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Research Note

Study on genetics of yield component traits under salt stress in two rice crosses raised using honeycomb selection design

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Abstract
Yield components related to grain yield were severely affected by salinity. For the agronomically important traits, precise phenotyping of crop plants is essential under stress where soil heterogeneity is aggravated for genetic analysis and developing improved crop varieties. A set of two F_2 populations obtained from ADT(R)45 × FL478 (A × F) and ADT(R)45 × Nona Bokra (A × N) crosses along with check variety CSR10 were raised in Honeycomb Selection Design (HSD) to study the genetics of yield component traits under salt stress. The study found that the trait, panicle weight in A × F exhibited normal distribution while the other traits exhibited non-normal distribution in both the crosses. Negative skewness was observed for panicle length in both the crosses and days to flowering in the A × N indicating duplicate (additive × additive) gene interactions. Whereas positive skewness was observed for plant height, number of tillers, productive tillers and single plant yield in both the crosses, days to flowering and spikelet sterility in A × F and panicle weight in A × N indicating complementary gene interactions. Leptokurtosis was observed for days to flowering in the A × F. While the remaining characters in both the crosses exhibited platykurtosis. Transgressive segregants in both directions (towards both the parents) were obtained for all the eight characters studied in both the crosses suggesting that all traits were governed by additive gene action. Honeycomb selection design (HSD) provides an excellent phenotyping framework for studying the genetics of any quantitative traits, particularly yield and its component traits, as well as for breeding varieties that will provide more consistent results in yield in a shorter time frame.

Keywords: Skewness, kurtosis, transgressive segregation, gene action

Rice, the world's most important food crop, forms the staple food for about four billion people *i.e.,* half of humankind on the planet. By 2050, rice production must increase by 87% due to the rising global population (Kromdijk and Long, 2016). Despite its significance, the production of rice is limited globally by several biotic and abiotic stresses, with salt being one of the most common abiotic stresses that significantly reduce the yield. (Wang *et al.,* 2012). Therefore, enhancing crops' salt tolerance would not only result in the efficient use of saline-alkaline land but would also promote sustainable agriculture and help to resolve the global food crisis (Qin *et al.,* 2020). Lands that have been affected by salt have highly variable salt concentrations, which is problematic since it increases the environmental components of variation compared to genetic variation, making evaluation and selection challenging. It also raises the question of whether selection can be carried out at high or low salt levels. Additionally, it is important to characterize salt stress to select the best field designs, either to minimize experimental error or to get rid of the confounding effects of soil heterogeneity.

Yield and its component traits were severely affected by salinity. Rice is reasonably tolerant at germination, but growth becomes extremely sensitive during the early seedling stage (1-3 weeks) and then becomes more tolerant during active tillering. When it comes to overall grain yield, the period from panicle initiation to flowering and fertilization is the most vulnerable (Singh *et al.,* 2021). In addition, salinity severely impacts the physiological and biochemical attributes of the plants (Govindaraju and Balakrishanan, 2002).

For genetic analysis (of traits) and breeding (selection) superior crop varieties, accurate and precise phenotyping (evaluation/screening) of crop plants is necessary to identify their agronomically significant qualities. Choosing the proper field designs can, to some extent, reduce experimental error as we handle the issue of soil heterogeneity, which is more troublesome in saline fields than it is in normal fields. Although many types of field plot designs, including RCBD and lattice designs, have been put forth for tackling spatial heterogeneity in breeding trials, they are restricted by several factors. Therefore, it is essential to select an appropriate unit of plant phenotyping in the field to maximize the effectiveness of selection in plant breeding programs and associated quantifiable genetic gain. After a systematic search for the obstacles that lower the effectiveness of early generation selection for yield, the honeycomb breeding approach emerged as a very effective way to address the confounding effects of spatial variation (Fasoula and Fasoula, 1997; Fasoula and Fasoula, 2000).

