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Study on Flag Leaf and its Penultimate Leaves for their Association with Grain Yield in Rice (*Oryza sativa* L.)

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ABSTRACT: Flag leaf contributes 45% of rice grain yield because it mostly provides photosynthetic products to the panicle so significant improvement in grain output is directly related with improving flag leaf features. A field test was carried out to see the link between paddy grain yield and the top three leaves. The 3rd leaf is longer than the 1st and 2nd leaves among the top three leaves. The first leaf is wider than the second and third leaves. Angle of third leaf is more compared to top two leaves. Correlation studies revealed that flag leaf length, thickness, area, chlorophyll content index (CCI) and SPAD has significantly positive correlation with grain yield. Flag leaf thickness has also shown significantly positive correlation with chlorophyll content index (CCI) and SPAD. JMS11B, CMS59B, and MTU1010 had the highest grain yields among genotypes studied. In our experiment, the high producing genotypes had short and intermediate leaf length and leaf width, and at least two of the top three leaf angles were erect or intermediate types.

Keywords: Chlorophyll content, Flag leaf, grain yield, Rice, SPAD.

INTRODUCTION

Rice is a member of the Oryza genus in the Gramineae family and is a staple meal for over half of the world's population.High yield has become a main goal in rice breeding programmes as the world's population grows. The main source of carbohydrate production is flag leaf and its penultimate leaves (Al-Tahir, 2014).Flag leaf contributes 45% of rice grain yield because it mostly provides photosynthetic products to the panicle. Plant breeding may result in a significant improvement in grain output by improving flag leaf features. The length of the flag leaf has long been considered as one of the most important variables in the development of novel rice plant ideotypes with high yielding potential (Vangahun, 2012). Leaf length varies greatly in rice and is closely related to leaf angle. Droopyness is related with long leaves, whereas erectness is associated with short and tiny leaves (Vangahun, 2012). The angle of the flag leaf had a significant impact on rice grain output because of light interception will change with the angle (Prakash *et al*. 2011). Through ideotype breeding, the ideal leaf length, width, thickness, angle, and area were addressed for generating high-yielding rice cultivars (Peng *et al*. 2008). As a result, we assessed the flag leaf and its penultimate leaves for their association with grain yield.

MATERIAL AND METHODS

During *kharif*, 2017 eleven CMS lines along with their maintainer lines were raised at Rice Research Center, PJTSAU, Hyderabad. All the lines were grown in three replications in randomized block design with spacing of 20 x 15 cm. To raise a successful crop, the entire suggested package of practices was followed. Eleven Cytoplasmic male sterile lines and five local varieties were utilized as test subjects for determining the leaf features of the top three leaves (1st leaf or flag leaf, 2nd leaf or penultimate leaf and 3rd leaf), *viz*., length, width, angle, thickness SPAD and CCI of the leaves. At reproductive stage, the lengths of the top three leaves on the main culm were measured in centimeters (cm) using a coded measuring device and classified as: very short (<21 cm), short (21–40 cm), intermediate (41–60 cm), long (61–80 cm) and extra long (>80 cm) (Jockson 2010). At reproductive stage, the width of the top three leaves was measured in cm at the widest portion of the leaf blade with a coded measuring instrument and classified as: narrow (<1 cm), intermediate (1–2 cm) and broad (>2 cm) (Jockson, 2010).

Palaniswamy and Gomez's (1972) formula was used to calculate the area of the flag leaf, second leaf, and third leaf:

Leaf Area = (length × width) × K Constant (K)= 0.75

The leaf angle was calculated by marking the position of each leaf's tip and collar on the paper against the main culm, which served as a vertical line. A line was drawn between the two spots, and an angle was measured with a protractor between the line and the vertical axis (Yoshida *et al*. 1976) and The angle of the flag leaf at full bloom determines the categorization, where the flag leaf angle ranges from 0-30 degrees for upright, 31-60degrees for intermediate, 61–90 degrees for horizontal, and 91 degrees or greater for downward. (Chang *et al*., 1965).

