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Variability in Size and Frequency of Stomata in Leaves of Rice Varieties and its Correlation in Drought Resistance*

by

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Introduction: For an understanding of water economy in the rice plant it is essential to study the mechanism by which water is transpired in the plant. Transpiration takes place through the cuticle as well as through the stomata. In general it is recognised that in mesophytes, stomatal transpiration is 80 to 97 percent whereas cuticular transpiration is only 2 to 3 per cent. The total stomatal apertures in spite of their large number, occupy only one to two per cent of the total leaf surface. 'The epidermis along with the stomata possesses the physical properties of a multi-perforate septum and the diffusion of gases through these minute apertures proceeds almost at the same rate as if the cutinised epidermis were actually removed and the cells of the mesophyll were in direct contact with the external atmosphere'. It may be seen therefore that the stomata do play a very great part in the water balance of plants. The present study is restricted to stomatal sizes and their frequency in leaves of rice varities.

Review of Literature: Darwin (1898) (quoted by Knight, 1917) considered that stomata play a predominant part in the control of transpiration. Lloyd (1908) concluded that though the change in the stomatal dimension is small, this does not exclude the possibility of the stomatal opening exerting any regulatory effect upon transpiration. Knight (1917) found no agreement between stomatal aperture and the rate of transpiration and concluded that the water content of the leaf was an important factor involved in the control of transpiration. Loftfield (1921) after an elaborate study of the behaviour of stomata under a variety of external conditions found that at certain specific aperture, the stomata control transpiration.

Eckerson (1908) found that marked variations in number and size of stomata occur not only in different varieties of the same species of common green house plants, but also in the same varieties grown under different conditions. Zalenski (1904) (reviewed by Maximov, 1929) studied the anatomical differences in leaves at

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different levels in the stem and found that the higher the leaf, the greater the number of stomata per unit area of the leaf surface. Yapp (1912) reports that the upper xeromorphic leaves of *Spiraea ulmaria* have 1300 stomata per square millimeter whereas the mesomorphic leaves of the same plant had about 300 stomata per square mm. Salisbury (1927) in an extensive study of the stomatal frequency of the woodland flora of England concluded that stomatal frequency is mainly dependent on the humidity of the environment, dry exposed conditions being associated with high frequencies and humid conditions with low frequencies. Miller (1938) found that the number of stomata varies greatly from year to year and most of the plants have more stomata on the lower surface than on the upper surface, when stomata occur on both surfaces.

Hirano (1931) found that the hardier varieties and species of *Citrus* trees are characterised by lower stomatal density, though there were some exceptions. In the material studied by him it was found that the density of stomata is governed by genetio factors. Yocum (1935) reported a maximum of 1900 stomata per sq. mm. in Spanish oaks and suggests that the action of stomata is very important in trees, where the soil water is limited. Kolkunov (1905) (reviewed by Maximov, 1929) and Pavlov (1931) found that the less drought resistant wheats had longer stomata than the most resistant wheats. Whiteside (1941) observed that the leaf size, the distance between the stomata and other morphological characters were affected when plants were grown under low moisture supply. Evans (1939) working on the drought resistance in sugarcane varieties dit not find any correlation between the rate of transpiration and the density of stomata. He found approximately double the number of stomata per unit area on the lower spidermis as compared to the upper epidermis. Lal and Mehrotra (1949) studying the 12 varieties of sugarcane with varying degress of drought resistance measured the length, width and number of stomata and found the stomatal index. They suggest that the lower the index, the greater was the drought resistant capacity of the cane.

Differences in anatomical structures have been observed in rice varieties which differed in their degree of resistance to drought. Yasuda (1924) studied the relation between amount of transpiration and the development of vascular system. He found that the most important internal factors concerned are the structure, the number and the function of stomata and the nature of the epidermal cells. Onodera (1930) observed that in lowland rice the 'Sterome' or mechanical tissue is well developed on the lower side of the leaf while in some of the upland rices its development is much feebler. Alam (1939) observed that the varieties which stood drought when grown under artifical conditions of irrigation had fewer stomata and in them the stomatal cells were also smaller.

