

# Stomata development variability of ten wheat genotypes under early water stress

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**Abstract** - Much is known about the physiological control of stomatal aperture to adjust water availability. In contrast, few studies were conducted on the stomatal development control to limit water loss. The objective of this study was to investigate stomatal density control in response to water deprivation for ten wheat genotypes: six varieties of Durum wheat and four varieties of Bread wheat. Three water regimes were applied. Separate statistical analysis of leaf area (LA) and stomatal density (SD) at three leaf stages showed significant differences between Durum wheat and Bread wheat and all varieties at each stage. Results revealed that SD increased with leaf stages. Drought reduced LA and induced various responses in terms of SD. In another hand, tolerance indices of SD was in concordance with results based on LA and revealed that only genotype "Mahmoudi" limited the stomatal development in response to water loss. These findings highlight the role that could be played to stomatal development during leaf expansion as a longer term to limit water loss from leaves. They also put the emphasis on the variability of stomatal response between genotypes. Finally, for eventual breeding program the key roles of SD should be considered to select better genotypes in terms of drought adaptability. Also, Mahmoudi should be recommended as potential genotype for drought tolerance.

**Keywords:** Water stress, Stomata density, Wheat, Variability.

## 1. Introduction

Genetics and physiology became linked to success plant breeding in environment stress adaptation (Monneveux, 1998). Physiological and anatomical reactions would be analyzed to understand adaptation mechanism to water stress and then to identify some interesting parameters to select best genotypes. Especially since the majority of Tunisian cereal crops are highly dependent on climatic conditions and they have to face frequent droughts (Slama, 2005). Among the morpho-physiological characters, stomata structure reflect both maximize photosynthetic efficiency and minimize water loss (Mehri et al., 2009). In fact, stomata control the exchange of water and carbon dioxide between the plant and the atmosphere (Loranger and Shipley, 2010). Several researches have focused on changes in light intensity and CO<sub>2</sub> concentrations effects on stomata mechanism (Stephen et al., 1993; Betts et al., 2007; Wagner et al., 2011), which has great significance given to the changes in global climate. However, the influence of other environmental parameters such as water deficit needs to be investigated as well as how plants integrate their stress signals to determine a developmental response. The rate of transpiration can be adjusted by the regulation of stomatal opening and density (Casson and Gray, 2008). Reduction of stomatal density seems to be a good water stress resistance indicator. The objectives of this work were to investigate the response of ten Tunisian wheat genotypes evaluated under water stress based on stomata density.

## 2. Material and methods

### 2.1. Vegetal material

Ten wheat genotypes were the subject of this work (Tab.1): six varieties of Durum wheat (*Triticum durum* L.) called : Karim, Nasr 99, Mahmoudi, Jneh-Khottifa, Baiadha and Souabaa-Aljia and four varieties of Bread wheat (*Triticum aestivum* L.) called Haidra, Utique 96, Vaga and Byrsa.

**Table 1:** Provenance of studied wheat genotypes.

<b>Genotypes</b>	<b>Provenance</b>
<b>Karim</b>	Selected by the "Institut National de Recherches Agronomiques de Tunisie" INRAT crossing (D21563/AA "S" // Fg "S" - CM9799-126M-1M4Y-OBJ)
<b>Nasr 99</b>	Made at ICARDA in 1985. It is derived from the cross (ICD 85-1340).
<b>Mahmoudi</b>	Originally introduced in Setif / Algeria. It is a selection made by Botanical Service of Tunis in a population of type Mahmoudi from the region of Kasserine.
<b>Jenah-Khottifa</b>	Selected by pedigree culture from cultivated populations in the Tunisian north.(INRAT).
<b>Souabaa Algia</b>	Improved by the INRAT
<b>Baiada</b>	Improved by the INRAT.
<b>Haidra</b>	Selected by the method of "bulk" in the INRAT (Bow "S" / Dougga 74- 83-89-osbj-OKF-oe-26kf169j-obj).
<b>UTIQUE 96</b>	From the cross (CM85836 (ND/VG9144//Kal/Bb/3/Yaco/4/Vee #5 CM85836-5oy-OM-oy-3M-oy-oBj).
<b>VAGA</b>	Improved by CIMMYT/ICARDA and distributed to the INRAT at 1992.
<b>BYRSA</b>	Released by CIMMYT and reached to INRAT in 1982 at F6 stage (CM34630-D)

