Fall 1974

VARIATION IN STOMATAL LENGTH AND FREQUENCY AND ITS RELATIONSHIP TO LEAF CHARACTERISTICS AND YIELD IN BROMUS INERMIS LEYSS

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by

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B.Sc., Nanyang University, 1965

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A THESIS

Submitted to the University of New Hampshire

In Partial Fulfillment of

The Requirements for the Degree of

Doctor of Philosophy

Graduate School

Department of Plant Science

November, 1974

This thesis has been examined and approved.

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Nov. 25, 1974

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ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude to his major professor, Dr. G. M. Dunn, for his advice in these studies and criticism of the manuscript. Gratitude is also expressed to the members of the graduate committee, Drs. L. C. Peirce, O. M. Rogers, F. K. Hoornbeek, and W. M. Collins.

The author also gratefully acknowledges the assistance and encouragement of his wife, Wai-Koon, throughout these studies and her assistance in typing of the manuscript.

The author is grateful to the New Hampshire Agricultural Experiment Station, University of New Hampshire for financial support under H116-NE 28.

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ABSTRACT

VARIATION IN STOMATAL LENGTH AND FREQUENCY AND ITS RELATIONSHIP TO LEAF CHARACTERISTICS AND YIELD IN BROMUS INERMIS LEYSS.

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Seven octoploid bromegrass varieties were examined for the extent of variability in stomatal length and frequency and for their differences in stomatal distributional patterns.

Significant differences were obtained among varieties for stomatal length (μ) and frequency (mm^{-2}) on the adaxial and abaxial surfaces and at five different leaf positions on the culm. The varieites 'Carlton' and 'Blair' had more but smaller stomata, whereas 'Saratoga' and 'Red Patch' had larger but fewer stomata. Stomatal length increased while frequency decreased from the first to the third leaves and leveled off from the third to the fifth leaves on the culm. The tip of the leaf had the largest but fewest stomata with the reverse for the base of the leaf. Varietal differences in stomatal length and frequency were found mainly associated with the central position of leaf surfaces.

The relatively consistent differences among varieties and among leaf positions on the culm and on the individual leaf suggested that stomatal length and frequency are likely under genetic control.

Genetic variation in stomatal length and frequency was investigated in 10 possible single crosses of a half-diallel cross involving five bromegrass genotypes.

The diallel analyses indicated a much higher general combining ability (GCA) value than the specific combining ability (SCA) for all characters. The SCA was not significant for any character. Relatively high narrow-sense heritability estimates were obtained for stomatal length (0.53) and frequency (0.74) and for other leaf characters. Estimates of SCA effects showed parent 5 to be the best combiner for stomatal characters, leaf length, width, area, and tiller dry weight.

Negative and significant genotypic correlations were obtained between stomatal length and frequency of the same leaf. The phenotypic and genotypic correlations obtained between stomatal characters and other traits showed that stomatal length was positively while stomatal frequency negatively correlated with leaf length, width, area, and tiller dry weight. These indicated that selection for larger and fewer stomata should tend to result in longer and wider leaves of larger areas and greater tiller dry weight. The highly significant negative genotypic correlation coefficient found for stomatal frequency (abaxial) and plant dry weight also indicated that selection for decreased stomatal frequency at the abaxial surface should increase the dry matter production per plant. However, such relationship was not significant between stomatal frequency and forage yield in drilled plots. No association seemed to

exist between plant dry weight and forage yield.

The findings of high heritability estimates, large GCA and the correlations showed among stomatal characters with other traits are indicative of genetic variability and relationships of the characters. Therefore, selection for high, or for low, stomatal frequency, and for size of stomata on the individual plant would be possible, and rapid response to selection would be expected in this population.

PART I

VARIETAL DIFFERENCES IN STOMATAL LENGTH AND FREQUENCY AND THEIR RELATION TO OTHER CHARACTERS IN *BROMUS INERMIS* LEYSS.

INTRODUCTION

Stomata are major apertures for the exit and entry of gases into a leaf. They regulate loss of water and uptake of carbon dioxide by leaves (Gaastra, 1959), and provide routes for invasion of leaves by pathogens (Rich, 1963) and gaseous pollutants (Mansfield, 1973). There is also the possibility that the two important plant processes, photosynthesis and transpiration, are influenced by stomatal frequency. A variety with low stomatal frequency and high total leaf resistance to water loss was reported (Heichel, 1971a) to have faster net photosynthesis than a variety with high stomatal frequency in maize. Miskin et al. (1972) reported that stomatal frequency did not influence the rate of photosynthesis, but it influenced stomatal diffusion resistances and transpiration rates. They found that lines of barley having low stomatal frequencies had higher stomatal resistances, and transpired less water than lines with more stomata. However, other workers reported no relationship between frequency of stomata and photosynthesis (Freeland, 1948) or transpiration (Muenscher, 1915). Low stomatal frequency was also found associated with greater drought tolerance in blue panicgrass (Dobrenz et al., 1969) and with improved winter hardiness in American holly (Ilex opaca) (Knecht and Orton, 1970). Therefore, investigation of the control of conductance of the leaf epidermis by the genetic manipulation of stomatal length and frequency may be rewarding.

REVIEW OF LITERATURE

Variation in stomatal size and frequency per unit area between and within species has been reported by a number of investigators (Cole and Dobrenz, 1970; Heichel, 1971b; Miskin and Rasmusson, 1970; Shearman and Beard, 1972 and Teare, Peterson and Law, 1971). Cultivars of alfalfa (Cole and Dobrenz, 1970) and clones of blue panicgrass (Dobrenz et al., 1969) varied significantly in stomatal frequency. Miskin and Rasmusson (1970) found that stomatal frequency varied by 2-fold among 649 barley cultivars from a world collection. The consistent differences in stomatal frequency found in the field and greenhouse and the significant differences of guard cell length among cultivars suggested that these characters may be subject to genetic manipulation and that a selection program for high and low stomatal frequency would be effective.

Intraplant variation in stomatal frequency has generally been observed in a number of species (Dobrenz et al., 1969; Miskin and Rasmusson, 1970; Shearman and Beard, 1972; Teare et al., 1971, and Tan and Dunn, 1974). Stomatal frequency and distribution varied with leaf position and leaf surface (Hunt and Christie, 1969; Miskin and Rasmusson, 1970; Teare et al., 1971, and Tan and Dunn, 1974). Stomatal number decreased progressively from the flag to lower leaves in alfalfa (Cole and Dobrenz, 1970), barley (Miskin and Rasmusson, 1970), bromegrass (Tan and Dunn, 1974) and wheat (Teare et al., 1971). A reverse pattern of stomatal distribution was demonstrated in blue panicgrass (Dobrenz et al., 1969).

Significant differences were reported in stomatal frequency at three different positions on the same leaf in bromegrass (Tan and Dunn, 1974) and in wheat (Teare et al., 1971), but not in blue panicgrass (Dobrenz et al., 1969).

The stomatal frequency was reported to be greater on the adaxial than on the abaxial surface in alfalfa (Cole and Dobrenz, 1970), bromegrass (Tan and Dunn, 1974), creeping bentgrass (Shearman and Beard, 1972) and wheat (Teare et al., 1971). Similar stomatal frequencies were reported on both leaf surfaces for barley (Miskin and Rasmusson, 1970) while blue panicgrass (Dobrenz et al., 1969) and maize (Heichel, 1971b) had higher stomatal frequency on abaxial than on the adaxial surface.

Stomatal length in bromegrass was reported (Tan and Dunn, 1973) to be positively correlated with ploidy level whereas stomatal frequency was inversely associated with ploidy. The differences between ploidies were consistent at different leaf positions on the culm and different positions on the individual leaf (Tan and Dunn, 1974).

Stomatal length on adaxial was found (Tan and Dunn, 1974) to be highly correlated with that on the abaxial surface for all leaf positions and both ploidy levels of bromegrass. A similar relationship was also found between stomatal frequency on both leaf surfaces. Stomatal length and frequency were, on the other hand, reported to be negatively correlated within each ploidy level.

The primary objectives of the present study were (a) to examine further the extent of variability in stomatal

length and frequency among seven octoploid bromegrass varieties and their differences in distributional patterns, and

(b) to study the relationship between stomatal characters and leaf length, width, area, and tiller dry weight.

MATERIALS AND METHODS

The seven octoploid bromegrass varieties involved in this study were as follows : 'Blair' (F.C.39,537), 'Carlton' (F.C. 40,039), 'Fox' (F.C.39,981), 'Manchar' (F.C.39,474), 'Sac' (F.C.38,981), 'Saratoga' (F.C.38,446), and 'Red Patch'. The first six were originally obtained from the Agricultural Research Service, USDA, Beltsville, Maryland, and the last from the Central Research Farm, Ottawa, Canada. These varieties were well established and were space-planted together with some synthetics in 1969 in a randomized complete block with four replications. Each plot contained 10 plants in each replicate.

Samples were taken from two replications. For each variety, three plants were randomly selected from each replicate for examination of stomatal and leaf characteristics. A total of six plants were measured from each of the seven varieties. Sampling was started approximately June 20, 1972 when anthesis began. One culm was taken from each plant. Five leaves were sampled from each culm: L1 to L5, where L1 designates first leaf, L2 the second, L3 the third, L4 the fourth and L5 the fifth leaf below the panicle. Each leaf was measured for length, width and area. Leaf width was measured at the widest part of the blade and length was measured from the base to the tip. Leaf area was obtained by tracing leaf outlines on paper, then taking planimeter measurements of the tracings. A 2-cm long section was sampled from the center of each leaf and prepared for stomatal measurements. For L1, the tip and base sections were also included. The sections

were boiled in 70% alcohol for 30 min, then cleared and softened in 90% lactic acid at 60°C according to Clark's method (Clark, 1960) with slight modification. The leaf samples were whole-mounted onto a slide in 90% lactic acid without removing epidermal, mesophyll and vascular tissue.