Large and heritable phenotypic variation and application of high selection pressure optimize response to selection. In order to achieve this, a Honeycomb Selection design (HSD) that maximizes phenotypic expression while minimizing soil variability, was used. In this design, the absence of competition is ensured by growing individual plants at a greater distance from one another, and each plant is taken into account as an experimental unit rather than a traditional plot. Further systematic entry allocation and multiple replicates (check variety) not only ensure effective sampling of spatial heterogeneity but also counteract its detrimental effects on selection efficiency. In light of these facts, the present study was conducted to study the genetics of yield and its component traits in two F_2 crosses raised using Honeycomb Selection Design under salt stress.

Plant Materials: Two F_2 populations from the crosses of ADT(R)45 × FL478 (A × F) and ADT(R)45 × Nona Bokra (A × N) as well as the check variety CSR10 grown in the Honeycomb Selection Design (HSD) in *Kharif* 2022, comprised the experimental material. The female parent was ADT(R)45, a well-known short-duration rice variety from Tamil Nadu with agronomically desirable characteristics but prone to salinity. FL478 was the male parent which is a F_2 derived F_8 recombinant inbred line developed using Pokkali, a salt-tolerant landrace and IR 29, an improved *indica* cultivar at IRRI. FL478 has a higher salt tolerance than or on par with its tolerant parent, Pokkali. West Bengal landrace known as Nona Bokra, the male parent in A × N is extremely salt tolerant and is frequently utilized in breeding for salt tolerance. The rice variety CSR10, which was developed from the rice line M40-431-24-114 (an F_1 mutant of CSR 1/IR 8) has a short duration (110–120 days), dwarf type (80–85 cm) and salt tolerance, was utilized as a check in this design.

Layout of honeycomb design: The honeycomb selection design (HSD) (UN-1) was used to raise 988 plants in each cross, with 847 F_2 plants (ADT(R)45 × FL478 and ADT45 × Nona Bokra) and 141 CSR10 plants per cross, at a wider spacing of 90 cm × 100 cm. The single check CSR10 (sodic tolerant early check) occupies 14.3% of the places in the unreplicated-1 (UNR-1) honeycomb design, accounting for 141 plants in each cross. The parents *viz.,* ADT(R)45, FL478 and Nona Bokra were raised with a greater spacing of 90 x 100 cm to resemble the F_2 field layout. For each of the crosses, a total of 26 rows with 38 plants each were grown. In the 841 plants of A × F and 838 plants of A × N, only 847 F_2 plants survived.

Phenotyping under salt stress condition: To induce the F_2 population to undergo salinity stress, borewell water was used to irrigate the field. To characterize the stress in both field designs as it specifies the tolerance level of the genotypes in the field, nine piezometer like structures were installed in the field, and a sample of the root zone water was taken for measuring EC and pH at fortnightly intervals. Overall, the root zone water's EC ranged from 0.74 to 1.92 dSm^{-1}, while its pH varied from 7.1 to 9.15. In terms of EC, the highest spatial range was found 95 days after sowing (0.99-1.90 dSm^{-1}), and in terms of pH, 60 days after sowing (7.45-9.15). While the highest temporal range was observed at piezometer spot 5 with 0.80-1.92 dSm^{-1} for EC and at piezometer spot 3 with 7.22-9.15 for pH. The nine places showed significant spatial and temporal heterogeneity over two weeks, demonstrating the field's extreme soil variability, which was aggravated stronger by salinity. There needs effective soil heterogeneity sampling to choose plants from low and high salinity areas. During the crop period, the weather was mostly bright with sporadic rains. The temperature ranged from 24.1°C to 27°C at its minimum point to 31.1°C to 37.4°C at the maximum. In the morning and evening, relative humidity readings of 72% to 92% and 47% to 74% were recorded, respectively. Between June and December, 1604 mm of rainfall in total, with 61 rainy days were recorded.

Observations and statistical analysis:The phenotypic observations such as days to flowering (DAF), plant height (PHT), number of tillers per plant (TNE), number of productive tillers per plant (PTL), panicle weight (PWT), panicle length (PNL), spikelet sterility (SS%) and single plant yield (SPY) (adjusted to 14 % moisture) were recorded from all the plants on single plant basis in both the crosses. The phenotypic observations were subjected to statistical analysis such as normality test, skewness, kurtosis and measure of transgressive segregation to find out the gene action behind the characters. IRRI's software, STAR 2.0.1 was used for the estimation of the above parameters.