*SOURCES : Rajaram and Hettel, 1995 ; Deghais et al., 2007*

## 2.2. Experiment installation

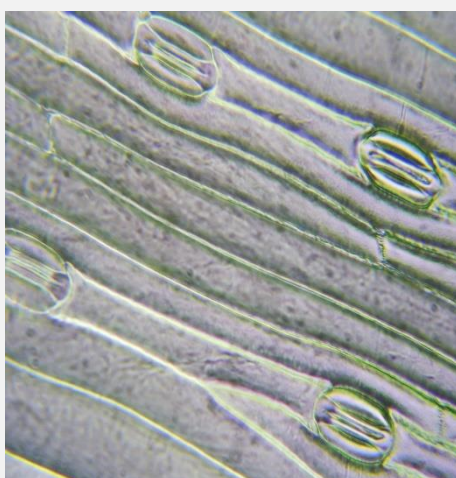
The experiment was taken under glasshouse and three water regimes were installed. The soil water content was maintained at 100% (T1), 50% (T2) and 30% (T3) of substrate water capacity. Pots have been weighed every 2 days in the morning, and water has been added until the initial weight of the pots was reached for each treatment. Treatments were applied between 2–3 and 6 leaf stages (48 days).

## 2.3. Measurements

Stomata density was calculated by optical microscopic observations performed on epidermic impressions found on a tape colorless. The method is to apply a coat of transparent polish over leaf area of almost 2 cm<sup>2</sup>. Once the varnish has become dry, clear tape is glued on top, then removed and placed on a microscope slide for observation. Three replications were carried out on both sides of wheat leaves and that for control plants and stressed plants. Given that counting the number of stomata per surface was done, microscope observation had a magnification equal to 400 times (40 x 10). Similarly, the leaf area on which stomata density counts were measured, was carried out using an optical planimeter.

