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Effects of salicylic acid and water stress on leaf chlorophyll and variation trend of different growth stages of wheat (*Triticum aestivum* L.)

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Abstract

In order to study effects of salicylic acid and water stress on leaf chlorophyll and variation trend of different growth stages wheat (*Triticum aestivum* L.) cultivar Chamran, two field experiments were conducted in 2010-2011 and 2011- 2013. Each experiment was carried out as split-plot factorial based on randomized complete block design with three replications. The results of combined analysis showed that year has not significantly affected leaf chlorophyll. Well-watered and water stress (Z55) conditions had the highest and lowest chlorophyll respectively. Salicylic acid application timing and different salicylic acid concentrations had significant effect on leaf chlorophyll. Leaf chlorophyll was significantly positive correlated with grain yield at vegetative and generative stages and was significantly negative correlated with grain yield at grain growth stage. Leaf chlorophyll was significantly positive correlated with 1000-seed weight at flowering stage and was significantly negative correlated with 1000-seed weight at grain growth stage. The data showed that salicylic acid application led to increase of leaf chlorophyll at vegetative and generative stages, so it was an important strategy to increased yield against detrimental effects of water stress.

Keywords: chlorophyll, grain yield, priming, salicylic acid.

Introduction

Not only does water deficit cause decrease of pigments, but also causes disorder of thylakoid membrane. Thus, water stress can be expected to decrease of chlorophyll (Legal *et al.*, 2000). Kulshrehtha *et al.* (1987) reported that water stress did not have effect on chlorophyll. El-Tayeb (2005) observed that salicylic acid application led to increase of chlorophyll. In a study salicylic acid caused an increase of chlorophyll in soybean plants (Leslie *et al.*, 1988). In a field experience, foliar application with salicylic acid (10 and 20 ml) led to increase of leaf chlorophyll of rapeseed (Ghai *et al.*, 2002). Also, foliar application with salicylic acid caused increase of leaf chlorophyll of wheat (Shehata *et al.*, 2000). Salicylic acid was decreased ethylene synthesis by membrane depolarization and increase of photosynthesis, therefore, chlorophyll was increased in soybean plants (Leslie *et al.*, 1988). Foliar application with salicylic acid led to increase of chlorophyll *a* and *b* in maize plants under salinity stress to control (Khodary, 2004). Moharekar *et al.* (2003) observed that total chlorophyll (*a* and *b*) and ratio of chlorophyll *a* to chlorophyll *b* have significantly decreased with increasing salicylic acid concentration (from 2 to 20 mg kg^{-1}) except for 10 mg kg^{-1}. In contrast, Ghai *et al.* (2002) reported that rapeseed leaf chlorophyll was increased by salicylic acid foliar application (10 and 20 μg). The chlorophyll of treated green gram plants by salicylic acid was lower than control and chlorophyll was significantly decreased

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Table 1. Combined analysis of variance for total chlorophyll at different growth stages

S.O.V	df	MS		
		Vegetative stage chlorophyll	Generative stage chlorophyll	Grain growth stage chlorophyll
Year (Y)	1	2425.3^{ns}	3328.8^{ns}	222.2^{ns}
Error (a)	4	18.7	8.9	157.1
Water Stress (WS)	2	0.89^{ns}	12.9^{ns}	29.4^{ns}
Y × WS	2	19.2^{ns}	22.7^{ns}	14.7^{ns}
Error (b)	8	3.09	9.6	152.6
Salicylic acid application Timing (ST)	2	11.2**	5.3^{ns}	0.30^{ns}
Salicylic acid Concentration (SC)	3	8.4**	0.68^{ns}	59.7**
Y × ST	2	6.7^{ns}	0.05^{ns}	0.63^{ns}
Y × SC	3	9.42^{ns}	2.7^{ns}	10.36^{ns}
ST × SC	6	4.5**	5.8^{ns}	12.3^{ns}
WS × ST	4	0.057^{ns}	5.2^{ns}	28.5^{ns}
WS × SC	6	4.3**	4.8^{ns}	33.9^{ns}
Y × WS × SC	6	7.4^{ns}	0.96^{ns}	16.9^{ns}
Y × WS × ST × SC	4	5.5^{ns}	1.8^{ns}	3.9^{ns}
WS × ST × SC	12	3.4^{ns}	2.8^{ns}	14.9^{ns}
Y × WS × ST × SC	18	2.7^{ns}	2.7^{ns}	4.8^{ns}
Error (c)	132	1.6	2.6	17.4
C.V (%)	C.V%	11	8	17

* and **: Significant at the 5% and 1% probability levels respectively. ns: Non- significant.

by increasing salicylic acid concentration (Pancheva *et al.*, 1996). The aim of this study was to assess variation and duration of chlorophyll in the leaf wheat cultivar Chamran as affected by salicylic acid application under water stress conditions.