Materials and Methods: The present study was made to find out the variability in stomatal size and stomatal frequency in rice varieties which exhibited different degrees of resistance to drought. Two parents and one drought resistant F_4 progeny have also been studied in detail for the same purpose. The arrangement of the stomata, the size of the stomata, length and breadth and the frequency of the stomata have been studied.

Fourteen varieties of rice viz., CEB. 24, Co. 13 (wet rices), MTU. 17, MTU. 18, Vedurusannam, PTB. 28, PTB. 29, PTB. 30, TKM. 1, TKM. 2 and Bairuvadlu (dry rices), T. 129, T. 740 and T. 1702 (Wild rices, *Oryza sativa* forma *spontanea*), were grown under uniform conditions in a wet field. The plants were 60 days old and the fixing of the material was done between 9 A. M. and 10 A. M. as followed by Eckerson (1908) when the stomatal pores would be wide open. For a detailed study of the stomata in parents and their progeny, the cross Co. 13 × T. 129 was chosen. These were grown under restricted irrigation and were 53 days old when the material was taken.

Formalin acetic alcohol (70% alcohol - 90 c.c.; acetic acid 4 c.c. and formalin 6 c.c.) was used for fixing the samples. Leaf portions from the middle of the leaf blade were cut and quickly dropped into the fixative to avoid any alterations in the stomatal dimensions. For the varietal study the first leaf from the top of the plant was taken and for intensive study with the two parents and a progeny the first, second and third leaf from the top were fixed. Scrapping with a sharp scalpel as done by Prat (1932) was followed in obtaining the epidermal peel. Acetic orcein (1 gm. orcein dissolved in hot 42 per cent acetic acid) was used as the mounting medium. This medium has the tendency to expand the stomata which would have undergone shrinkage while getting fixed. A spencer's microscope with eye piece 10 × fitted with a micrometer with 100 divisions and objective with 4 M. M. - N.A.O. 6 - 44 × was used. Readings were taken to the nearest micrometer division.

In a preliminary study it was found that in the regions adjacent to the mid rib the parenchymatous cells are more and the veins are far apart, whereas towards the margin the parenchymatous cells are fewer in rows and the veins are closer. Moreover, it was found that the epidermal peels, both of the lower and upper epidermis, were easily obtained in the mid-rib region. Hence uniformly in all the samples epidermal peels in the mid-rib region alone were taken for study. Both the upper and lower epidermis were studied in all the varieties since stomata are found in both the epidermis. For obtaining the upper epidermis the scraping was begun from the lower layer and vice versa for the lower epidermis.

Size of the stomata was determined by measuring both length and breadth of the guard cells. Hundred stomata were measured for their length and breadth in each of the variety studied and the means calculated. The frequency of the stomata was measured by counting the number of stomata which are included in 100 divisions of the micrometer in a row. Twenty five readings were taken in the lower and the upper epidermis for each variety and the mean calculated. Rows of stomata adjacent to the mid-rib were taken for measuring the frequency. The arrangement of the stomata was studied with a view to find the distance between the stomatal rows, as they occur in linear rows alternating with longitudinal cells. The distance between the two veins was measured in three places where the stomatal rows were counted and the mean calculated. The distance between individual rows was found by dividing the number of rows.

Observation: In the internal structure of the rice leaf, which is exposed to the light of the sun on both sides, the mesophyll presents no differentiation into palisade and spongy parenchyma. The whole of the mesophyll consists of compactly arranged parenchymatous cells. The cells of the upper epidermis have large motor cells which help in the rolling of the leaf (plate No. 1). The epidermis is a continuous layer consisting of flattened cells with their cell walls distinctly cutinised. The continuity of the epidermis is interrupted by stomata, the guard cells of which are dumbbell shaped.

Rice leaf being an isobilateral monocot leaf has stomata on both lower and upper epidermis. This feature introduces a special problem in the study, as to which surface is more important for comparison. Therefore for comparing the varieties in their size and number of stomata, details have been given for both the sides separately.