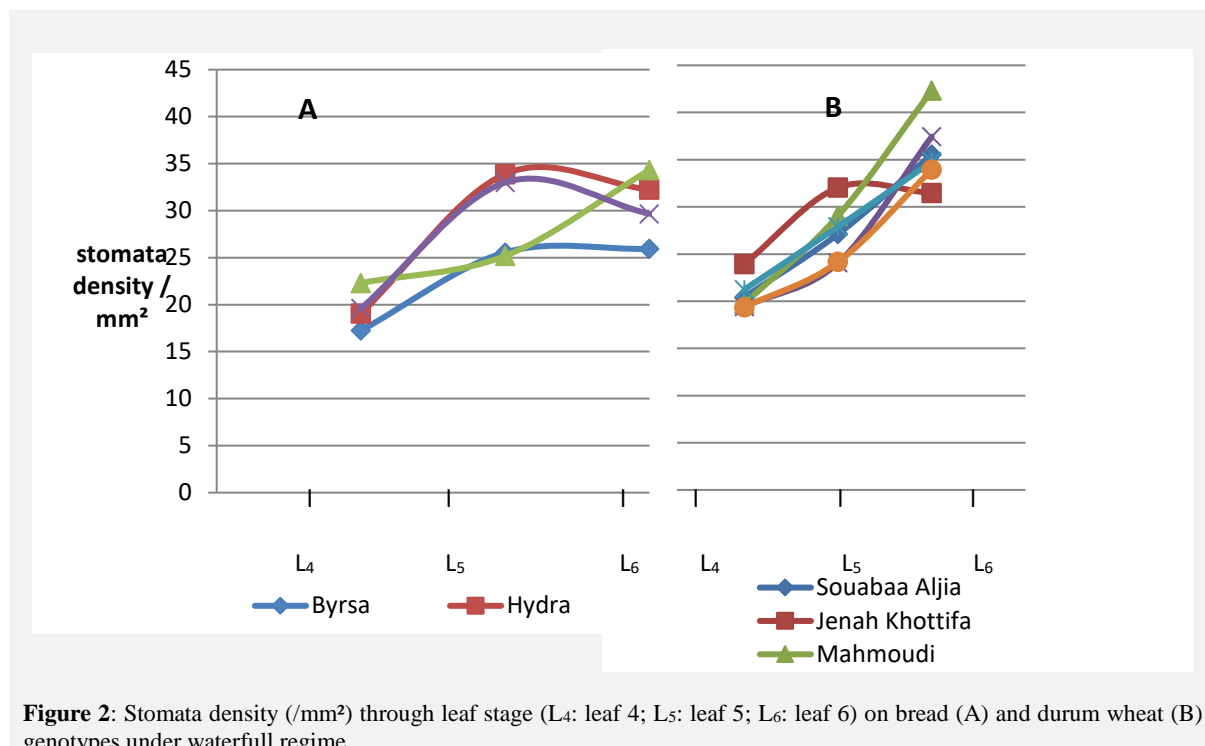
## 3. Results and discussion

The regular and linear array of stomata throughout the cellular structure of the epidermal leaf were observed (figure 1).



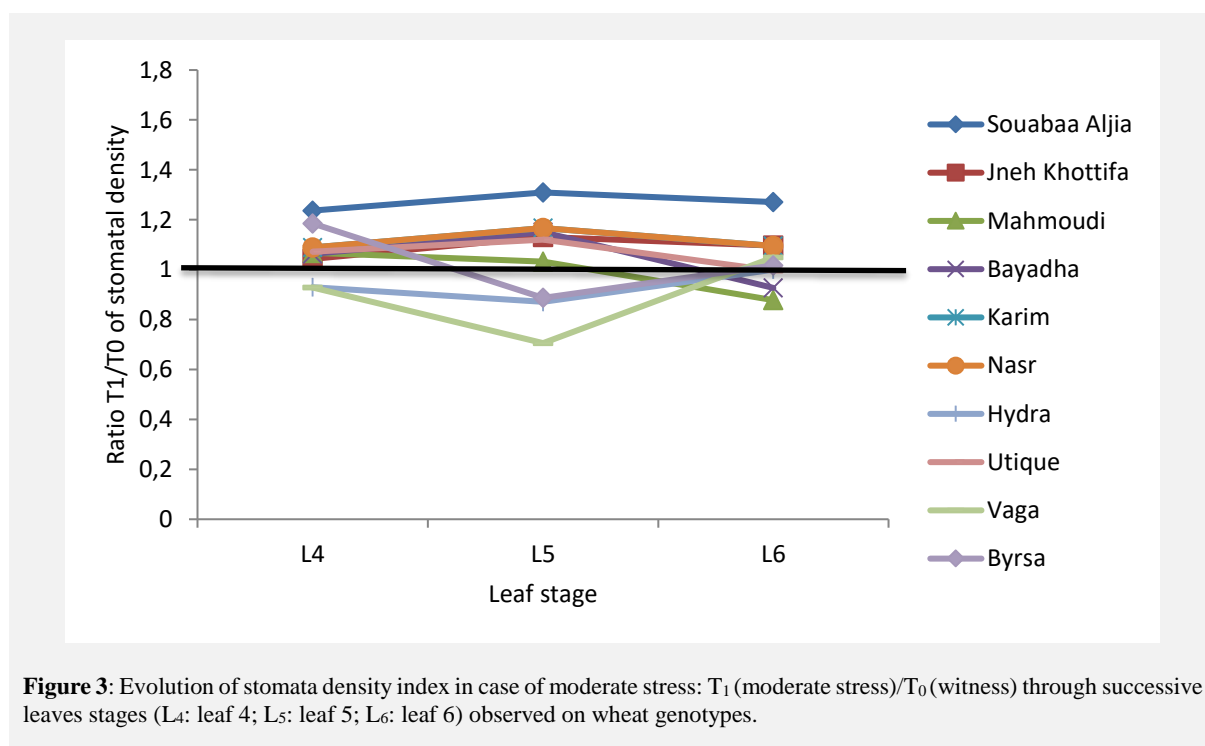
**Figure 1:** Epidermal peels (x40) showing examples of both ordered and random abaxial stomatal patterning of wheat leaf.

