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GENETIC PARAMETERS OF TWO BC_2F_1 POPULATIONS FOR DEVELOPMENT OF SUPERIOR MALE STERILE LINES PERTAINING TO MORPHO-FLORAL TRAITS FOR AEROBIC RICE (*Oryza sativa* L.)

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ABSTRACT

The behaviour of introgressed male-sterilility with respect to various morphological traits is based on its superior performance in segregating backcross populations. In this study a maiden attempt has been made to identify male sterile lines suitable for developing aerobic rice hybrids. Two BC_2F_1 populations derived from IR70369A x MAS 99 and KCMS31A x MAS 99 crosses were evaluated for various morpho-floral traits. Pollen and spikelet fertility test revealed that all plants of these populations were completely male sterile. Genetic variability studies indicated high heritability for most of the traits (>60%). Narrow variation between Phenotypic Co-efficient of variation (PCV) and Genotypic Coefficient of Variation (GCV) was observed for most traits. Stigma exertion, Panicle exertion, Tiller number and Spikelet per panicle recorded moderate heritability with wider differenced between PCV and GCV. Negatively skewed platykurtic distribution was observed for most of the traits in both BC_2F_1 populations. The identified new male sterile lines will be stabilised, and they could serve as potential A lines for development of rice hybrids for aerobic condions.

Keywords: Aerobic rice, backcross, cytoplasmic male sterility, floral traits, variability.

INTRODUCTION

CMS (cytoplasmic male sterility) is the cornerstone of hybrid rice technology (Ahmadikhah et al., 2015) by which heterotic hybrids are developed. Rice breeders need to develop wide range of CMS lines for successful exploitation of heterosis breeding for various situations (Virmani,

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et al., 1997). In the process of new CMS line development, introgression of CMS trait from existing male sterile lines into desired background can diversify CMS lines. Though there are many CMS lines developed, they are mostly suitable for irrigated puddled ecosystem. Off late due to the shortage of rainfall, water for irrigated rice in the present form of cultivation of rice is becoming a limiting factor. Under these circumstances, rice cultivation has to be modified in order to keep the pace of rice production with limited water available. Aerobic rice cultivation is one such choice wherein the water required is half of that of irrigated puddled rice, without compromising the productivity (Gandhi et al., 2011a). Though varieties have been developed exclusively for aerobic cultivation (Gandhi et al., 2011a; Gandhi et al., 2011b), developing hybrids for such situation would further increase the water productivity and boost the rice yields in the receding water situation (Naresh 2010), Hence, an attempt has been made to convert the existing CMS lines in the background of MAS 99, an elite aerobic rice variety by backcross breeding using two popular A lines. The two backcross populations were advanced to BC_2F_1generation and evaluated for various genetic factors. Variation in new CMS lines could be useful for further selection to develop superior hybrids in rice (Das et al., 2013). Behaviour of CMS lines with respect to various morphological traits is judged precisely (Ali et al., 2013) for selection of superior and the suitability of CMS lines. The success of hybrid rice breeding depends on the extent of natural out crossing on CMS lines (Hittalmani and Shivashankar, 1987). Rice, being an autogamous plant, does not encourage out crossing naturally. Floral morphology and flowering behaviour of CMS lines is important (Ali et al., 2013; Ghadi et al., 2013). Out crossing depends on number of floral characters, viz. percentage of panicle exertion, style length, stigma length, stigma area and stigma exertion etc. Hence, assessing and understanding the genetic variability and the inheritance pattern of floral traits is essential for proper choice of CMS lines (Sheeba et al., 2006). On the other hand, variability parameters viz. range, phenotypic coefficient of variation (PCV), genotypic co-efficient of variation (GCV) and heritabilitywill be useful for efficient exploitation of variability (Kishore et al, 2015). Hence, the present investigation was undertaken to study the variability in the two male sterility introgressed BC_2F_1 populations of rice there by selecting superior male sterile plants in back cross generations.

MATERIAL AND METHODS

Plant material and experimental site

The study was conducted in experimental fields of University of Agricultural Sciences, GKVK, Bangalore, India. The two BC_2F_1 populations along with parents were evaluated for morpho-floral traits in *Kharif* 2014. The experimental material composed of 150 BC_2F_1 progenies, among them 100plants from IR70369A×MAS99 cross and other 50 pants were derived from KCMS31A×MAS99 cross. IR70369A and KCMS31A are popular male sterile lines, while MAS 99 is a promising short duration aerobic rice genotype. Procedure followed to develop BC_2F_1 population is shown in figure 1.

Aerobic rice cultivation

Aerobic rice cultivation involves direct sowing of seeds in un-puddled land with a spacing of 30 cm × 25 cm. Irrigation was given at 5 days interval to maintain soil moisture at field capacity throughout the crop growth. The soil is maintained under aerobic condition unlike the anaerobic condition in puddled soils with standing water in irrigated transplanted rice. During reproductive stage the irrigation is given once in 3 days interval. Remaining cultural practices were done as per recommended package of practices for aerobic rice developed by UAS, Bangalore (Gandhi et al., 2011b).

