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Antioxidant and yield responses of wheat and clover in intercropping system to late season drought stress induced by partial root-zone irrigation regime

Hamdollah Eskandari[1], Ashraf Alizadeh-Amraie[2] and Bakhtiar Lalehgani[3]

1-Associate Professor, Department of Agriculture, Payame Noor University, Tehran, Iran
2- Instructor, Department of Agriculture, Payame Noor University, Tehran, Iran
3-Assistant Professor, Department of Agriculture, Payame Noor University, Tehran, Iran
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Abstract

A field experiment was conducted to evaluate the effect of planting pattern and irrigation system on antioxidant responses of wheat and Persian clover. The experiment was carried out as a two-factor factorial with three replications. The first factor was irrigation system (conventional and partial root-zone irrigation) and the second was planting pattern (sole wheat, sole Persian clover, alternate-row intercropping and within-row intercropping). The results indicated that RWC of wheat and clover leaves decreased with lowering water supply. Both crops had higher RWC in intercropping compared to the sole cropping. Before irrigation, wheat and clover had lower proline in intercropping systems (15% and 9% lower for wheat and clover, respectively) compared to the sole cropping. SOD activity in wheat and Persian clover increased in partial root zone irrigation compared to conventional irrigation system. Under partial root-zone irrigation, SOD of wheat and Persian clover was increased up to 10% and 46% compare to the conventional irrigation. The MDA content of wheat and Persian clover leaves under partial root-zone irrigation was more than that of conventional irrigation. Regarding MDA content, wheat experienced a higher stress under the sole cropping compared to intercropping system. The highest activity of POD was recorded in Persian clover under partial root zone irrigation system which was 20% more than that of wheat. Partial root-zone irrigation reduced the grain yield of wheat and Persian clover down to 27% and 36%, respectively, compared to conventional irrigation. Land equivalent ratio of intercropping in partial root-zone irrigation was significantly ($P \leq 0.01$) more than that of conventional irrigation, suggesting that intercropping reduced the negative effect of lower water supply. Generally, partial root-zone irrigation induced higher antioxidant activity of wheat and Persian clover. However, partial root-zone irrigation may have a good potential for reducing water consumption in intercropping systems.

Key words: Antioxidant responses, Irrigation system, Land equivalent ratio, Multiple Cropping, Water deficits.

Introduction

Intercropping, the cultivation of two or more crops simultaneously on the same field, is one of the multiple cropping systems practiced in many parts all over the word. In comparison with the sole crop systems, numerous advantages have been reported for intercropping such as higher environmental resource exploitations i.e. radiation, nutrient and water and land use efficiency (Yang *et al*., 2011).

Due to decreasing agricultural water resources, the importance of water-saving methods and techniques have been increasing in recent years (Hu *et al*., 2010). There are several irrigation patterns such as limited irrigation (Kang *et al*., 2002) and partial root-zone irrigation (PRI) (Yang *et al*., 2011) which are used for optimizing water consumption in agricultural systems. Partial root-zone irrigation (PRI) is an agronomic practice in which half of root system is irrigated while the remaining is exposed to dry soil. This may result in increasing the abscisic acid (ABA) hormone, stomatal closure and decreasing the transpiration process (Tardieu *et al*., 1993). As a result, partial root-zone irrigation method, may improve water use efficiency of crops without occurring high yield reduction (Yang *et al*., 2012), and thus, it is proposed for more efficient use of limited water resources (Wang *et al*., 2009). Field and greenhouse researches revealed that partial root-one irrigation reduce water consumption by 30–50% without much yield loss (Li *et al*., 2010).