At waterfull regime, stomata density depended upon species and leaf stages. It varied on durum wheat, from  $20.75 \pm 2.8$  to  $36.1 \pm 6$  stomata /mm<sup>2</sup> at fourth and sixth leaves stages, respectively (figure2). Therefore, on bread wheat, it was slightly lower and varied from  $19.6 \pm 2.8$  to  $30.4 \pm 6$  stomata /mm<sup>2</sup> on the same leaves stages.



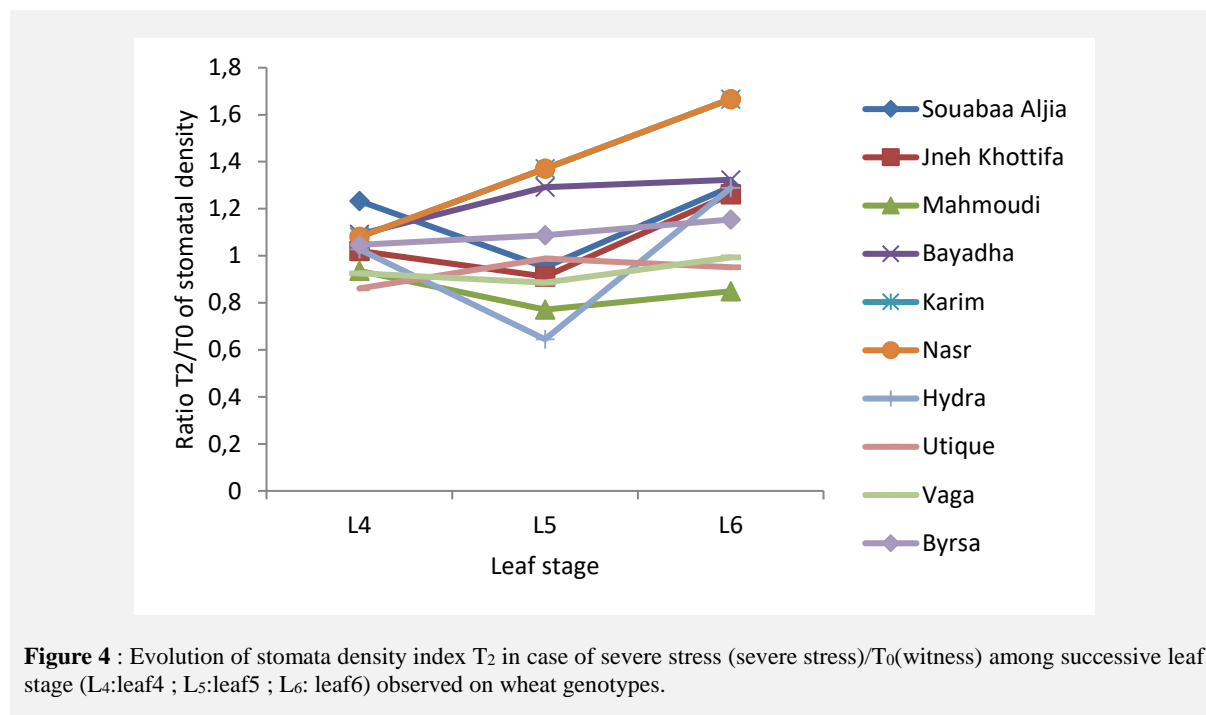
**Figure 2:** Stomata density (/mm<sup>2</sup>) through leaf stage (L4: leaf 4; L5: leaf 5; L6: leaf 6) on bread (A) and durum wheat (B) genotypes under waterfull regime.