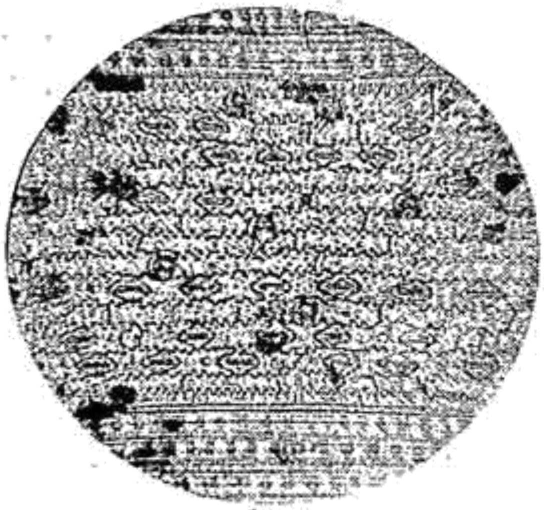

PLATE 1

PLATE 2

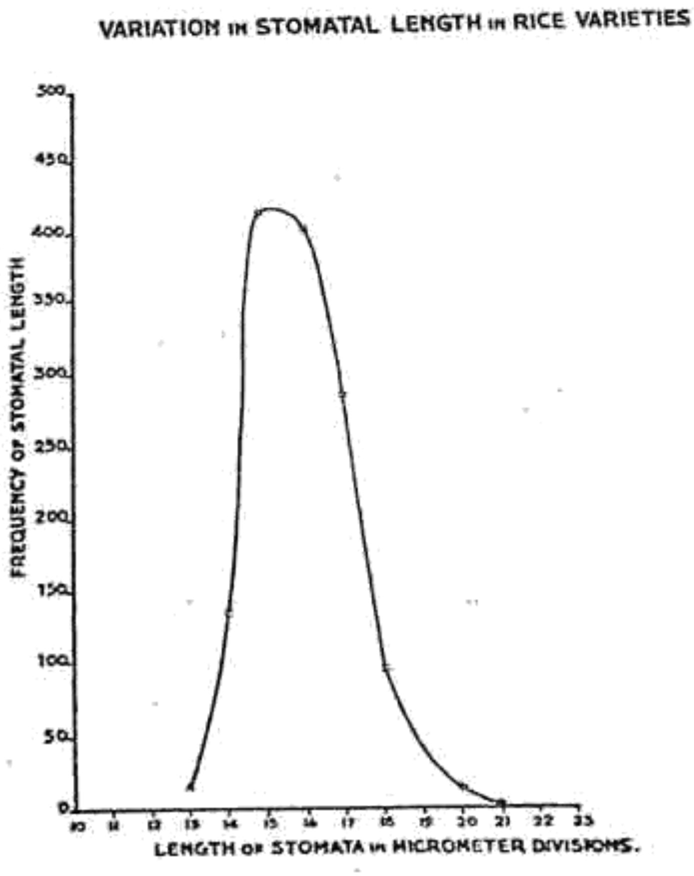

PLATE 3

The epidermis of the rice leaf shows veins running parallel to each other. The main conducting vessel is the midrib and the veins are parallel to the midrib. In between two veins, stomata are found in rows alternating with bands of long cells which have distinctive ripple walls (plate No. 2). The distance between two veins is larger in the midrib region and gets reduced towards the margin of the leaf.

The number of rows of stomata depends upon the distance between the two adjacent veins where they are distributed. Table 1 gives the distance between the two veins and the number of rows of stomata in between the two veins in the 14 rice varieties studied. The distance between the two rows of stomata is greater in the upper epidermis than in the lower epidermis showing that there are fewer rows of stomata in the upper epidermis than in the lower epidermis. This fact was observed in all the varieties studied. Table 3 which gives the data for the two parents and the F. 4 progeny also indicate the same features in the first, second and third leaf of the rice plant. Hence it is confirmed that the upper epidermis has fewer stomata than the lower epidermis in the rice leaf.

