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# Analysis of the Effects of Stomatal Frequency and Size on Transpiration and Yield of Wheat (*Triticum aestivum* L.)

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**Abstract:** In order to determine the effects of stomatal size and frequency on transpiration rate and yield in wheat, an experiment based on RCBD was performed in glasshouse condition using 21 drought tolerant cultivars. Stomatal frequency was measured in microscopic fields of leaf impressions taken from middle parts of flag leaves. Transpiration rate was expressed as the slope of the linear relationship between detached leaf weight loss (adjusted based on the leaf area) and time after cutting. Another experiment was conducted in the field condition in order to determine the yield of the same cultivars. Significant differences were found among cultivars in terms of transpiration rate, stomatal frequency, stomatal size and yield. However, stomatal characteristics were found to be poorly correlated with both yield and transpiration rate. Transpiration rate was found to be weakly correlated with stomatal size while yield was poorly correlated with stomatal number. More than 90% of total variation of transpiration rate and yield was attributed to unknown sources of variation and could not be explained by variation in stomatal characteristics.

**Key words:** Transpiration % Stomatal frequency % Stomatal size % Yield % Wheat

## INTRODUCTION

Water stress is a crucial climatic variable in wheat growing areas of the world under such conditions. Plant features which increase their capabilities to sustain their physiological processes may increase their growth and productivity [1-3]. Maintaining gas exchange properties in normal rates is among those features which may increase plant growth and yield [4].

Stomata are the major gates for gas exchange of leaves [5-7]. Guard cells that surrounds the stomata contain chloroplasts thus are able to increase their sugar concentration which in turn causes water absorption and swelling of the cells [8, 9]. This makes stomata to show movements during the daytime and change the amount of water transpired and $CO_2$ absorbed. Transpiration however, is not a totally detrimental process. It is normally takes place at leaf surfaces and has a cooling effect and drives the needed force to take up water and nutrients from soil solution and pump them through the plant body [10, 11]. It has been estimated that more than 95% of water entering the plant is lost by transpiration [12, 13].

To cope better with dry condition, stomata must open to allow $CO_2$ uptake and close during drought conditions to minimize water loss by leaves [14]. Stomata can adjust stomatal conductance under environmental conditions to optimize $CO_2$ uptake and transpiration rates [4, 15]. They are responsive to environmental factors such as light, relative humidity, $CO_2$ concentration and plant water status [4, 9]. However, different cultivars of crop plants may have different gas exchange capabilities because they have various numbers of stomata per unit of leaf area and various stomatal sizes [16, 17]. Significant differences have been found among plant species in the responses of their stomata to changing environment [6].

During the past decades several researchers have tried to correlate stomatal frequency and size to transpiration rate as well as yield [16, 18, 19]. It has been shown that Stomatal density of transgenic *Arabidopsis thaliana* plants over-expressing the stomatal density and distributions gene was reduced to 40% and in the sdd1-1 mutant increased to 300 % of the wild type. However, $CO_2$ assimilation rate and stomatal conductance of over- expressers and the sdd1-1 mutant measured under light conditions were unchanged compared

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with wild types [14]. They concluded that lower stomatal density was compensated for by stomatal aperture and increased stomatal density by reducing stomatal aperture.

Leaf permeability has been shown to be significantly rank-correlated with short-term transpiration, with short-term photosynthesis and with grain yield, but not with water consumption [19]. It has been suggested that wheat cultivars having wider somatal aperture produce higher yields without consuming more water [20]. Koy *et al.* [21] found a 25% decrease in frequency of stomata reduced transpiration rates by about 24%. They found that stomatal frequency did not influence the rate of photosynthesis. Hence, the possibility exists of altering transpiration without altering photosynthesis by selecting varieties with fewer stomata. Gaskell and Pearce found that maize hybrids having higher $CO_2$ exchange rate (CER) capabilities and greater stomatal frequency than low-CER hybrids [22]. They found that stomatal density was negatively correlated with grain yield and with stomatal size. Stomatal frequency in wheat was shown to be greater on the adaxial than on the abaxial surface. Mean ratios (abaxial/adaxial) were 0.748 for the first leaf and 0.728 for the second leaf [19].