Digital callipers were used to measure leaf thickness, which was given in millimeters (Kiran *et al*., 2013). SPAD and CCI were captured at the blossoming stage utilisingMC-100 chlorophyll concentration meter.

Panicles from 1 m^2 were collected at physiological maturity, sun dried, threshed, cleaned, and the weight of grains was recorded and expressed in g m^{-2} then computed to q.ac^{-1} (Yang *et al*., 2007).

RESULTS AND DISCUSSION

The angle of the third leaf was greater than the angles of the second and first leaves among the top three leaves in CMS lines and checks (Table 1). CMS 64B has the highest flag leaf angle of 46.67^o among the CMS lines and checks, followed by CMS 23B (33.33^o) and CMS 59B (21.67^o). JGL11470 had the maximum angle of 33.33^o in the second leaf, followed by CMS64B (30^o). The third leaf angle was the greatest in CMS 23B (43^O) followed by RNR15048(41.67^o). Among the genotypes CMS 23B and JGL18047 were intermediate type and remaining were had erect type flag leaves. Correlation studies revealed angle of flag leaf has significantly negatively associated with grain yield (Table 2). This could be because the most effective arrangement for optimal photosynthesis is erect leaves. When LAI is large or sunlight is abundant, erect leaves are the most efficient arrangement for maximum photosynthesis. They have a higher leaf area index, which increases light interception for photosynthesis, and more upright leaves, which allow solar energy to penetrate into the lower levels of the aerial structure of plants (Vangahun, 2012).

Table 1: Mean performance of CMS lines and rice varieties for top three leaf characters and yield.

Treatments	An 1st	An 2nd	An 3rd	Ln 1st (cm)	Ln 2nd (cm)	Ln 3rd (cm)	wd1st (cm)	wd 2nd (cm)	wd 3rd (cm)
CMS 11B	20.00	28.33	31.67	31.85	29.90	34.83	1.13	0.97	0.93
CMS 14B	16.33	23.33	36.67	34.21	43.47	47.33	1.73	1.27	1.27
CMS 23B	43.33	30.00	43.00	22.22	41.43	34.33	1.63	1.37	1.23
CMS 46B	18.33	21.67	21.67	32.25	38.17	45.43	1.47	1.13	0.97
CMS 59B	13.00	20.00	36.67	35.87	45.50	48.13	1.33	0.87	0.87
CMS 64B	21.00	30.00	40.00	31.76	48.37	47.20	1.47	1.17	1.23
JMS 11B	11.67	25.00	28.33	36.60	40.63	40.00	1.43	1.07	0.77
JMS 13B	14.67	30.00	33.33	34.91	49.40	48.10	1.93	1.43	1.07
JMS 14B	14.67	28.33	33.33	34.54	47.90	51.70	1.57	1.27	1.13
JMS 17B	16.67	15.00	25.00	33.45	35.20	40.67	1.77	1.30	1.30
JMS 18B	22.33	25.00	33.33	26.98	39.63	41.63	1.83	1.30	1.33
RNR 15048(C)	16.33	25.00	41.67	33.90	35.00	38.27	1.57	1.13	0.83
JGL 18047 (C)	38.33	18.33	28.33	25.66	30.27	31.23	1.07	0.83	0.80
JGL 11470 (C)	14.00	33.33	36.67	35.13	40.10	43.80	1.60	1.53	1.30
JGL 1798 (C)	18.67	30.00	33.33	33.00	37.83	39.13	1.67	1.30	0.90
MTU 1010 (C)	14.33	20.00	36.67	35.71	35.73	36.30	0.97	0.97	0.93
Mean	**19.60**	**25.21**	**33.73**	**32.38**	**39.91**	**41.76**	**1.51**	**1.18**	**1.05**
C.D. 5%	2.69	7.98	9.18	4.15	10.34	6.13	0.22	0.25	0.25
S.E ±	0.93	2.76	3.18	1.44	3.58	2.12	0.08	0.09	0.09

An 1st: Flag leaf Angle Ln 1st: Flag leaf length wd 1st: Flag leaf width
An 2nd: Second leaf Angle Ln 2nd: Second leaf length wd 2nd: Second leaf width
An 3$^{rd:}$ Third leaf Angle Ln 3rd: Third leaf length wd 3rd: Third leaf width

Table 1 (Cont..)