Observations recorded

The observations were recorded as per Standard Evaluation system (SES) for rice (1996). Plant growth parameters recorded were, plant height after 45 days (cm), plant height at maturity (cm), total number of tillers, days to 50% flowering and days to maturity. Floral characters included were stigma length(mm), pollen sterility (%), style length (mm), spikelet fertility (%), panicle exertion (cm), stigma exertion (%), grain related traits, number of panicles plant^{-1}, panicle length (cm), number of spikelets panicle^{-1} . Soil Plant Analysis Development (SPAD) using chlorophyll meter reading was carried out to determine the chlorophyll content.

Statistical analysis

Descriptive statistics such as mean, range and genetic parameters PCV, GCV and heritability (Robinson, 1949) were estimated using MS Excel program. Skewness and kurtosis were estimated as per Snedecor and Cochran (1994) to understand the nature of distribution of BC_2F_1 population for growth and related traits under aerobic condition. The mean values of quantitative traits of above cross was used to estimate coefficients of skewness and kurtosis using 'SPSS' software program. Kurtosis indicates the relative number of genes controlling the traits (Robson, 1956). Three types of kurtosis are recognized based on the kurtosis value which depends on distribution curve. If kurtosis value is 3 it is Mesokurtic. If kurtosis value > 3 it is Leptokurtic, if kurtosis value < 3 it is Platykurtic. Similarly, the lack of symmetry i.e., skewness was recognized based on the co-efficient of skewness values which range from -3 to +3. The type of distribution based on the skewness values were as follows. If skewness value is zero then, it is called as symmetrical distribution, if skewness value is negative, it is negatively skewed distribution and if skewness value is positive then it is positively skewed distribution.

RESULTS AND DISCUSSION

Success of any plant breeding programme largely depends upon genetic variability present for the character under improvement. The genotypic coefficient of variation measures the range of variability available in a crop and also enables to compare the amount of variability present in different characters. The phenotypic expression of the character is the result of interaction between genotype and environment. Hence, the total variance needs to be partitioned into heritable and non-heritable components to assess the inheritance pattern of the particular character under study. The pollen and spikelet fertility test revealed that all the plants were completely male sterile in both the BC_2F_1 populations.

Mean and range

Among the traits studied wide range was observed for spikelets panicle^{-1}, 90.6 to 154.6 with the mean of 128.3 in cross A and 105.2 to 151.2 with the mean of 130.28 in cross B. Stigma exertion % 18.51 to 43.46% with the mean of 30.19% in cross A, 15.31% to 30.38% with the mean of 24.78% in cross B was observed. Between these two populations, cross A had wider range for most of the traits. The range was very narrow for stigma length i.e. 0.79mm to 1.29mm with the mean of 1.07mm in cross A and 0.85mm to1.27mm with the mean of 1.06mm in cross B. The details of range and mean for each trait of BC_2F_1 populations derived from both the crosses are presented in table 1. Studies on range indicated existence of variability among the CMS plants. Banumathy et al. (2002); Hasan et al. (2011) and Ingle et al. (2008) also found that the variability for various traits existed in the rice CMS lines.

PCV and GCV

The calculated PCV and GCV values for characters under study were presented in table 2. The PCV and GCV values were found highest for panicles plant^{-1} i.e. 16.99% and 14.59% in cross A, 18.95% and 17.78% in cross B. Followed by total tillers recorded PCV and GCV of 15.65% and 12.99% in cross A, 19.09% and 16.36% in cross B indicated presence high degree of variability for these traits. Whereas lowest PCV and GCV was recorded for Days to 50% flowering i.e. 1.92% and 1.43% in cross A and 1.74 % &1.47% in cross B indicated presence of lower degree of variability. These results are in accordance with Chauhan (1996), Nath and Talekar, (1997).The narrow difference between PCV and GCV was found for plant height at 45 days, days to 50% flowering, style length, stigma length, SPAD reading, days to maturity, plant height at maturity in both crosses indicated that expression of these traits were least influenced by environment. Similar results were observed by Ali et al. (2013), Kishore et al. (2015), Rama Rao (1990), Sathyanarayana et al. (2005) and Singh et al. (2005). Relatively higher difference between PCV and GCV was found for traits such as stigma exertion, SPAD reading, total tillers, panicles plant^{-1}, panicle length, panicle exertion, spikelet panicle^{-1}in both crosses suggested that the expression of these traits were influenced by environment. Similar results were reported by Sheeba et al. (2006).

