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Elucidating parent-progeny regression among the M_4 and M_5 mutants of Kodo millet (*Paspalum scrobiculatum* L.)

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Abstract
Kodo millet is one of the important small millet rich in nutrition and has the ability to withstand extreme climatic conditions. Since. It is a highly self pollinated crop variability in this crop is limited. Mutation and selection is one of the breeding approach carried out to induce variability and helps in the selection of better performing mutants for the future use. The present investigation was carried out to study the relationship between progenies of 67 families of M_4 and M_5 mutants of Kodo millet. Observation on the traits, Stomatal Length (µm), Stomatal number, Chlorophyll Index, Culm thickness (cm) and Yield (g) were the key traits for selection. The regression analysis shows that there was a strong relationship between two generations M_4 and M_5. Intergeneration correlation was positively significant among M_4 and M_5 generations revealing traits are heritable to the further generations. Higher narrow sense heritability was observed all the traits except Stomatal length and Chlorophyll index which showed moderate heritability. This suggests the traits are governed by additive gene action. Selection on these generations based on the traits would be rewarding and focus on the improvement of the population

Keywords: Kodo millet, regression analysis, intergenerational correlation, narrow sense heritability, selection

Introduction
Mutation breeding is one of the breeding methods that deploy in inducing variation in crops where the recombination breeding is difficult to perform. Kodo millet is one such crop which is a wonder cereal and is highly self-pollinated owing to its cleistogamous flowers (Hariprasanna, 2017) [6]. The frequency of attaining desirable mutants in a population is fairly low and this reinstates the importance of selecting and forwarding desirable progenies in each generation of mutants. In a mutant population, it is reported that each individual is a genotype and a maximum population is screened under M2 generation in order to identify the desirable plant types (Jency *et al*. 2020) [7]. Thus, handling segregants of mutants are rather a unique process than the conventional and marker-aided methods in plant breeding. For a selection to be effectively carried out the genetic variance is to be analysed, other than that to study the impact of mutagens the progeny regression analysis for the desired traits has to focused on dissecting elite mutants. This predicts the overall performance of the progeny for the particular trait and also yields the major selection indices for the response trait. Similar studies by Lalitha *et al*. (2018) [9] presented the significant regression for days to 50 percent flowering, flag leaf length and panicle length in rice. The progeny analysis in the segregants in addition depicts the indicative performance of the farther generation on comparison to the older ones. The studies on progeny analysis further the presents the attainment of homozygosity in the later generations and this presents the overall inheritance of traits across generations (Banumathy *et al*. 2017; Ananthi *et al*. 2018) [3, 1].
Subsequently, progeny regression analysis thereby was also effectively carried out on three modes of selection in three F2 populations of wheat that revealed the efficacy of selection of plants based on individual traits and traits with higher expression than the control (Singh *et al*. 2001) [13]. Hence, it could be understood that the progeny regression analysis depicts the inter-generation trait association, selection gain and trait fixation (Dubey *et al* 2019). In a view of this, progeny regression analysis and progeny inheritance analysis were carried in the M_4 and M_5 mutants of Kodo millet for photosynthetic traits and yield. This study would bring about the effectiveness of the mutagens on inducing the desirable traits and also would reveal the best strategy for selection in kodo millet mutants.

Materials and Methods

The present investigation was carried out using M_4 and M_5 mutants of kodo millet obtained by inducing CO 3 variety to physical mutagen (Gamma rays) and chemical mutagen (Ethyl methanosulfonate) (Jency *et al*. 2020) [7]. The selection was carried in 242 mutants from 67 families of M_4 mutants were grown in the Millet Breeding Station, TNAU, Coimbatore during Kharif 2020. From these 136 mutants with positive homozygous progenies from 67 families were forwarded to M_5 generations which was raised during summer 2021. Quantitative traits *viz*., Stomatal Length (µm), Stomatal number, Chlorophyll Index, Culm thickness (cm) and Yield (g) were recorded on the progenies of these mutants. Average of progenies on the each mutant families was taken and subjected to Intergenerational correlation and Parent progeny regression analysis using XLSTAT. Skewness, Kurtosis and narrow sense heritability was calculated using Microsoft Excel 2019.

Result and Discussion

The mean performance of 66 families of M_4 and M_5 mutants of Kodo millet along with control CO 3 was given in Table 1. Increased mean performance was observed in M_5 generation from M_4 generation for Stomatal length, Chlorophyll Index and Yield. The selection for parents for the M_5 generations is based on the positive homozygous progenies of M_4 generation plays a major role in improvement of the trait performance of Stomatal length, Chlorophyll Index and Yield in M_5 generations. The traits *viz*., Stomatal Number and Culm thickness were highly environment responsive traits which were affected during M_5 generations. Similar reports given by Jeeva *et al*. (2022) where the traits Stomatal number is highly influenced by environment. Overall mean, range, skewness and Kurtosis is given in the Table 2 and frequency distribution is given in the Figure 1.