Materials and Methods

Experimental design and agronomic applications

The experiments were conducted at the Agricultural Research Station in Safiabad-Iran in 2010 and 2012. Each experiment was carried out as split-plot factorial based on randomized complete block design with three replications. Water stress as the main plot was imposed at three levels including well-watered conditions (control), halt irrigation at flowering stage (Z55) and halt irrigation at early grain filling stage (Z85). Different concentrations of salicylic acid as the sub plot were applied at four levels including 0, 0.7, 1.2 and 2.7 mM. Salicylic acid application timing as the sub plot was applied at three levels including seed priming, foliar application at tillering stage (Z25) and foliar application at early stem elongation stage (Z31). The toein surfactant 40% (Merck, Co.) was applied to increase absorption area. The experiments were covered by plastic in order to protect the rainfall. Chlorophyll was measured by chlorophyll meter (SPAD). For this purpose, a fully expanded young leaf (flag leaf of five plants) was selected from each treatment and replication at the mid-canopy position after two weeks foliar application (Whetherley, 1950).

Statistical analysis

The recorded data were statistically analyzed using the software MSTAT-C and SAS. 9.2. Mean comparisons were calculated using Least Significant Difference (LSD) at $P \leq 0.05$.

Results

Chlorophyll

Water stress did not significantly affect chlorophyll at different growth stages (Table 1). The results of mean comparisons showed that the highest chlorophyll was found under well-watered conditions and the lowest chlorophyll was observed at flowering stage under water stress (Table 2). Salicylic acid application timing, interactions between salicylic acid application timing in salicylic acid concentrations and water stress in salicylic acid concentrations had significant effect on chlorophyll at vegetative growth stage. Salicylic acid concentrations have significantly affected chlorophyll at vegetative growth and grain growth stages, but it did not have significant effect on chlorophyll at generative

Table 2. Mean comparisons of effects of salicylic acid concentration (SC), salicylic acid application timing (ST) and water stress (WS) on total chlorophyll

Factor	Vegetative stage chlorophyll (g mg^{-1})	Generative stage chlorophyll (g mg^{-1})	Grain growth stage chlorophyll (g mg^{-1})
Salicylic acid Concentration (mmol)			
0	11.4 b	19.6 a	25.2 a
0.7	12.4 a	19.5 a	23 b
1.2	11.7 b	19.5 a	25.1 a
2.7	11.8 b	19.4 a	25.4 a
LSD (5%)	0.48	0.60	1.6
ST			
Priming	12.1 a	19.7 a	24.6 a
Vegetative Stage (Z25)	11.4 b	19.8 b	24.6 a
Generative Stage (Z31)	12 a	19.5 ab	24.5 a
LSD (5%)	0.48	0.52	1.4
Water Stress			
Control	11.9 a	19.3 b	23.8 a
Flowering Stage (Z55)	11.7 a	19.9 a	25 a
Grain Filling Stage (Z85)	11.8 a	19.5 ab	25 a
LSD (5%)	0.48	0.52	1.4

Means, in each column and for each factor, followed by at least one letter in common are not significantly different at the 5% probability level-using Least Significant Difference Test.

Table 3. Mean comparisons of interaction between salicylic acid concentration (SC) and salicylic acid application timing (ST) on total chlorophyll

Interaction		Vegetative stage chlorophyll (g mg^{-1})	Generative stage chlorophyll (g mg^{-1})	Grain growth stage chlorophyll (g mg^{-1})
SC (mmol)	ST (phenological stages)			
0	Priming	12.2 ab	20 a	25.2 ab
	Vegetative Stage (Z25)	11.6 cde	19.6 ab	25.1 ab
	Generative Stage (Z31)	11 de	19.3 ab	24.7 abc
0.7	Priming	12.7 a	19 b	22.4 c
	Vegetative Stage (Z25)	12.1 ab	19.4 ab	23 bc
	Generative Stage (Z31)	12.8 ab	20 a	23.5 abc
1.2	Priming	12.6 ab	20.2 a	26.2 a
	Vegetative Stage (Z25)	10.6 e	18.8 b	24.2 abc
	Generative Stage (Z31)	12.3 ab	19.3 ab	24.8 abc
2.7	Priming	12 ab	19.7 ab	24.5 abc
	Vegetative Stage (Z25)	11.6 bcd	19 b	26 a
	Generative Stage (Z31)	11.8 bc	19.5 ab	25 abc
LSD (5%)	-	0.84	1.3	2.7