Test of normality: The Shapiro-Wilk test is often used to verify the normality of data distribution since it has superior statistical characteristics than a variety of alternative tests. From the results of the study, it was found that only the trait, panicle weight in A × F exhibited normal distribution (Pr(<W) = 0.05) while the remaining traits in both the crosses exhibited non-normal distribution (**Table 1**). Similar reports of non-normal distribution were reported by Yoglakshmi *et al.* (2022). Departure from normality shows that, in most cases, polygenes operating additively but with allele interactions at some loci, influenced yield traits during salt stress (Singh *et al.,* 2001). The non-normal distribution may be due to sterile F_2 seeds, natural selection, meiotic distortion, linkage drag and linkage disequilibrium (Girish *et al.,* 2006). According to Fasoulas (1988), the expected normal distribution of the F_2 population depends on the number of harmful genes present, which could cause the normal distribution to deviate as indicated by a significant negative kurtosis. Additionally, Fasoulas (1988) demonstrated the significance of additive gene effects over dominant or masking side effects of harmful genes in heterozygosity as they are heritable and improve distribution characteristics, resulting in a more normal curve.

Skewness and kurtosis:The distribution of the data is described by four cumulants: the mean, which indicates the location of the distribution's center; the variance which describes the distribution of the data; the skewness, which indicates how far the distribution deviates from symmetry; and the kurtosis, which describes the distribution's peaking behavior. Accordingly, these four cumulants are also denoted as first, second, third and fourth-order statistics respectively. Despite the importance of third and fourth-degree statistics being emphasized by Fisher *et al.* (1932), the majority of our present quantitative genetic theories are based on first and second-degree statistics. As skewness and kurtosis provide information about the nature of gene action (Fisher *et al.,* 1932) and the number of genes regulating the traits (Robson, 1956), respectively, the genetics of the traits can be studied with third and fourth-degree statistics in segregating generations of rice.

According to Roy (2000), the occurrence of skewness may be due to lethal genes, incomplete linkage, epistasis, non-additive genetic effects, G x E interaction, genes showing multiplicative effects, competition and environmental factors. In this experiment, G x E interaction, competition and environmental factors such as stress and fertility are prominent and are still aggravated under salt stress conditions.

In the present study, negative skewness was observed for panicle length in both the crosses and days to flowering in the A × N indicating duplicate (additive x additive) gene interactions. Whereas positive skewness was observed for plant height, number of tillers, productive tillers and single plant yield in both the crosses, days to flowering and spikelet sterility in A × F and panicle weight in A × N indicating complementary gene interactions

Table 1. Descriptive statistics for two F_2 crosses

Characters	Cross	W value	Pr (<W)	Skewness	T – value	Kurtosis	T – value
Days to flowering	A × F	0.87	0.00	1.69**	20.11	5.04**	29.84
	A × N	0.95	0.00	-0.41**	-4.87	-0.70**	-4.16
Plant height	A × F	0.95	0.00	0.86**	10.22	2.23**	13.19
	A × N	0.99	0.00	0.41**	4.87	-0.11	-0.65
Number of tillers	A × F	0.96	0.00	0.82**	9.80	1.43**	8.45
	A × N	0.97	0.00	0.63**	7.49	0.13	0.77
Productive tillers	A × F	0.96	0.00	0.75**	8.94	1.01**	5.92
	A × N	0.93	0.00	1.01**	12.01	0.92**	5.47
Panicle weight	A × F	0.99	0.05	-0.01	-0.11	-0.05	-0.33
	A × N	0.96	0.00	0.86**	10.22	0.81**	4.81
Panicle length	A × F	0.98	0.00	-0.39**	-4.66	1.27**	7.55
	A × N	0.99	0.00	-0.40**	-4.76	0.28	1.66
Spikelet sterility	A × F	0.92	0.00	0.81**	9.68	-0.16	-0.95
	A × N	0.96	0.00	-0.04	-0.48	-1.14**	-6.78
Single plant yield	A × F	0.96	0.00	0.54**	6.50	0.34*	2.02
	A × N	0.86	0.00	1.39**	16.52	2.04**	12.13

