

Full Length Research Paper

Relationship between leaf rolling and some physiological parameters in durum wheat under water stress

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Durum wheat is an important staple food in Morocco. Wheat and barley are mostly grown in drought prone areas in this country. Drought is the most limiting factor under Mediterranean climate. Leaf rolling ability has been identified as a potential selection criterion for drought tolerance in cereals. The objective of this study is to test this hypothesis. The role of leaf rolling in water stress tolerance was assessed in eleven Moroccan wheat cultivars. Trials were conducted under greenhouse conditions and three watering regimes simulating different drought levels were applied. Leaf rolling, leaf area, leaf specific weight and relative water content were recorded. The results indicated that the studied cultivars have shown significant differences in their leaf rolling (LR) under water deficit. The varieties 2777 and *Irden* showed the highest LR scored under these constraints. The variety *Marjana* showed the lowest LR score and expressed no LR ability under non limited irrigation treatment. Strong correlation have been observed between LR and relative water content (RWC) ($r=0.923$), and negative correlation was observed between LR and leaf area (LA, $r=-0.783$). Cultivars with high LR scores showed their capacity to control the negative impacts of water stress by keeping their physiological traits to adequate levels in order to maintain the main physiological activities of the plants. This result showed the important role of LR in water stress tolerance in the studied durum wheat cultivars.

Key words: Water stress, agro physiological traits, drought tolerance, leaf rolling, Morocco.

INTRODUCTION

In Morocco, durum wheat is one of the oldest cultivated cereal species. It is highly appreciated by Moroccan

consumers, mainly for the preparation of bread and traditional products with an average consumption of

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around 90 kg/person/year (Taghouti et al., 2017). Morocco is ranked third in the Mediterranean region and first in the North Africa and Middle East region in terms of durum wheat acreage (Nsarellah et al., 2011). This crop is sown over 1.0 million hectares annually and 45% of which are in the arid and semi-arid regions. However, water availability for agriculture in these regions is an issue of growing concern because of the high evaporative demand (about 1500 mm/year) and the low and irregular rainfall (200-300 mm/year) (Bouizgaren et al., 2013). Furthermore, climate change is expected to increase the extents of drought and temporal climatic variation in the Mediterranean region (IPCC, 2007). Water stress limits wheat productivity in the drought-prone areas of Morocco where the average grain yield is low and variable, ranging from 0.5 to 1.2 t/ha (Jouve, 1988). Drought is the most important stress factor.

A viable solution for crop production in these areas is to develop drought tolerant varieties. In this respect, selection of durum wheat is mainly aimed at the creation of new genotypes with adaptive morphological and physiological characteristics, which could provide protection against drought (Sarieva et al., 2010). Leaf rolling is among morphological traits envisaged to maintain yield under water-limited conditions. It is considered as an important drought avoidance mechanism (Richards, 1996). Leaf rolling allows the plant under water stress to reduce its exposed leaf area and to reduce transpiration and gas exchange through the stomata. In addition, it is hypothesized that optimal operation of photosynthesis under drought could be sustained by changes of the rolling of the leaf blade (Price et al., 2002). Selection of cultivars with a capability for leaf rolling, which provides and increased drought tolerance, was used for rice (Dingkuhn et al., 1989), maize (Premachandra et al., 1993), sorghum (Corlett et al., 1994) and wheat (Omarova et al., 1995). In Morocco, durum wheat breeding program has been undertaken since the beginning of the twentieth century. Genetic improvement has led to the development of multiple modern durum wheat cultivars that are highly productive (Nsarellah et al., 2005). However, more information related to drought tolerance of these new cultivars is needed, in particular, the physiological role of leaf rolling which remains insufficiently studied.

The present study aims to assess water deficit effects on leaf rolling and some physiological parameters in eleven Moroccan durum wheat cultivars (*Triticum durum Desf.*), and to determine the relationship between several morphological and physiological traits under water deficit conditions.