Broad sense heritability

Heritability indicates the relative degree at which a character is transmitted from parent to offspring. High heritability values indicate that the characters under study are less influenced by environment in their expression and such characters could be improved by adopting simple selection methods. Heritability values calculated is presented in table 2. Highest heritability was found for style length i.e. 96.48% & 95.31% in cross A and cross B respectively followed by days to maturity i.e. 96.37% & 96.12%. However, stigma length recorded heritability of 93.81% and 97.53% in cross A and B respectively indicated that these traits were least influenced by environment. Whereas lowest heritability was recorded for panicle exertion (30.72% & 35.36%) and stigma exertion (21.88% & 55.24%) inferred that these traits were most influenced by environment.

Third (Skewness) and fourth (Kurtosis) degree statistics in BC_2F_1 back cross populations

The study on distribution properties such as coefficients of skewness and kurtosis provides insight about the nature of gene action and number of genes controlling the traits respectively. They are more powerful than first and second degree statistics which reveal interaction genetic effects. The skewness and kurtosis values for each trait is presented in table 3. It infers that for most of traits frequency distribution was negatively skewed and platykurtic (<3) in both crosses except for style length, SPAD reading, total tillers were positively skewed and platykurtic (<3). Whereas for panicle length distribution was positively skewed (0.97) in cross A but negatively skewed (-0.096) in cross B. For panicles plant-1 negatively skewed (-0.31) for cross A but positively skewed (0.44) in cross B (Table 3; Figure 2; Figure 3; Figure 4). The difference in the skewness and kurtosis values for each traits and for each population is the result of linkage and variable crossing over leads to varied genome recovery of parents. Negatively skewed platykurtic distribution indicated that these traits were governed by large number of genes and majority of them displaying dominant and dominant based duplicate epistasis.Hence, mild selection is expected to result in rapid genetic gain for that traits. Whereas positively skewed platykurtic distribution suggested that these traits were governed by large number of genes and majority of displaying dominant and dominant based complementary epistasis. Hence, intense selection is required for rapid genetic gain in these traits (Pooni et al., 1977).

CONCLUSIONS

Back cross breeding is one of the powerful methods for trait introgression. It helps to recover the genome as well as create some variability in the subsequent generations so that a more desirable genotype can be selected. In this study it was found that there was presence of adequate genetic variability for various morpho-floral traits. The individuals of backcross populations with the introgression of male sterility and possessing superior floral characters were identified that will help further in identifying new R lines for hybrid seed production in rice for aerobic situation. Based on the phenotypic acceptance of male sterile plants with highest stigma exertion, panicle exertion and short duration,plants *viz.,* A09, A27, A42, A45, A78 in cross A and B16, B22, B31, B43, B48 in cross B were selected as superior male sterile plants and advanced to BC_3F_1generation. The identified new male sterile plants will be stabilised and further they could be utilised to develop rice hybrids suitable for aerobic condition with suitable R lines.

REFERENCES

Ahmadikhah, A., Mehdi, M., Mohammad, H. P. and Leila, N. 2015. Marker-assisted backcrossing (MABC) using CMS-specific, SSR and ISSR markers to develop an elite CMS line in rice. *The Plant Genome*, 31:1-78

Ali, M., Hossain M. A., Kulsum M. U., Sharma N. and Mian, M. A. K. 2013. Variability in quasi CMS lines of aromatic rice (*Oryza sativa* L.) in BC_3 generation and their phenotypic acceptability. *Eco-friendly Agriculture Journal*, 6 (05): 78-82

Banumathy, S., Thiyagarajan, K. and Vaidyanathan, P. 2002. Genetic variability for floral traits and their association with outcrossing in rice, *Crop Research*, 23(3): 507-509.

Chauhan, J. S. 1996. Segregation analysis and estimation of genetic parameters for some quality traits in F_2 generation in rice. *Oryza*, 33: 168-173

Das, P., Chand K. S., Sankar M. B., Mukherjee and Tapash D. 2013. Genetic variability in cytoplasmic male sterile lines in rice (*Oryza sativa* L.). *Journal of Agriculture and Veterinary Science*, 3(5): 95-100

Gandhi, V. R., Shivamurthy, M., Rudresh, N. S. and Hittamani, S. 2011a. Performance and adaption of new aerobic rice variety MAS946-1 (Sharada) in southern Karnataka. *Karnataka Journal of Agricultural Sciences*, 25(1):5-8

Gandhi, V. R., Hittamani, S. and Shivamurthy, M. 2011b. Performance and adaption of Direct seeded aerobic rice variety MAS26 (Onasiri) in Southern Karnataka. *Journal of Extension Education*, 23(3): 4684-4690

Ghadi, A., Babaeian, J. N., Bagheri. 2013. Evaluation of improved rice CMS lines according sterility, fertility and allogamy characteristics (*Oryza sativa* l.) *Modern Genetics*, 31(7): 397- 408

Hasan, M. J., Umma Kulsum, M. H., Rahman, M., Hazrat and Julfiquar, A. W. 2011. Genetic variability of some cytoplasmic male sterilelines (CMS) of rice *(Oryza sativa* l.) genotypes, *Bangladesh Journal Agricultural Research*, 36(2): 263-270