*Significance at 5 % level (1.963)
**Significance at 1 % level (2.582)

(**Table 1**). This positive skewness, which was observed in the current study, is caused by the transposition of low-yielding plants from the left to the right tail of the curve, which reflects the magnitude of selection efficiency in the absence of competition, and the transposition of low yielders and strong competitors from the left to the right tail of the distribution, which reflects the magnitude of the negative correlation between yield and competitive ability in the presence of competition as stated by Kyriakou and Fasoula (1985), Pasini and Bos (1990) and Yoglakshmi *et al.* (2022) in a study comparing the effectiveness of traditional and honeycomb selection designs in studies on winter rye, spring rye and rice respectively. While Fasoulas (1988) believed that skewness and kurtosis were signs of undesirable genes that a breeder needed to eliminate during the selection process.

As previously stated, kurtosis describes the distribution's peaking, with platykurtosis occurring when the kurtosis value is less than 3 and leptokurtosis occurring when the kurtosis value is greater than 3. In this present study, leptokurtosis was observed for days to flowering in the A × F. While the remaining characters in both the crosses exhibited platykurtosis. Positively skewed leptokurtic distribution observed for days to flowering in A × F suggests that the expression of the trait is controlled by a few number of genes with complementary epistasis and hence the intense selection is required for a rapid genetic gain while positively skewed platykurtic distribution observed for plant height, number of tillers, productive tillers and single plant yield in A × F and productive tillers, panicle weight and single plant yield in A × N suggests that these traits are controlled by a large number of genes but they also exhibit complementing epistasis, so strong selection is necessary to achieve a rapid genetic gain. Negative skewness with platykurtic distribution observed for panicle length in the A × F and days to flowering in the A × N suggested that these traits are controlled by a large number of genes but displaying duplicate (additive x additive) epistasis and hence a mild selection is required for a rapid genetic gain. Similar study related to skewness and kurtosis was reported in segregating populations of rice by Savitha and Kumari (2015); Priyanka *et al.* (2019); Fathima *et al.* (2021) and Yoglakshmi *et al.* (2022).

Transgressive segregation: Traditional knowledge suggests that transgressive phenotypes can result from any combination of the following: positive or negative complementation of additive alleles, epistatic interaction of unique parental traits, unmasking of recessive genes from a heterozygous parent, or any one of these mechanisms individually. A significant number of transgressive segregants can readily be identified since the honeycomb selection design increases the level of genotypic expression, which improves phenotypic expression. (Rieseberg *et al.,* 1999). The ability to distinguish between homozygotes and heterozygotes makes it simple to detect transgressive segregants, which are a result of additive genetic variance, at a wider spacing.

Table 2. Transgressive segregants for yield and its components traits in two F_2 crosses

Characters	Cross	F_2 cross				Parents		No. of Transgressive segregants	
		Total no. of plants scored	Mean	Highest plant value	Lowest plant value	ADT45	FL478/ Nona Bokra	Higher than highest parent	Lower than lowest parent
Days to flowering	A × F	841	82.60	125.00	71.00	78.20	92.60	83	203
	A × N	838	100.60	135.00	71.00	78.30	113.10	147	42
Plant height	A × F	841	84.56	136.00	52.00	82.30	108.90	21	386
	A × N	838	106.24	179.00	45.00	83.30	138.00	88	128
Number of tillers	A × F	841	20.27	54.00	3.00	18.20	13.50	479	157
	A × N	838	29.16	88.00	4.00	17.80	30.30	356	194
Productive tillers	A × F	841	17.90	56.00	1.00	20.50	24.80	181	529
	A × N	838	29.15	93.00	2.00	19.70	18.90	496	239
Panicle weight	A × F	841	2.22	4.76	0.06	1.64	2.42	351	194
	A × N	838	2.17	7.20	0.20	1.51	4.45	26	305
Panicle length	A × F	841	21.86	35.20	8.20	19.46	23.31	262	156
	A × N	838	23.62	33.20	11.50	19.47	25.91	90	224
Spikelet sterility	A × F	841	35.59	100.00	0.00	39.88	34.31	309	485
	A × N	838	52.61	100.00	0.00	41.32	16.02	539	73
Single plant yield	A × F	841	15.19	55.94	0.00	7.17	21.17	215	191
	A × N	838	20.72	116.32	0.09	6.47	79.81	11	275