MATERIALS AND METHODS

Plant material

The experiment was conducted on 11 cultivars of *Triticum durum* (*Irden*, 2777, *Sebou*, *Yassmine*, *Oumrabiaa*, *Islly*, *Marouane*,

Massa, *Jawhar*, *Marjana*, *Korifla*). Ten varieties were selected and provided by the durum wheat breeding program at the National Institute for Agricultural Research of Morocco (INRA). One variety was selected by the International Center for Agricultural Studies in the Dry Areas (ICARDA) (Table 1).

Experimental conditions and design

Seeds of the studied durum wheat cultivars were surface disinfected with 5% sodium hypochlorite for 5 min and rinsed with deionized water several times, and then germinated at 25°C and total darkness in Petri dishes containing two imbibed layers of filter paper. Seedlings that were 6 to 7 days old were transferred to plastic pots that were 12 cm high and with a 6 cm diameter. The pots were previously filled with 5.0 kg of sterilized clay-loam soil and peat with a proportion of 3:1 (on dry weight basis). Pots were then placed in the greenhouse with a temperature of 27/20° (day/night), 49 to 70% of relative humidity and 16 h photoperiod (21 Klux).

A total of 66 pots containing 8 plants each were subjected to two irrigation treatments: the first treatments is a non-limiting water condition and at soil field capacity (FC) with a frequency of twice a week. The second treatment consists of water stressed conditions through stopping irrigation for one week from the end of the stem extension to the beginning of heading stages. The experiment was conducted during 5 weeks in three replications under homogeneous conditions. The plants at three leaves growth stage were harvested and several parameters were assessed.

Parameters assessed

Leaf rolling

Leaf rolling (LR) was assessed visually in stressed and non-stressed plants using 0 to 7 scales (Table 2): 0 = no leaf rolling, 1 to 3 = low rolling, 4 to 5 = intermediate rolling and 6 to 7 = complete rolling (Figure 1).

Leaf area

Leaf area (LA) was measured by using an area meter (LI-3100C AREA METER) and expressed in cm². The measurements were performed at the beginning of the heading stage on flag leaf.

Specific leaf weight

The specific leaf weight (SLW, mgcm⁻²) is the ratio between the leaf dry weight (LDW) and the leaf area (LA). This is an indicator of leaf thickness or of leaf weight per unit leaf area, it is calculated as follows (Figure 1):

$$SLW = LDW A^{-1}$$

Relative water content (RWC)

Relative water content (RWC) was determined in 0.1 g of fresh leaflets weight (FW) from separate plants. The turgid weight (TW) of the samples were measured after keeping them for 4 h in deionized water, followed by complete drying in a hot air oven until a constant weight was reached then their dry weight (DW) were determined. RWC was calculated by using the following equation:

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

Table 1. The studied wheat varieties and their main characteristics and adaptation zone.

Cultivar	Adaptation zone	Hessian fly resistance (S: Sensitive; R: Resistant)
<i>Irden</i>	Semi-arid, drought tolerant	R
<i>2777</i>	Favorable	S
<i>Sebou</i>	Semi-arid + favorable	S
<i>Yasmine</i>	Large	S
<i>Oum rabiaa</i>	Semi-arid	S
<i>Isly</i>	Large	S
<i>Marouane</i>	Semi-arid, drought tolerant	R
<i>Massa</i>	Large, rainfed	S
<i>Jawhar</i>	Large, Irrigated	S
<i>Marjana</i>	Large	S
<i>Korifla</i> (selected by ICARDA)	Large – Drought tolerant	S

Table 2. Leaf rolling scores utilized in this study.

Rolling class	Rolling index	Description
No rolling	0	Absence of rolling
Weak rolling	1	The tip of the leaf rolls up
	2	A quarter of the leaf rolls up
	3	One third of the leaf rolls up
Medium rolling	4	Half of the leaf rolls up
	5	More than half of the leaf rolls up
High rolling	6	Two third of the leaf rolls up
	7	Thorn shape of leaf fully rolled

Statistical analysis

Statistical analysis consists of a two-way analysis of variance (ANOVA II) and Tukey's grouping test. It was performed by using the SPSS 21.0 software (SPSS, Chicago, Illinois, USA 2012). Significant differences were determined at 0.05 probability level ($P < 0.05$), 0.01 probability level ($P < 0.01$) and at 0.001 probability level ($P < 0.001$).