The samples were examined microscopically with the aid of an ocular micrometer. Length of guard cells was measured for five stomata from the center section of each of the five leaves, and on five microscopic fields, each one mm^2, the number of stomata was determined for each leaf surface. The base and tip sections of L1 were examined in the same manner.

Four tillers were sampled from each plant for the determination of dry weight per tiller by oven drying for 24 hr at 100°C.

In statistical analyses, all factors were considered to be fixed rather than random except variety. The varieties were considered a random sample from a restricted population of smooth bromegrass because they had not been previously selected for stomatal characters. In the analyses of variance (Tables 1, 4 and 6), replication, variety and variety x leaf (or variety x position) were tested against error, whereas leaf (or position) was tested against variety x leaf (or variety x position).

The varietal means were tested according to Tukey's w-procedure (hsd) (Steel and Torrie, 1960). Simple correlation coefficients were computed among characters.

RESULTS AND DISCUSSION

Stomatal Characters

An analysis of variance for stomatal length and frequency for all varieties (Table 1) indicated highly significant ($P<0.001$) varietal differences in size and frequency of stomata on both leaf surfaces. Stomatal length and frequency at five leaf positions on the culm also differed significantly ($P<0.001$). The highly significant interactions ($P<0.01$ and $P<0.001$) between variety and leaf position indicated that the differences among the seven varieties in stomatal length and stomatal frequency were not the same at the five leaf positions on the culm.

Mean stomatal length at each leaf position for the seven varieties are presented in Table 2. On the adaxial surface of L1, for instance, stomatal lengtbs ranged from 48 to 57 μ, and from 48 to 58 μ on the adaxial surface. In general, 'Carlton' and 'Blair' had consistently shorter stomata at different positions on culm, and 'Saratoga' and 'Red Patch' had significantly longer stomata among the varieties examined.

At either leaf surface, the lower leaves on the culm had significantly longer stomata than did the upper leaves (Table 2). Differences of stomatal length among the three lower leaves, L3, L4 and L5, were non-significant, whereas stomatal length of the three leaves differed significantly from that of L1 and L2. Again, L2 had slightly longer stomata than L1, but the difference was statistically significant for the abaxial surface only.

Varietal differences were apparent in frequency of stomata at different leaf positions on the culm (Table 3, Figs. 1 and 2).

Table 1. Analysis of variance for stomatal characters at five leaf positions on culm of seven varieties of bromegrass.

Source	df	Mean Squares			
		Stomatal Length (μ)		Stomatal Frequency (mm^{-2})	
		Adaxial	Abaxial	Adaxial	Abaxial
Replication	1	14.301*	16.914**	68.864**	9.413
Variety (V)	6	64.546***	91.394***	483.477***	409.953***
Leaf (L)	4	342.918***	320.450***	2825.849***	617.078***
V x L	24	5.223**	5.283***	36.548***	38.040***
Error	34	1.943	1.468	6.757	7.064

* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Table 2. Mean stomatal length (μ) in seven varieties of Bromus inermis in relation to the adaxial and abaxial surfaces for five leaf positions on the culm. /

Varieties	Leaf positions on culm				
	L1	L2	L3	L4	L5
			Adaxial		
Blair	49.1 b*	52.1 c	59.3 ab	60.0 b	61.7 ab
Carlton	48.5 b	51.0 c	56.0 b	61.3 ab	59.2 b
Fox	50.2 ab	53.1 bc	56.5 b	61.2 ab	61.9 ab
Manchar	53.4 ab	58.0 ab	62.7 ab	62.1 ab	65.3 a
Red Patch	54.3 ab	59.3 a	64.5 a	64.8 ab	64.8 a
Sac	50.5 ab	53.6 bc	63.6 a	64.6 ab	63.3 ab
Saratoga	57.0 a	57.3 ab	64.0 a	65.6 a	61.6 ab
Mean	51.9 B	54.9 B	60.9 A	62.8 A	62.5 A
			Abaxial		
Blair	48.5 d	54.1 cd	60.1 abc	60.9 bc	62.4 ab
Carlton	48.1 d	50.5 e	56.6 c	59.4 c	57.1 b
Fox	50.1 cd	53.6 cd	58.1 bc	59.6 c	61.1 ab
Manchar	54.1 b	57.4 b	63.9 ab	61.4 bc	62.9 ab
Red Patch	54.0 b	59.9 a	65.6 a	66.1 a	64.0 a
Sac	52.3 bc	54.5 c	64.2 ab	66.1 a	66.4 a
Saratoga	58.0 a	60.2 a	65.9 a	64.7 ab	63.1 a
Mean	52.2 C	55.7 B	62.1 A	62.6 A	62.4 A

* Means not followed by letters in common (lower case - columns, upper case - rows) are significantly different at the 5% level of probability, according to Tukey's w-procedure (hsd).

/ Each value is the mean of 30 stomata.

On the adaxial surface of L1 (Table 3 and Fig. 1), the seven varieties could be separated into four distinct groups with significantly different stomatal frequencies. Comparing the two extreme groups, 'Carlton' had 30% more stomata than 'Saratoga'. On the abaxial surface of L1 (Table 3 and fig. 2), 'Blair' had twice as many stomata as 'Manchar'. With a few exceptions, 'Carlton' and 'Blair' had the highest stomatal frequency at different leaf surfaces and positions on culm, whereas 'Manchar', 'Red Patch' and 'Saratoga' had the lowest. The adaxial surface had consistently more stomata than the abaxial surface of the same leaf at a corresponding position.

Stomatal frequency declined sharply from the first (L1) to the third (L3) leaf on the culm and leveled off below the third leaf (Figs. 1 and 2). Therefore, the frequency of stomata of the three upper leaves (L1 - L3) were significantly affected by leaf position on the culm. Positional effect played a less significant role on the lower leaves (L3 - L5).

From the analysis of variance (Table 4), it was determined that varietal differences in stomatal length and frequency at three positions on both leaf surfaces of L1 were highly significant ($P<0.001$). Variation due to positional effect on the individual leaf was also significant ($P<0.001$). Interactions between varieties and positions on a leaf were significant at $P<0.05$ for stomatal length and at $P<0.001$ for stomatal frequency on either leaf surface.

Mean stomatal lengths and frequencies at three positions of L1 were shown in Table 5. Varietal differences in stomatal length

Table 3. Mean stomatal frequency (mm^{-2}) in seven varieties of Bromus inermis in relation to the adaxial and abaxial surfaces for five leaf positions on the culm. ⁄

Varieties	Leaf positions on culm				
	L1	L2	L3	L4	L5
			Adaxial		
Blair	90.9 a*	69.5 ab	53.9 a	51.1 a	48.1 ab
Carlton	92.6 a	78.6 a	56.7 a	46.5 ab	53.6 a
Fox	79.7 b	64.2 abc	56.2 a	48.2 a	43.3 abc
Manchar	70.7 c	51.0 c	42.2 b	43.9 ab	35.7 c
Red Patch	70.5 c	54.4 c	41.0 b	38.7 b	38.7 bc
Sac	71.7 c	65.4 abc	48.1 ab	45.7 ab	46.4 abc
Saratoga	64.6 d	55.1 bc	46.0 ab	37.9 b	44.5 abc
Mean	77.2 A	62.6 B	49.2 C	44.6 C	44.3 C
			Abaxial		
Blair	64.7 a	53.1 a	41.9 a	41.2 a	39.0 a
Carlton	47.5 bc	44.3 ab	33.8 ab	34.1 ab	39.0 a
Fox	43.0 cd	42.8 ab	36.7 ab	32.6 ab	31.1 ab
Manchar	32.4 d	27.9 c	25.2 b	29.5 b	29.9 b
Red Patch	45.2 c	35.9 bc	27.7 b	27.2 b	29.1 b
Sac	59.5 ab	48.0 a	34.3 ab	30.5 b	32.6 ab
Saratoga	38.4 cd	33.0 bc	28.8 b	28.0 b	33.6 ab
Mean	47.2 A	40.7 AB	32.6 C	31.9 C	33.5 BC

* Means not followed by letters in common (lower case - columns, upper case - rows) are significantly different at the 5% level of probability, according to Tukey's w-procedure (hsd).

⁄ Each value is the mean number of stomata from 30 microscopic fields.