Hittalmani, S. and Shivashankar, G. 1987. Out crossing on male sterile lines of rice. *Mysore Journal of Agriculture Sciences*, 21:158-160

Ingale, B. V., Waghmode, B. D. and Hodawadekar, S. S., 2008. Identification of restorers and maintainers for different CMS lines of rice. *Madras Agricultural Journal*, 95(7/12): 266-277

Kishore, N. S. Srinivas, T. Nagabhushanam, U. Pallavi, M. and Sameera, S. K. 2015. Genetic variability, correlation and path Analysis for yield and yield components in Promising rice (*oryzasativa*l.) Genotypes. *SAARC Journal of Agriculture,* 13 (1):99-108

Nath and Talukdar, P. 1997. Genetic variability and correlation studies in segregating populations of indigenous scented x high yielding non scented crosses of rice. *Oryza.* 34: 91-93

Naresh Babu, N. 2010. Identification of Restorers and maintainers for the development of aerobic rice hybrids (*Oryza sativa* L.) and Validation of SSR markers linked to fertility (*Rf*) locus. *M.Sc. Thesis*, University Agricultural Sciences, Bangalore

Pooni, H. S., Jinks, J. L. and Cornish, M. A. 1977. The causes and consequences of non-normality in pretending the properties of recombinant inbred lines. *Heredity*, 38: 329-338

Rama Rao, M. 1990. Genetic analysis for yield and its components in rice crosses (*Oryza sativa* L.). M. Sc. (Ag) thesis, Acharya N. G. Ranga Agricultural University, Rajendranagar, Hyderabad (India).

Robinson, H. F., Comstock, R. E. and Harvey, P. H. 1949. Estimation of heritability and degree of dominance in Corn. *Agronomy Journal*, 41: 353-359

Robson, D. S. 1956. Application of K_4 statistics to genetic variance component analysis. *Biometrics*, 12: 433-444

Satyanarayana, P. V., Srinivas T, Reddy, P. R., Madhavilatha, L. and Suneetha, 2005. Studies on variability, correlation and path coefficient analysis for restorer lines in Rice. *Research on Crops*, 6 (1): 80-84

Sheeba, A.,Vivekanandan P. and Ibrahim, S. M. 2006. Genetic variability for floral traits influencing outcrossing in the CMS lines of rice, *Indian Journal of Agricultural Research*, 40 (4) : 272 – 276

Singh, I., Dey, K., Sanjeev, S. and Shani, J. P. 2005. Variability, heritability, genetic advance and genetic divergence in induced mutants of irrigated basmati rice (*Oryza sativa* L.). *Oryza*, 42(3): 210-213

Snedecor, G. W. and Cochran, W. G. 1994. statistical methods. Fifth edition. Iowa State University Press. Ames. Iowa, USA.

Standard Evaluation System for Rice. 1996. Prepared by INGER – Genetic Resources Center, International Rice Research Institute, Manila, Philippines. Pp.1-39.

Virmani, S. S., Viraktamath, B. C., Casal C. L., Toledo, R. S., Lopez, M. T. and Manalo, J. O. 1997. Hybrid Rice Breading Manual. Int. Rice Research Institute. *Los Banos*,Leguna, Philippines. pp.155

Table 1. Estimates of range and mean of two BC_2F_1 populations derived from IR70369A × MAS99 and KCMS31A × MAS99 crosses in rice

	Traits	IR70369A × MAS99 (cross A)			KCMS31A × MAS99 (cross B)		
		Range			Range		
		Min	Max	Mean	Min	Max	Mean
1)	Plant height at 45 days (cm)	31	44	37.54	27	42	36.46
2)	Days to 50% flowering	89	98	94.78	94	100	96.9
3)	Style length (mm)	0.68	1.24	0.93	0.75	1.17	0.93
4)	Stigma length (mm)	0.79	1.29	1.07	0.85	1.27	1.06
5)	Stigma exertion (%)	18.51	43.46	30.19	15.31	30.38	24.78
6)	SPAD chlorophyll meter reading	28.1	48.5	34.65	27.5	42.1	33.24
7)	Days to maturity	121	134	128.2	126	136	132.38
8)	Total tillers	14	30	22.01	15	32	22.06
9)	Panicles plant^{-1}	7	28	19.93	13	29	20.02
10	Plant height at maturity (cm)	59	83	73.68	64	79	72.53
11	Panicle length (cm)	14.38	24.66	17.73	12.36	22.82	17.66
12	Panicle exertion (cm)	-8.68	-3.6	-6.22	-7.58	-4.92	-6.32
13	Spikelet panicle^{-1}	90.6	154.6	128.3	105.2	151.2	130.28

Table 2. PCV, GCV and heritability of two BC_2F_1 populations derived from IR70369A × MAS99 and KCMS31A × MAS99 crosses in rice