RESULTS

Effect of water deficit on leaf rolling (LR)

Under non limited water conditions, none of the studied wheat cultivars have shown any leaf rolling. A wide variation in LR scores has been noted among all of the studied cultivars in response to limited water conditions (Figure 2). *Marjana* cultivar showed the lowest LR score (LR = 1). The maximum LR scores were reached by *2777* and *Irden* cultivars. In contrast, *Massa*, *Oum rabiaa*, *Marouane*, *Jawhar* and *Isly* cultivars showed low or moderate leaf-rolling abilities under stressed conditions.

Effect on leaf area (LA)

The results showed that water deficit has significantly ($P < 0.001$) reduced the leaf area of flag leaves in all of the studied wheat cultivars with significant variations among them (Table 3). Under non water limited conditions, *Irden* and *Oum Rabiaa* cultivars showed the highest LA values of 15.3 ± 0.73 and 14.4 ± 0.82 cm², respectively. The lowest LA score, of 10.9 ± 0.68 cm² have been recorded on the variety *Marjana* under water limited conditions. This variety showed the lowest LA reduction rate (10.3%). The LA reduction rates were more pronounced in *Irden* and *2777* cultivars and were as high as 57.2 and 49.7%, respectively.

Effect on specific leaf weight (SLW)

The SLW was significantly ($P < 0.001$) reduced under water stressed treatments in all of the studied cultivars (Table 3). *Isly* cultivar showed the highest SLW values of 2.62 ± 0.02 and 2.04 ± 0.03 mg cm⁻² under well-watered