Water deficit is an environmental stress reducing crop growth and production. However, crops react to drought stress through some physiological changes such

*Corresponding Author, Email: ehamdollah@pnu.ac.ir

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as accumulation of compatible solutes (i.e. proline) or antioxidant enzymes level (Akbarian and Arzani, 2016). In this case, Li *et al*., (2010) reported that partial root zone irrigation increased proline and malondialdehyde (MDA) in maize and reduced relative water content of leaves. A strong relationship between crop tolerance to the oxidative stress and an increase in the concentration of oxidative enzymes has been reported (Mittler, 2002; Cruz de Carvalho 2008). Lascano *et al*. (2001), working on the response of two wheat genotypes (tolerant and sensitive) to drought stress under field conditions, declared that in tolerant genotype, the level of antioxidant enzymes was increased while no increase in enzyme activity was recorded in sensitive genotype. Another research on wheat seedling reported that low superoxide dismutase (SOD) activity resulted in weak performance of seedlings under drought stress (Esfandiari *et al.,* 2010). In rapeseed, a positive correlation was observed between tolerance of water deficit and proline production, where tolerant genotypes had higher proline content under drought conditions (Ansari, 2010).

Crop reactions to limited irrigation in terms of physiological responses such as photosynthesis or transpiration; have been studied in numerous researches (Aganchich *et al*., 2007; Zamani *et al*., 2013; Esfandiari *et al*., 2009; Hu *et al*., 2010). However, there is limited information on antioxidant and grain yield responses of wheat and Persian clover intercropping to partial root-zone irrigation. Therefore, this research was aimed to evaluate the effects of different irrigation methods on antioxidant defense system of wheat and Persian clover under the sole and intercropping systems.

Materials and methods

Experimental materials: The experiment was conducted during 2015-2016 growing seasons in a research field in Selseleh city, Lorestan province, west-Iran (33°51′N, 48°15′E). The experimental site was located in moderate climate with mean annual temperature and precipitation of 12.7°C and 440.3mm, respectively. Some physical and chemical properties of soil in the experimental site is presented in Table1.

Wheat (Chamran cultivar) and Persian clover (local cultivar) were simultaneously sown in 24 Nov, 2015. Each plot consisted of six meter-long rows, with 30 cm between rows. All plots were fertilized with the same amount of fertilizer, containing 150 kg ha^{-1} ammonium phosphate. The seeds were sown at high density to ensure adequate emergence. After seedling establishment, plots were thinned to 400 and 800 plants m^{-2} for Persian clover and wheat, respectively (Table 1).

Experimental design: The research was conducted as a 2×4 factorial experiment based on randomized complete block design (RCBD) with three replications. The first factor was planting patterns (W= sole wheat, C= sole clover, WC_a= alternate-row intercropping of wheat and clover and WC_w= within-row intercropping of wheat and clover) and the second factor was irrigation regime (C=control in which every row was irrigated during each irrigation and FPRI= fixed partial-root irrigation regime in which one of the two neighboring furrows was irrigated constantly during each watering). In alternate-row intercropping system, wheat and Persian clover were planted in every other row in which three rows were allocated to wheat and three other rows were assigned to Persian clover. In within-row intercropping, planting was based on 2:1 ratio of wheat and Persian clover, respectively, where, for each seed of wheat, two seeds of clover were cultivated. Irrigation regime started after the end of seasonal precipitations at the onset of wheat flowering. The last irrigation was applied in 26 Jun, 2016 when wheat was at the end of seed filling period and clover was at the mid of seed filling period.

Relative water content (RWC): RWC was measured using a 5cm×5cm surface of newly fully expanded leaves (flag leaf and lower leaf) of wheat and clover (means of five upper phytomers). At first, fresh weight of samples was determined and then, samples were floated on distilled water for 7 hours in darkness. The turgid weight was measured. The samples were oven-dried at 75°C for 48 hours for determining their dry weight. RWC was measured using the following equation (Smart and Bingham, 1974):

$$RWC\ (\%) = [(FW-DW) / (TW-DW)] \times 100$$

In which FW is fresh weight, DW is dry weight and TW is turgid weight. RWC was measured at two stages of 12 hours before and 12 hours after irrigation.