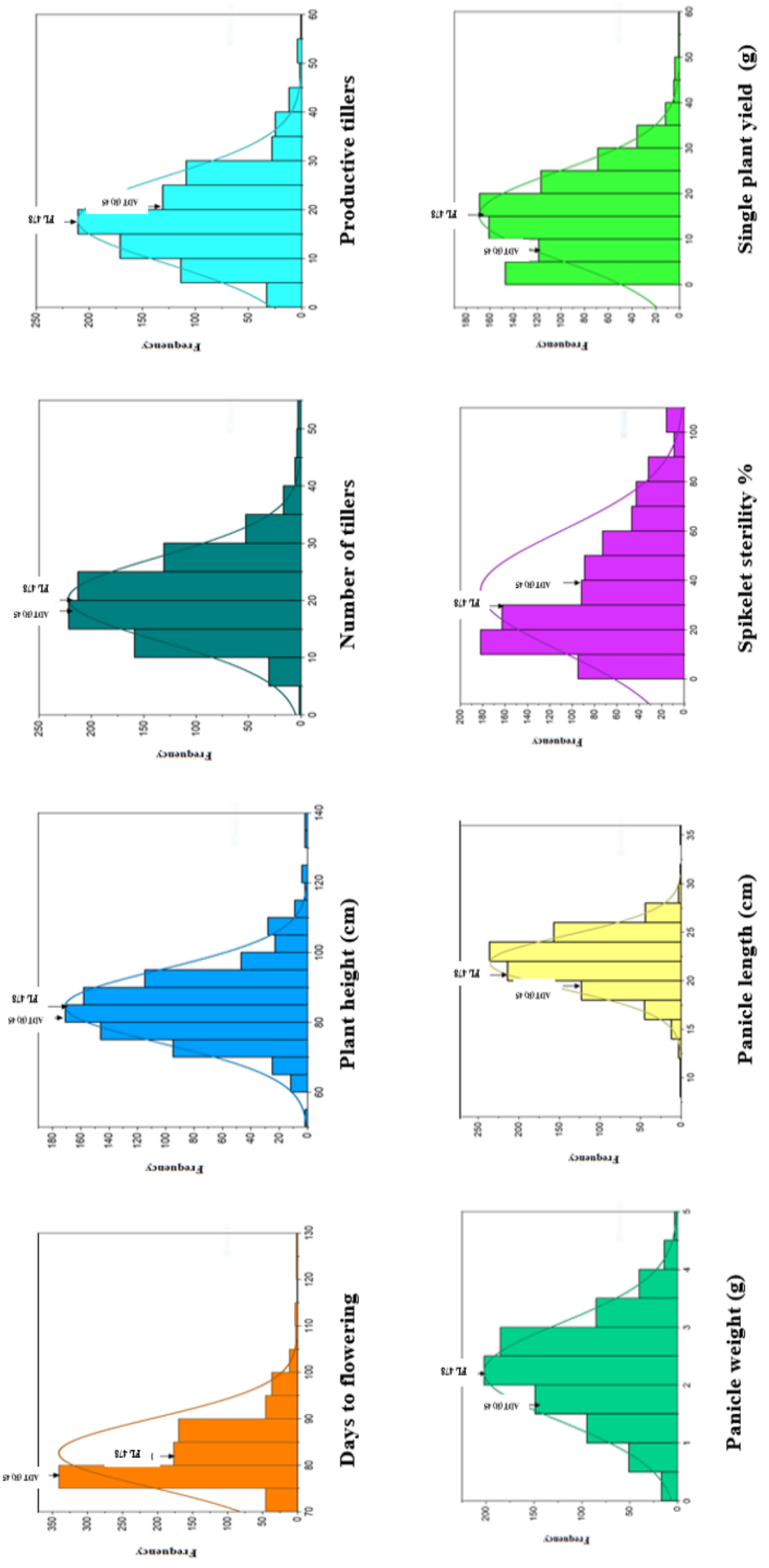

Fig. 1. Graphical representation of frequency distribution of F_2 population