Treatments	Th 1st (mm)	Th 2nd (mm)	Th 3rd (mm)	LA1	LA2	LA3	CCI	SPAD reading	Yield (q/ac)
CMS 11B	0.14	0.15	0.12	32.93	21.56	24.45	14.80	31.83	28.63
CMS 14B	0.16	0.15	0.15	36.40	41.15	44.88	16.60	32.17	37.06
CMS 23B	0.14	0.08	0.17	22.02	42.52	31.43	16.00	29.53	25.62
CMS 46B	0.15	0.15	0.15	33.68	32.16	32.93	15.03	30.87	29.76
CMS 59B	0.20	0.17	0.18	41.13	29.69	31.45	18.63	34.20	46.98
CMS 64B	0.18	0.14	0.17	33.25	42.45	43.73	14.40	30.67	28.38
JMS 11B	0.23	0.14	0.15	43.33	31.53	23.06	18.83	34.63	47.04
JMS 13B	0.18	0.13	0.15	38.93	53.32	38.94	16.93	32.90	38.41
JMS 14B	0.13	0.14	0.14	37.13	45.28	43.92	13.83	26.43	37.29
JMS 17B	0.15	0.16	0.17	35.41	34.58	39.55	13.67	24.78	34.34
JMS 18B	0.17	0.16	0.16	33.12	38.80	41.57	14.30	29.60	26.57
RNR15048(C)	0.16	0.14	0.14	36.03	29.48	23.83	16.07	31.70	36.02
JGL18047 (C)	0.14	0.15	0.16	23.80	18.91	18.46	13.90	29.70	26.37
JGL11470 (C)	0.18	0.14	0.16	40.02	47.24	42.54	17.30	33.13	38.83
JGL 1798 (C)	0.15	0.16	0.17	34.60	36.98	27.00	15.30	31.00	33.72
MTU 1010 (C)	0.19	0.17	0.17	40.54	26.12	25.38	16.33	33.13	39.34
Mean	**0.16**	**0.14**	**0.16**	**35.14**	**35.74**	**33.32**	**15.75**	**31.02**	**34.65**
C.D. 5%	0.02	0.01	0.02	3.39	12.58	9.31	3.01	3.16	9.98
S.E ±	0.01	0.00	0.01	1.17	4.36	3.22	1.04	1.09	3.45

Th 1st: Flag leaf thickness Th 2nd: Second leaf thickness Th 3rd: Third leaf thickness
LA1: Flag leaf area LA2: Second leaf areaLA3: Third leaf area CCI: Chlorophyll Content Index

The length of the third leaf was greater than the length of the second and first leaves among the top three leaves in CMS lines and checks (Table 1). JMS11B had the longest flag leaf of 36.60 cm among the CMS lines and checks, followed by CMS59B (35.87 cm) and MTU1010 (35.71 cm). JMS13B had the longest second leaf (49.40 cm), followed by CMS64B (48.37 cm). The longest 3rd leaf was present in JMS14B (51.70 cm), followed by CMS59B (48.13 cm). Because mutual shading is reduced and light interception is more efficient, many breeders discard lines with unusually long flag leaves extending 30 cm or more. Short leaves are more erect and evenly distributed throughout the canopy, so mutual shading is reduced and light interception is more efficient (Vangahun, 2012). The association between flag leaf length and yield was positive (r=0.832) and very significant; plants with longer flag leaf length may have elongated panicles, resulting in more primary and secondary rachis, and thus more grain in the panicle, which improved the cultivar's production (Rahman *et al*., 2013).