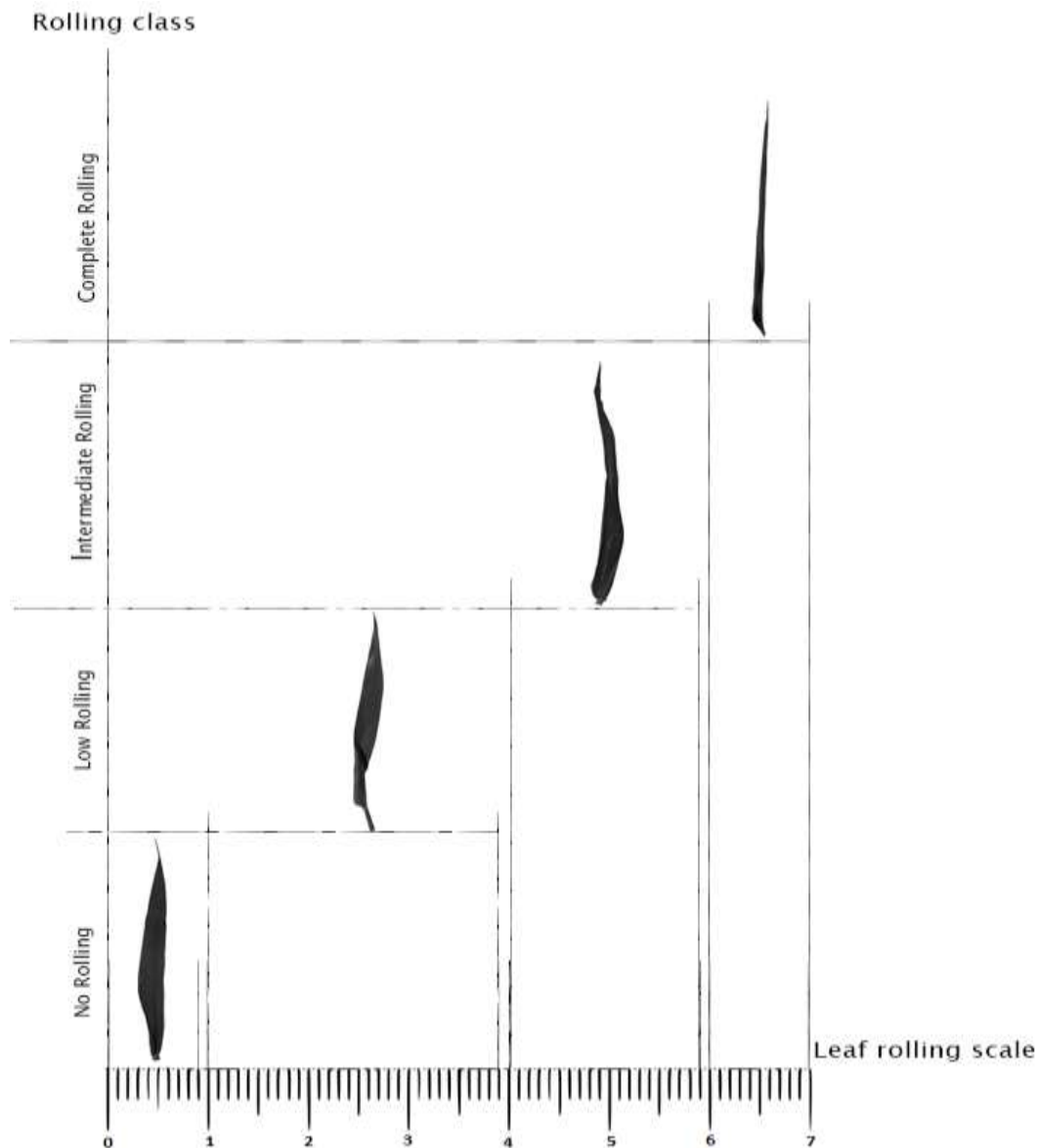


Figure 1. Visual leaf rolling ranking.

and stressed conditions, respectively. However, in terms of reductions under stress conditions, *2777* and *Irden* cultivars presented the lowest values (6.8 and 10.3%, respectively) while, *Marjana* variety has shown the highest reduction rate (33.1%).

Effect of drought stress on relative water content (RWC)

Water stress has exerted a negative effect on RWC in all of the studied cultivars under water deficit (Tables 3). Significant differences have been noted in the behavior of each studied cultivar. The highest RWC values of $81.8 \pm$

5.5 and $79.2 \pm 2.2\%$ were recorded for *Irden* and *2777* cultivars under water stress, respectively. Meanwhile, the lowest RWC values of 21.3 ± 4.4 and $45.8 \pm 2.3\%$ were recorded for *Marjana* and *Isly* cultivars under the same conditions.

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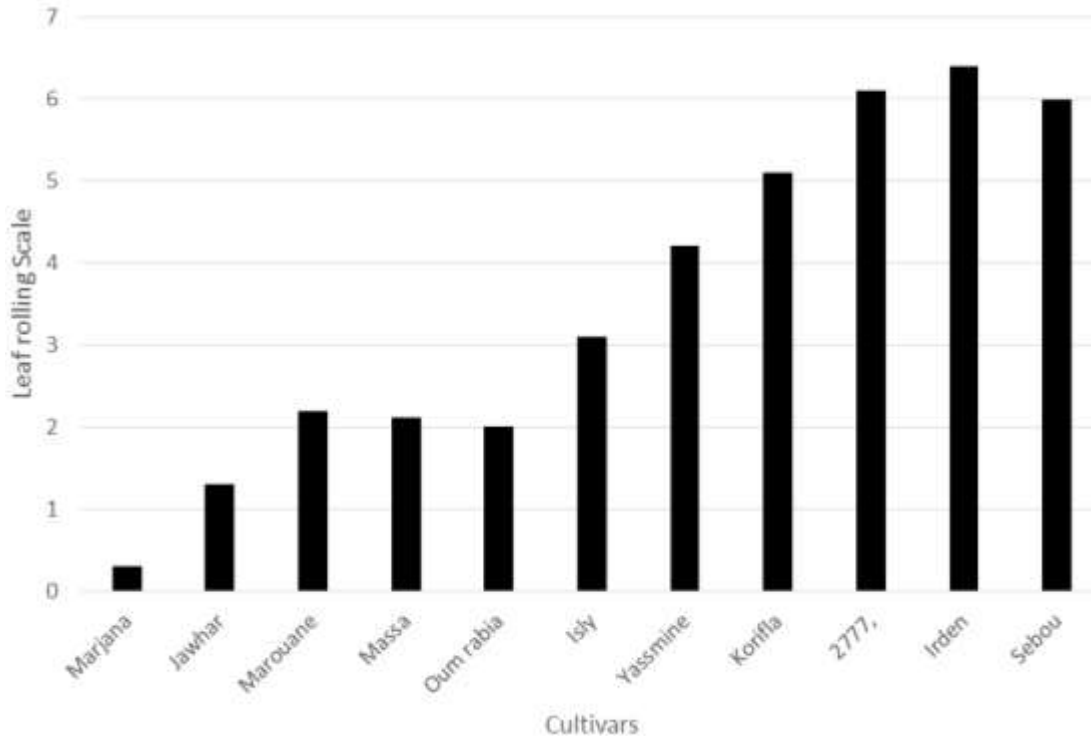