of ADT(R)45 × FL478

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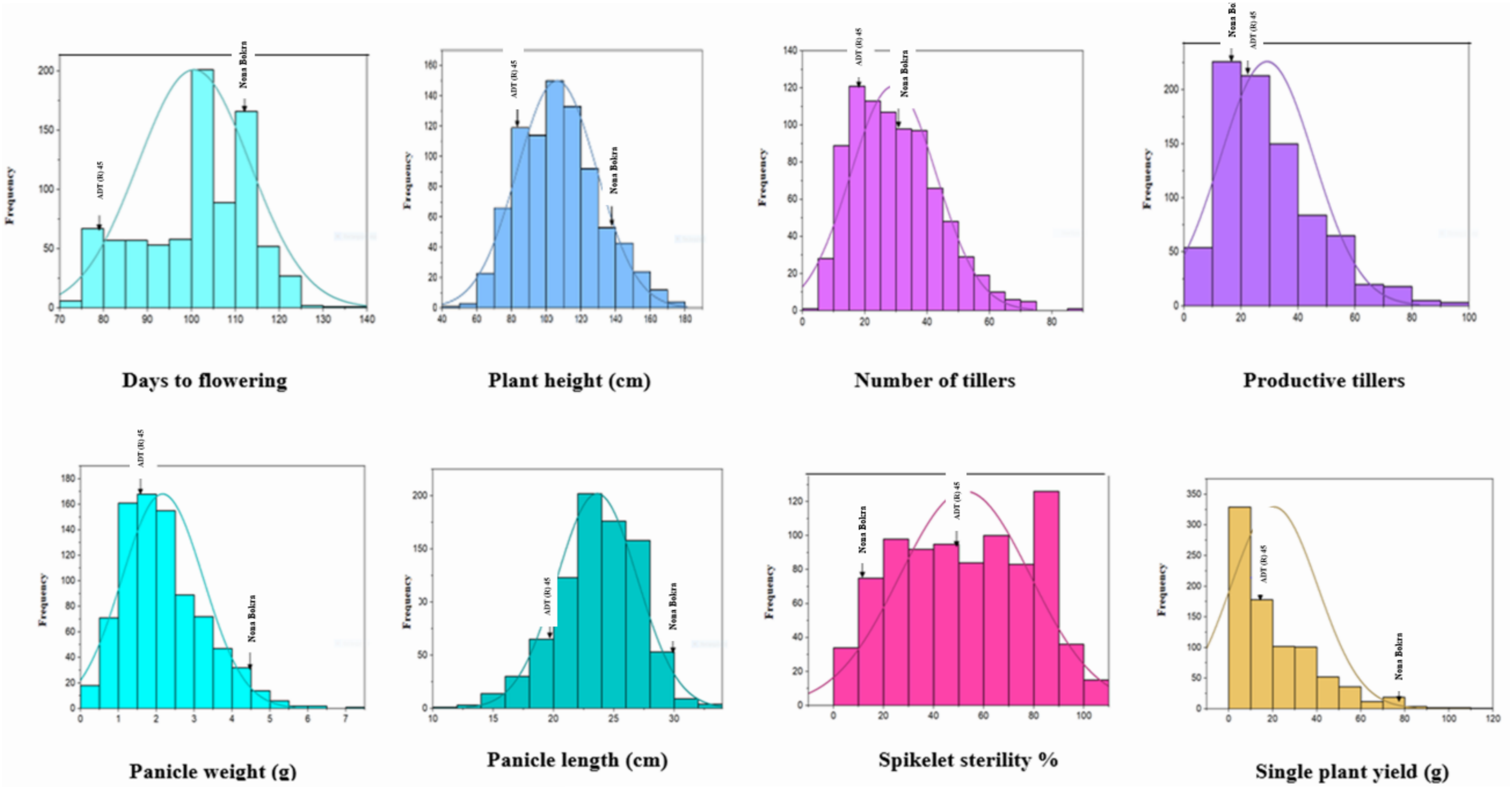