Means, in each column and for each factor, followed by at least one letter in common are not significantly different at the 5% probability level-using Least Significant Difference Test.

growth stage (Table 1). The results of mean comparisons showed that priming with salicylic acid had the highest chlorophyll at generative growth stage (Table 2). Low concentration of salicylic acid (0.7 mmol) had the highest chlorophyll at vegetative growth stage, but it had the lowest chlorophyll at grain growth stage (Table 2). The results of interactions between all of factors showed that the highest chlorophyll was found under seed priming and application of 0.7 mmol salicylic acid concentrations at vegetative growth stage and the lowest chlorophyll was observed under application of 1.2 mmol salicylic acid concentrations at middle of tillering stage (Table 3). The results of interactions between water stress and salicylic acid concentrations showed that the highest chlorophyll was found under well-watered conditions and low concentration of salicylic acid (0.7 mmol) at vegetative growth stage and the lowest chlorophyll was observed under well-watered conditions and without application of salicylic acid (Table 4).

Table 4. Mean comparisons of interaction between salicylic acid concentration (SC) and water stress (WS) on total chlorophyll

Interaction SC (mmol)	WS	Vegetative stage chlorophyll (g mg^{-1})	Generative stage chlorophyll (g mg^{-1})	Grain growth stage chlorophyll (g mg^{-1})
0	Control	11 c	19.1 bc	23.5 cd
	Flowering Stage (Z55)	11.6 bc	20.4 a	24.3 bcd
	Grain Filling Stage (Z85)	11.5 bc	19.37 bc	27.2 a
0.7	Control	13 a	19.5 abc	23.7 cd
	Flowering Stage (Z55)	11.8 bc	19.4 abc	22.5 d
	Grain Filling Stage (Z85)	12.3 ab	19.4 abc	22.8 d
1.2	Control	12 b	19 c	24 bcd
	Flowering Stage (Z55)	11.8 bc	20.3 a	26.3 abc
	Grain Filling Stage (Z85)	11.1 c	19.2 bc	25 abcd
2.7	Control	12.1 ab	18.6 c	24 bcd
	Flowering Stage (Z55)	11.5 bc	19.4 abc	26.6 ab
	Grain Filling Stage (Z85)	11.8 bc	20.1 ab	24.8 abcd
LSD (5%)	-	0.84	1.2	2.7

Means, in each column and for each factor, followed by at least one letter in common are not significantly different at the 5% probability level-using Least Significant Difference Test.
Correlation between chlorophyll productions at different growth stages

Variation trend of chlorophyll

Before imposing water stress, chlorophyll was equal at early flowering stage, but it increased in well-watered conditions when water stress was imposed (Fig. 1). Seed priming with salicylic acid had the highest chlorophyll before applying foliar spray with salicylic acid. Seed priming and foliar application with salicylic acid had the highest chlorophyll at early stem elongation stage (generative phase), when foliar spray was applied. Salicylic acid application had the lowest chlorophyll at early tillering stage (vegetative phase). Low concentration of salicylic acid (0.7 mmol) had the highest chlorophyll at vegetative growth stage, but chlorophyll was equal in all of salicylic acid concentrations at vegetative stage until early grain filling stage and it has the lowest chlorophyll at grain filling stage (Fig. 3).

Discussion

Chlorophyll

Legal *et al.* (2000) reported that chlorophyll has decreased by water stress due to increase of membrane lipid peroxidation, enzyme inhibition and increasing accumulation of reactive oxygen species. The results of the present study are in agreement with the conclusions of Waseem (2006) that the total chlorophyll and chlorophyll *a/b* were decreased by water stress. Hamada and Al-Hakimi (2001) stated that seed priming with 100 ppm salicylic acid led to increase of chlorophyll in wheat plans. The results of this study are in agreement with the conclusions of Waseem (2006) that seed priming with salicylic acid has been increased photosynthetic pigments under well-watered and water stress conditions. It seems that low concentration of salicylic acid (0.7 mmol) can cause increase of growth and leaf