Enzyme activity: Lipid peroxidation of wheat and Persian clover was determined by estimating the MDA content in terms of nmol g^{-1} FW. MDA is the final product of lipid peroxidation, introducing a good estimate of lipid peroxidation (Bandeoglu *et al.,* 2004). The method of Heath and Packer (1968) was used to determine MDA content. Free proline (Pro) content in terms of mg g^{-1} FW was measured by the method of Bates *et al* (1973). Activities of peroxidas (POD) and SOD in terms of nmol g^{-1} FW were assayed according to Bergmeyer (1974) and Giannopolitis and Ries (1977), respectively. Enzymes's activity was measured at two stages: 12 hours before each irrigation and 24 hours after each irrigation.

Grain yield: At maturity (wheat was considered as matured when its seeds moisture content reached 14% and Persian clover seeds were harvested in 30 days after flowering when its highest seed quality was attained (Eskandari and Alizadeh-Amraie, 2017)), all plot area was harvested after removing side effects and the grain yield of wheat and Persian clover was determined.

Land equivalent ratio (LER): The profitability of intercropping for grain yield was calculated by determination of LER using the following equation:

$$LER = [(Yab / Yaa) + (Yba / Ybb)]$$

In which:

Yab and Yba are the grain yield of first and second crop in intercropping, respectively. Yaa and Ybb are the grain yield of first and second crop in the sole cropping

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Table1. Some physio-chemical properties of experimental site.

Depth (cm)	Silt (%)	Loam (%)	Clay (%)	Soil texture	pH	EC (dS m^{-1})	Total nitrogen (g)	Organic matter (g)
0-60	35	38	27	Silt-loam	7.79	1.4	0.78	17.20

system.

Data analysis: All statistical procedures were carried out using MSTATC statistical software. Data were checked for normal distribution and homogeneity of variance before analysis. Mean comparison was performed using Duncan's multiple range test at significant level of P≤0.05.

Results

RWC: In all cropping systems, RWC of wheat and Persian clover leaves decreased significantly with lowering water supply induced by partial root-zone irrigation (Table2). Clover showed higher increase in RWC after rewatering compared to wheat. Both crops had 7 percent higher RWC in intercropping compared to their sole cropping systems, where wheat and Persian clover showed 7 percent increase in RWC in intercropping system. Intercropping resulted in increased RWC of wheat and clover under conventional and partial root-zone irrigations. Furthermore, intercropping diminished the negative effects of water deficit, where RWC of wheat and clover in intercropping systems under partial root-zone irrigation did not show significant (P≤0.05) difference with their values in the sole cropping under conventional irrigation (Table2).

Proline content: Proline content of wheat and Persian clover was significantly (P≤0.05) affected by irrigation systems and planting pattern interaction. Wheat and clover had lower proline content in partial root-zone irrigation compared with conventional irrigation (Table 3). Before the irrigation stage, wheat and clover had lower proline in intercropping systems compared to sole cropping (15% and 9% lower for wheat and clover, respectively). After the irrigation, proline content of wheat and clover had a 19% and 31% decrease, respectively (Table 3).

Lipid peroxidation: As shown in Table 4, MDA content of wheat and Persian clover leaves increased in water deficit treatment. In the after-irrigation stage, the MDA content of wheat leaves under partial root-zone irrigation was 84% more than the conventional irrigation. However, before the irrigation, MDA content of wheat and clover showed only a slight increase (about 8 percent) indicating that wheat and clover experienced stress conditions before the irrigation.

After rewatering, the MDA content of wheat and Persian clover decreased greatly, where wheat and Persian clover showed 28% and 50% reduction in MDA content (Table4). The MDA content of wheat leaves in sole cropping and intercropping systems had significantly decreases down to 36% and 24% (P≤0.05) respectively, suggesting that wheat experienced a higher stress under sole cropping. The MDA content of Persian clover leaves showed 58% and 46% reduction in sole cropping and intercropping systems, indicating that intercropping resulted in significant (P≤0.05) moderated stress condition for Persian clover. Because of higher MDA content of leaves, Persian clover was more susceptible to water shortage than wheat. Furthermore, the results showed that lipid peroxidation was closely related to irrigation method and the irrigation was beneficial for higher tolerance against oxidative stress.