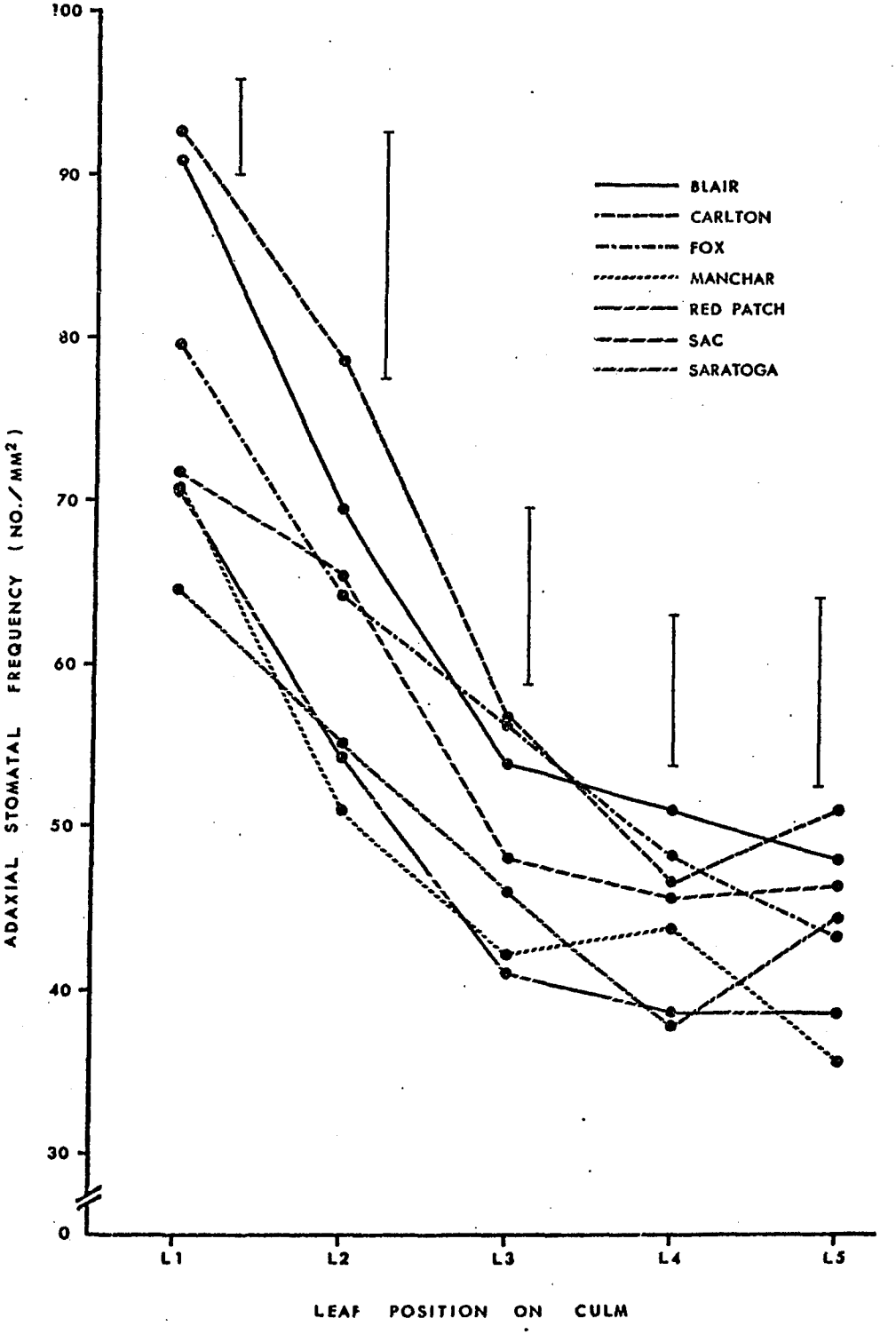

Fig. 1. Distribution of adaxial stomatal frequency (mm^{-2}) at five positions on the culm for seven varieties of Bromus inermis. Tukey's 5% LSR for seven means in a comparison is indicated above each set of seven varieties.

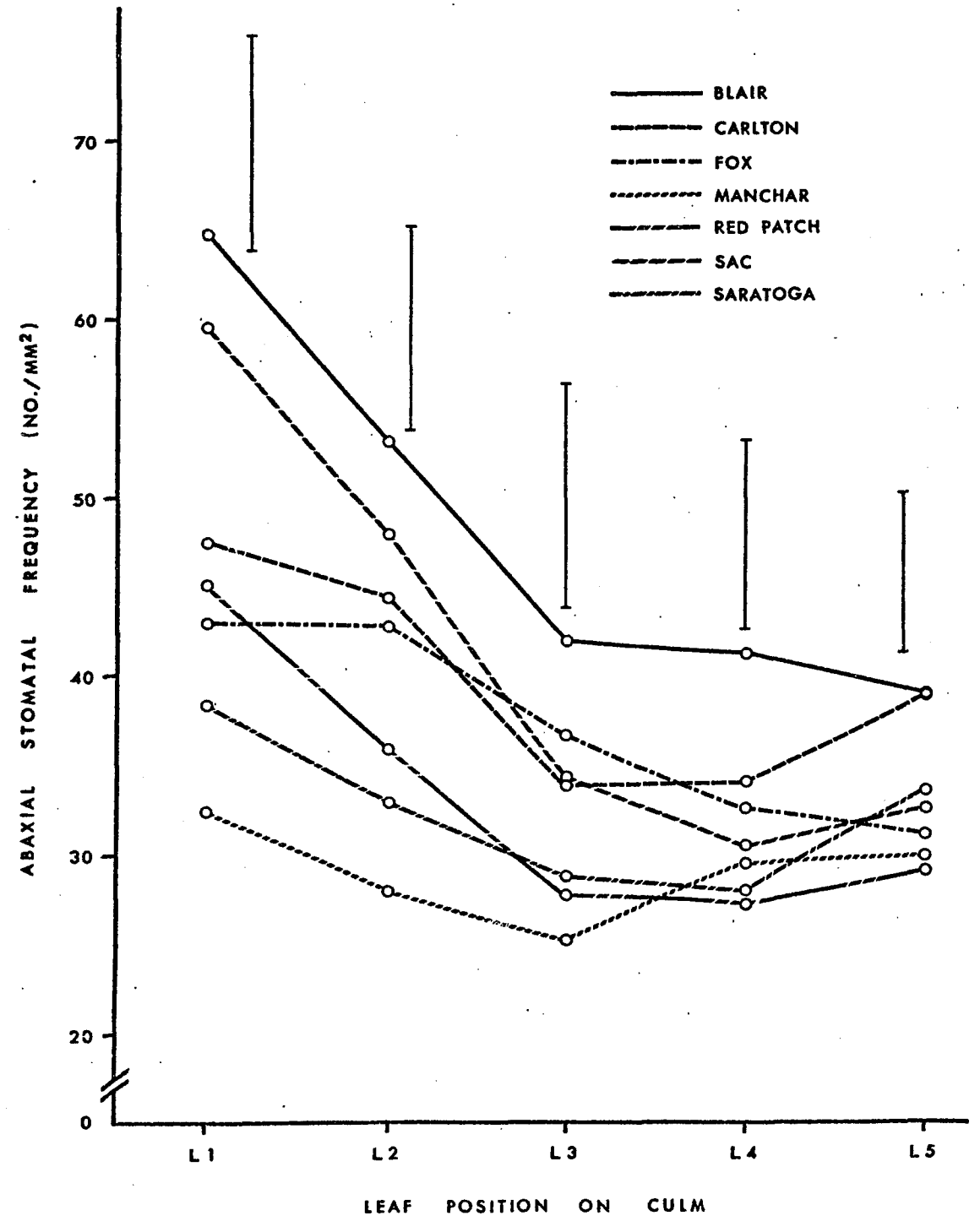

Fig. 2. Distribution of abaxial stomatal frequency (mm^{-2}) at five positions on the culm for seven varieties of Bromus inermis. Tukey's 5% LSR for seven means in a comparison is indicated above each set of seven varieties.

Table 4. Analysis of variance for stomatal characters from three leaf positions on the first leaf (L1) of seven varieties of bromegrass.

Source	df	Mean Squares			
		Stomatal Length (μ)		Stomatal Frequency (mm^{-2})	
		Adaxial	Abaxial	Adaxial	Abaxial
Replication	1	7.628	1.704	45.739	10.162
Variety (V)	6	26.902***	33.674***	346.329***	282.531***
Position (P)	2	151.873***	127.897***	5759.430***	886.979**
V x P	12	4.615*	4.349*	89.166***	111.338***
Error	20	2.005	1.561	13.627	14.569

* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

were found mainly associated with the central position of leaf surfaces. Differences in stomatal length among varieties were non-significant at the tip position on both surfaces and the basal position on abaxial surface. The tip of leaf had the largest stomata, and the base the smallest.

Stomatal frequency was significantly different at three positions of L1 on the adaxial leaf surface, with the base having the most, the center intermediate and the tip the least stomata per mm^2 leaf area (Table 5). At the abaxial surface, the differences in stomatal frequency between tip and center was non-significant, but both differed significantly from the basal position of the leaf.

Leaf Dimension and Tiller Dry Weight

Significant varietal differences ($P<0.001$) in leaf length, width and area for leaves at five positions on the culm are indicated in Table 6. Leaves at different positions were also significantly different ($P<0.001$) in length, width and area. The variety-leaf-position interaction was, however, not significant for any leaf character which indicated that all varieties having the smallest leaf area of L1 are likely to have smaller leaf areas at other positions on the culm.

Tables 7, 8 and 9 show mean comparisons of length, width and area of five leaves on the culms of seven varieties, respectively. The variety 'Red Patch' consistently had the longest and widest leaf and the largest leaf area of the seven varieties, followed by 'Saratoga'. For L1, 'Red Patch' exhibited about 30% longer leaf, 35% wider leaf and over 50% greater leaf area than 'Carlton'.

Table 5. Mean stomatal length (μ) and frequency (mm^{-2}) in seven varieties of Bromus inermis in relation to the adaxial and abaxial surfaces for three positions on the first leaf.

Varieties	Leaf-blade position			Leaf-blade position		
	Tip	Center	Base	Tip	Center	Base
	Stomatal Length≠ (μ)			Stomatal Freq.≠ (mm^{-2})		
			Adaxial			
Blair	54.1 a*	49.1 b	45.2 c	46.6 ab	90.9 a	105.1 a
Carlton	51.7 a	48.5 b	49.2 abc	58.4 a	92.6 a	88.6 ab
Fox	54.4 a	50.2 ab	47.4 abc	48.5 ab	79.7 b	98.3 ab
Manchar	56.1 a	53.4 ab	50.3 ab	40.4 b	70.7 c	78.1 b
Red Patch	57.3 a	54.3 ab	50.2 ab	45.9 b	70.5 c	77.2 b
Sac	56.4 a	50.5 ab	46.8 bc	48.2 ab	71.7 c	84.9 ab
Saratoga	56.4 a	57.0 a	51.2 a	50.3 ab	64.6 d	79.8 b
Mean	55.2 A	51.9 AB	48.6 B	48.3 C	77.2 B	87.4 A
			Abaxial			
Blair	54.9 a	48.5 d	46.4 a	41.9 a	64.7 a	75.4 a
Carlton	51.3 a	48.1 d	48.8 a	51.0 a	47.5 bc	56.4 d
Fox	53.6 a	50.1 cd	47.1 a	52.4 a	43.0 cd	58.9 cd
Manchar	56.5 a	54.1 b	51.3 a	41.8 a	32.4 d	53.0 d
Red Patch	56.8 a	54.0 b	49.7 a	55.2 a	45.2 c	65.6 bc
Sac	56.2 a	52.3 bc	48.8 a	55.2 a	59.5 ab	73.2 ab
Saratoga	57.1 a	58.0 a	52.0 a	57.0 a	38.3 cd	54.4 d
Mean	55.2 A	52.2 AB	49.2 B	50.7 B	47.2 B	62.4 A

* Means not followed by letters in common (lower case - columns, upper case - rows) are significantly different at the 5% level of probability according to Tukey's w-procedure.