The distance between the rows of stomata varies and there are indications that some of the varieties have their stomata in rows farther apart. In the short duration varieties, it is seen, that some of the drought resistant types have their stomatal rows farther apart in both the epidermis. In the medium duration group it is noticed that except in the case of the lower epidermis of the wild rice T. 740, all the other drought resistant types have fewer stomatal rows than in the case of the drought susceptible type CEB. 24.

Similarly, the drought resistant progeny No. 3115 and the parent T. 129 have their stomatal rows farther apart than in the case of the drought susceptible parent Co. 13 as seen in table 3. (I leaf).

The size of stomata was studied by measuring the length as well as the breadth of the guard cells. The data are presented in tables 2 and 4. It is seen that the breadth of the stomata is less variable than the length. The average breadth in the lower epidermis mostly ranges between 9.5 and 10.5 micrometer divisions, i, e. 15.2 μ and 16.8 μ with a few exceptions as seen in table 4, where the maximum breadth is 11.4 micrometer divisions. The breadth of stomata is not directly proportional to the length but the trend seems to be for breadth to increase with length. The breadth of the stomata is greater in the upper epidermis than in the lower epidermis. The difference in breadth between the varieties and between the parents and progeny is not marked.

There is a great range of variation in the length of the stomata. The average length of the stomata in the lower epidermis ranges between 13 and 21 micrometer divisions which corresponds to 20.8 μ and 33.1 μ. Table 5 and the graph (plate No. 3) give the frequency of stomatal length in rice varieties, in the lower epidermis of the first leaf. It shows that most of the stomata have a length of 15 to 16 micrometer divisions or 24.0 μ to 25.6 μ. Stomata in the upper epidermis are longer than those in the lower epidermis. The differences in stomatal length between the varieties apparently show that they do not bear any relation to the drought resistant habit of the rice varieties studied. The length of the stomata in the three leaves studied in the two parents and progeny show that there is a gradual increase in length of the stomata from the first leaf to the third leaf as seen from table 4 the only exception being in the lower epidermis of T. 129. The breadth of the stomata closely follows the length in this respect.

However, the length of the stomata varies with varieties, with different leaves in the same plant and with conditions under which it is grown. The effect of change in environment of stomatal length and breadth is seen in varieties Co. 13 and T. 129 (table 2 and 4) which record different sizes. Thus it is noted that in the first instance (table 2), Co. 13 has a stomatal length of 15·7 micrometer divisions and a breadth of 10·3 micrometer divisions in the lower epidermis whereas the length is 16·5 and breadth 9·9 micrometer divisions in the first leaf of the same variety in the second case (table 4). A similar difference is noticed in the upper epidermis also. Another variety, T. 129 which was also studied in two instances (table 2 and 4) has different sizes of stomata. Leaves were taken for study from plants grown under two different conditions and the size of stomata is seen to vary in the different environments.

Apart from the environmental effect, there appears to be a genetic difference between the varieties in the size of stomata as seen from table 4, where the data for the two parents Co. 13 and T. 129 and the progeny number 3115 are given. It is seen that size of stomata of the progeny is different from that of both the parents n the lower and the upper epidermis in all the three leaves studied. An analysis of the data of the third leaf of the lower epidermis shows that the progeny number 3115 has significantly greater stomatal ength than both the parents. The difference of length of stomata n the progeny in highly significant when compared to the parents table 6). The progeny has a mean stomatal length of 19·10 nicrometer divisions as compared to a mean length of 16·81 micrometer divisions in the cultivated rice, Co. 13 and a mean length of 15·70 micrometer divisions in the case of the wild rice T. 129. The ength of stomata in Co. 13 is also significantly greater than that of T. 129 which shows that in this case the drought resistant rice has smaller stomata.

The frequency of stomata was measured by counting the number of stomata in 100 divisions of the micrometer at 25 places which is given in columns 5 and 8 of table 2 and 4. The varieties studied do not show that the difference in frequency between them has any bearing on their drought resistant and drought susceptible habits.