Traits		Phenotypic coefficient of variation		Genotypic coefficient of variation		Broad sense heritability	
		IR70369A × MAS99	**KCMS31A × MAS99**	**IR70369A × MAS99**	**KCMS31A × MAS99**	**IR70369A × MAS99**	**KCMS31A × MAS99**
1)	Plant height at 45 days (cm)	8.19	8.13	7.52	7.68	84.15	89.18
2)	Days to 50% flowering	1.92	1.74	1.43	1.47	54.89	71.7
3)	Style length (mm),	11.72	10.18	11.51	9.94	96.48	95.31
4)	Stigma length (mm),	9.74	9.82	9.43	9.7	93.81	97.53
5)	Stigma exertion (%)	15.78	13.69	7.38	10.17	21.88	55.24
6)	SPAD chlorophyll meter reading	9.26	9.49	7.99	8.59	74.5	81.85
7)	Days to maturity	2.24	1.92	2.2	1.88	96.37	96.12
8)	Total tillers	15.65	19.09	12.99	16.36	68.83	73.49
9)	Panicles plant^{-1}	16.99	18.95	14.59	17.78	73.82	88.06
10)	Plant height at maturity (cm)	5.79	4.25	5.46	3.7	89	75.8
11)	Panicle length (cm)	10.76	12.11	8.64	10.08	64.38	69.35
12)	Panicle exertion (cm)	13.81	9.51	7.65	5.66	30.72	35.36
13)	Spikelet panicle^{-1}	10.35	7.52	7.25	5.1	49.07	46.04

Table 3. Skewness and Kurtosis of BC_2F_1 populations derived from IR70369A × MAS99 and KCMS31A × MAS99 crosses in rice

	Traits	Skewness		Kurtosis	
		IR70369A × MAS99	**KCMS31A × MAS99**	**IR70369A × MAS99**	**KCMS31A × MAS99**
1)	Plant height at 45 days (cm)	0.01	-0.774	-0.47	1.54
2)	Days to 50% flowering	-0.41	-0.132	0.02	-0.94
3)	Style length (mm)	0.36	0.485	0.34	0.04
4)	Stigma length (mm)	-0.56	-0.215	-0.03	-0.92
5)	Stigma exertion (%)	0.15	-0.486	0.1	0.23
6)	SPAD chlorophyll meter reading	0.86	0.912	2.35	0.95
7)	Days to maturity	-0.39	-0.86	-0.19	0.45
8)	Total tillers	0.15	0.39	-0.57	-0.41
9)	Panicles $plant^{-1}$	-0.31	0.443	1.1	-0.2
10)	Plant height at maturity (cm)	-0.56	-0.401	0.84	0.13
11)	Panicle length (cm)	0.97	-0.096	1.66	0.46
12)	Panicle exertion (cm)	-0.05	-0.024	0.48	-0.11
13)	Spikelet $panicle^{-1}$	-0.27	-0.299	-0.156	0.29

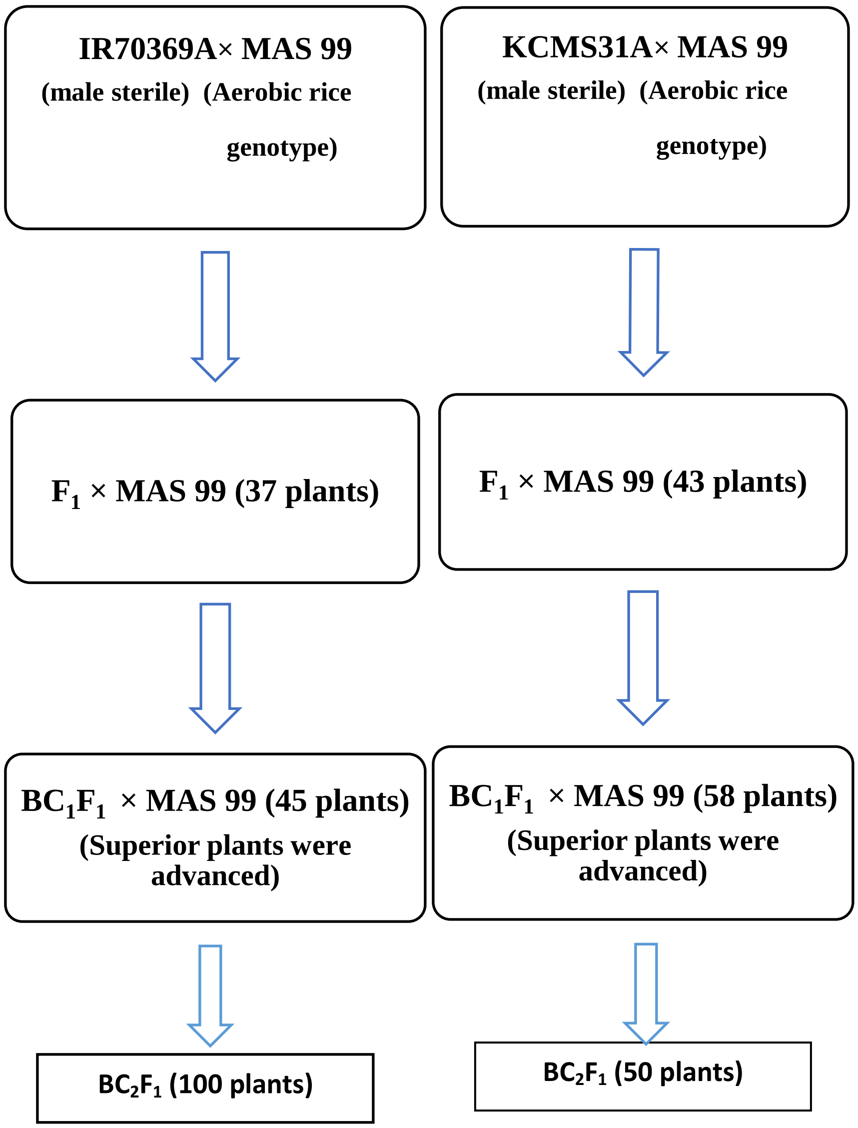
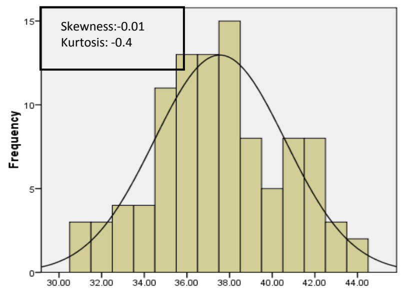
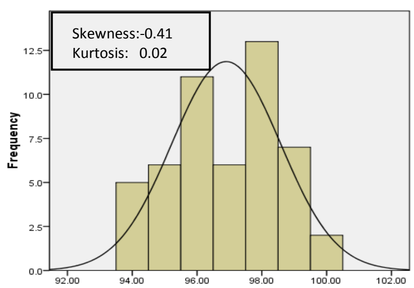


