Stomatal characteristics, heritability and their relationship to grain yield in a double haploid bread wheat population

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ABSTRACT

Assessment of heritability of stomatal characteristics and their relation to grain yield is useful in formulating a breeding strategy and reliable selection in crop improvement especially where water supply is limited**.** A set of 99 doubled-haploid lines was developed from a cross between two Canadian bread wheat lines ES32 and P8911-G1D3, their parents and 9 Iranian bread varieties were evaluated in a 2-year field study, to ascertain and compare heritability for stomata frequency, size, length, width and area in abaxial and adaxial flag leaf surfaces. Moreover, abaxial/adaxial stomata frequency ratio, grain yield and the correlation between these traits were assessed. The results showed that, there were highly significant differences among doubled haploid lines, the parents and the Iranian varieties for all the considered characters with the exception of abaxial/adaxial stomata frequency ratio. The stomata were significantly ($P<0.01$) more frequent on abaxial surface (ranged from 43.38 to 66.81) than the adaxial surface (ranged from 30.18 to 49.20). Stomatal length means were significantly higher ($P<0.01$) for the lower surface (ranging from 45.52 to 61.46) than the upper surface (ranging from 43.35 to 61.20). The stomatal area followed a similar pattern, where means were significantly higher for the lower surface than upper surface. Heritability were moderate to low for all the traits measured, where stomatal frequency of adaxial surface was highest (36.16%) and abaxial/adaxial stomata frequency ratio was lowest (4.44%). There was a negative correlation between abaxial stomatal frequency with stomatal length (r = –0.62**), stomatal area (r = –0.48**) and stomatal width (r = –0.16*) and similar relations were observed for adaxial surface stomatal characteristics. Among stomatal characteristics, abaxial surface stomatal width (r = 0.21**), adaxial stomatal length (r = 0.22**) and stomatal area (r = 0.18*) showed significant correlation with grain yield. Stepwise regression appeared also confirm relationship of these stomatal characteristics with grain yield.

Keywords: Stomatal characteristics, heritability, bread wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most widely grown crop in the world cultivated on approximately one sixth of the total arable land. More efficient wheat breeding methods are needed to meet demand for wheat from expected population growth in developing countries. Flag leaf characteristics provide a potential target for selection (4). In this regard, stomata are the portals for gas exchange between the leaf mesophyll cells and the environment. Their frequency and size have often been used as morphological markers for identifying ploidy level in many plant species (12) and also have been used as an indicator of water loss. The relationship between stomatal characteristics and stomatal resistance is also important in determining water status of crops under water-stress conditions. The number, distribution, and morphology of stomata on the wheat leaf surfaces may be an important trait in the adaptation of wheat cultivars to fluctuating CO_2 levels. In sorghum, the abaxial leaf surface had a significantly higher stomatal frequency than the adaxial side (10). Bkagwat and Bhatia (2) showed a range of 42% to 81% of stomatal frequency in bread wheat. Stomata on abaxial surfaces are longer than those on adaxial surfaces (10). It is reported that, stomatal frequency mean ratios (abaxial/adaxial) ranged from 1.12 to 1.134 in *Vicia faba* (13) and 1.4 to 4.0 in *Amphistomatous* hybrid cultivars (15).

Heritability of a character describes the extent to which it is transmitted from one generation to the next generation. The knowledge of heritability thus guides the plant breeder to predict the behaviour of the succeeding generation, making purposeful selection and predicting the magnitude of genetic advance possible. Although stomatal characteristics are one of the main factors that determine photosynthetic ability, the genetic basis for stomatal frequency is still poorly understood. Moderate to low narrow-sense heritability is reported for stomatal frequency based on parent-progeny regression (2; 14). Elucidation of relationships between stomatal characteristics and their relation to grain yield would be valuable in the design of strategies for stomatal manipulation aimed to increasing environmental fitness and grain yield. Positive correlation coefficients between stomatal frequency (r = 0.39 P<0.05) and grain yield per plant are reported (3). Khan et al. (9) found a positive direct effect of stomatal frequency on grain yield using path coefficient analysis. However, it seems that the relationship between stomata frequency and yield is tenuous. According to Teare et al. (17), no general relation occurs between grain yield and number of stomata/cm^2 of leaf surface or between grain yield and stomata length and stomata per unit of adaxial leaf area. The present study has been conducted to ascertain the heritability, and interrelationships of flag leaf stomatal characteristics with grain yield in a doubled haploid population of bread wheat.