For moderate water stress, at five leaf stage, stomata density decreased for the three bread wheat genotypes (Vaga, Hydra and Byrsa). For the durum wheat genotypes, Mahmoudi stomata density declines slightly at sixth leaf. However, on the majority of studied genotypes, an increase were observed about 10 - 20 % of stomata density ( $T_1/T_0 > 1$ ) due to this rate of water regime (figure 3).



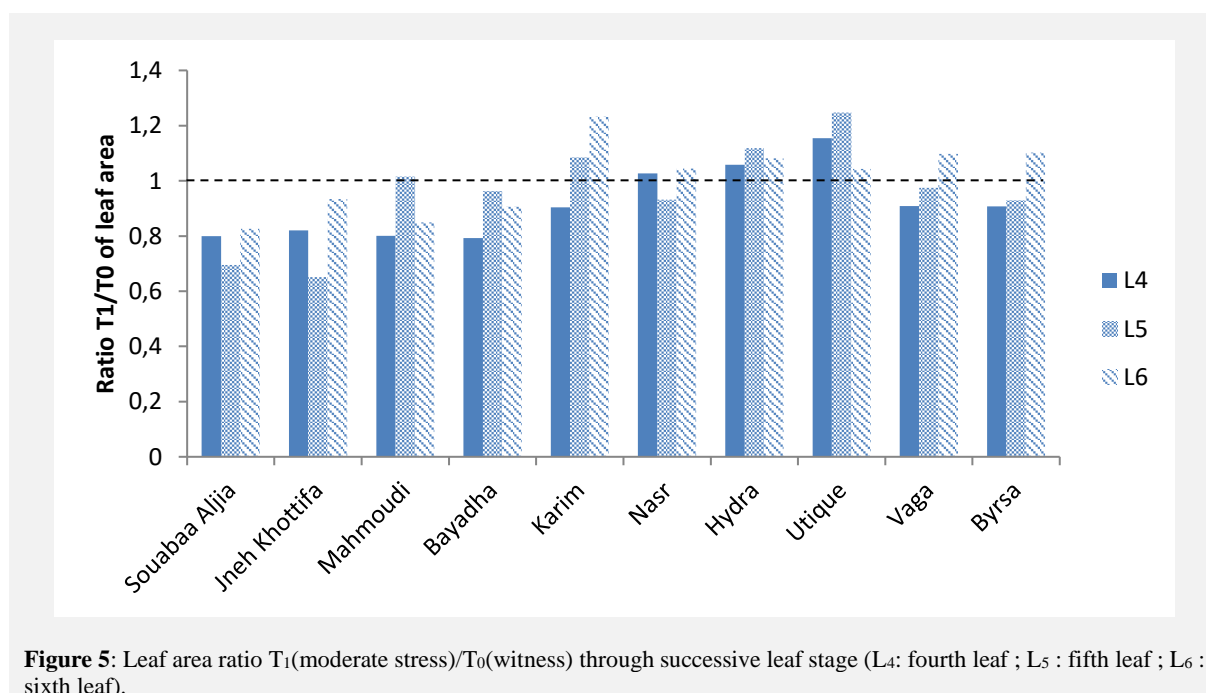
**Figure 3:** Evolution of stomata density index in case of moderate stress:  $T_1$  (moderate stress)/ $T_0$  (witness) through successive leaves stages (L4: leaf 4; L5: leaf 5; L6: leaf 6) observed on wheat genotypes.