≠ Each value is the mean of 30 stomata.

≠ Each value is the mean number of stomata from 30 microscopic fields.

Table 6. Analaysis of variance for the leaf characters of five leaves on the culms of seven varieties of bromegrass.

Source	df	Mean Squares		
		Leaf Dimension		
		Length (cm)	Width (mm)	Area (cm^2)
Replication	1	0.862	1.032	0.003
Variety (V)	6	49.944***	30.514***	214.579***
Leaf (L)	4	122.453***	80.437***	502.270***
V x L	24	1.113	1.130	4.544
Error	34	1.438	0.742	3.575

*** $P<0.001$.

Table 7. Mean leaf length (cm) of five leaves on the culms of seven varieties of *Bromus inermis*. ⁄

Varieties	Leaf positions on culm				
	L1	L2	L3	L4	L5
Blair	15.8 bc*	20.7 ab	22.5 bc	22.3 ab	20.1 ab
Carlton	14.3 c	17.0 b	22.0 c	20.2 b	17.9 b
Fox	17.6 abc	24.1 ab	26.3 ab	25.9 a	24.6 a
Manchar	17.7 abc	24.5 a	26.2 ab	25.8 a	24.2 a
Red Patch	20.2 a	25.9 a	26.9 a	25.7 a	25.1 a
Sac	17.1 abc	22.7 ab	24.4 abc	24.4 ab	23.3 a
Saratoga	18.1 ab	23.5 ab	25.5 abc	24.4 ab	21.9 ab
Mean	17.3 B	22.6 A	24.8 A	24.1 A	22.4 A

* Means not followed by letters in common (lower case - columns, upper case - rows) are significantly different at the 5% level of probability, according to Tukey's w-procedure (hsd).

⁄ Each value is the mean of 6 leaves.

Table 8. Mean leaf width (mm) of five leaves on the culms of seven varieties of *Bromus inermis*. ⁄

Varieties	Leaf positions on culm				
	L1	L2	L3	L4	L5
Blair	6.4 b*	7.1 b	11.5 bc	11.1 cd	10.5 bc
Carlton	5.7 b	8.9 ab	10.4 c	10.0 d	9.3 c
Fox	6.7 ab	10.0 ab	11.4 c	11.1 cd	11.2 abc
Manchar	6.9 ab	10.7 ab	11.8 bc	11.9 bcd	12.0 abc
Red Patch	8.7 a	13.4 a	15.7 a	15.9 a	14.0 a
Sac	7.8 ab	12.6 a	14.9 ab	15.0 ab	12.9 ab
Saratoga	6.9 ab	11.8 ab	13.9 abc	14.2 abc	12.7 ab
Mean	7.0 B	10.6 A	12.8 A	12.7 A	11.8 A

* Means not followed by letters in common (lower case - columns, upper case - rows) are significantly different at the 5% level of probability, according to Tukey's w-procedure (hsd).

⁄ Each value is the mean of 6 leaves.

Table 9. Mean leaf area (cm^2) of five leaves on the culms of seven varieties of *Bromus inermis*. ≠

Varieties	Leaf positions on culm				
	L1	L2	L3	L4	L5
Blair	7.96 b*	15.58 b	19.45 bc	19.16 bc	17.19 bc
Carlton	7.01 b	14.34 b	17.74 c	16.28 c	13.44 c
Fox	9.43 b	18.84 b	23.49 bc	23.10 abc	21.63 ab
Manchar	9.98 b	19.38 b	24.80 bc	25.09 abc	23.56 ab
Red Patch	14.91 a	29.28 a	34.16 a	32.90 a	29.23 a
Sac	10.46 ab	20.81 b	27.08 ab	26.46 abc	23.43 ab
Saratoga	11.03 ab	21.66 b	28.43 ab	27.19 ab	21.59 b
Mean	10.11 B	19.99 A	25.02 A	24.31 A	21.44 A

* Means not followed by letters in common (lower case - columns, upper case - rows) are significantly different at the 5% level of probability, according to Tukey's w-procedure (hsd).

≠ Each value is the mean of 6 leaves.

Averages for leaf length, width and area of the seven varieties showed that L1 was significantly smaller than the other leaves on the culm.

Mean area per leaf of the seven varieties increased from L1 to L3 with the L1 having about 40% the area of L3. From L3 downward, area per leaf decreased gradually but the differences among L2, L3, L4 and L5 were non-significant at the 5% hsd test.

Tiller dry weight and yield per hectare are shown in Table 10. 'Red Patch', together with 'Saratoga', exhibited the highest average tiller dry weight and 'Blair' and 'Fox' the lowest. 'Saratoga' out-yielded 'Fox' by almost two-fold on a per tiller basis. 'Carlton', 'Manchar' and 'Sac' were intermediate in dry weight per tiller, and were not significantly different from the two extreme groups.

There was no correlation ($r = -0.077$) between dry weight per tiller and yield per hectare. 'Blair' and 'Fox', which had the lowest dry weight per tiller, were among the highest yielding varieties (Table 10). The relatively low dry weight per tiller for the two varieties may have been compensated for by producing more tillers per plant. Number of tillers per plant was not examined in the present study.

Interrelationship of Characters

Correlation coefficients among mean values for stomatal characters, leaf dimensions of L1 and tiller dry weight for the seven varieties are presented in Table 11. L x f, the product of stomatal guard-cell length (L) and frequency (f), a character which is relatively comparable to l x f (the total pore opening

Table 10. Tiller dry weight and yield of seven bromegrass varieties.

Varieties	Dry wt per tiller (g)	Yield≠ (tons/ha)			
		1970	1971	1972	Avg
Blair	2.322 b*	8.48 a	7.74 a	5.62 a	7.28 a
Carlton	2.594 ab	7.98 a	6.99 a	4.91 a	6.62 a
Fox	1.814 b	7.68 a	7.50 a	4.93 a	6.70 a
Manchar	2.489 ab	6.86 a	6.83 a	4.38 a	6.02 a
Red Patch	3.554 a	-	-	-	-
Sac	2.940 ab	3.97 b	7.25 a	5.08 a	5.43 a
Saratoga	3.565 a	8.76 a	7.74 a	4.67 a	7.05 a

* Means not followed by letters in common are significantly different at the 5% level of probability, according to Tukey's w-procedure (hsd).

≠ Each value is the mean of 24 tillers.

≠ Each value is the mean of 4 replications.

per unit leaf area) was also included.

Length of stomata on the adaxial and length on the abaxial surface of L1 of the seven varieties were positively correlated ($P<0.01$). The stomatal frequencies on the adaxial and abaxial surfaces of L1 were also positively correlated ($P<0.01$). Stomatal length and frequency were negatively correlated on the adaxial ($P<0.01$) and abaxial ($P<0.05$) surface. The inter-relationship among stomatal characters are in close agreement with those reported earlier (Tan and Dunn, 1974) for two ploidy levels in this species, but the magnitude of the correlation coefficients was reduced. This may be explained by the fact that variability for stomatal characters associated with two ploidies is more diverse than variability among varieties within one ploidy level.

Adaxial stomatal length of L1 was positively correlated with leaf length ($P<0.01$), width ($P<0.05$), area ($P<0.01$) and tiller dry weight ($P<0.01$). Highly positive correlations were obtained ($P<0.01$) between stomatal frequency and L x f of the corresponding leaf surface. Therefore, L x f was affected more by stomatal frequency than stomatal length on a leaf surface. Stomatal frequency showed strong and consistent correlations ($P<0.01$) with leaf length, width and area and tiller dry weight on the adaxial surface, but was non-significantly correlated with the above traits on the abaxial surface.

Leaf area was closely associated with leaf length and width (Table 11). Leaf length was also significantly related to leaf width. There were also significant positive correlations between leaf length, width, area and tiller dry weight. Additional

Table 11. Simple correlation coefficients among stomatal and leaf characters of the first leaf and tiller dry weight in seven varieties of Bromus inermis. ≠

Character	2≠	3	4	5	6	7	8	9	10
1	.797**	-.672**	-.403**	-.252	-.114	.500**	.368*	.544**	.605**
2		-.678**	-.318*	-.389*	.067	.525**	.297	.542**	.615**
3			.461**	.882**	.224	-.535**	-.507**	-.608**	-.536**
4				.359*	.916**	-.162	-.065	-.176	-.064
5					.233	-.382*	-.425**	-.458**	-.322*
6						.014	.032	.002	.138
7							.408**	.844**	.608**
8								.754**	.425**
9									.646**

* $P<0.05$; ** $P<0.01$.

≠ With 40 degrees of freedom, coefficients of .304 and .393 are significant at the 5% and 1% level, respectively.

≠ 1 = Stomatal length (L)(adaxial), 2 = Stomatal length (L)(abaxial), 3 = Stomatal frequency (f) (adaxial), 4 = Stomatal frequency (f)(abaxial), 5 = L x f (adaxial), 6 = L x f (abaxial), 7 = Leaf length, 8 = Leaf width, 9 = Leaf area, 10 = Tiller dry weight.

information was obtained on the estimation of leaf area from width x length measurements. Regression of actual leaf areas on the width-length products gave a regression coefficient of 0.79. The formula, width x length x 0.79, is therefore recommended for use in converting width-length measurements to leaf area in octoploid bromegrass.

The inter-relationship among the various characters suggested that a variety with fewer stomata per unit leaf area is likely to have larger stomata, longer and wider leaves of larger areas, and greater dry weight per tiller than a variety with more stomata. This does not necessarily imply that such a variety would give higher yield per hectare than those varieties with greater stomatal frequency.