Figure 2. Leaf rolling score of studied durum wheat cultivars noted under water stress in greenhouse experiments.

Correlations between leaf rolling (LR) and agrophysiological traits and preservation of their performance under water stress

The correlations between leaf rolling and studied agrophysiological traits are shown in Table 4. A strong positive correlation is observed between LR and RWC ($r=0.923$, $P<0.001$). Strong negative correlations are shown between LR and LA ($r= -0.783$, $P<0.001$), and between LR and SLW ($r= -0.407$, $P<0.001$).

DISCUSSION

The irregular occurrence of drought periods influences the growth and yields of wheat cultivars grown in drought-prone regions of Morocco. Cultivation of drought adapted cultivars is the best approach to avoid yield loss under water deficit conditions. One of the essential defense adaptation mechanisms in plants to avoid drought damage is the maintenance of osmotic adjustment in the cells. This can be achieved by acting on some plant attributes such as the reduction of leaf size, or leaf rolling under a short-term water deficiency (Nemeskéri et al., 2012). In our experiment, the eleven wheat cultivars were visually assessed for leaf rolling ability under drought stress conditions as an indicator of plant water status. There was a marked variation among cultivars for this

trait, and two cultivars 2777 and *Irden* were highly scored. This increased leaf rolling under drought stress would have the advantage of preventing water loss, respiratory losses, and of promoting a cooler leaf temperature and avoiding radiation damage (Pandey and Shukla, 2015). It may be considered as drought adaptive mechanism in wheat (Sarieva et al., 2010). Bogale et al. (2011) reported that genotypes with high LR presented more grain yield and high-water productivity in comparison with those of lower scores. This may lead to the conclusion that leaf rolling has positive effects on controlling the leaf expansion and stomatal water loss under water limited conditions. In this study, *Marjana* cultivar presented low LR score and expressed no ability of leaf rolling under both water treatments. This may lead to classify it as a sensitive cultivar to drought stress. According to the leaf rolling scores presented in Figure 2, the other cultivars were classified as moderately tolerant or moderately sensitive to drought stress.

The results showed that leaf area (LA) was negatively correlated to LR trait ($r= -0.783$, $p<0.001$) and to RWC ($r=-0.580$, $P<0.001$). Larbi and Mekliche (2004) reported that genotypes possessing the ability to maintain green leaf area duration "stay green" and high RWC traits throughout grain filling are potential candidates to assure yield in semi-arid regions. The importance of flag leaf in grain filling is well recognized. For grain filling to occur under drought, either a relatively uncompromised or a

Table 3. Effect of water limited conditions on leaf area (LA), specific leaf weight (SLW) and relative water content (RWC) of the Moroccan durum wheat cultivars.