The frequencies of stomata in the first, second and third leaf in the two parents and the progeny (table 4) clearly show that there are definitely fewer stomata per unit length in the third leaf than in the top leaf of the same plant. The number stomata per unit length gets reduced gradually from the top leaf to the third leaf in almost all the cases in both the epidermis. An exception is noticed in the upper epidermis of T. 129. As the size of stomata becomes larger from the top leaf to the bottom leaf, the number gets reduced and this fact is noticed from the data presented. A typical example of this is noticed in the progeny number 3·15. In the lower epidermis, the length of stomata is 16·2, 18·8 and 19·1 micrometer divisions in the first, second and third leaf respectively whereas the number of stomata in 25 X 100 micrometer divisions are 74, 67 and 60 which shows a gradual reduction from the top leaf to the third leaf.

Discussion: Among the anatomical features which characterise drought resistance are the structure and size of cells, hairiness of stem and leaves and development of 'Sterome' or mechanical tissue. Differences in anatomical structures in rice varieties which differed in their degree of resistance to drought have been observed by Onodera (1930) and Alam (1939).

In the present investigation the stomatal studies in rice have been made on fairly large samples. The study has been made in one of the drought resistant hybrid progenies, its parents and some of the reputed drought resistant rice varieties.

The results obtained show that stomata are fewer in the upper epidermis because of the stomatal rows being fewer and farther apart in the upper epidermis than in the corresponding lower epidermis. Rice leaf bears stomata on both the upper and lower epidermis since it is equally illuminated on both the sides owing to the isobilateral disposition of the leaf. The general arrangement of the stomata in rows parallel to veins has facilitated the adoption of a definite method in studying them. The distance between the two rows gives an idea whether the stomatal rows are closer or farther apart. When the distance between rows is more, there are fewer rows of stomata in between two adjacent veins. Thus it has been possible to find out that the upper epidermis has fewer stomata than the lower epidermis. The differences between the drought resistant and susceptible rice varieties as well between the two parents their progeny are not constant though some of the reputed dry rices and wild rices have their stomata farther apart. Hence it has not been possible to associate their drought resistance with fewer stomata.

In the detailed study of the two parents and their progeny where the first, second and third leaf of the same plant have been examined, it has been found that the distance between rows in the lower and upper epidermis has a constant variability. The distance was the least in the wet rice Co. 13 and the wild parent and the drought resistant progeny have their stomatal rows very far apart compared to the wet rice. In both the epidermis the drought resistant progeny has fewer rows of stomata.

In studying the size, both length and breadth have been taken into account. It has been found that the breadth is less variable than the length. Though the breadth is not directly proportional to length, the tendency is for it to increase with the length. There is a greater variation in length of the stomata as shown in tables 2 and 4. The length ranges from $20{\cdot}8\mu$ to $33{\cdot}1\mu$ in the lower epidermis of the 14 rice varieties studied. The study of the first, second and third leaf of the rice plant revealed that in the same plant, the length of stomata increases from the top leaf to the third leaf in both the lower and upper epidermis. This corresponds to what zalenski (1905) (quoted by Maximov 1929) and Yapp (1912) found in their studies on stomata in relation to the height of insertion. They came to the conclusion that the upper leaves are more Xeromorphic than the lower ones.

Size of stomata seems to be a varietal character and the variation in size is due to the genetic make-up of the plant. A comparison of the stomatal length in the lower epidermis of the third leaf of Co. 13, T. 129 and the hybrid progeny No. 3115 has shown that the progeny has a significantly longer stomata than both the parents. The study has shown that the size of stomata does not indicate the drought resistance in the rice plant. It varies from leaf to leaf and between varieties.

The frequency of stomatal distribution in the epidermis of the rice plant shows that the number of stomata in an unit distance decreases from the top to the bottom leaf in both the epidermis. This is due to the fact that the size of the stomata increases from the top leaf to the bottom leaf as has already been stated. The study of the varieties has not given any indication to signify their varietal drought resistance.

Summary: The arrangement, size and frequency of stomata in two parents and a progeny and 14 varieties studied showed that the stomata are fewer in the upper epidermis than in the lower epidermis. No significant difference in stomatal size is noticed between the drought resistant and drought susceptible types though it has been observed that the progeny has a significantly greater stomatal length than both the parents. It has also been established that the size of stomata is a genetic character and found to be affected by environments. The size of stomata was also found to increase from the top leaf to the third leaf whereas the frequency decreased and this is in confirmation with what other workers have found in some other crops.