SOD activity: In general, the SOD activity in wheat and Persian clover increased in water deficit treatment. As shown in table 5, no significant difference was observed in the activity of SOD in wheat leaves between two irrigation methods before the irrigation. After rewatering, SOD activity was decreased in wheat (19%) and Persian clover (37%). Under partial root-zone irrigation, SOD activity of wheat and Persian clover had 10% and 46% increases compared with the conventional irrigation. Intercropping had no significant effect on SOD activity of wheat and Persian clover leaves.

POD activity: Effects of plant type, irrigation system and the interaction of plant type× irrigation system were significant (P≤0.05) on POD activity. Before irrigation, the highest activity of POD was recorded in Persian clover under partial root zone irrigation system which was 20% more than that of wheat (Table 6). POD activity planting pattern (intercropping and sole cropping) had no significant effect on POD activity.

Grain yield and yield advantage: Due to lower plant population, wheat had lower grain yield in intercropping compared to its sole cropping system. However, under the partial root zone irrigation system, the grain yield of wheat was not lower than that of sole cropping (Table 7). The highest grain yield of Persian clover was achieved under the conventional irrigation system. Partial root zone irrigation resulted in significant (P≤0.01) grain yield reduction of Persian clover. Regarding yield reduction (27% and 36% for wheat and Persian clover, respectively), Persian clover was more susceptible to water deficit compared to wheat.

Wheat-Persian clover intercropping system had much higher LER (P≤0.01) under partial root-zone irrigation (Table 7). However, LER of wheat-Persian clover intercropping under conventional irrigation was also more than unity, indicating the advantage of intercropping in both irrigation systems (Table 1).

Discussion

Lower RWC and higher proline, as well as MDA content and SOD activity indicated that plants were exposed to environmental stress. In respect to the

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Table 2. RWC of wheat and Persian clover leaves in the sole and intercropping systems before and after each irrigation under conventional (W_1) and partial root-zone (W_2) irrigation regimes (the data are means of RWC measured before and after each irrigation).

Irrigation method	Before irrigation						After irrigation					
	Wheat			Persian Clover			wheat			Persian Clover		
	Wh	WC_a	WC_w	Cl	WC_a	WC_w	Wh	WC_a	WC_w	Cl	WC_a	WC_w
W_1	59.3b	62.8a	64.3a	57.0c	61.0b	62.3a	68.3de	73.0c	78.3b	78.6b	82.3a	84.3a
W_2	56.3c	59.6b	61.3ab	55.0c	59.6b	62.0a	68.0e	71.3cd	75.6bc	77.0b	80.3ab	82.0a

Wh: sole wheat; WCa: alternate-row intercropping; WCw: within-row intercropping; Cl: sole clover. Means with different letters indicate significant difference (P≤0.05) with respect to experimental stage (before and after irrigation) and irrigation method.

Table 3. Proline content (mg g^{-1} FW) of wheat and Persian clover in the sole and intercropping systems before and after irrigation under conventional (W_1) and partial root-zone (W_2) irrigation regimes (the data are means of proline content measured before and after each irrigation).

Irrigation method	Before irrigation						After irrigation					
	Wheat			Persian Clover			wheat			Persian Clover		
	Wh	WC_a	WC_w	Cl	WC_a	WC_w	Wh	WC_a	WC_w	Cl	WC_a	WC_w
W_1	0.611a	0.566f	0.551f	0.678^{d}	0.634e	0.618e	0.424f	0.485e	0.519d	0.299h	0.363g	0.492e
W_2	0.809a	0.758b	0.709c	0.778^{b}	0.763b	0.722c	0.607^{ab}	0.581bc	0.564c	0.617a	0.580^{bc}	0.538d

Wh: sole wheat; WCa: alternate-row intercropping; WCw: within-row intercropping; Cl: sole clover. Means with different letters indicate significant difference (P≤0.05) with respect to experimental stage (before and after irrigation) and irrigation method.

Table 4. MDA (nmol g^{-1} FW) content of wheat and Persian clover in the sole and intercropping systems before and after irrigation under conventional (W_1) and partial root-zone (W_2) irrigation regimes.