Cultivar	Leaf area (LA) (cm ²)			Specific leaf weight (SLW) (mg cm ⁻²)			Relative water content (RWC) (%)		
	Treatments			Treatments			Treatments		
	Non-limited water	Water stress	Reduction (%)	Non-limited water	Water stress	Reduction (%)	Non-limited water	Water stress	Reduction (%)
<i>Marjana</i>	10.9 ± 0.68 ^a	9.81 ± 0.79 ^{de}	10.3	2.49 ± 0.05 ^g	1.66 ± 0.03 ^f	33.1	84.8 ± 9.3 ^a	21.3 ± 4.4 ^{de}	75.0
<i>Massa</i>	13.1 ± 0.45 ^{bc}	10.77 ± 0.28 ^{ef}	18.2	1.94 ± 0.02 ^d	1.50 ± 0.03 ^{de}	22.3	91.5 ± 0.8 ^{cd}	52.5 ± 8.2 ^{ef}	42.5
<i>Oum Rabiaa</i>	14.4 ± 0.82 ^c	11.78 ± 0.43 ^f	18.3	2.04 ± 0.09 ^e	1.57 ± 0.08 ^e	23.1	98.8 ± 0.4 ^{de}	53.5 ± 5.2 ^f	45.9
<i>Marouane</i>	12.1 ± 0.36 ^{ab}	9.49 ± 0.41 ^{cd}	21.2	1.69 ± 0.01 ^c	1.30 ± 0.01 ^b	23.5	86.5 ± 3.0 ^{ab}	47.8 ± 1.5 ^{cd}	44.5
<i>Isly</i>	13.7 ± 0.81 ^c	10.81 ± 0.75 ^{ef}	21.5	2.62 ± 0.02 ^h	2.04 ± 0.03 ^h	22.1	93.5 ± 2.4 ^d	45.8 ± 2.3 ^{ef}	50.7
<i>Jawhar</i>	13.2 ± 0.78 ^{bc}	10.44 ± 0.81 ^{de}	21.5	2.14 ± 0.05 ^f	1.68 ± 0.04 ^g	21.0	95.7 ± 1.4 ^{cd}	56.5 ± 4.2 ^{de}	41.0
<i>Yassmine</i>	11.3 ± 0.74 ^a	8.67 ± 0.60 ^c	23.8	1.68 ± 0.04 ^{bc}	1.42 ± 0.04 ^c	15.6	85.7 ± 4.8 ^a	66.0 ± 6.2 ^c	23.3
<i>Korifla</i>	13.6 ± 0.77 ^c	9.66 ± 0.68 ^{cd}	29.3	1.27 ± 0.03 ^a	1.08 ± 0.02 ^a	15.1	93.7 ± 3.1 ^d	69.0 ± 5.9 ^{cd}	26.5
<i>Sebou</i>	13.6 ± 0.17 ^c	7.19 ± 0.05 ^b	47.5	1.63 ± 0.05 ^{bc}	1.38 ± 0.07 ^c	15.5	91.0 ± 3.2 ^d	69.3 ± 4.8 ^b	23.8
<i>2777</i>	12.0 ± 0.72 ^{ab}	6.05 ± 0.43 ^a	49.7	1.62 ± 0.02 ^{bc}	1.50 ± 0.03 ^{de}	6.8	89.2 ± 2.1 ^{ab}	79.2 ± 2.2 ^a	11.2
<i>Irden</i>	15.3 ± 0.73 ^d	6.60 ± 0.51 ^{ab}	57.2	1.60 ± 0.01 ^b	1.44 ± 0.01 ^{cd}	10.3	90.7 ± 3.9 ^e	81.8 ± 5.5 ^{ab}	10.0
	df	F		df	F		df	F	
Irrigation	1	1267,8***		1	2618,35***		1	1760,15***	
Cultivar	10	42,2***		10	687,86***		10	52,03***	
Interactions	11	39,6***		11	43,61***		11	43,61***	

*Significance at 0.05 probability level. **Significance at 0.01 probability level. ***Significance at 0.001 probability level; NS: not significant at 0.05. Values are means of six replicates ± standard errors and superscript letters represent significance of Tukey's test at 0.05 probability level.

favorably reprogrammed function of flag leaf is required to maintain synthesis and transport of photo-assimilates (Pandey and Shukla, 2015).