Fig. 2. Graphical representation of frequency distribution of F_2 population of ADT(R)45 × Nona Bokra

In the present investigation, a high frequency of favorable transgressive segregants was observed for number of tillers, panicle weight, panicle length and single plant yield in the A × F, whereas the A × N recorded a higher number of transgressive segregants for days to flowering, number of tillers and productive tillers (**Table 2**). Transgressive segregants with a lower value than the lowest parent were high in number for days to flowering, plant height, productive tillers and spikelet sterility for A × F. In the A × N, plant height, panicle weight, panicle length and single plant yield recorded a higher number of lowest value transgressive segregants (**Fig. 1 and Fig. 2**). Observation of transgressive segregation is highly anticipated and indicative of the predominance of additive gene action because yield and its components were shown to be (polygenic) quantitative in nature. This further confirmed the additivity of the characters studied in addition to the results of normality. In this study, all eight of the characters were found to have transgressive segregants in both directions (towards both parents). This indicates that these traits were controlled by numerous genes or alleles and that these traits appeared to work additively, demonstrating polygenic inheritance (Reddyyamini *et al.,* 2019). Such plants are produced by the accumulation of favorable genes from both parents as a consequence of segregation and recombination. According to Kshirsagar *et al.* (2013), transgressive segregation can be used to produce genotypes with favorable traits from both parents. Such transgression may occur in a certain F_2 population as a result of the accumulation of favorable alleles from both parents at multiple loci (Tanksley, 1993) and the unmasking of recessive deleterious alleles due to inbreeding (Rick and Smith, 1953). From this study, it was observed that honeycomb selection design (HSD) is regarded as the best phenotyping platform for studying the genetics of any quantitative traits, notably yield and its components, as well as for breeding varieties for a higher and more stable yield in a shorter period.

REFERENCES

Fasoula, D.A. and Fasoula,V.A. 1997. Competitive ability and plant breeding. *Plant Breed. Rev.*, **14**: 89–138. [Cross Ref]

Fasoula, D.A. and Fasoula, V.A. 2000. Honeycomb breeding: principles and applications. *Plant Breed. Rev.*, **18**: 177–250. [Cross Ref]

Fasoulas, A.C. 1988. The Honeycomb Methodology of Plant Breeding; Department of Genetics and Plant Breeding, Aristotle University of Thessaloniki: Thessaloniki, Greece.

Fathima, M.A., Geetha, S., Amudha, K. and Uma, D. 2021. Genetic variability, frequency distribution and association analysis in ADT (R) 48 x Kavuni derived F_2 population of rice (*Oryza sativa* L.). *Electron. J. Plant Breed.*, **12**(3): 659-666. [Cross Ref]

Fisher, R.A., Immer, F.R. and Tedin, O. 1932. The genetical interpretation of statistics of the third degree in the study of quantitative inheritance. *Genetics.*, **17**: 107- 124. [Cross Ref]

Girish, T.N., Gireesha, T.M., Vaishali, M.G., Hanamareddy, B.G. and Hittalmani, S. 2006. Response of a new IR50/Moroberekan recombinant inbred population of rice (*Oryza sativa* L.) from an *indica×japonica* cross for growth and yield traits under aerobic conditions. *Euphytica.*, **152**(2): 149–161. [Cross Ref]

Govindaraju, P. and Balakrishanan, K. 2002. Effect of salinity in certain enzyme activity, physiological traits and yield of rice cultivars. *Madras Agric. J.*, **89**(1-3): 67-69. [Cross Ref]