The present study was not designed to obtain an estimation of genetic variance. It does provide information that there are differences among bromegrass varieties for the characteristics which were studied. Such differences were observed consistently over replications and leaf positions. It would seem, as suggested by Miskin and Rasmusson (1970) and Heichel (1971b) in other species, that stomatal characteristics may be subject to genetic manipulation in octoploid bromegrass.

SUMMARY

1. Seven varieties of octoploid bromegrass were evaluated for the extent of variability associated with stomatal length and frequency and for their relationships with leaf length, width, area, tiller dry weight, and yield.

2. The varieties differed significantly in stomatal length and frequency on the adaxial and abaxial surfaces at five leaf positions on the culm. The varieties 'Carlton' and 'Blair' had consistently smaller stomata with greater frequency, whereas 'Saratoga' and 'Red Patch' had consistently larger stomata with less frequency.

3. The seven varieties showed a similar distributional pattern for stomatal length and frequency at five leaf positions on the culm and three different positions on the individual leaf. Stomatal length increased while frequency decreased from L1 to L3 and leveled off from L3 to L5. The tip of the leaf had the largest but fewest number of stomata with the reverse for the base of the leaf. Varietal differences in stomatal length and frequency were mainly associated with the central position of leaf surfaces.

4. Significant differences were also found among the seven varieties for length, width and area of the five leaves on the culm. The variety 'Red Patch' consistently had the longest and widest leaves of greatest area, whereas 'Carlton' had the smallest leaves at all positions on the culm. Mean area per leaf of the seven varieties increased from L1 to L3 with the L1 having about

40% the area of L3. From L3 downward, area per leaf decreased gradually but differences among L2, L3, L4 and L5 were not significant.

5. The formula, leaf width x length x 0.79 was recommended for converting width-length measurements to leaf area for future leaf area estimations in octoploid bromegrass.

6. Dry weight per tiller differed significantly among the seven varieties, but yield did not. Hence, there appears to be no correlation between dry weight per tiller and yield per hectare.

7. The adaxial and abaxial surfaces of L1 were highly positively correlated for both stomatal length and frequency. The two stomatal characters were, however, negatively correlated. L x f, the product of stomatal guard cell length and stomatal frequency, was affected more by stomatal frequency than length on the same leaf surface.

8. At the adaxial surface of L1, stomatal length was positively, whereas stomatal frequency was negatively correlated with leaf length, width, area and tiller dry weight. Significant positive correlations were also found among the leaf characters and tiller dry weight. The inter-relationship among characters suggested that a variety with fewer stomata is likely to have larger stomata, longer and wider leaves of larger areas, and greater dry weight per tiller than a variety with more stomata.

9. The results of this study indicated that stomatal length and frequency may be subject to genetic manipulation in this species.

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PART II

GENETIC VARIATION IN STOMATAL LENGTH AND FREQUENCY, LEAF CHARACTERISTICS AND DRY MATTER PRODUCTION IN *BROMUS INERMIS* LEYSS.

INTRODUCTION

The relatively consistent differences among varieties of Bromus inermis in our previous study indicated that genotype is prominent in determining stomatal length and frequency and, hence, that it may be possible to modify these characters through breeding. Likewise, the significant intraplant variation in stomatal length and frequency, i.e. successively lower leaves had consistently larger but fewer stomata, is likely under genetic control.

The preceding experiment was, however, unable to provide information on the magnitude of genetic differences for these characters which may be utilized by selection. The present experiment was therefore designed to provide information on the importance of general and specific combining ability and on heritabilities of stomatal length and frequency, and their phenotypic and genotypic correlations with leaf length, width and area, tiller dry weight and dry matter production in a half-diallel cross of five genotypes of Bromus inermis.

REVIEW OF LITERATURE

Considerable data have been reported from combining ability and heritability studies for seed and forage yield and other agronomic characters in smooth bromegrass (Bromus inermis Leyss.) (e.g. Thomas and Kernkamp, 1954; Knowles, 1955; Grissom and Kalton, 1956; Nielson and Kalton, 1959; Timothy et al., 1959; Drolsom and Nielsen, 1970; Dunn and Wright, 1970; Mishra and Drolsom, 1972 and 1973; Sleper et al., 1973, and Sleper and Drolsom, 1974).

In a number of quantitative characters, such as forage and seed yield and yield components in bromegrass, general combining ability (GCA) was reported to be larger than specific combining ability (SCA) (Timothy et al., 1959; Dunn and Wright, 1970, and Mishra and Drolsom, 1972). For other characters such as green forage weight, plant type and height, diameter and vigor, SCA had been reported more important than GCA by Drolsom and Nielsen (1970). However, there were also contrasting results showing that GCA was of greater importance than SCA for plant height (Timothy et al., 1959; Ross et al., 1970, and Mishra and Drolsom, 1972).

Stomatal frequency was found to be under the control of few rather than many genes (Heichel, 1971) from a cross between inbreds of corn with low and high stomata frequencies. Martin (1970) obtained similar data from a bean cross. He expressed the data as a stomatal index (stomatal no./stomatal no. plus epidermal cell no.). Broad sense heritabilities (H_b) for both upper and lower stomatal indices were about 60%, and narrow sense heritability (H_n) values ranged from 25 to 11% in F_2 and F_3 generations, respectively.

In a study of the inheritance of stomatal frequency in barley, Miskin et al. (1972) reported heritabilities of stomatal frequency ranged from 22 to 74%, estimated both by the parent-progeny regression method and realized heritabilities.

Wilson (1971) concluded from the selection response after divergent selection for stomatal length and frequency and other leaf characters in Lolium perenne L. that much of the variation in stomatal characters was genetic and additive. Heritability of stomatal frequency was 52%, about twice that of stomatal pore length and of frequency x length. He also reported that stomatal frequency responded negatively to selection for stomatal length, but selection for frequency had no significant effect on stomatal length. Response to selection for frequency x length was based mainly on a response by frequency. Stomatal frequency was also found phenotypically correlated with leaf length ($r = -0.30$). However, this relationship was not reflected in a correlated response to selection. Heritability estimates based on parent-progeny regression for leaf length, width and area were approximately 60-70%.

In bromegrass, Mishra and Drolsom (1972) reported that general combining ability was highly significant for length of the third leaf from the top. Specific combining ability was less prominent, whereas the reverse was true for width of the third leaf. For leaf length, the heritability estimate in the broad sense ($H_b = 0.52$) was reported equal to that in the narrow sense. The heritability estimates for leaf width were 0.63 in the broad sense, and 0.32 in the narrow. Morphological traits such as

plant height, leaf length, culm diameter, panicle length and number of spikelets per panicle of smooth bromegrass were reported to be highly correlated among one another both phenotypically and genotypically (Mishra and Drolsom, 1973). The correlations of leaf width with these traits were, however, low and non-significant except with culm diameter. There have been no previous reports concerning combining ability or heritability for stomatal characters and their genotypic correlations with other morphological characters in bromegrass.

MATERIALS AND METHODS

The 10 single crosses of the 5-parent half-diallel were obtained from 15 original single crosses of a 6-parent half-diallel first established in 1959. The 6-parent half-diallel had previously been reported by Dunn and Wright (1970). Sufficient seeds were available from only five of the six original parents. The five open-pollinated bromegrass genotypes were originally selected on the basis of their phenotypes for resistance to brown leaf spot, leafiness, and aftermath growth, plus a limited amount of polycross progeny testing for yield and leafspot reaction. The pedigree and sources of the five genotypes used as parents are shown in Table 12. All five parents were highly self-sterile under bags.

The 10 possible single crosses were originally made in isolated crossing blocks in the field without emasculation or bagging. These were established with six clones of each parent.

After 12-13 years of storage in a refrigerator, the seed had an average germination of over 60% in petri dishes in 1971 for all single crosses except two, for which germination was under 50%. The seedlings were planted singly in 4½-inch plastic pots and were maintained in the greenhouse for four months during the winter season to favour leaf and root development before establishment in the field.

Individual plants were transplanted to the field and spaced 92 cm apart within and between rows in a randomized complete block design of six replications in April, 1972. The 10 single crosses

Table 12. Pedigree and source of each parent used in the five-parent diallel.

Code	Original serial number	Pedigree	Source
1	1669	NY 47-94	A polycross progeny from Dr. R. P. Murphy, Cornell University, 1952.
2	3771	29-774	Plant grown from open-pollinated seed from single plant selection from strain 46-141. Strain 46-141 was a polycross progeny from Dr. R. P. Murphy, Cornell University.
3	3404	29-992	Plant grown from open-pollinated seed from single plant selection within variety 'Achenbach'.
4	4228	PI198064	From Plant Introduction Station, Ames, Iowa, 1954.
5	1273	IV-2	A polycross progeny obtained from Dr. H. L. Carnahan, Pennsylvania State University, 1953.

were randomly assigned within replicates with 5 plants of each cross per replicate.

Sampling was started in July, 1972 when anthesis began. Two leaves - the first (L1) and the second (L2) below the panicle - were collected from each of two culms from each plant. These leaves were measured for length and median width. Leaf size was then estimated as length x width x 0.79 (see Part I). Central portions of the first leaves were killed in boiling water and treated with ethyl alcohol and lactic acid according to Clark's (1960) method with slight modification for stomata examination as described in Part I. From each plant, two tillers were sampled for the determination of dry weight by oven-drying for 24 hr at 100°C. Plants were harvested individually around the end of July for the evaluation of dry matter production by oven-drying for 48 hr at 100°C.

The data were subjected to analyses of variance, and Griffing's Method 4 Model II (1956) of diallel analysis. Model II was chosen since plant selection was random in terms of the stomatal and leaf characteristics which were evaluated. The variance components for GCA, SCA and error were obtained by equating the calculated mean squares to observed expectations as given by Griffing (1956) (Table 13).