Skewness and Kurtosis are two parameters used to analyse the variations in the segregating populations (Nadarajan *et al*. 2016) [10]. Skewness provides the gene interaction and kurtosis provides the information about the gene controlling the traits. Negatively skewness was observed for Stomatal length and Stomatal number which explains the duplicate epistatic gene action in M_4 generation whereas in M_5 generation it is found only in Stomatal length. The traits, Chlorophyll index, culm thickness and Yield showed positive skewness in both M_4 and M_5 generations. Similar report for yield was reported by Harijan *et al* (2021) [5], Seeli *et al*. (2021) [12]. This suggested that these traits are governed by complimentary epistatic gene action. In this case, genetic gain will be faster in mild selection and less is intense selection. Negative kurtosis was observed for all the traits except of Chlorophyll index in both the M_4 and M_5 generations. Yield showed positive kurtosis in M_5 generations where it is found to be negative in M_4 generation. All the traits showed leptokurtic curve in M_5 generations suggest that relatively fewer number of genes controlling the traits comparing the M_4 generations where the traits Stomatal number and Culm thickness showed mesokurtic curve. Regression, Intergenerational correlation and narrow sense heritability was given in the table 3. Parent progeny Regression coefficient was given in the figure 2.

Regression analysis between the parent and progeny suggests the influence of environment on the selection traits. Parent progeny regression analysis was estimated using the progeny mean values of 66 families of M_4 generations and M_5 generations. The result showed strong associations in the traits between the generations. Highly significant regression coefficient was found for Stomatal length, stomatal number, Chlorophyll Index, Culm thickness and Yield. This suggests the lesser influence of environment and selection based on these traits in this generations will be rewarding. Similar results was obtained for single plant yield by Anilkumar *et al*. (2011) [2].

Intergenerational correlation provides the extent of genetic potential obtained in the further generations (Kumar *et al*. 2020, Rani *et al*. 2021) [8, 11]. Positive significant intergenerational correlation was observed in all the traits showing that these traits are highly heritable in these generations (Vinothini *et al* 2021, Seeli *et al* 2021) [14, 12].

Narrow sense heritability estimated based on the parent progeny regression and intergenerational correlation (Dubey *et al* 2019). High narrow sense heritability was observed in the traits Culm thickness (52.84%), Stomatal number (47.03%) and Yield (45.02%). Significant regression and high heritability shows additive gene action and the trait is heritable to the next generations. The selection based on these traits will be rewarding.

Table 1: Mean performance of 66 families of M_4 and M_5 progenies

	M_4 generation					M_5 generation				
	STL	STN	CI	CT	YLD	STL	STN	CI	CT	YLD
100-1-4	7.44	53.13	20.99	1.01	12.06	8.38	47.85	28.77	1.19	11.82
100-2-5	8.31	53.71	28.97	0.98	11.89	8.42	42.78	33.74	1.13	9.49
100-3-3	7.71	51.60	31.28	0.93	7.74	7.26	35.20	25.47	0.84	5.83
100-5-2	8.39	55.00	21.55	1.00	10.74	7.56	64.78	16.95	1.08	14.29
100-7-1	8.49	60.22	50.01	1.02	10.94	9.41	65.28	49.23	1.18	13.35
100-8-2	9.07	41.75	33.93	0.99	13.35	10.53	41.78	50.07	0.58	15.75
100-9-3	9.35	59.67	32.80	1.00	13.77	8.78	52.13	29.75	0.79	16.19
100-10-3	9.08	65.70	31.63	0.99	18.38	8.50	68.76	50.41	1.12	20.25
100-12-5	9.41	42.33	33.83	1.01	15.78	9.93	31.14	39.10	0.91	14.63
100-13-2	9.01	45.00	32.97	1.00	23.36	9.10	38.50	30.37	1.14	18.98
100-16-5	9.10	59.33	31.58	1.15	22.90	11.60	70.73	42.42	1.12	17.17
100-17-1	7.80	58.00	25.72	1.24	10.42	10.09	74.58	24.91	1.15	12.71
200-1-2	7.32	68.00	27.01	1.46	14.86	6.83	57.76	24.79	1.20	17.89
200-2-4	7.13	59.00	36.40	1.56	15.25	7.97	71.06	32.70	1.29	21.65
200-3-1	8.25	68.38	33.01	1.51	14.15	6.00	64.43	48.67	1.16	19.11
200-4-5	8.38	58.89	29.86	1.50	14.38	8.64	59.28	35.91	1.67	15.77
200-5-5	8.11	57.70	28.81	1.49	17.58	10.86	52.53	20.09	1.56	16.32