Kromdijk, J. and Long, S.P. 2016. One crop breeding cycle from starvation. How engineering crop photosynthesis for rising CO_2 and temperature could be one important route to alleviation. *Proceedings of the Royal Society B: Biological Sciences.*, **283** (1826): 20152578. [Cross Ref]

Kshirsagar, D.B., Bhalekar, M.N., Patil, R.S., Kute, N.S. and Patil, S.B. 2013. Transgressive segregation in F_3 generation of intervarietal crosses of tomato (*Solanum lycopersicon* L.). *Veg. Sci.*, **40**(2): 240-242.

Kyriakou, D.T. and Fasoulas, A.C. 1985. Effects of competition and selection pressure on yield response in winter rye (*Secale cereale* L.). *Euphytica.*, **34**: 883–895. [Cross Ref]

Pasini, R.J. and Bos, I. 1990. The effect of interplant distance on the effectiveness of honeycomb selection. I. Results of the first selection cycle. *Euphytica.*, **49**: 121–130. [Cross Ref]

Priyanka, A. R., Gnanamalar, R. P., Banumathy, S., Senthil, N. and Hemalatha, G. 2019. Genetic variability and frequency distribution studies in F_2 segregating generation of rice. *Electron. J. Plant Breed.*, **10**(3): 988-994. [Cross Ref]

Qin, H., Li, Y. and Huang, R. 2020. Advances and challenges in the breeding of salt-tolerant rice. *Int. J. Mol. Sci.*, **21**(21): 8385. [Cross Ref]

Reddyyamini, B., Hariprasad Reddy, K., Lakshmi Narayana Reddy, V., Ramesh Babu, P. and Sudhakar, P. 2019. Transgressive segregation for yield and its component traits in rice (*Oryza sativa* L.). *Int. J. Curr. Microbiol. Appl. Sci.,* **8**(06): 2450-2455. [Cross Ref]

Rick, C.M. and Smith, P.G. 1953. Novel variation in tomato species hybrids. *Am. Nat.*, **87**(837): 359-373. [Cross Ref]

https://doi.org/10.37992/2024.1504.099

Rieseberg, L.H., Archer, M.A. and Wayne, R.K. 1999. Transgressive segregation, adaptation and speciation. *Heredity.*, **83**: 363-372. [Cross Ref]

Robson, D.S. 1956. Application of K_2 statistics to genetic variance component analysis. *Biometrics.*, **12**: 433-444. [Cross Ref]

Roy, D. 2000. Plant breeding: Analysis and exploitation of variation. NAROSA Publishers, New Delhi.

Savitha, P. and Kumari, R.U. 2015. Studies on skewness, kurtosis and parent progeny regression for yield and its related traits in segregating generations of rice. *Oryza.*, **52**(2): 80-86.

Singh, R. K., Mishra, B. and Jetly, V. 2001. Segregations for alkalinity tolerance in three rice crosses. *SABRAO. J. Breed. Genet.*, **33**: 31-34.

Singh R.K, Kota, S. and Flowers, T.J. 2021. Salt tolerance in rice: seedling and reproductive stage QTL mapping come of age. *Theor. Appl. Genet.*, **99**: 120-134. [Cross Ref]

Tanksley, S.D. 1993. Mapping polygenes. *Annu. Rev. Genet.*, **27**(1): 205-233. [Cross Ref]

Wang, Z., Chen, Z., Cheng, J., Lai, Y., Wang, J. and Bao, Y., *et al*. 2012. QTL Analysis of Na^+ and K^+ Concentrations in Roots and Shoots under Different Levels of NaCl Stress in Rice (*Oryza sativa* L.). *Plos one.*, **7**(12): e51202. [Cross Ref]

Yoglakshmi, C., Pearl, R.I., Fasoula, V.A., Karthick, J. and Thirumeni, S. 2022. Efficiency of honeycomb selection design in early segregating generation in ADT45 × Nona Bokra cross under salt stress. *Indian J. Exp. Biol.,* **60**: 463-470.