Wheat is cultivated over a wide range of regions around the world which are characterized by semi-arid climatic condition. Therefore, it usually exposed to drought stress during the growth. The aim of this study was to analyze the relationship between stomatal characteristics, transpiration rate and yield in drought tolerant wheat cultivars and to determine the direct and indirect effects of them on transpiration rate and final crop performance.

Table 1: Characteristics of wheat cultivars used in the experiment

| Cultivar | Response to environmental stresses | Yield (t/ha) |
|---|---|---|
| Navid | Semi-tolerant to cold stress | 5.0 |
| Kavir | Tolerant to salt and terminal drought stress | 6.3 |
| Rowshan | Tolerant to salt and drought stress | 4.0 |
| Chamran | Tolerant to heat and drought stress | 6.3 |
| Alvand | Tolerant to salt, cold and drought stress | 6.5 |
| Azar2 | Tolerant to drought and relatively tolerant to cold stress | 4.0 |
| Shahryar | - | 2.7 |
| Gascojen | - | 4.0 |
| Mahdavi | Tolerant to salt stress | 7.0 |
| Rowshan BC (winter) | - | 4.5 |
| Zarin | Relatively tolerant to cold stress | 6.4 |
| Rowshan BC (spiring) | - | 3.5 |
| Darab2 | - | 5.9 |
| Falat | - | 4.5 |
| Tajan | - | 6.3 |
| Niknejad | Tolerant to drought stress | 6.7 |
| Atrak | - | 5.8 |
| Hirmand | Tolerant to salt and drought stress | 5.0 |
| Shiraz | Tolerant to drought stress | 7.4 |
| Hamoun | Tolerant to drought and salt stress | 6.6 |
| Dez | Tolerant to terminal heat stress | 6.0 |

## MATERIALS AND METHODS

**a) Transpiration rate:** A pot experimental was conducted in the experimental field of S.B. University of Kerman under controlled conditions using 21 drought tolerant wheat cultivars. Table 1 shows some plant features, responses to the environmental stresses and origins of these cultivars. The experiment was a randomized complete block design with three replications. Five seeds were planted per each Wagner pot (1/2000) containing (5-6) kg of mixed soil (1/3 fine sand, 2/3 soil, pH: 7, EC: 4.2) which was fertilized with sufficient amount of N-P-K fertilizer. Plants were grown under mild water stress condition.

After heading a small segment (5 cm in size) from the middle part of the flag leaves was sampled and immediately transferred into an electronic analytical balance (ALE-40SM- Shimatzu (accuracy 0.00001 g)) and the cabinet windows were closed. Changes of leaf weight were recorded using a video camera which was started right after closing the window for three minuets while the time recorder set to "ON". Leaves were illuminated using two common 100 W lamps which were placed 50 cm far from the windows. Videos were then inspected and leaf weights at 5 second intervals were hand-recorded. Three samples were taken from each pot. It was assumed that leaf transpiration remains intact for 3 minutes after leaves were excises. All samples were taken between 1000 and 1100 hrs. Leaf transpiration rate in each case was expressed as the slope of the line fitted to the data between leaf weight and time (Fig. 2). Line slopes were then compared using student t-test as described by Steel and Torrie [23] as fallows:

$$t = \frac{b1 - b2}{\sqrt{s_p^2\left[1/\sum(X_{1j}-\overline{X_{1.}})^2 + 1/\sum(X_{2j}-\overline{X_{2.}})^2\right]}} \quad (1)$$

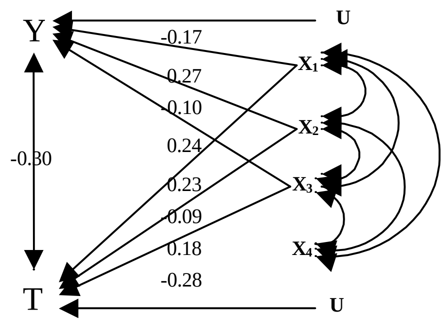


Fig. 1: Path diagram of in common correlated cause's model showing the directs and indirect effects of stomatal characteristics on yield and transpiration