Irrigation method	Before irrigation						After irrigation					
	Wheat			Persian Clover			wheat			Persian Clover		
	Wh	WC_a	WC_w	Cl	WC_a	Wh	WC_a	WC_w	Cl	WC_a	WC_w	
W_1	0.121^{cd}	0.115^{d}	0.108^{e}	0.119^{d}	0.098^{e}	0.057^{de}	0.074^{bc}	0.081^{b}	0.040^{f}	0.049^{ef}	0.050^{ef}	
W_2	0.131^{bc}	0.120^{cd}	0.122^{cd}	0.144^{a}	0.133^{b}	0.105^{a}	0.101a	0.096^{a}	0.071^{bc}	0.071^{bc}	0.064^{cd}	

Wh: sole wheat; WCa: alternate-row intercropping; WCw: within-row intercropping; Cl: sole clover. Means with different letters indicate significant difference (P≤0.05) with respect to experimental stage (before and after irrigation) and irrigation method.

Table 5. SOD activity (nmol g^{-1} FW) of wheat and Persian clover in the sole and intercropping systems before and after irrigation under conventional (W_1) and partial root-zone (W_2) irrigation regimes.

Irrigation method	Before irrigation						After irrigation					
	Wheat			Persian Clover			wheat			Persian Clover		
	Wh	WC_a	WC_w	Cl	WC_a	Wh	WC_a	WC_w	Cl	WC_a	WC_w	
W_1	2.07^{ab}	2.04^{ab}	1.90^{b}	1.10^{cd}	1.07^{d}	1.62^{b}	1.66^{b}	1.70^{ab}	0.90^{c}	0.95^{c}	0.97^{c}	
W_2	2.19^{a}	2.18^{ab}	2.06^{ab}	1.92^{b}	1.19^{c}	1.85^{a}	1.83^{a}	1.75^{ab}	1.00^{c}	0.99^{c}	0.94^{c}	

Wh: sole wheat; WCa: alternate-row intercropping; WCw: within-row intercropping; Cl: sole clover. Means with different letters indicate significant difference (P≤0.05) with respect to experimental stage (before and after irrigation) and irrigation method.

Table 6. POD activity (nmol g^{-1} FW) of wheat and Persian clover in the sole and intercropping systems before and after irrigation under conventional (W_1) and partial root-zone (W_2) irrigation regimes.

Irrigation method	Before irrigation						After irrigation					
	Wheat			Persian Clover			wheat			Persian Clover		
	Wh	WC_a	WC_w	Cl	WC_a	WC_w	Wh	WCa	WCw	Cl	WCa	WCw
W_1	0.590^{d}	0.546^{d}	0.752^{c}	0.844^{b}	0.865^{b}	0.847^{b}	0.542^{d}	0.642^{c}	0.562^{d}	0.662^{c}	0.656^{c}	0.863^{a}
W_2	0.786^{c}	0.760^{c}	0.566^{d}	0.977^{a}	0.940^{a}	0.941^{a}	0.556^{d}	0.592^{c}	0.501^{d}	0.832^{a}	0.786^{b}	0.789^{b}

Wh: sole wheat; WCa: alternate-row intercropping; WCw: within-row intercropping; Cl: sole clover. Means with different letters indicate significant difference (P≤0.05) with respect to experimental stage (before and after irrigation) and irrigation method.

irrigation method, these factors were higher in sole cropping compared to intercropping system. Wheat and

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Table 7. Grain yield and land equivalent ratio of wheat and Persian clover under different cropping and irrigation system.