For severe stress, at five leaves stage the stomata density decrease for Hydra, Mahmoudi, Vaga and Jneh Khottifa. Therefore, at six leaf stage the decrease is recovered for all aforementioned genotypes at exception of Mahmoudi who maintain its decline. For the other genotypes, we register an increase of stomata density varying from 10 to 80% (figure 4).



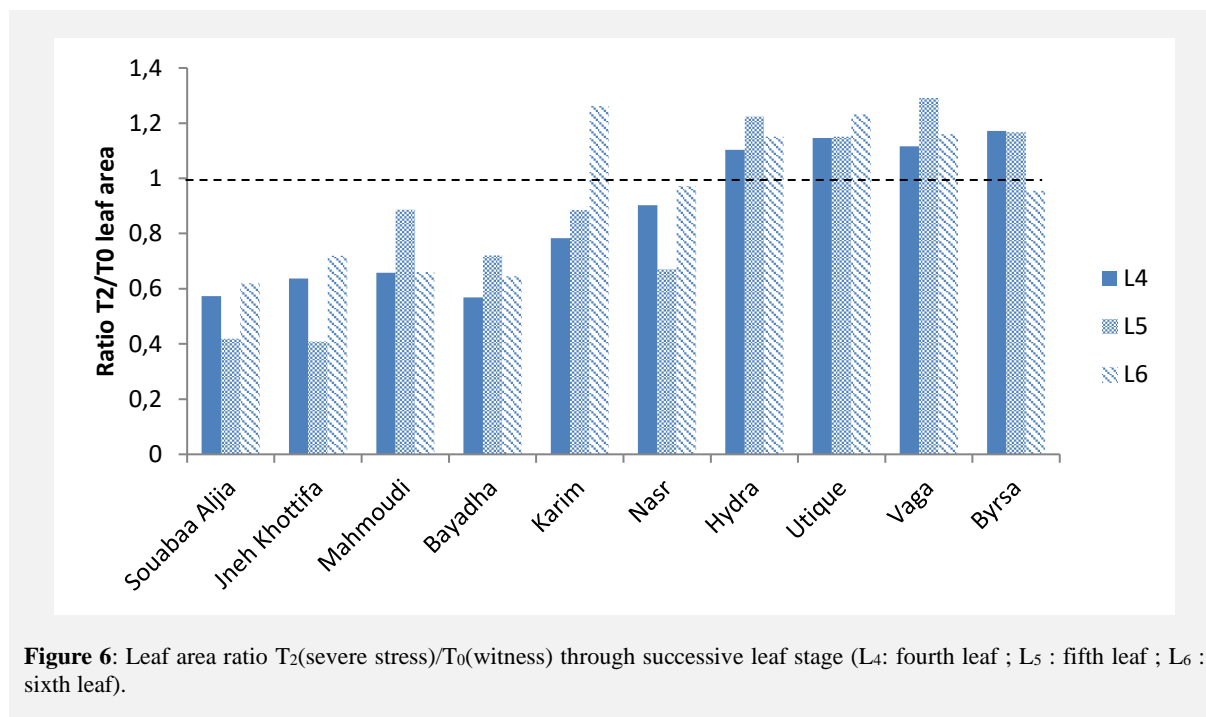
**Figure 4** : Evolution of stomata density index  $T_2$  in case of severe stress (severe stress)/ $T_0$ (witness) among successive leaf stage (L<sub>4</sub>:leaf4 ; L<sub>5</sub>:leaf5 ; L<sub>6</sub>: leaf6) observed on wheat genotypes.

Leaf Area ratio ( $T_1/T_0$ ) of ten genotypes calculated at 3 leaves stages and under moderate water stress was presented at figure 5. Leaf area declined for the majority of bread wheat genotypes (Souabaa Aljia, Jneh Khottifa, Bayadha), at exception of Mahmoudi on fifth leaf. For the other genotypes we observed an increase tendency compared to full irrigated conditions (figure 5).