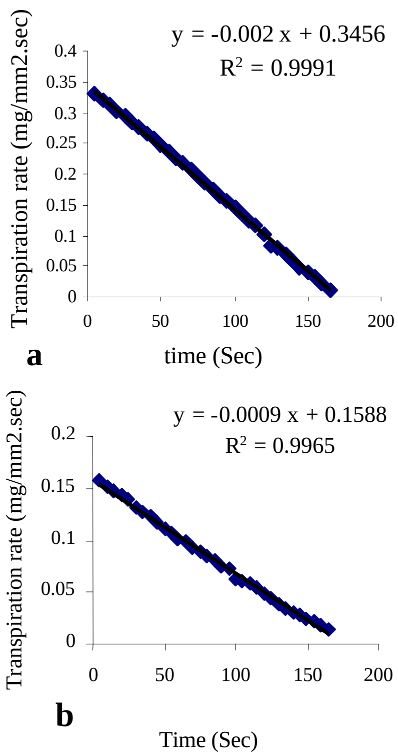


Fig. 2: Linier relationship between leaves weight and time in Navid (a) and Alvand (b) cultivars

In which $b_1$ and $b_2$ are line slopes (transpiration rates) for the two cultivars being compared and $\sum_j \left(x_{1j} - \overline{x_1}\right)^2$ and $\sum_j \left(x_{2j} - \overline{x_2}\right)^2$ are sum of squares for the first and second cultivars leaf weight values, respectively. $½^{2}_{Ñ}$ is the estimated error variance (pooled over two samples) which was obtained as fallows:

$$s_p^2 = \frac{\left\{\Sigma\left(Y_{1j} - \overline{Y}_{1.}\right)^2 - \left[\Sigma\left(X_{1j} - \overline{X}1.\right)\left(Y_{1j} - \overline{Y}_{1.}\right)\right]^2 / \Sigma\left(X_{1J} - \overline{X}_{1.}\right)^2\right\} + \left\{\Sigma\left(Y_{2j} - \overline{Y}_{1.}\right)^2 - \left[\Sigma\left(X_{2j} - \overline{X}_{2.}\right)\left(Y_{2j} - \overline{Y}_{2.}\right)\right]^2 / \Sigma\left(X_{2j} - \overline{X}_{2.}\right)^2\right\}}{n_1 - 2 - n_2 - 2} \quad (2)$$

$n_1$ and $n_2$ are the sample size for the two samples.

Path analysis [24] was performed to find the direct and indirect effects of stomatal characteristics on yield and transpiration, using the fallowing common correlated causes model (Fig. 1).

In which ($X_1$, $X_2$) and ($X_3$, X4) are stomatal frequency and size on adaxial and abaxial leafe surfaces, respectively. Y and T are yield and transpiration rate, respectively. Dirrect effects (P vector) were calculated by solution of the following matrix equation:

$$P = \left(X^{/}X\right)^{-1} X^{/}Y \quad (3)$$

Where; P and Y are the vectors of path coefficients and the correlation coefficients of yield and transpiration with stomatal characteristics, respectively. X is the correlation matrix. Unexplained variations in yield and transpiration were computed using the following equation:

$$r^2uy = 1 - \begin{pmatrix} p^2{}_{1y} + p^2{}_{2y} + p^2{}_{3y} + p^2{}_{4y} + 2p_{1y}p_{2y}r_{12} + 2p_{1y}p_{3y}r_{13} \\ +2p_{1y}p_{4y}r_{14} + 2p_{2y}p_{3y}r_{23} + 2p_{2y}p_{4y}r_{24} + 2p_{3y}p_{4y}r_{34} \end{pmatrix} \quad (4)$$

Where; y is substituted for yield or respiration and 1, 2, 3 and 4 subscripts are referring to stomatal frequencies and size on adaxial and abaxial leave surfaces. u is a dummy variable, which represents the effect of all other unknown sources of variation in yield and transpiration.