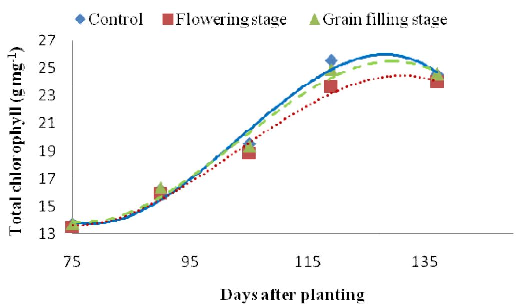

Fig. 1. Effect of water stress on variation trend of total chlorophyll

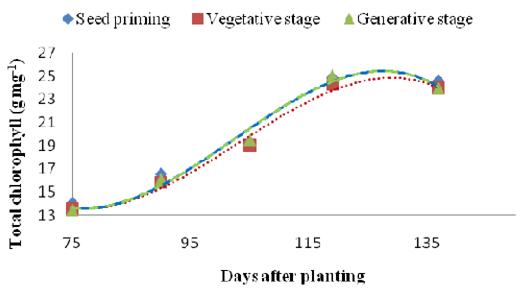

Fig. 2. Effect of salicylic acid application timing on variation trend of total chlorophyll

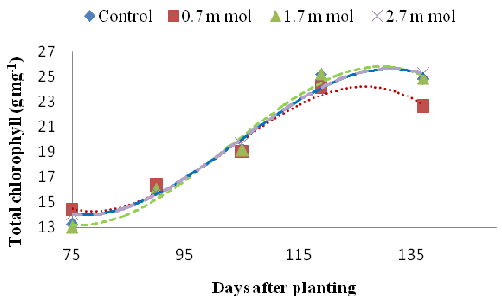

Fig. 3. Effect of salicylic acid concentration on variation trend of total chlorophyll

production, which ultimately culminate in chlorophyll production. Hayat *et al.* (2005) reported that seed priming with low concentration of salicylic acid produced more chlorophyll compared with high concentration of salicylic acid in wheat plants, which is somehow in accordance with the results of this experiment. Maibangsa *et al.* (2000) observed that treated plants with low concentration of salicylic acid produced more chlorophyll. Salicylic acid application produced more chlorophyll due to increase of leaf area. The results of this study are in agreement with the conclusions of Sivakumar *et al.* (2002). Siddique *et al.* (1999) stated that increasing chlorophyll is associated with environmental stresses and therefore chlorophyll variations can cause presence of water stress.

Variation trend of chlorophyll

Photosynthetic pigments were the highest at flowering stage due to increases of growth rate and leaf area, therefore, imposing water stress led to severe decrease of chlorophyll. Seed priming with salicylic acid caused increases of germination rate and growth rate, which ultimately culminate in increase of chlorophyll. Also, salicylic acid application led to increase of chlorophyll at generative growth stage because of increasing leaf area index. Therefore, it can cause production of photosynthetic pigments in particular chlorophyll (Fig. 2).

At vegetative growth and flowering stages, chlorophyll was significantly positive correlated with grain yield and grain growth stage, it was significantly negative correlated with grain yield (Table 5). It seems that chlorophyll production at vegetative growth and flowering stages is increased due to presence of young and fresh leaves, also, photosynthetic pigments are high at the same stages, because leaf area index is increased. Thus, chlorophyll production leads to increase of photosynthetic production, which is attributable to current photosynthesis; consequently, it has a positive effect on grain yield. In contrast, chlorophyll was decreased at grain growth stage due to leaf senescence and plant respiration; consequently, it has a negative effect on grain yield. At vegetative growth stage, chlorophyll was significantly positive correlated with chlorophyll at flowering and grain growth stages and it was significantly negative correlated with 1000-seed weight. At flowering growth stage, chlorophyll was significantly positive correlated with chlorophyll at grain growth stage and 1000-seed weight. At grain growth stage, chlorophyll was significantly negative correlated with 1000-seed weight.

Conclusion

The data showed that salicylic acid application timing and salicylic acid concentration were significantly affected chlorophyll. Low concentration of salicylic acid (0.7 mmol) led to increase of chlorophyll, which ultimately culminate in increasing photosynthesis; consequently, increasing grain yield under water stress conditions. We concluded that salicylic acid application led to a decrease of detrimental effects of water stress due to the regulatory effects.

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Table 5. Correlation between grain yield (GY), 1000-seed weight (1000-SW) and leaf chlorophyll (LCh) of wheat cultivar Chamran

Traits	1	2	3	4	5
1- Grain yield	1				
2- Vegetative stage chlorophyll	0.37*	1			
3- Generative stage chlorophyll	0.45*	0.84**	1		
4- Grain growth stage chlorophyll	-0.47*	0.37*	0.46*	1	
5- 1000-seed weight	0.54*	-0.54**	0.62**	-0.45*	1

* and **: Significant at the 5% and 1% probability levels respectively. ns: Non- significant.

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