameters can serve as useful markers for screening wheat genotypes and identifying drought-tolerant genotypes. Our result showed that ABA application significantly reduced the stomatal conductance and decreased photosynthetic system performance. It clearly was associated with an increase in non-photochemical quenching. On the contrary, spraying with cytokinin could significantly increase g_s, Fv/Fm and Φ_{PSII}. Also the principle component analysis (PCA) showed a high positive correlation between the three mentioned parameters. In this context, the Fv/Fm analysis and stomatal conductance are attractive tests because they allow one to monitor the photosynthetic performance and capacity to maintain a functional PSII after phytohormone application. Findings of this study are however, in need of reconfirmation in order to identify suitable dose and critical stage of hormone spraying.

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