**b) Stomatal frequency and size:** Leaf segments which were selected from the middle parts of the flag leaves were immersed in a solution of 2K- Acryllacke[1] and after removing the excess amount of the solution from leaf tip were dried. Leaf epidermis imprints formed on adaxial and abaxial leaf surfaces were then removed using transparent sticky-tape and placed on a microscopic slide. Stomatal frequency was expressed as the number of stomata per microscopic field at (10×40) X magnification. Stomatal size was measured using an ocular ruler. All data were

[1]25-50% n-butly acetate, 25-50% Aliphatic polyisocyanat, 2.5-10% 2-methoxy-1-methylethyl acetate, 2.5-10% 2-butoxyethyl acetate, < 2.5% solvent naphtha

mean of 5 observations. Data were subjected to analysis of variance using Excel computer software. Linear correlation coefficients were calculated and used for determining path coefficients in a diagram relating stomatal frequency and size to transpiration rate and yield (Fig 1).

**c) Grain yield:** In order to compare wheat cultivars yield under drought stress condition an experiment was conducted in the field of agricultural research station of Kerman University (latitude, 57°:10', longitude; 30°:20' altitude; 1750). According to Koupen method, Kerman climate has been classified as dry (average annual precipitation 180mm). The soil was silty sand with PH, 7.8 and saturated soil extracts electrical conductivity at 2.41 ds/m. The experiment was a randomized complete block design with three replications. Needed amount of nitrogen and phosphorous fertilizers were calculated based on soil test and added to the soil before planting. Each plot consisted of 5 rows which were 200 cm in size and distanced 20 cm from each other. All plots were irrigated after planting to assure a full crop establishment. Irrigation was withheld before stem elongation to simulate what is happening under drought stress condition. Full mature plants were harvested from the middle parts of the rows and grain yield was measured, based on the $1m^2$ land area.

## RESULTS AND DISCUSSION

Results of analysis of variance of the data regarding stomatal characteristics are shown in Table 2. As this Table shows, cultivar has a significant effect on stomatal frequency on both surfaces and stomatal size on abaxial surfaces of flag leaves, suggesting that genetic variation exists among wheat cultivars in terms of the above mentioned characteristics.

Difference between cultivars in terms of stomatal frequency on both adaxial and abaxial surfaces of flag leaves was also significant. Generally all cultivars had more stomata on the adaxial compared to abaxial surface (Fig. 3a). On the other, hand cultivars with more stomata showed smaller guard cell size. Rowshan BC (winter type) and Rowshan BC (spring type) and Chamran cultivars had