**Figure 5**: Leaf area ratio  $T_1$ (moderate stress)/ $T_0$ (witness) through successive leaf stage (L<sub>4</sub>: fourth leaf ; L<sub>5</sub> : fifth leaf ; L<sub>6</sub> : sixth leaf).

Leaf area ratio ( $T_2/T_0$ ) of ten genotypes calculated at 3 leaves stages and under severe water stress was presented at figure 6. Leaf area decreased for all autochthon genotypes (Soubaa Aljia, Jneh Khouttifa, Bayadha and Mahmoudi). In contrast, we observed an upsurge relatively to full irrigated conditions for all other genotypes at exception of Nasr (figure 6).



**Figure 6:** Leaf area ratio  $T_2$ (severe stress)/ $T_0$ (witness) through successive leaf stage (L4: fourth leaf ; L5 : fifth leaf ; L6 : sixth leaf).

For all varieties combined, the correlation between leaf area and stomata density was not significant ( $r = 0.018$ ). However, with separate analysis by genotype (table 2), a correlation between these two parameters becomes more significant and tends to be negative but remains very heterogeneous intra-species.

Moreover, leaf area index and stomata density index seems to be not correlated in despite of significant values for certain genotypes especially at leaf four stage.

Significant differences of stomata density between leaf stages were showed for the tow species and varied from  $20.75 \pm 2.8$  at fourth leaf to  $36.1 \pm 6$  stomata / $\text{mm}^2$  at sixth leaf on durum wheat. Then, it is slightly lower on bread wheat, varying from  $19.6 \pm 2.8$  to  $30.4 \pm 6$  stomata/ $\text{mm}^2$  at the same leaves stages. This finding was in concordance with the results reported by Poole et al.(1996). They demonstrate that a high level of variation in stomatal characters occurs both within and between leaves. Correlations between stomatal density, epidermal cell density and stomatal index were found on tree leaves (Poole et al., 1996). Those indicate that the variation in stomatal density within leaves arose primarily from local differences in stomatal differentiation, rather than from local differences in leaf expansion. Similar results found by Araus et al. (Araus et al., 1996) concluded that complex relationship between different morpho-physiological characteristics probably control stomata conductance differences among genotypes and that these relationships differ for each different plant part.

The plant reacts differently depending on the genotypes and the intensity of stress considered. Three responses cases were registered depending upon genotypes. The improved genotypes of durum wheat (Nasr and Karim) and all bread wheat genotypes increased their stomatal densities and maintain a good leaf growth as a result of stress. Other genotypes increased their stomatal densities but they are affected in term of leaf area, it was the case of Soubaa Aljia, Jneh ElKhouttifa, Bayadha. Finally, Mahmoudi was the unique genotype showing a decrease in stomata density and leaf area.

Among the studied genotypes, Mahmoudi appears very interesting. In fact, his stomatal density decreases progressively depending upon the level of stress. It seems to be a strategic response to reduce leaf water loss and ensure a more efficient use of available water resources. These results were similar to other reported by Zhenzhu and Guangsheng (2008) who indicate high flexibilities in stomatal density and guard cell size changing in response to water status in a perennial grass.

**Table 2** : Correlation values between leaf area (LA) and stomata density (SD) in full irrigated conditions (witness) and tow stressful conditions (moderate and severe), and between tolerance index (leaf area and stomata density ratio :  $T_1/T_0$ ,  $T_2/T_0$ ) in tow water stressed conditions through three successive leaf stages (L<sub>4</sub>, L<sub>5</sub>, L<sub>6</sub>)