Figure 1. Schematic diagram showing crosses effected to develop two BC_2F_1 Populations

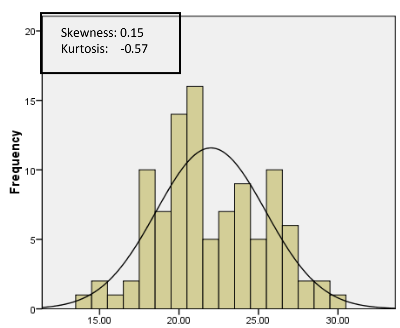
IR70369A × MAS99



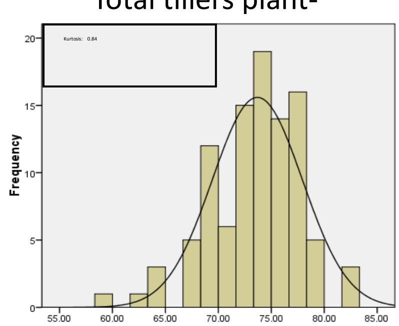
Plant height at 45 days (cm)



Days to 50% flowering

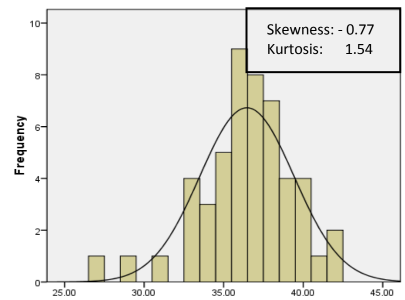


Total tillers plant-1

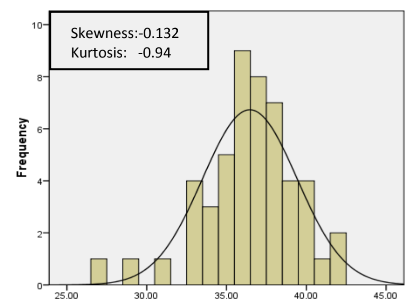


Plant height at maturity (cm)

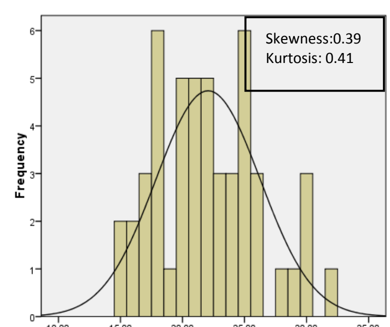
KCMS31A × MAS99



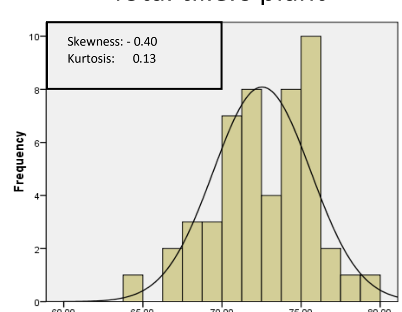
Plant height at 45 days (cm)



Days to 50% flowering



Total tillers plant-1



Plant height at maturity (cm)