MATERIALS AND METHODS

The present study was conducted at the research farm of Shahrekord University, Iran, during 2006-2007. The genetic material comprised of a set of 99 doubled-haploid lines which was developed from a cross between two Canadian bread wheat lines ES32 and P8911-G1D3. The parents and 9 local Iranian check varieties were included in the test in a triple 10×11 lattice design.

At anthesis, four fully expanded flag leaves of the main stem in each plot was used to estimate abaxial and adaxial surface stomata characteristics with the impression method (12). Impressions were viewed with a light microscope (40 × objective). Observations were made on an average of 10 fields mm^{-2} for each leaf and measurements were made with a calibrated eyepiece micrometer. Stomatal length and stomatal width were measured and then stomatal size was converted to µm (12). The characteristics including, stomatal frequency of abaxial leaf surface (SFAb), stomatal frequency of adaxial leaf surface (SFAd), stomatal length of abaxial leaf surface (SLAb), stomatal width of abaxial leaf surface (SWAb), mean stomatal area of abaxial leaf surface (SAAb), stomatal length of adaxial leaf surface (SLAd), stomatal width of adaxial leaf surface (SWad), and mean stomatal area of adaxial leaf surface (SAAd), were measured. Grain yield was determined as g m^{-2}. The SFAb/SFAd ratio was calculated.

Heritability on a plot mean basis was estimated using variance components (7). Because the genetic variance among doubled haploid lines equals $2\sigma_A^2$ (additive variance), thus the estimate of heritability is equivalent to the narrow-sense heritability. Direct and indirect path coefficients were calculated as in Wright (18). Stepwise regression was used to estimate the contribution of each character to the yield (3). All the analyses were computed using MINITAB (11) and MSTATC statistical software.

RESULTS AND DISCUSSION

The univariate ANOVA indicated a significant difference (P<0.01) between genotypes for grain yield and all the stomatal characters except SFAb/SFAd ratio (data not shown). These results also showed that, there were highly significant differences among the parents, doubled haploid lines, and the Iranian varieties for all the stomata characteristics with the exception of SFAb/ SFAd ratio.

The SFAb was significantly (*P*<0.01) more than SFAd (Table 1). These results are in agreement with those of Liang et al. (10), who showed that the abaxial leaf surface had a significantly higher stomatal frequency than the adaxial leaf in grain sorghum. Also the stomata were significantly (P<0.01) longer on lower than upper flag leaf sides (Table 1). This appears to be inconsistent with an earlier study in sorghum that suggested stomata on abaxial surfaces were longer than those on adaxial surfaces (10). Abaxial and adaxial stomata surface area relation followed such as stomatal length trend.

The heritability estimates (phenotypic variance due to additive genetic variability) are tabulated in Table 1. For stomatal characteristics they differed from as high as 36.16% for SFAb to 4.44% for SFAb/ SFAd ratio. Bkagwat and Bhatia (2) reported a moderate heritability of 42% for leaf stomatal frequency. Moreover, Quarrie and Jones (14) found narrow-sense heritability to be moderate (h^2= 36.3% - 49.7%) in two populations. In contrast our analysis revealed that the grain yield showed a low heritability of h^2= 20.4%. These results indicated that most of the traits were under additive genetic control; however, the phenotyping may not have been sufficiently precise, there was a strong environmental influence or a large genotype by environment interaction was involved in variability of these traits.

The correlation coefficients between the characters showed a significant association between almost all of the traits (Table 2). Notably, there was a significant negative association between stomatal size and length at both abaxial (r = -0.62**) and adaxial (r = -0.47**) surfaces. Similarly other researchers report a negative correlation between stomatal frequency and size (1; 16). A Strong positive correlation was observed between adaxial and abaxial surfaces for pairs stomatal frequency (*r* = 0.58**), stomatal length (*r* = 0.60**), stomatal area (*r* = 0.58**) and other characters (Table 2). This indicates that genotype evaluation based on one side of the leaf is sufficient. Because of higher heritability of adaxial stomatal frequency and length (Table 1), adaxial characters would be best for progress in selection of these traits in succeeding generations.