Water regime	Witness (T <sub>0</sub> )			Moderate stress(T <sub>1</sub> )						Severe stress (T <sub>2</sub> )					
	L <sub>4</sub> A-SD	L <sub>5</sub> A-SD	L <sub>6</sub> A-SD	L <sub>4</sub> A-SD T <sub>1</sub>	L <sub>4</sub> A-SD T <sub>1</sub> /T <sub>0</sub>	L <sub>5</sub> A-SD T <sub>1</sub>	L <sub>5</sub> A-SD T <sub>1</sub> /T <sub>0</sub>	L <sub>6</sub> A-SD T <sub>1</sub>	L <sub>6</sub> A-SD T <sub>1</sub> /T <sub>0</sub>	L <sub>4</sub> A-SD T <sub>2</sub>	L <sub>4</sub> A-SD T <sub>2</sub> /T <sub>0</sub>	L <sub>5</sub> A-SD T <sub>2</sub>	L <sub>5</sub> A-SD T <sub>2</sub> /T <sub>0</sub>	L <sub>6</sub> A-SD T <sub>2</sub>	L <sub>6</sub> A-SD T <sub>2</sub> /T <sub>0</sub>
Souabea Algia	-0.67	0.23	0.22	0.73	-0.08	-0.32	-0.39	0.36	0.07	0.03	-0.81	-0.86	-0.42	-0.51	0.13
Jenah Khottifa	-0.17	0.80	0.48	-0.44	-0.79	-0.23	0.07	-0.62	-0.97	-0.48	-0.28	0.07	-0.12	-0.66	0.41
Mahmoudi	0.89	0.82	1.00	-0.78	0.29	-0.63	0.72	-0.49	-0.84	-0.06	0.55	-0.64	0.90	-0.96	0.71
Baiadha	0.48	0.57	-0.37	-0.31	-0.02	-0.01	0.02	-0.14	-0.14	0.75	0.81	0.47	0.60	-0.43	-0.83
Karim	-0.23	-0.63	0.72	-0.29	0.44	0.05	-0.10	-0.45	0.13	-0.61	-0.10	-0.08	-0.18	-0.15	-0.04
Nasr	-0.36	-0.59	0.75	0.49	0.63	0.12	-0.72	0.65	0.53	-0.17	0.73	0.42	-0.21	0.34	0.35
Hydra	-0.23	0.10	-0.28	0.54	-0.33	0.88	-0.30	-0.26	-0.22	0.61	0.38	0.88	0.58	-0.11	-0.63
Utique	-0.70	0.02	0.03	-0.07	0.82	0.43	-0.04	-0.78	-0.87	-0.27	-0.66	-0.66	-0.04	0.39	0.14
Vaga	-0.98	0.51	-0.40	0.65	0.40	0.38	0.65	-0.44	-0.17	-0.67	-0.16	0.24	0.37	-0.14	-0.41
Byrsa	-0.70	0.84	0.93	-0.38	0.29	-0.88	0.78	0.27	0.04	0.07	-0.22	-0.75	0.38	-0.75	0.04

*T<sub>0</sub> : witness ; T<sub>1</sub> : moderate stress and T<sub>2</sub> : severe stress.*

At normal condition, the analyses of correlation indicated a non-significant relationship between stomata density and leaf area when we consider all genotypes combined. In opposite to Loranger and Shipley (2010), who found a significant negative relationship. However, when we dissect it genotype by genotype, the aforementioned correlation and the correlation between stress tolerance indexes varied with leaf stage and stress intensity and became more frequently significant. These results indicate an intra-species variability of stomata development reaction to stress and highlight the alteration of stomatal development under water loss. These findings are in concordance with results of Hamanishi et al. (2012). Otherwise, other factors could alter this correlation; specifically, leaf dry mass per unit area (MA) and foliage density (mass per unit volume) and leaf death may also be initiated by a plant, for example in response to some phenological cue or to shading (Wright and Cannon, 2001; Wright et al., 2004).

#### 4. Conclusion

Stomata development varied in response to water deficit. Increasingly, the intra-species variability of this parameter was emphasized: some genotypes increase stomata density, others maintain the same density and some interesting genotypes decrease it in response to water deficit. These results demonstrate that this parameter play a crucial role to distinguish tolerant genotypes and should be considered by breeder. Mahmoudi appears as a potential genotype recommended for genetic improvement of drought tolerance programs.

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