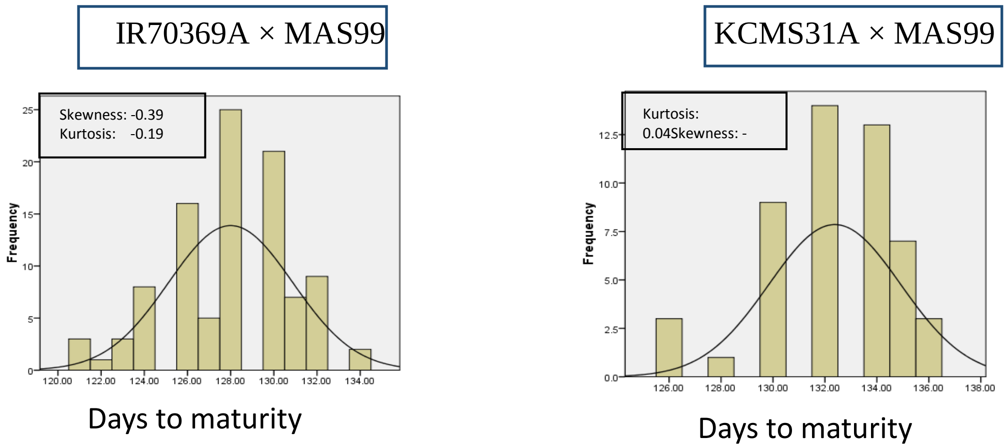


Figure 2. Distribution pattern of growth characters *viz*., Plant height at 45 days, days to 50% flowering,Total tillers,Plant height at maturity and days to maturity in BC_2F_1 populations derived from IR70369A × MAS99 and KCMS31A × MAS99 crosses