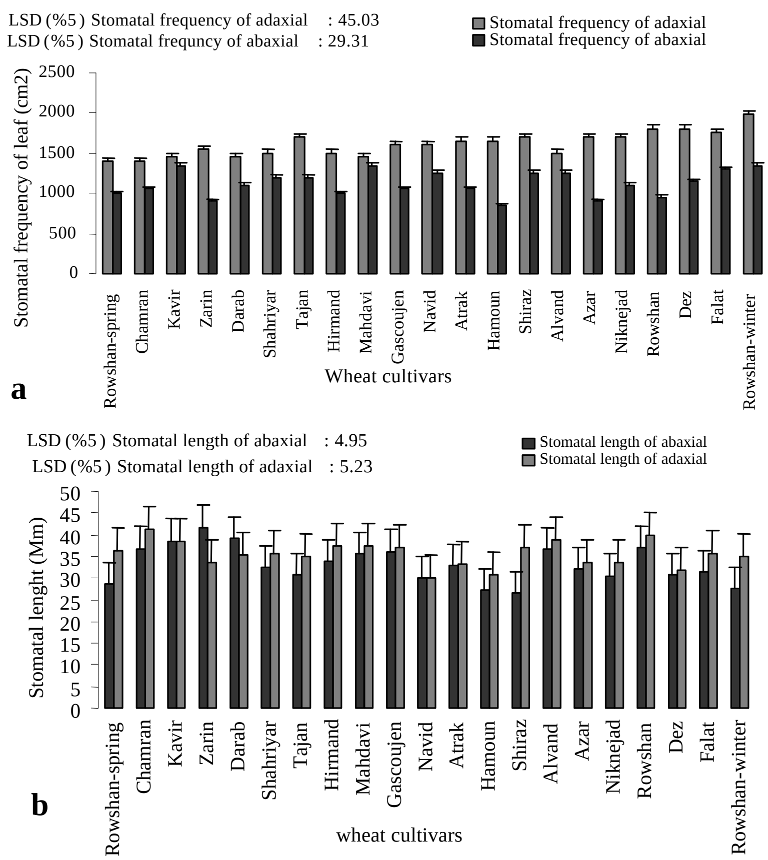


Fig. 3: Mean values of stomatal frequency (a) and size (b) on abaxial and adaxil sides of flag leaves in wheat cultivars

Table 2: Mean squares in the analysis of variance of stomatal frequency, stomatal size and yield of wheat cultivars

| Mean Square | | | | | | |
|---|---|---|---|---|---|---|
| | | Stomatal frequency ($cm^2$) | | Stomatal size (Mm) | | |
| ANOVA | DF | abaxial | Adaxial | abaxial | adaxial | Grain yield |
| Cultivar | 20 | $1316.64^{**}$ | $618.10^{**}$ | $35.03^{ns}$ | $29.3^{**}$ | $55190.55^{**}$ |
| Error | 40 | 744.68 | 315.64 | 10.06 | 9.03 | 61644.55 |

maximum and minimum stomatal frequency on the adaxial surface of flag leaves, respectively. Meanwhile, Kavir and Hamoun had maximum and minimum stomatal frequency on the abaxial surface of flag leaves, respectively (Fig. 3a). Mahdavi, Kavir and Alvand cultivars showed more stomatal frequency on both abaxial and adaxial surfaces compared to the others. Stomatal size on adaxial surface of flag leaves was the highest in Rowshan and the lowest in Navid, respectively (Fig. 3a). Meanwhile, Zarin and Shiraz had maximum and minimum stomatal size on the abaxial surface of flag leaves, respectively (Fig. 3b). Differences between varieties have also been shown to exist in other crop plants such as barley [21], maize hybrids [22], sorghum [25], alfalfa [18], wheat [19, 20], soybean [16], *Vicia faba* [26] and various spices of weed plants.

Negative correlation coefficients were found between stomatal frequency and size on both adaxial ($-0.42^{**}$) and abaxial ($-0.36^{**}$) surfaces of flag leaves. Values of stomatal frequency on adaxial and abaxial surfaces of flag leaves were also correlated whit each other ($0.46^{**}$) (Table 4).

Significant linear relationships were found between leaf weight loss and time after cutting (Fig. 2). Assuming that the amount of water released from the cutting edges of the leaves is negligble; leaf weight loss could be attributed to the amount of water transpired by the leaves through the stomata if light condition and leaf water status remain almost to the same levels as were in intact leaves during the first three minutes after leaf excision. Line slopes (transpiration rate) for all possible pairs of cultivars were compared and the results are given in Table 3, indicating that genetic variation exist among wheat cultivars in terms of transpiration rate.

Cultivar also showed significant effect on the yield (Table 2) so that significant differences were found among them. Cultivars Rowshan and Navid showed the highest (333.82 $g/m^2$) and the lowest (122.35 $g/m^2$) grain yield, respectively. Transpiration rate was poorly correlated with stomatal frequency on both adaxial ($0.23^{*}$) and abaxial ($0.18^{*}$) surfaces of flag leaves. Meanwhile, correlations was not significant between transpiration rate and stomatal size on adaxial ($-0.09^{ns}$) surface of flag