The additive genetic (σ^2_a) and non-additive genetic (σ^2_{na}) variances can be estimated from the combining ability components by $\sigma^2_a = 2\sigma^2_g$ and $\sigma^2_{na} = \sigma^2_s$ (Griffing, 1956). The genotypic and phenotypic variances may be estimated as

Table 13. Expected mean squares for the diallel analysis.

Source	df	Expected mean squares *
General Combining Ability	P - 1	$\sigma^2_e + \sigma^2_s + (P-2)\sigma^2_g$
Specific Combining Ability	P(P-3)/2	$\sigma^2_e + \sigma^2_s$
Error	m	σ^2_e

* Where σ^2_g = GCA variance component; σ^2_s = SCA variance component;
σ^2_e = error variance component; P = No. of parents, and
m = (R - 1)(F - 1); R = No. of replications, and F = P(P - 1)/2.

$$\sigma^2_G = 2\sigma^2_g + \sigma^2_s \text{ , and}$$
$$\sigma^2_P = \sigma^2_G + \sigma^2_E ;$$

where

σ^2_g = general combining ability (largely additive effects),

σ^2_s = specific combining ability (largely non-additive effects),

σ^2_G = genotypic variance,

σ^2_P = phenotypic variance,

σ^2_E = environmental variance.

In the present experiment these variances were used to provide estimates of heritability in the broad-(H_b) and narrow-(H_n) sense for each character. In all computations, negative estimates of variance components were treated as 0. The heritability estimates were obtained as follows:

$$H_b = \frac{\sigma^2_G}{\sigma^2_P} \quad \text{and} \quad H_n = \frac{\sigma^2_a}{\sigma^2_P} \text{ .}$$

A covariance analysis was used to calculate genotypic, phenotypic and environmental correlations. The genotypic correlation was calculated as follows:

$$r_G = (\sigma_{G_{xy}}) / \left[(\sigma^2_{G_x})(\sigma^2_{G_y})\right]^{-\frac{1}{2}}$$

where

$\sigma_{G_{xy}}$ = genotypic covariance between variables x and y.

$\sigma^2_{G_x}$ = genotypic variance of the independent variable.

$\sigma^2_{G_y}$ = genotypic variance of the dependent variable.

The phenotypic correlation was calculated as:

$$r_P = (\sigma_{G_{xy}} + \sigma_{E_{xy}}) / \left[(\sigma^2_{G_x} + \sigma^2_{E_x})(\sigma^2_{G_y} + \sigma^2_{E_y})\right]^{-\frac{1}{2}}$$

where

$\sigma_{E_{xy}}$ = error covariance between variables x and y.

$\sigma^2_{E_x}$ = error variance of the independent variable.

$\sigma^2_{E_y}$ = error variance of the dependent variable.

The environmental correlation was calculated as:

$$r_e = (\sigma_{E_{xy}}) / \left[(\sigma^2_{E_x})(\sigma^2_{E_y})\right]^{-\frac{1}{2}} .$$

RESULTS

The mean, standard error of the mean, and coefficient of variation for each of the 12 characters over all 10 single crosses are shown in Table 14. Lowest coefficients of variation (CV) were obtained for stomatal lengths at both leaf surfaces. High CVs were observed for leaf areas and dry weight per tiller and per plant. The values of some characters of most interest such as stomatal length ranged from 50 to 59 μ at the adaxial surface of L1 and from 52 to 61 μ at the abaxial surface; and stomatal frequency from 62 to 89 per mm^2 and from 43 to 70 per mm^2 of the adaxial and abaxial leaf surfaces, respectively (Appendix Table 1).

Analyses of variance (Table 15) revealed significant ($P<0.01$) differences among genotypes (crosses) in all characters measured. The genotypic variation was then partitioned into variation due to General Combining Ability (GCA) and Specific Combining Ability (SCA) in the combining ability analyses (Table 16).

GCA was found significant or highly significant for all characters whereas SCA was not significant for any character. The ratios of GCA mean squares to SCA mean squares (Table 16) ranged from 6:1 for both leaf length and dry weight per plant to 211:1 for leaf width, indicating that genotypic variation due to GCA predominated. These results were interpreted to mean that additive gene action was relatively important in controlling the level of expression of each character.

The estimates of variance components for general and specific combining ability, and residual are presented in Table 17. It is

Table 14. Mean ($\bar{x}$), standard error of the mean ($S\bar{x}$) and coefficient of variation (CV) for 12 characters of smooth bromegrass progenies derived from a diallel mating.

Characters	$\bar{x}$	$S\bar{x}$	CV (%)
Stomatal Character:			
Stomatal length (L1-Adaxial)(μ)	55.2	0.32	8.9
Stomatal length (L1-Abaxial)(μ)	56.5	0.34	9.1
Stomatal frequency (L1-Adaxial)(mm^{-2})	76.3	0.92	18.3
Stomatal frequency (L1-Abaxial)(mm^{-2})	54.6	0.75	20.9
Leaf Dimension:			
Leaf length (L1) (cm)	18.7	0.26	21.4
Leaf width (L1) (mm)	7.7	0.11	22.6
Leaf area (L1) (cm^2)	11.8	0.32	41.2
Leaf length (L2) (cm)	22.8	0.27	18.3
Leaf width (L2) (mm)	11.2	0.16	22.2
Leaf area (L2) (cm^2)	20.8	0.49	36.2
Dry Weight:			
Tiller dry wt (g)	2.31	0.06	36.6
Plant dry wt (g)	36.65	1.03	44.5

Table 15. Mean squares from randomized-block analyses of variance for 12 characters of bromegrass diallel cross progenies.

Source	df	Character					
		Stomatal Length (L1 - Adaxial)	Stomatal length (L1 - Abaxial)	Stomatal Freq. (L1 - Adaxial)	Stomatal Freq. (L1 - Abaxial)	Leaf Length (L1)	Leaf Width (L1)
Replication	5	56.203**	25.056	39.625	41.251	6.143	2.396
Crosses	9	253.882**	266.731**	3005.589**	1968.361**	165.848**	34.926**
Rep x Crosses	45	18.764*	23.474*	153.077**	98.265**	16.350**	2.522**
Bet. full sibs, within plots	172	12.739	15.013	63.908	46.422	8.603	1.547

* $P<0.05$; ** $P<0.01$.

Table 15. (Continued)

Source	df	Character					df	
		Leaf Area (L1)	Leaf Length (L2)	Leaf Width (L2)	Leaf Area (L2)	Tiller Dry wt		Plant dry wt
Replication	5	16.345	5.670	6.814	45.487	0.475	5	289.247
Crosses	9	261.347**	144.762**	61.186**	560.553**	5.618**	9	914.986**
Rep x Crosses	45	20.225**	18.791**	5.335*	51.240*	0.536	45	210.694
Bet. full sibs, within plots	172	12.435	10.762	3.564	32.366	0.515	190	248.851

* $P<0.05$; ** $P<0.01$.

Table 16. Mean squares for general (GCA) and specific (SCA) combining ability and ratio of GCA:SCA for 12 characters estimated from the 10 diallel cross progenies of bromegrass.

Source	df	Character					
		Stomatal Length (L1 - Adaxial)	Stomatal Length (L1 - Abaxial)	Stomatal Freq. (L1 - Adaxial)	Stomatal Freq. (L1 - Abaxial)	Leaf Length (L1)	Leaf Width (L1)
GCA	4	22.301**	24.339**	281.183**	192.603**	14.110*	3.492**
SCA	5	0.628	1.143	4.963	7.497	1.297	0.022
Error	45	0.808	1.011	6.598	4.235	0.704	0.108
Ratio GCA:SCA		35:1	21:1	56:1	25:1	11:1	156:1

* $P<0.05$; ** $P<0.01$.

Table 16. (Continued)

Source	df	Character					
		Leaf Area (L1)	Leaf Length (L2)	Leaf Width (L2)	Leaf Area (L2)	Tiller Dry Wt	Plant Dry Wt
GCA	4	23.656**	11.706*	5.827**	50.220**	0.530**	72.520*
SCA	5	0.564	1.770	0.027	1.460	0.024	11.171
Error	45	0.871	0.809	0.229	2.208	0.023	8.427
Ratio GCA:SCA		42:1	6:1	211:1	34:1	21:1	6:1

* $P<0.05$; ** $P<0.01$.

Table 17. Variance component estimates of general (σ^2_g) and specific (σ^2_s) combining ability and error (σ^2_e), and broad- (H_b) and narrow- (H_n) sense heritability estimates for 12 characters of bromegrass diallel cross progenies.

Characters	Estimate of Variance Component			Heritability	
	σ^2_g	σ^2_s	σ^2_e	H_b	H_n
Stomatal Length (L1-Adaxial)	7.224	-0.180	12.739	0.53	0.53
Stomatal Length (L1-Abaxial)	7.731	0.132	15.013	0.51	0.50
Stomatal Freq. (L1-Adaxial)	92.073	-1.634	63.908	0.74	0.74
Stomatal Freq. (L1-Abaxial)	61.702	3.261	46.422	0.73	0.71
Leaf Length (L1)	4.270	0.592	8.603	0.51	0.48
Leaf Width (L1)	1.156	-0.086	1.547	0.59	0.59
Leaf Area (L1)	7.697	-0.306	12.435	0.55	0.55
Leaf Length (L2)	3.312	0.960	10.762	0.41	0.36
Leaf Width (L2)	1.933	0.202	3.564	0.51	0.51
Leaf Area (L2)	16.253	-0.747	32.366	0.50	0.50
Tiller Dry Wt	0.168	0.001	0.515	0.39	0.39
Plant Dry Wt	20.449	2.743	248.851	0.15	0.14

recognized that all estimates were confounded with locations and years, since data were from one location and one year. The importance of the GCA components is evident from the large and positive estimates that exceeded the SCA variance components which were either negative or very small for all characters. The residual variance component estimate was the larger of the three estimates for each character except stomatal frequencies at both surfaces of L1.