The width of the first leaf is greater than the width of the second and third leaves among the top three leaves (Table 1). Among the CMS lines and checks, wider flag leaf was presentinJMS13B (1.93 cm), JMS17B (1.77 cm) and CMS14B (1.73 cm) that was on par with each other. In the instance of JGL11470, the width of the second leaf was the widest (1.53 cm) followed by CMS23B (1.37 cm), JMS17B (1.30 cm) JMS18B (1.30 cm) that was on par with each other. JMS18B has the widest 3rd leaf, measuring 1.33 cm. According to Tari *et al*. (2009), the flag leaf must be wide and upright in order to increase rice grain yield.

Among the CMS lines and checks, the thickest flag leaf was present in JMS11B (0.23 mm), followed by CMS59B (0.20 mm). Flag leaf thickness has shown significantly positive correlation with SPAD, CCI (chlorophyll content index) and grain yield. This may be due to thicker leaves may accommodate more chlorophyll content per unit area which results in improved photosynthesis. Guru *et al.* (2017) also reported similar results. In rice, leaf thickness has a positive relationship with single-leaf net photosynthetic rate (Pn), while the Pn of the flag leaf after heading has a positive relationship with grain yield (Vangahun 2012).

Flag leaf thickness has also shown significantly positive correlation with flag leaf length (r=0.557) and significantly negative correlation with flag leaf angle (r=-0.553). Liu *et al*. (2014) observed that leaf length and leaf thickness have a significant positive correlation, it showed that thicker leaves were good for increasing single leaf area and that leaf thickness was inversely linked with leaf angle, implying that thicker leaves were favorable to the upright canopy.

Among the CMS lines and genotypes highest flag leaf area of 43.33 cm^2 was recorded in JMS11B, followed by CMS59B (41.13 cm^2) and MTU1010 (40.54 cm) which were on par. Flag leaf area has shown significantly positive correlation with grain yield. Flag leaf area was picked by Tari *et al*. (2009) as a factor for boosting rice grain output.

Flag leaf chlorophyll content index and SPAD values has recorded maximum in JMS11B followed by CMS59B and JGL11470 (Table 1). Flag leaf chlorophyll content index (CCI) and SPAD values has shown that grain yield and flag leaf thickness have a substantial positive correlation (Table 3) significant differences were observed among the genotypes with respect to grain yield (Table 1). The genotype with the highest grain yield out of all the genotypes was JMS11B (47.04 q.ac^{-1}), followed by CMS59B (46.98 q.ac^{-1}) and MTU1010 (39.34 q.ac^{-1}), which was on par and significantly superior to other genotypes. Flag leaf morphological parameters like size and shape, as well as physiological traits like chlorophyll content index and SPAD value, have long been thought to be major predictors of grain output in cereals (Xue *et al*., 2008).

Table 2: ANOVA summary of top three leaf traits.

Treatments	**Df**	An 1st	An 2nd	An 3rd	Ln 1st (cm)	Ln 2nd (cm)	Ln 3rd (cm)	wd1st (cm)	wd 2nd (cm)	wd 3rd (cm)
Replicates	2	4.021	6.771	10.333	2.938	50.061	71.299 *	0.076 *	0.051	0.015
Treatments	15	234.099 ***	79.861 **	101.521 **	49.008 ***	107.310 **	105.019 ***	0.226 ***	0.122 ***	0.121 ***
Treatment Error	30	2.599	22.882	30.333	6.196	38.472	13.512	0.017	0.023	0.022
Total	47	76.542	40.381	52.202	19.721	60.935	45.176	0.086	0.056	0.054
General mean		19.604	25.208	33.729	32.377	39.908	41.756	1.510	1.181	1.054
C.V.		8.223	18.976	16.329	7.688	15.542	8.803	8.608	12.796	14.172

Table 2 (Cont.)