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TABLE 1

Spacing of Stomatal rows in Rice Varieties

(Mean values)

S. No.	Name of variety	Lower epidermis: Number of rows of stomata between two veins	Lower epidermis: Distance between two veins (Micro-meter divisions)	Lower epidermis: Distance between rows of stomata (Micro-meter divisions)	Upper epidermis: Number of rows of stomata between two veins	Upper epidermis: Distance between two veins (Micro-meter divisions)	Upper epidermis: Distance between rows of stomata (Micro-meter divisions)
1	2	3	4	5	6	7	8
Short Duration:							
1.	Co. 13 (wet rice)	8·1	110	13·8	6·0	120	20·0
2.	MTU. 17 (dry rice)	14·0	200	14·3	6·7	183	27·4
3.	MTU. 18 do.	8·7	120	13·8	7·0	170	24·3
4.	PTB. 28 do.	10·0	110	11·0	8·5	135	15·9
5.	PTB. 29 do.	10·3	140	14·0	6·7	160	23·9
6.	PTB. 30 do.	8·3	150	18·0	5·3	136	25·5
7.	Vedurusannam do.	8·5	185	21·8	3·7	130	35·4
Medium Duration:							
8.	GEB. 24 (wet rice)	8·0	115	14·4	7·0	115	16·4
9.	TKM. 1 (dry rice)	8·5	135	15·9	8·5	150	17·7
10.	TKM. 2 do.	7·0	150	21·4	4·0	115	28·8
11.	Bairuvadlu do.	7·7	115	14·9	6·3	115	18·2
12.	T. 129 (wild rice) *Oryza sativa* forma *spontanea*	6·7	135	20·4	4·3	117	27·0
13.	T. 740 do.	11·3	138	12·2	6·3	130	20·5
14.	T. 1702 do.	6·0	125	20·8	5·3	152	28·5

TABLE 2

Size and frequency of stomata in rice varietie

(Mean values)

S. No.	Name of variety	Lower epidermis: Length of stomata (Micro-meter divisions)	Lower epidermis: Breadth of stomata (Micro-meter divisions)	Lower epidermis: Number of stomata in 25 x 100 (Micro-meter divisions)	Upper epidermis: Length of stomata (Micro-meter divisions)	Upper epidermis: Breadth of stomata (Micro-meter divisions)	Upper epidermis: Number of stomata in 25 x 100 (Micro-meter divisions)
1	2	3	4	5	6	7	8
Short Duration:							
1.	Co. 13 (wet rice)	15·7	10·3	74	15·7	10·4	79
2.	MTU. 17 (dry rice)	15·2	9·9	60	15·3	10·1	68
3.	MTU. 18 do.	14·9	9·8	69	16·4	9·9	64
4.	PTB. 28 do.	14·5	9·5	74	14·8	9·9	64
5.	PTB. 29 do.	14·7	9·8	79	15·6	9·9	76
6.	PTB. 30 do.	16·2	10·2	70	15·5	10·3	77
7.	Vedurusannam do.	18·5	10·3	68	17·6	10·0	71
Medium Duration:							
8.	GEB. 24 (wet rice)	15·8	9·9	70	14·7	10·2	64
9.	TKM. 1 (dry rice)	16·4	10·1	70	15·9	10·2	74
10.	TKM. 2 do.	16·8	10·3	69	16·7	10·3	67
11.	Bairuvadlu do.	15·2	9·4	64	15·5	9·8	72
12.	T. 129 (wild rice) *Oryza sativa* forma *spontanea*	15·9	10·0	65	16·4	10·3	59
13.	T. 740 do.	15·3	9·7	75	15·2	9·9	81
14.	T. 1702 do.	16·7	10·1	78	16·4	10·0	72

TABLE 3

Spacing af stomatal rows in parents and progeny of drought resistant cross Co. 13 X T. 129
(Mean values)