Both broad- and narrow-sense heritability estimates for each of the 12 characters are also shown in Table 17. The heritability estimates in the broad sense corresponded very closely to those in the narrow sense. This occurred due to the relatively small and certain negative SCA variance components that were treated as 0. Stomatal frequency was more heritable than stomatal length. The latter was 53% and the former 74%. Heritability of leaf dimensions was approximately 50%. Relatively lower heritability estimates were obtained for dry weight per tiller (39%) and dry weight per plant (14%).

A GCA effect is a measure of the summed contributions of genes with additive effects and parts of interactions between genes with additive effects. If genic contributions are equal, lines with positive GCA effects possess larger than average concentrations of genes with positive effects while lines with negative GCA effects possess smaller than average concentrations of gene with positive effects. Both negative and positive GCA effects are therefore of interest since either the ability to decrease or increase a character may be desired (Poneleit, 1968).

The estimates of the GCA and SCA effects for stomatal characters and other traits are presented in Tables 18 and 19. Parent 5 had the largest positive GCA effects for stomatal length and the largest negative GCA effects for stomatal frequency at both surfaces (Table 18). Parent 4 tended to have smaller stomata among the parents, whereas parent 3 followed by parent 4 tended to have greater stomatal frequency. Parent 5 also had the greatest effect on increasing leaf length, width and area, and dry weight per tiller while parents 3 and 4 were more important in reducing leaf length, width and area. Dry weight per plant was increased most by parents 1 and 5, and was decreased by parents 3 and 4.

SCA effects were calculated for each trait (Table 19) although the genotypic variation attributed to SCA was very small. The standard errors of the difference between two SCA effects are also given in the table. The cross of 4x5 had the largest positive effect for stomatal length, but had the largest negative effect for stomatal frequency. Large positive SCA effects for leaf length, width and area and tiller dry weight were manifested in cross 1x2. Large estimate of SCA effects for dry weight per plant was found in cross 2x5, followed by crosses 3x5, 1x4 and 1x2.

Phenotypic, genotypic and environmental correlation coefficients for 9 characters, measured on the diallel progenies, are presented in Table 20. Stomatal frequency, at either adaxial or abaxial leaf surface, showed negative and highly significant phenotypic correlations with stomatal length, leaf length, leaf width, leaf area, and dry weight per tiller and per plant. The corresponding genotypic correlations were also significant and of

Table 18. Estimates of general combining ability effects for 12 characters of 10 F_1 genotypes.

Parent	Character											
	Stomatal Length (L1 - Adaxial)	RANK	Stomatal Length (L1 - Abaxial)	RANK	Stomatal Freq. (L1 - Adaxial)	RANK	Stomatal Freq. (L1 - Abaxial)	RANK	Leaf Length (L1)	RANK	Leaf Width (L1)	RANK
1	-1.98	4	-1.68	4	1.86	4	-1.32	4	-0.72	3	-0.29	2
2	-0.43	3	-1.14	3	3.64	3	-0.22	3	1.74	2	-0.36	3
3	0.07	2	-0.44	2	6.90	1	8.81	1	-2.28	5	-0.52	4
4	-2.20	5	-1.73	5	4.60	2	5.06	2	-1.51	4	-0.72	5
5	4.55	1	5.01	1	-17.00	5	-12.32	5	2.78	1	1.90	1
$SE(g_i-g_j)$*	0.32		0.36		0.93		0.75		0.31		0.12	

* Standard error of the difference between two GCA effects.

Table 18. (Continued)

Parent	Character											
	Leaf Area (L1)	RANK	Leaf Length (L2)	RANK	Leaf Width (L2)	RANK	Leaf Area (L2)	RANK	Tiller Dry Wt	RANK	Plant Dry Wt	RANK
1	-0.97	3	-0.47	3	-0.28	2	-1.01	3	-0.21	4	5.35	1
2	0.50	2	1.20	2	-0.61	4	-0.06	2	-0.02	2	1.39	3
3	-2.21	5	-2.11	5	-0.38	3	-2.53	4	-0.29	5	-5.61	5
4	-1.96	4	-1.37	4	-1.14	5	-3.33	5	-0.20	3	-4.68	4
5	4.64	1	2.75	1	2.42	1	6.95	1	0.73	1	3.55	2
$SE(g_i-g_j)$*	0.34		0.32		0.17		0.54		0.05		1.06	

* Standard error of the difference between two GCA effects.

Table 19. Estimates of specific combining ability effects for 12 characters of 10 F_1 genotypes.

Genotypes	Character					
	Stomatal Length (L1 - Adaxial)	Stomatal Length (L1 - Abaxial)	Stomatal Freq. (L1 - Adaxial)	Stomatal Freq. (L1 - Abaxial)	Leaf Length (L1)	Leaf Width (L1)
1 x 2	0.79	1.13	-0.77	-2.46	1.32	0.08
1 x 3	0.28	0.24	-1.73	-1.74	0.70	-0.05
1 x 4	-0.60	-0.73	-0.84	1.56	-0.92	0.05
1 x 5	-0.47	-0.64	3.34	2.63	-1.09	0.08
2 x 3	0.04	0.17	0.53	0.90	-0.95	-0.18
2 x 4	-0.58	-0.62	1.17	-0.10	-0.14	0.01
2 x 5	-0.26	-0.68	-0.93	1.67	-0.22	0.08
3 x 4	0.06	-0.19	1.64	1.84	0.00	0.08
3 x 5	-0.39	-0.22	-0.44	-1.00	0.24	0.15
4 x 5	1.12	1.55	-1.97	-3.30	1.07	-0.15
$SE(s_{ij}-s_{ik})$*	0.32	0.36	0.93	0.75	0.30	0.12
$SE(s_{ij}-s_{kl})$**	0.23	0.26	0.66	0.53	0.21	0.08

* Standard error of a difference between two SCA effects with one common parent.
** Standard error of a difference between two SCA effects with no common parent.

Table 19. (Continued)

Genotypes	Character					
	Leaf Area (L1)	Leaf Length (L2)	Leaf Width (L2)	Leaf Area (L2)	Tiller Dry Wt	Plant Dry Wt
1 x 2	0.83	1.34	0.18	1.38	0.21	1.56
1 x 3	0.38	0.97	-0.05	0.72	0.04	0.55
1 x 4	-0.41	-0.87	-0.08	-0.74	-0.09	1.59
1 x 5	-0.80	-1.45	-0.05	-1.36	-0.16	-3.71
2 x 3	-0.87	-0.93	-0.21	-1.12	-0.12	-4.01
2 x 4	-0.25	-0.09	0.05	-0.21	-0.06	-0.61
2 x 5	0.29	-0.32	-0.01	-0.04	-0.02	3.06
3 x 4	0.32	-0.42	0.11	-0.02	0.02	0.91
3 x 5	0.16	0.38	0.15	0.42	0.05	2.54
4 x 5	0.34	1.38	-0.08	0.98	0.13	-1.89
$SE(s_{ij}-s_{ik})$*	0.10	0.32	0.17	0.54	0.05	1.06
$SE(s_{ij}-s_{kl})$**	0.24	0.23	0.12	0.38	0.03	0.74

* Standard error of a difference between two SCA effects with one common parent.
** Standard error of a difference between two SCA effects with no common parent.

Table 20. Phenotypic (r_P), genotypic (r_G) and environmental (r_e) correlation coefficients for nine characters measured on 10 F_1 bromegrass progenies derived from a diallel mating.

Characters		2/	3	4	5	6	7	8	9
1	r_P	0.935**	-0.896**	-0.739**	0.620**	0.789**	0.744**	0.667**	0.190
	r_G	0.981**	-0.873**	-0.730*	0.701*	0.929**	0.847**	0.922**	0.267
	r_e	0.820**	-0.666**	-0.801**	0.434**	0.391**	0.465**	0.059	0.083
2	r_P		-0.955**	-0.799**	0.587**	0.740**	0.695**	0.618**	0.305*
	r_G		-0.930**	-0.788**	0.696*	0.966**	0.856**	0.844**	0.424
	r_e		-1.063**	-0.867**	-0.387**	0.082	0.244	0.064	0.139
3	r_P			0.895**	-0.698**	-0.883**	-0.829**	-0.828**	-0.451**
	r_G			0.933**	-0.769**	-0.986**	-0.920**	-0.975**	-0.571
	r_e			0.725**	-0.510**	-0.498**	-0.506**	-0.392**	-0.332*
4	r_P				-0.879**	-0.828**	-0.850**	-0.778**	-0.600**
	r_G				-0.883**	-0.898**	-0.919**	-0.957**	-0.768**
	r_e				-0.917**	-0.561**	-0.602**	-0.221	-0.367*
5	r_P					0.720**	0.884**	0.691**	0.444**
	r_G					0.753*	0.885**	0.865**	0.596
	r_e					0.644**	0.892**	0.297*	0.242
6	r_P						0.914**	0.414**	0.410**
	r_G						0.921**	0.471	0.521
	r_e						0.889**	0.261	0.257
7	r_P							0.798**	0.491**
	r_G							0.951**	0.583
	r_e							0.394**	0.383**
8	r_P								0.399**
	r_G								0.435
	r_e								0.368*

* $P<0.05$; ** $P<0.01$.

/ 1 = Stomatal Length (L1-Adaxial), 2 = Stomatal Length (L1-Abaxial), 3 = Stomatal Freq. (L1-Adaxial), 4 = Stomatal Freq. (L1-Abaxial), 5 = Leaf Length (L1), 6= Leaf Width (L1), 7 = Leaf Area (L1), 8 = Tiller Dry Wt, 9 = Plant Dry Wt.

similar sign. Environmental correlations involving stomatal frequency were negative and mostly significant, whereas those involving dry weight per tiller and per plant were generally small and positive. Stomatal length at the adaxial surface was, on the other hand, positively and highly significantly correlated with all other characters both genotypically and phenotypically except plant dry weight. Plant dry weight had relatively low correlations with most other characters.