Irrigation system	Planting Pattern	Wheat Grain Yield (kg ha^{-1})	Persian clover grain yield (kg ha^{-1})	LER
I_1	Wh	6393^{a}	---	---
	C	---	453^{a}	---
	WC_a	5377^{b}	420^{ab}	1.77 ^{b}
	WC_w	5541^{b}	394^{b}	1.74 ^{b}
I_2	W	4649^{d}	---	---
	Cl	---	290^{d}	---
	WC_a	4935^{cd}	298^{d}	2.09 ^{a}
	WC_w	5132^{bc}	337^{c}	2.26 ^{a}

I_1: Conventional irrigation; I_2: partial root-zone irrigation; Wh: sole wheat; WCa: alternate-row intercropping; WCw: within-row intercropping; Cl: sole clover; LER: land equivalent ratio. Means with different letters indicate significant difference (P≤0.01).

Persian clover experienced stress condition when cultivated under a partial root-zone irrigation system. Since physiological processes of a plant are greatly dependent on the water content, RWC has been considered an important factor for evaluating the resistance to drought stress (Graca *et al*., 2010). It has been also reported that tolerant plants maintain more water in their tissues resulting in higher RWC (Silva *et al*.., 2007). Before irrigation, wheat had higher RWC in two irrigation methods, suggesting that wheat was a more drought tolerant plant compared with Persian clover. However, both wheat and Persian clover had more RWC in intercropping system, indicating that intercropping alleviates the effects of drought stress on wheat and Persian clover growth under low water availability (the partial root zone irrigation method), supporting by proline content of wheat and Persian clover, where wheat and Persian clover had higher concentration of proline when exposed to water deficit conditions. Drought stress increased the content of Proline in *Melissa officinalis* (Abbas-zadeh *et al*., 2008) which is in line with the results of our experiment. Since SOD plays an important role in the reactive oxygen species (ROS) scavenging, its activity is increased under adverse environmental conditions (Aganchich *et al*., 2007). Both wheat and Persian clover showed a lower SOD content after rewatering, indicating the alleviation of drought stress effects on wheat and Persian clover after irrigation.

In bean (*Phaseolus vulg*aris L.) plant, higher MDA production under adverse environmental conditions has been reported (Zlatev *et al.,* 2006). Plants with high tolerance to environmental stress have low lipid peroxidation in their cells (Campos *et al*., 2003). In tolerant plants with lower MDA production, more unsaturated fatty acids exist in cell membrane structure which diminishes membrane destruction under stress condition (Mafakheri *et al.,* 2016). Wheat and Persian clover had higher MDA in sole cropping systems, suggesting that intercropping alleviated drought effects on these crops. This can be resulted from more shading by wheat and more soil surface covering by Persian clover, resulting in lower evaporation.

Under both conventional and partial root zone irrigation, LER was achieved more than once, indicating the profitability of intercropping system for wheat and Persian clover grain production. The profitability of intercropping compared to the sole crop has been reported for many agronomical systems including wheat-chickpea (Mandel *et al.,* 1996), maize-bean (Geren *et al.,* 2008) and wheat-rapeseed (Koocheki *et al.,* 2014), supporting the findings of this experiment.

LER of intercropping in partial root zone irrigation system was much higher (P≤0.01) than that of conventional irrigation, indicating that intercropping partly compensated the negative effects of low water supply. Since RWC of wheat and Persian clover in the intercropping system was higher than that of sole cropping systems (Table 2), wheat and Persian clover were less affected by drought stress in intercropping. Furthermore, yield reduction of wheat and Persian clover in intercropping under partial root zone irrigation was lower than that of sole cropping, resulting in improving LER. These findings are in line with the findings of Yang *et al* (2011) for the wheat-maize intercropping system.

Conclusion

Limited irrigation resulted in oxidative stress in both wheat and Persian clover through increasing the membrane peroxidation and decreasing leaf water content. However, under low water availability, antioxidant activity of wheat and Persian clover increased through an increase in proline and SOD activity. Intercropping alleviated water shortage effects, suggesting that partial root-zone irrigation may have a good potential for reducing water consumption in agricultural systems. In this research, partial root zone irrigation system resulted in grain yield reduction. However, regarding the reduction of water consumption in partial root zone irrigation, the aggregate impact of yield reduction and water consumption was positive. As a final remark, partial root zone irrigation can be considered as an appropriate method which can keep grain yield at acceptable level while reducing the water consumption.

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