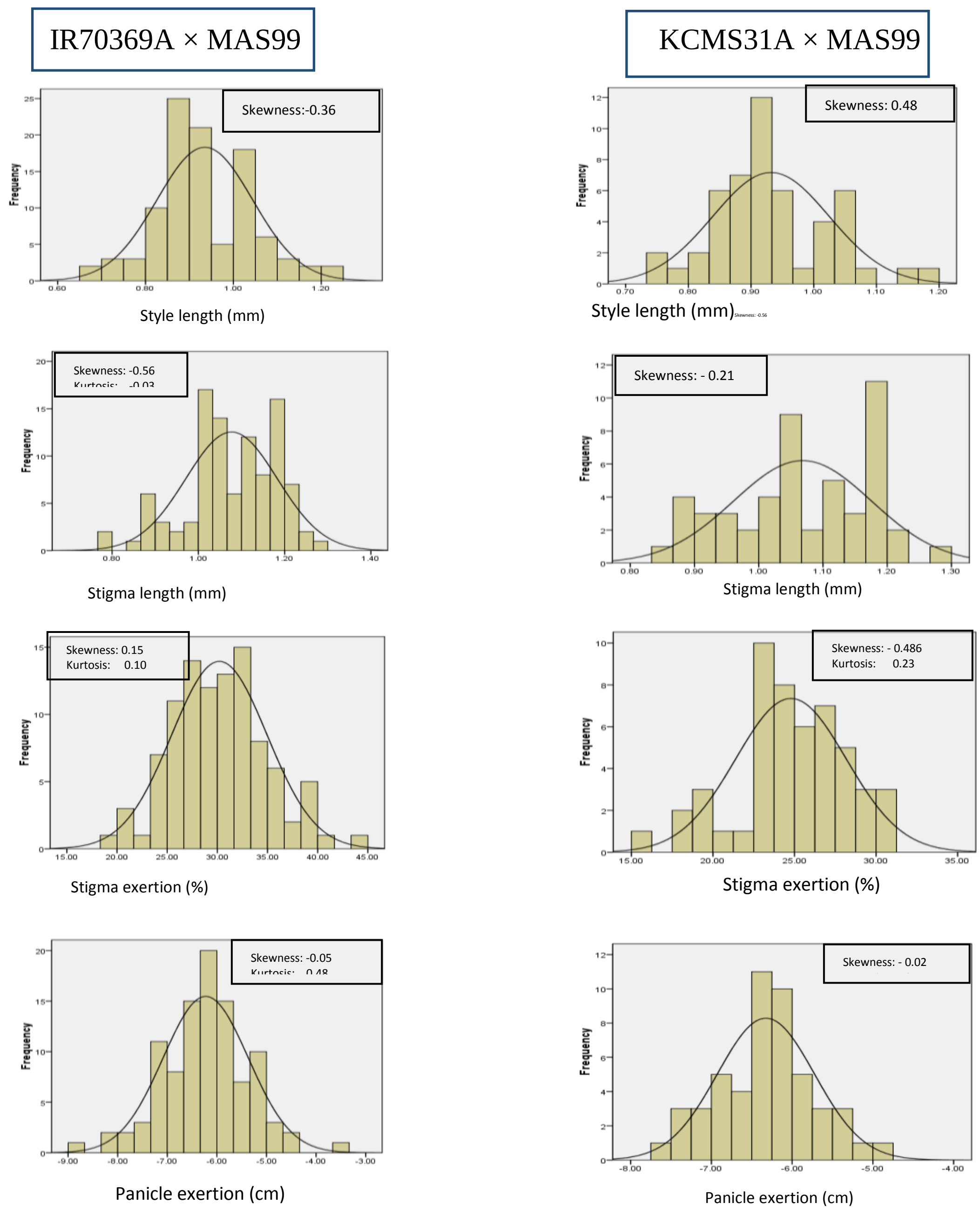


Figure 3. Distribution pattern of Floral traits *viz.*, Style length, Stigma length Stigma exertion, and Panicle exertion in BC_2F_1 populations derived from IR70369A × MAS99 and KCMS31A × MAS99 crosses

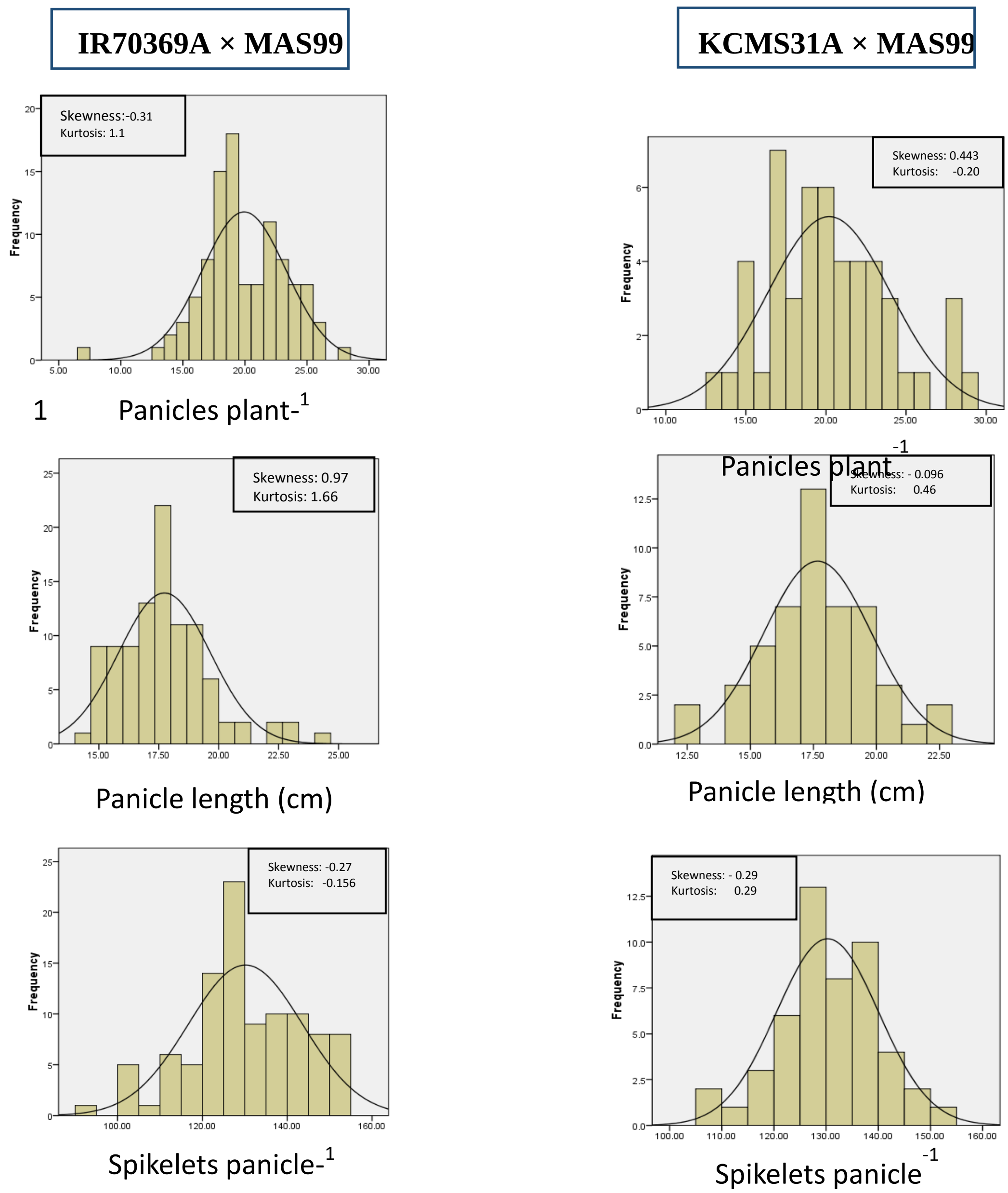


Figure 4. Distribution pattern of yield related traits *viz.*, Panicle length, Productive tillers per plant and Spikelets per panicle in BC_2F_1 populations derived from IR70369A × MAS99 and KCMS31A × MAS99 crosses