DISCUSSION

The genotypic differences in stomatal characteristics confirm results from earlier studies on seven varieties of smooth bromegrass (see Part I). The ratio of GCA:SCA and the magnitude of the narrow-sense heritability estimates indicated that much of the variation in stomatal length and frequency in this population was genetic and additive. These results agree fairly well with those reported in corn (Heichel, 1971), perennial ryegrass (Wilson, 1971), and barley (Miskin et al., 1972). Selection for high, or for low, stomatal frequency and for size of stomata on the individual plant would therefore be possible, and rapid response to selection would be expected in this population.

Leaf length, width and area were also strongly influenced by additive gene action. However, contrasting results were reported for leaf length and width in this species: leaf length, which was found to be influenced more by GCA in an earlier report by Mishra and Drolsom (1972), was later reported to be under the influence of SCA (Sleper and Drolsom, 1974). For leaf width SCA was reported more important than GCA earlier (Mishra and Drolsom, 1972), but a reversed result was obtained for the same trait later (Sleper and Drolsom, 1974). A more complicated selection method, such as reciprocal recurrent selection for the leaf characters, would be necessary since two types of gene action seem to be involved.

The phenotypic and genotypic correlations between stomatal length and stomatal frequency at both leaf surfaces were negative and highly significant, indicating that selection for greater stomatal size would result in fewer stomata per unit leaf area.

However, according to Wilson (1971), such negative correlations might not prove to be a barrier to independent selection if heritabilities of the two characters differed.

Highly significant negative genotypic correlations of stomatal frequency with leaf length, width, area, and dry weight per tiller indicated that a genotype with fewer stomata per unit leaf area may have longer and wider leaves of larger areas as well as greater dry weight per tiller. This shows good agreement with earlier results for seven varieties of bromegrass (Part I).

Both phenotypic and genotypic correlations between stomatal length at the adaxial leaf surface and dry weight per plant was small and non-significant. The phenotypic correlations of stomatal length (abaxial) and frequency (adaxial) with plant dry weight were low even though significant. The genotypic correlation coefficients for the above characters were not significant. A highly significant negative genotypic correlation coefficient found for the association between stomatal frequency at the abaxial surface and plant dry weight indicated that selection for decreased stomatal frequency at the abaxial surface should increase the dry matter production per plant. Since only spaced plants were available in this study, forage yields were not obtained. However, considerable yield data were obtained previously (Dunn and Wright, 1970) on the original 6-parent half-diallel. Table 21 shows the simple correlation coefficients computed between stomatal and leaf characters of the present experiment and the average forage yield of the corresponding single crosses of the previous experiments. Stomatal length was positively while stomatal frequency negatively correlated

Table 21. Simple correlation coefficients for stomatal characters and yield.[1]

Characters	Avg Forage Yield[2]	
	Syn 1	Syn 2
Stomatal Length (L1-Adaxial)	0.094	0.321
Stomatal Length (L1-Abaxial)	0.180	0.464
Stomatal Frequency (L1-Adaxial)	-0.322	-0.570
Stomatal Frequency (L1-Abaxial)	-0.300	-0.436
Leaf Length (L1)	0.227	0.162
Leaf Width (L1)	0.238	0.463
Leaf Area (L1)	0.256	0.374
Tiller Dry Wt	0.293	0.468
Plant Dry Wt	-0.119	0.150

[1] With 8 degrees of freedom, 0.632 and 0.765 are significant at the 5% and 1% level respectively.

[2] Mean forage yield (tons/ha) over 3 years (1965-67); data adopted from Dunn and Wright (1970), Experiment 3, where crosses 2x3, 2x4, 2x5, 2x6 ..... are equivalent to crosses 1x2, 1x3, 1x4, 1x5 ....., respectively, of the present experiment.

with forage yield, represented by both syn-1 and syn-2 in drilled plots. The correlation coefficients were, however, not significant. There was no association between plant dry weight and yield in drilled plots. None of the leaf characters were significantly correlated with yield.

In general, genotypic correlations were higher or slightly higher than phenotypic correlations. The rough agreement between the phenotypic and genotypic correlations indicated greater importance of genetic variability with common effects than the environmental variability with common effects. According to Falconer (1960), estimates of higher genotypic correlations are usually subject to rather large sampling errors and are therefore seldom precise.

The environmental correlations involving dry weight per tiller and per plant were generally smaller than that involving stomatal and leaf characters. These may be indicating that the correlations between two characters involving dry weight per tiller or per plant, if of any significance, are due more to genetical than to environmental causes. Some of the high environmental correlations exhibited among the stomatal lengths and among leaf characters indicated that the environment was exerting a common influence on such traits in their development.

The genotypic associations of stomatal length and frequency with leaf length, width and area, and dry weight are high and significant, indicating that positive selection for stomatal length or negative selection for stomatal frequency should bring a substantial improvement in other traits as well. However, achieving high

yielding clones by applying selection pressure on stomatal characters alone should not be expected. Wallace *et al.* (1972) warned that 'a high correlation existing between a physiological component and yield did not indicate that all genetic variability affecting yield was centered in that component'. Each component will make a small, and not necessarily all important, contribution to yield.

If the role of stomata in whole plant growth can be clearly defined, and if they show useful response to selection without too many undesirable correlations, then these stomatal characteristics might be of value as one of the selection criteria in a forage grass breeding program.

SUMMARY

Genetic variation of stomatal length and frequency was studied in 10 possible single crosses of a half-diallel cross involving five Bromus inermis genotypes. The progenies were space-planted in a randomized complete block of six replications. Diallel analyses were carried out according to Griffing's Method 4 Model II (1956).

1. The results of the analyses revealed significant or highly significant GCA mean squares for stomatal length and frequency, leaf length, width and area, dry weight per tiller and per plant. None of the SCA mean squares were significant in any analysis. The ratio of GCA:SCA ranged from 6:1 to 211:1 for all characters, which meant that additive gene action was relatively important in controlling the level of expression of each character.

2. Parent 5 had the highest positive and lowest negative GCA effects for stomatal length and stomatal frequency, respectively, and had the greatest effect on increasing leaf length, width, area and dry weight per tiller.

3. Heritability estimates in the narrow-sense were high for adaxial stomatal frequency (0.74), adaxial stomatal length (0.53), leaf length (L1) (0.48), leaf width (L1) (0.59), leaf area (L1) (0.55) and dry weight per tiller (0.39), but low for dry weight per plant (0.14). Broad-sense heritabilities were either equal to or slightly greater than narrow-sense heritabilities.

4. The preponderance of GCA, which is based on additive gene action, and the high narrow-sense heritability estimates suggest that individual plant selection for stomatal characters would be

effective. Selection for leaf characters will, on the other hand, require a more complicated method such as reciprocal recurrent selection.

5. Highly significant phenotypic and genotypic correlations between stomatal characters and other traits indicated that a genotype with fewer stomata per unit leaf area is likely to have larger stomata, longer and wider leaves of larger areas as well as greater dry weight per tiller.

6. The phenotypic correlations of stomatal length (both adaxial and abaxial) and frequency (adaxial) with dry weight per plant were either non-significant or low even though significant. The genotypic correlations among them were higher than the phenotypic correlations in general, but were not significant. Among the morphological characters, abaxial stomatal frequency had the largest genotypic correlation coefficient with plant dry weight indicated that selection for decreased stomatal frequency should increase the dry matter production on an individual plant basis. Correlations between stomatal frequencies and forage yield on drilled plot basis (based on 3 years data provided by Dunn and Wright, 1970) were, however, not statistically significant. There was no correlation between spaced plant yield and yield in drilled plots.

7. Environmental correlations involving dry weight per tiller and per plant were generally smaller than that involving stomatal and leaf characters, whereas those involving stomatal frequencies were highly significant and negative in sign. High environmental correlations among other traits such as stomatal length and leaf

characters indicated that the environment was exerting a common influence on such traits in their development.

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APPENDIX

Appendix Table 1. Means of the 12 morphological characters for the 10 bromegrass diallel cross progenies.

Genotypes	Character					
	Stomatal Length (L1 - Adaxial)	Stomatal Length (L1 - Abaxial)	Stomatal Freq. (L1 - Adaxial)	Stomatal Freq. (L1 - Abaxial)	Leaf Length (L1)	Leaf Width (L1)
	(μ)	(μ)	(mm^{-2})	(mm^{-2})	(cm)	(mm)
1 x 2	53.60	54.84	81.08	50.67	21.09	7.1
1 x 3	53.60	54.65	83.39	60.43	16.44	6.8
1 x 4	50.43	52.38	81.97	59.99	15.59	6.7
1 x 5	57.32	59.22	64.55	43.68	19.71	9.2
2 x 3	54.90	55.12	87.43	64.17	17.25	6.6
2 x 4	52.00	53.03	85.77	59.41	18.83	6.6
2 x 5	59.08	59.71	62.05	43.81	23.05	9.3
3 x 4	53.15	54.16	89.50	70.40	14.95	6.5
3 x 5	59.46	60.87	65.81	50.17	19.49	9.2
4 x 5	58.70	61.37	61.97	44.12	21.09	8.7

Appendix Table 1. (Continued)

Genotypes	Character					
	Leaf Area (L1)	Leaf Length (L2)	Leaf Width (L2)	Leaf Area (L2)	Tiller Dry Wt	Plant Dry Wt
	(cm^2)	(cm)	(mm)	(cm^2)	(g)	(g)
1 x 2	12.166	24.88	10.5	21.115	2.284	44.95
1 x 3	9.008	21.19	10.5	17.991	1.852	36.93
1 x 4	8.460	20.08	9.7	15.722	1.795	38.91
1 x 5	14.675	23.63	13.3	25.396	2.667	41.84
2 x 3	9.222	20.96	10.0	17.082	1.865	28.41
2 x 4	10.094	22.54	9.5	17.199	2.019	32.74
2 x 5	17.250	26.44	13.0	27.657	2.989	44.66
3 x 4	7.954	18.89	9.8	14.920	1.838	27.26
3 x 5	14.412	23.83	13.4	25.658	2.799	37.13
4 x 5	14.838	25.56	12.4	25.416	2.971	33.62

BIOGRAPHICAL DATA

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