Significant variation in specific leaf weight (SLW) has been observed in the studied wheat cultivars subjected to non-limited and deficit water conditions. Similar results were reported by Singh and Rajan (2009). Also, Semcheddine and Hafsi (2014) conducted an experiment on 10 durum wheat (*Triticum durum* Desf.) genotypes under rainfed conditions of Eastern Algeria and observed that SLW significantly differed among water treatments at heading and grain filling stages suggesting the possibility of selecting tolerant genotypes for drought tolerance under

semi-arid condition. They reported that the variation in SLW was due to enhanced photosynthate translocation efficiency which is a function of leaf dry matter content. In this study and in relation with rolling leaf trait, low reduction in SLW was recorded under drought stress for *2777* and *Irden* cultivars which are characterized by their high leaf rolling ability. The two parameters were significantly and negatively correlated ($r = -0.407$, $P = 0.001$) showing the role of LR in maintaining SLW under water limited conditions. A previous study indicated that flag leaf area and its active duration during grain filling has been considered as an essential trait in determining the grain yield (Khaliq et al., 2008).

Richards et al. (2002), Wu (2009), and Zhang et al. (2009) reported that leaf rolling in rice (*Oryza sativa* L.) reduces LA, improves photosynthetic efficiency and delays senescence by reducing water loss via the regulation of transpiration through stomata and optimizing light transmission and by consequence increasing grain yield.

Water stress has produced a negative effect on RWC; thus, under stress conditions, all cultivars lost much more water than under normally irrigated treatment. Water deficit caused 10 to 75% RWC reductions of the leaves.

The studied cultivars were significantly varied in maintaining a stable RWC under both water stress and non-limited water conditions. This reduction

Table 4. Correlations between agro-physiological traits and rolling leaf measured under drought stress treatment in the 11 assessed durum wheat cultivars.

Correlation	Relative water content	Leaf area	Specific leaf weight	Rolling leaf
Relative water content	1			
Leaf area	-0,580** (P<0.001)	1,000		
Specific leaf weight	-0,414** (P=0.01)	0.334** (P=0.006)	1,000	
Rolling leaf	0.923** (P<0.001)	-0.783** (P<0.001)	-0.407** (P=0.001)	1,000

**Significant at P<0.05

under stress was also reported by Kumar et al. (2014). The reduction might be due to rapid decline in cell division and leaf elongation under drought. This result confirms the findings of a previous study on durum wheat (Mekliche et al., 1992), showing the effect of water stress on RWC in wheat plants. Under stressed conditions, cultivars with high leaf rolling ability (2777 and *Irden*) showed their ability to restrict water loss. Significant positive correlation was observed between leaf rolling and RWC ($r= 0.923$, $P<0.001$). According to El Jaafari (2000), the ability of the plant to survive severe water deficit depends on its ability to restrict water loss through the leaf epidermis after the stomata have reached minimum aperture. During drought stress, plant water balance is disrupted and as a result, the RWC and water potential of leaves decreases (Bajjii et al., 2001). Changes in the RWC of leaves are considered to be a sensitive indicator of drought stress and to be a more useful integrator of plant water balance than the leaf water potential (Strauss and Agenbag, 2000). It has been reported also that highly leaf rolling rice genotypes presented high osmolyte accumulation and leaf RWC under drought stress (Swapna and Shylaraj, 2017). In addition to morphological responses, tolerant genotypes may accumulate more osmolytes in order to preserve the structural and the functional integrity of their cells (Mouradi et al., 2016). By using more than 200 rice genotypes, Cal et al. (2019) reported that several genotypes continue transpiration when rolling. They demonstrated that this mechanical response by bulliform cells to drought stress is not directly related to physiological process in rice. It could be useful to understand in detail of the LR relations by comparing large number of wheat genotypes in terms of several other physiological and biochemical traits before these traits could be used in drought tolerant genotypes selection.

Conclusion

Drought tolerance is a complex phenomenon involving many adaptation mechanisms. Among these mechanisms, leaf rolling induces a significant positive effect on RWC and on photosynthetic activity under stress conditions. Therefore, it is an important drought-avoidance mechanism and represents an important tool

to be used in wheat-breeding program to select cultivars adapted to drought-affected environments.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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