Table 3: Results of pair wise comparison of regression coefficient (transpiration rate) of wheat cultivars

| | Hirmand | Gascoujen | Mahdavi | Rowshan Bc (Spring type) | Kavir | Tajan | Niknejad | Azar | Darab | Alvand | Rowshan Bc (Winter type) | Shahriyar | Rowshan | Zarin | Chamran | Shiraz | Navid | Atrak | Flat | Dez |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gascoujen | ns | | | | | | | | | | | | | | | | | | | |
| Mahdavi | ns | ns | | | | | | | | | | | | | | | | | | |
| Rowshan Bc (Spring type) | ns | ns | ** | | | | | | | | | | | | | | | | | |
| Kavir | ** | ** | ** | ** | | | | | | | | | | | | | | | | |
| Tajan | ns | ** | ** | ** | ** | | | | | | | | | | | | | | | |
| Niknejad | ns | ns | ns | ns | ** | ns | | | | | | | | | | | | | | |
| Azar | ns | ns | ns | ns | ** | ns | ** | | | | | | | | | | | | | |
| Darab | ns | ns | ns | ** | ** | ns | ** | ** | | | | | | | | | | | | |
| Alvand | ** | ** | ** | ** | ns | ** | ** | ** | ** | | | | | | | | | | | |
| Rowshan Bc (Winter type) | ns | ** | * | ** | ** | ns | ** | ** | ** | ** | | | | | | | | | | |
| Shahriyar | ns | ** | ** | ** | ** | ns | ** | ** | ** | ** | ** | | | | | | | | | |
| Rowshan | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ns | ** | | | | | | | | |
| Zarin | ns | * | ** | ** | ** | ns | ** | ** | ** | ** | ** | ** | ** | | | | | | | |
| Chamran | ns | ns | ** | ** | ** | ns | ** | ** | ** | ns | ns | ns | ns | ns | | | | | | |
| Shiraz | ns | ns | ** | ** | ** | ns | ** | ** | ** | ns | ns | ns | ** | ns | ns | | | | | |
| Navid | ns | ** | ** | ** | ** | ns | ** | ** | ** | ns | ns | ns | ** | ns | ns | ** | | | | |
| Atrak | ns | ns | * | ** | ** | ns | ** | ** | ** | ns | ns | ns | ** | ns | ** | ns | ns | | | |
| Flat | ns | ns | ns | ns | ns | ns | ** | ** | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | | |
| Dez | ns | ns | ns | ns | ns | ns | ** | ** | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ** | |
| Hamoun | ns | ns | ns | ns | ns | ns | ** | ** | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ** | ** |

*and**: significant at 5% and 1%probability level, respectively. ns: Non-significant

Table 4: Correlation coefficients between transpiration rate and stomatal frequency and size with yield in wheat cultivars

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1: Stomatal frequency Adaxial | 1 | | | | | |
| 2: Stomatal size Adaxial | $-0.42^{**}$ | 1 | | | | |
| 3: Stomatal frequency Abaxial | $0.46^{**}$ | $0.17^{ns}$ | 1 | | | |
| 4: Stomatal size Abaxial | $-0.48^{**}$ | $0.08^{ns}$ | $-0.36^{**}$ | 1 | | |
| 5: Grain yield | $-0.17^{ns}$ | $0.27^{*}$ | $-0.10^{ns}$ | $0.24^{*}$ | 1 | |
| 6: Transpiration (gr/mm$^2$ sec) | $0.23^{*}$ | $-0.09^{ns}$ | $0.18^{ns}$ | $-0.28^{*}$ | $-0.30^{*}$ | 1 |

$^{*}$and$^{**}$: significant at 5% and 1%probability level, respectively. $^{ns}$: Non-significant

Table 5: Direct and indirect effects of stomatal characteristics on the yield of wheat cultivars

| | Indirect effect | | | | |
|---|---|---|---|---|---|
| Treat | 1 | 2 | 3 | 4 | Direct effect |
| 1: Stomatal frequency Adaxial | --- | -0.02909 | 0.003637 | -0.05633 | -0.23641 |
| 2: Stomatal size Adaxial | 0.0292994 | --- | 0.001361 | 0.009484 | 0.234745 |
| 3: Stomatal frequency Abaxial | -0.1092082 | 0.040582 | --- | -0.0423 | 0.007874 |
| 4: Stomatal size Abaxial | 0.1157063 | 0.019345 | -0.00289 | --- | 0.115091 |