Grain yield demonstrated a positive and significant correlation with some of stomatal characters including, SWAb, SLAd and SAAd (Table 2). However it seems that these correlations are not adequately high (up to *r*=0.22) to use them in indirect grain yield breeding. In addition the heritabilities of these traits are not high enough (Table 1), for effective indirect selection.

Table 1. Means of parents, the doubled haploid (DH) population and control varieties, range of the DH population and heritability (h^2) of stomatal characters and grain yield (g m^{-2}).

Trait	P8911-G1D3	ES32	DH population mean	DH population range	Control	h^2 (%)
SFAb	51.2	51.1	52.0	43.4 - 66.8	55.8	30.7
SFAd	36.0	38.2	38.4	30.2 - 49.2	42.2	36.2
SLAb	53.4	51.1	53.6	45.9 - 61.2	47.6	32.8
SLAd	56.6	56.4	55.1	45.5 - 61.5	51.9	35.7
SWAb	27.8	31.0	28.4	22.7 - 33.5	27.8	33.7
SWAd	28.7	30.1	28.2	24.5 - 32.6	29.4	26.3
SAAb	2471.2	2638.5	2533.0	1897.5 - 3161.2	2198.2	32.4
SAAd	2702.3	2814.3	2590.0	2050.8 - 3165	2556.1	31.4
SFAb/ SFAd	1.42	1.34	1.37	1.14 - 1.65	1.33	4.44
GY	303.5	326.6	320.9	174.4-479.5	297.4	20.4

We also examined the effect of stomatal characters on the economic trait grain yield. The stepwise regression revealed the adaxial stomatal area and frequency were more influential characters on the grain yield m^{-2}. Moreover all the stomatal characters had a significant linear pattern effect on grain yield. The path coefficients analysis (Figure 1) did not show a significant direct effect of stomatal adaxial length and area on grain yield. Though, the direct effect of adaxial stomatal area (0.221) was higher than its indirect effects (0.026), the converse was true for adaxial frequency (-0.108 to -0.054). Our work suggests that stomatal characteristics are not an appropriate approach to breed high yielding wheat varieties. Perhaps cultivar's association with other traits such as water use efficiency, dry matter and photosynthesis should be the target area of research. It is reported that stomatal frequency had a positive/increasing effect on grain yield (5; 9). According to Teare et al. (17), no general relation was found between grain yield and number neither of stomata cm^{-2} nor between grain yield and bn (the product of stomata length and stomata per unit of adaxial leaf area). Heursel et al. (8) also concluded that, utilizing stomatal characteristics as a selection tool for an increased crown diameter (yield) in azalea may not be feasible.

Table 2. Correlation coefficients between stomatal characters and grain yield[1].

	GY	SFAb	SLAb	SWAb	SAAb	SFAd	SLAd	SWAd	SAAd
SFAb	0.04								
SLAb	−0.03	−0.62							
SWAb	0.21	−0.16	0.22						
SAAb	0.12	−0.48	0.76	0.80					
SFAd	0.10	0.58	−0.53	−0.11	−0.40				
SLAd	0.22	−0.53	0.60	0.26	0.54	−0.47			
SWAd	0.10	−0.24	0.20	0.50	0.44	−0.22	0.39		
SAAd	0.18	−0.44	0.46	0.46	0.58	−0.40	0.80	0.86	
SFAb−SFA	−0.11	0.35	−0.02	−0.03	−0.03	−0.58	−0.03	−0.01	−0.02

[1]: The coefficients greater than 0.18 and 0.22 are significant at 0.05 and 0.01, respectively. SFA implies to SFAd.

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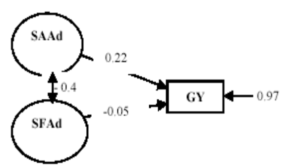

Figure 1. Path analysis for adaxial stomatal area (μm^2) and frequency on grain yield. Simple and double headed arrows indicate correlation and path coefficients.

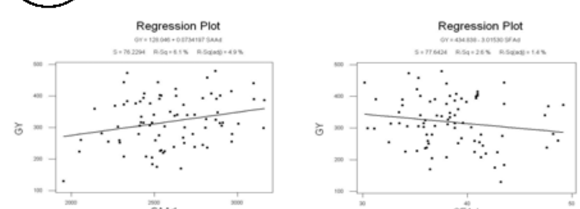

Figure 2. Regression of stomatal area (SAAd) and frequency (SFAd) on grain yield (GY).