Treatments	**Df**	Th 1st (mm)	Th 2nd (mm)	Th 3rd (mm)	LA1	LA2	LA3	CCI	SPAD reading	Yield (q/ac)
Replicates	2	0.000	0.000	0.000	9.758	199.224 *	104.862 *	7.823	7.730	72.320
Treatments	15	0.002 ***	0.001 ***	0.001 ***	98.486 ***	269.649 ***	236.568 ***	7.966 *	20.842 ***	137.934 ***
Treatment Error	30	0.000	0.000	0.000	4.138	56.940	31.171	3.260	3.587	35.788
Total	47	0.001	0.000	0.000	34.488	130.881	99.859	4.956	9.270	69.942
General mean		0.163	0.145	0.155	35.145	35.735	33.320	15.746	31.017	34.647
C.V.		6.522	5.330	6.594	5.788	21.116	16.756	14.022	11.466	6.106

An 1st: Flag leaf Angle Ln 1st: Flag leaf length Th 1st: Flag leaf thickness wd 1st: Flag leafwidth LA1: Flag leaf area
An 2nd: Second leaf Angle Ln 2nd: Second leaf length Th 2nd: Second leaf thickness wd 2nd: Second leafwidth LA2: Second leaf area
An 3$^{rd:}$ Third leaf Angle Ln 3rd: Third leaf length Th 3rd: Third leaf thickness wd 3rd: Third leaf width LA3: Third leaf area
CCI: Chlorophyll Content Index

Table 3: Correlation analysis between top three leaf characters and yield.

	An 1st	An 2nd	An 3rd	Ln 1st (cm)	Ln 2nd (cm)	Ln 3rd (cm)	Th 1st (mm)	Th 2nd (mm)	Th 3rd (mm)	LA1	LA2	LA3	CCI	SPAD reading	Yield (q/ac)
An 1st	1	0.012	0.165	-0.952*	-0.281	-0.594*	-0.643*	-0.575 *	0.129	-0.958**	-0.165	-0.259	-0.384	-0.317	-0.742**
An 2nd		1	0.487	-0.040	0.402	0.198	-0.083	-0.544*	-0.213	0.001	0.609*	0.276	0.146	0.238	-0.102
An 3rd			1	-0.129	0.322	0.006	-0.007	-0.401	0.179	-0.079	0.293	0.131	0.259	0.254	0.017
Ln 1st (cm)				1	0.260	0.525*	0.721**	0.528*	-0.100	0.942**	0.100	0.127	0.474	0.388	0.832**
Ln 2nd (cm)					1	0.836**	0.294	-0.205	0.295	0.327	0.797**	0.681**	0.289	0.099	0.337
Ln 3rd (cm)						1	0.274	0.170	0.086	0.531*	0.653**	0.765**	0.158	0.004	0.409
Th 1st (mm)							1	0.288	0.273	0.726**	0.039	-0.044	0.770**	0.683*	0.732**
Th 2nd (mm)								1	0.119	0.504*	-0.402	-0.032	-0.044	0.115	0.314
Th 3rd (mm)									1	-0.006	0.197	0.167	0.137	-0.037	0.121
LA1										1	0.171	0.180	0.580*	0.464	0.869**
LA2											1	0.795**	0.104	-0.100	0.088
LA3												1	-0.160	-0.315	-0.019
CCI													1	0.838**	0.775**
SPAD														1	0.512*
Yield (q/ac)															1

An 1st: Flag leaf Angle Ln 1st: Flag leaf length Th 1st: Flag leaf thickness LA1: Flag leaf area
An 2nd: Second leaf Angle Ln 2nd: Second leaf length Th 2nd: Second leaf thickness LA2: Second leaf area
An 3$^{rd:}$ Third leaf Angle Ln 3rd: Third leaf length Th 3rd: Third leaf thickness LA3: Third leaf area

CONCLUSION

As a result, the highest grain yields were found in JMS11B, CMS59B, and MTU1010. In our experiment, the high producing genotypes had short and intermediate leaf length and leaf width, and at least two of the top three leaf angles were erect or intermediate types. As a result, selecting these features is beneficial to yield improvement programmes.

FUTURE SCOPE

As these three leaves *i.e.*, flag leaf and its penultimate leaves characters mostly contribute for catching high light intensity which directly influences the grain yield. So by selecting above characters breeders may ultimately aim for high yielding varieties. As of now there is less research in this area so this manuscript may help future researches.

Conflict of Interest. None.

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