Table 6: Direct and indirect effects of stomatal characteristics on the transpiration of wheat cultivars

| | Indirect effect | | | | |
|---|---|---|---|---|---|
| Treat | 1 | 2 | 3 | 4 | Direct effect |
| 1: Stomatal frequency Adaxial | --- | 0.00979 | 0.037321 | 0.098573 | 0.087957 |
| 2: Stomatal size Adaxial | -0.0109008 | --- | 0.013967 | -0.0166 | -0.07899 |
| 3: Stomatal frequency Abaxial | 0.0406308 | -0.01366 | --- | 0.074015 | 0.080792 |
| 4: Stomatal size Abaxial | -0.0430484 | -0.00651 | -0.02969 | --- | -0.2014 |

leaves and the coefficient was significant between transpiration rate and stomatal size on abaxial ($-0.28^{**}$) surface (Table 4). Suggesting that higher transpiration rate may causes the soil water content to be decreased and plants goes under more sever drought stress at grain filling stage.

Weak correlation coefficients were found between grain yield and stomatal size on both adaxial ($0.27^{*}$) and abaxial ($0.24^{*}$) surfaces of flag leaves, while there were no correlations between grain yield and stomatal frequency on both adaxial ($-0.17^{ns}$) and abaxial ($-0.10^{ns}$) surfaces of flag leaves (Table 6). Negative significant correlation coefficient was found between transpiration rate and grain yield ($-0.30^{**}$) (Table 4).

Path coefficients showing direct and indirect effects of stomatal characteristics on yield and transpiration rate are given in Table 5 and 6. As these results are showing, direct effect of stomatal size of adaxial leaf surface (0.23) is higher than the same effect of stomatal frequency on yield. Meanwhile, the indirect effect of stomatal size of adaxial surface on yield via stomatal frequency was very small and the indirect effects via stomatal frequency and stomatal size were very small though difference in sign. Results also showing that stomatal size on abaxial surface has the highest direct effect on transpiration rate (-0.20), while the effects the other characteristics are almost the same. Meanwhile, stomatal size on abaxial leaf surface showed the highest indirect effect on transpiration via stomatal frequency on abaxial surface of leaves.

Theses results are showing that stomatal characteristics are poorly correlated with both grain yield and transpiration. As with many other morpho-physiological characteristics. stomatal features are not in close relation with yield. This may be due to polygenic nature of yield efforts.

During the past decodes efforts, has been focused on finding plant characteristics which are closely correlated to yield particularly under environmental stresses such as drought. This would mean that changing those features makes it possible to change the yield. However, because genetic basis of yield is extremely complex, breeding improved genotypes for drought stressed conditions

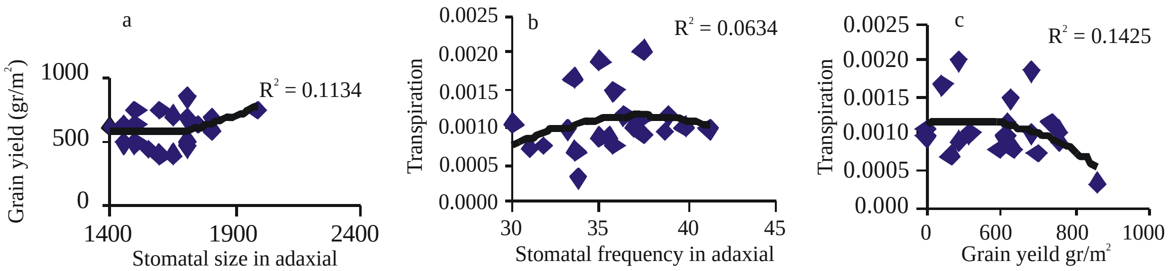


Fig. 4: Relationships between stomatal size of adaxial surface and grain yield (a), relationships between stomatal frequency of adaxial surface and transpiration (b) and relationships between transpiration and yield (c) in wheat cultivars used in this experiment