S. No.	Name of variety	Lower epidermis			Upper epidermis		
		Number of rows of stomata between two veins	Distance between two veins (Micro-meter divisions)	Distance between rows of stomata (Micro-meter divisions)	Number of rows of stomata between two veins	Distance between two veins (Micro-meter divisions)	Distance between rows of stomata (Micro-meter divisions)
1	2	3	4	5	6	7	8
1.	Co. 13 (wet rice):						
	I leaf	7·3	125	17·1	6·0	100	16·7
	II leaf	8·0	140	17·5	4·5	110	24·4
	III leaf	5·7	125	21·9	3·0	110	36·7
2.	3115 (P, progeny):						
	I leaf	3·0	105	35·0	2·0	80	40·0
	II leaf	6·5	155	23·9	2·7	125	46·8
	III leaf	7·5	115	15·3	3·0	115	38·3
3.	T. 129 (wild rice) *Oryza sativa* forma *spontanea*:						
	I leaf	3·8	105	28·0	4·0	115	28·8
	II leaf	7·7	130	16·9	2·0	67	33·5
	III leaf	4·3	119	25·4	4·0	110	27·5

TABLE 4

Size and frequency of stomata in parents and progeny of drought resistant cross Co. 13 x T. 129
(Mean values)

S. No.	Name of variety	Lower epidermis			Upper epidermis		
		Length of stomata (Micro-meter divisions)	Breadth of stomata (Micro-meter divisions)	Number of stomata in 25 x 100 (Micro-meter divisions)	Length of stomata (Micro-meter divisions)	Breadth of stomata (Micro-meter divisions)	Number of stomata in 25 x 100 (Micro-meter divisions)
1	2	3	4	5	6	7	8
1.	Co. 13 (wet rice):						
	I leaf	16·5	9·9	73	16·0	10·1	79
	II leaf	16·7	10·1	67	17·1	10·2	79
	III leaf	16·8	11·4	64	17·3	10·4	75
2.	3115 (F_4 progeny):						
	I leaf	16·2	10·4	74	15·8	10·2	86
	II leaf	18·8	10·8	67	16·9	10·1	77
	III leaf	19·1	11·0	60	18·7	11·2	70
3.	T. 129 (wild rice) *Oryza sativa* forma *spontanea*:						
	I leaf	13·2	9·3	81	17·4	10·0	60
	II leaf	17·3	10·8	69	17·7	10·6	57
	III leaf	15·7	9·9	68	18·9	10·7	60

TABLE 5

Frequencies of stomata length in rice varieties
First leaf–Lower epidermis

Serial No.	Name of variety	Length of stomatat in micrometer divisions								
		13	14	15	16	17	18	19	20	21
1.	Co. 13			5	23	61	11			
2.	MTU. 17		11	59	29	1				
3.	MTU. 18		18	69	11	2				
4.	PTB. 28	10	46	43	1					
5.	PTB. 29	4	30	58	8					
6.	PTB. 30		2	8	42	39	8	1		
7.	Vedurusannum				3	16	35	33	12	1
8.	GEB. 24			29	60	11				
9.	TKM. 1			7	49	39	5			
10.	TKM. 2			4	24	52	18	2		
11.	Bairuvadlu		12	56	28	4				
12.	T. 129		6	27	44	20	3			
13.	T. 740		10	45	43	2				
14.	T. 1702			5	37	39	17	2		
	Total ..	14	135	415	402	286	97	38	12	1

TABLE 6

Comparison of length of stomata between parents and a progeny of drought resistant cross Co. 13 x T. 129

Mean length of stomata in micro-meter divisions:

Co. 13 (cultivated parent) = 16·81
T. 129 (wild parent) = 15·70
F_4 progeny No. 3115 = 19·10

S. No.	Particulars of comparison	Difference between means (d)	Standard error of the difference between means (Ed)	$\frac{d}{Ed}$
1.	Co. 13 and 3115	2·29	0·131	17·46*
2.	Co. 13 and T. 129	1·11	0·123	9·06*
3.	3115 and T. 129	3·40	0·130	26·08*

* Highly significant