Table 7: Partitioning the correlation coefficient between transpiration rate and yield into its components relating the two characteristics in a path analysis based on a common correlated causes model

| | Component | Coefficient |
|---|---|---|
| 1 | $p_{y1}\ p_{t1}$ | -0.0300 |
| 2 | $p_{y2}\ p_{t2}$ | -0.0190 |
| 3 | $p_{y3}\ p_{t3}$ | 0.0007 |
| 4 | $p_{y4}\ p_{t4}$ | -0.0400 |
| 5 | $p_{y1}p_{t2}r_{12}$ | -0.0030 |
| 6 | $p_{y2}p_{t1}r_{12}$ | 0.0030 |
| 7 | $p_{y1}p_{t3}r_{13}$ | -0.0090 |
| 8 | $p_{y3}p_{t1}r_{13}$ | 0.0004 |
| 9 | $p_{y2}p_{t3}r_{23}$ | 0.0040 |
| 10 | $p_{y3}p_{t2}r_{23}$ | -0.0002 |
| 11 | $p_{y1}p_{t4}r_{14}$ | -0.0400 |
| 12 | $p_{y4}p_{t1}r_{14}$ | -0.0030 |
| 13 | $p_{y2}p_{t4}r_{24}$ | -0.0040 |
| 14 | $p_{y4}p_{t2}r_{24}$ | -0.0008 |
| 15 | $p_{y3}p_{t4}r_{34}$ | 0.0006 |
| 16 | $p_{y4}p_{t3}r_{34}$ | -0.0040 |

was not successful. Finding mechanisms which somehow affecting yield was the focus of many researches works [2]. To be convinced, researchers tried to use correlation coefficient as good estimates of the effects of physiological mechanisms behind the relationships on yield. Considering these coefficients as values explaining the phenomenon as cause and effects and partitioning them into the components which can describe their interactions with each other, may even provide them with a more clear determination of the effects. The advantage of path analysis over correlation coefficient is that they make it possible to separate the direct effects of each stomatal characteristic on transpiration and yield from their indirect effects via each others. On the other hand, what is expected from the functions of stomata is that they should have a close correlation with yield and transpiration rate. To increase photosynthesis and yield stomatal number and size should be increased. However, results of this experiment do not show this clearly as the direct and indirect effect values are veru smoll. Besides, unexplained variations in Y (0.92) and T (0.91) are considerably higher than explained variation. Less than 12% and 7% of total variation in yield and transpiration could be explained by stomatal characteristics (Fig. 4). Negative significant correlation coefficient was obtained between transpiration rate and yield. This is not in accordance with what is expected from Passioura [27] explanation of yield under dry condition. One reason for this may be the effect of high transpiration rate on the amount soil water content at grain filling stage.

Different environmental factors such as light and temperature and also genotypic factors like photosynthetic capacity and enzymes activity affecting plant growth and yield. Genetically, cultivars used in this experiment are not the same, even though they were grown under the same environmental conditions. It is expected that significant interactions to be developed so that to prevent the stomatal number and size effects on yield not to be expressed. This in turn may change the photosynthesis rate and yield. To obtain the pure effect of such characteristic it is necessary to develop isogene lines which are only different from each other in terms of stomatal characteristics. This in turn may be a hard task because genetic basis of stomatal characteristics is not yet well known.

Correlation coefficient between transpiration rate and yield was partitioned into path coefficients and the results are showing in Table 7. Generally the effects are very small as was expected because the total amount was also small. However, the contribution of $p_{y1}p_{t1}$, $p_{y4}\ p_{t4}$ and $p_{y1}p_{t4}r_{14}$ in total correlation was considerably higher than the others, suggesting that stomatal frequency on adaxial and stomatal size on abaxial surface have more contribution in explaining the cause of changes of yield due to transpiration. It should be noted that the sum of the effects of all possible components is not the same as the correlation between transpiration rate and yield due to rounding errors.

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