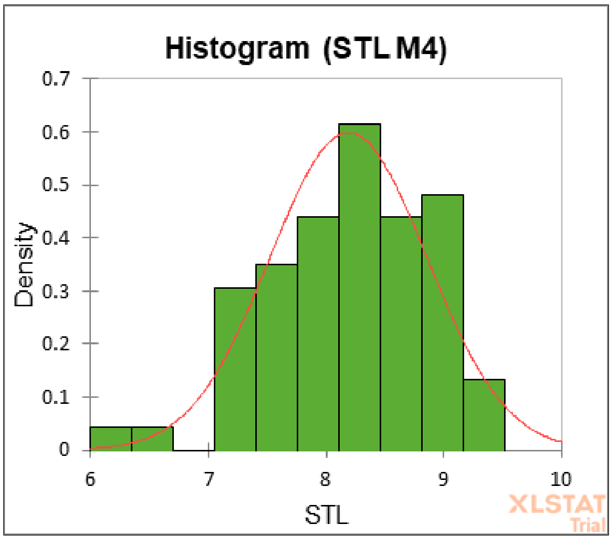
200-6-5	8.70	63.50	24.96	1.54	8.45	8.87	69.35	28.97	1.88	11.38
200-8-2	7.41	59.60	30.32	1.52	14.30	11.16	68.11	20.18	2.00	19.23
200-10-2	7.84	62.00	41.13	1.52	15.01	8.59	57.30	41.67	1.13	16.40
200-13-2	7.81	58.60	46.59	1.50	9.75	9.43	48.06	50.56	1.85	9.56
200-14-4	8.52	56.50	36.63	2.21	14.41	8.83	50.82	24.14	2.39	17.29
200-17-1	7.93	70.10	39.75	2.28	12.76	9.68	52.19	35.43	1.64	16.54
200-19-4	8.20	49.70	27.16	2.15	11.31	9.01	31.49	27.72	2.09	12.95
200-22-5	8.11	57.00	31.68	2.14	15.94	6.98	53.55	24.40	1.95	13.52
200-23-1	8.61	63.33	25.37	2.13	7.60	7.13	56.73	22.47	2.55	10.55
200-24-5	7.73	41.20	31.36	2.26	12.56	7.93	45.62	36.53	2.51	11.75
200-25-3	6.32	56.40	32.06	2.25	8.86	6.78	42.36	30.82	2.01	10.59
200-27-4	8.51	51.63	32.90	3.13	22.93	7.81	42.78	44.36	2.67	23.29
200-27-5	8.66	47.70	29.07	2.97	22.64	8.10	57.28	25.99	2.69	24.47
300-1-4	6.70	59.13	31.50	2.87	9.17	7.11	59.79	25.53	2.67	8.07
300-2-2	7.25	59.00	28.38	3.14	9.92	6.63	42.17	21.24	3.58	10.22
300-3-4	7.69	70.40	32.25	2.33	7.66	7.32	70.44	37.13	2.29	8.68
300-4-4	7.46	72.75	29.08	2.03	9.38	5.91	68.71	27.32	2.05	9.40
300-5-4	7.40	63.10	25.81	2.14	10.43	7.14	55.73	26.45	2.35	11.48
300-6-2	7.93	60.30	25.48	2.10	7.62	5.38	58.34	25.21	1.86	5.71
300-3-5	7.69	61.40	31.94	2.19	19.06	7.26	59.03	34.29	2.25	15.35
300-4-1	8.00	66.75	32.63	2.21	22.70	6.86	61.08	32.46	2.56	15.93
300-5-2	8.40	53.60	27.96	2.06	18.72	7.62	52.59	27.08	1.76	17.61
300-6-4	8.81	62.80	24.98	2.08	17.24	9.61	64.00	27.54	1.87	19.56
300-7-3	9.22	66.40	25.28	2.18	21.52	9.45	66.13	21.58	2.32	16.81
400-1-4	8.16	58.57	30.07	2.12	11.59	7.11	42.34	34.93	2.09	11.41
400-2-2	8.06	43.43	25.01	2.25	7.17	8.04	41.98	30.17	2.33	10.12
400-3-1	8.88	61.33	27.93	2.31	9.15	7.71	55.31	23.14	1.70	7.00
0.3-1-3	8.24	50.83	25.67	0.86	12.83	9.15	60.69	34.78	1.12	16.67
0.3-2-5	9.02	52.86	31.84	0.82	14.64	8.52	50.82	34.18	0.85	19.48
0.3-3-2	8.67	51.67	28.00	0.85	15.77	7.43	62.76	27.86	0.98	18.01
0.3-4-5	8.20	52.80	25.48	0.77	5.74	7.44	51.80	24.68	0.66	5.40
0.3-6-2	8.23	57.50	31.95	0.86	8.48	8.98	55.22	35.22	0.77	9.61
0.3-7-1	7.83	47.80	30.15	0.82	12.81	10.13	44.42	25.49	0.80	16.41
0.3-8-2	7.08	69.00	32.34	0.87	12.44	6.97	41.56	29.70	0.67	15.69
0.3-9-2	8.37	54.33	33.72	0.80	11.85	7.90	49.85	30.91	0.57	10.59
0.3-11-2	7.21	42.60	35.04	0.97	11.10	9.58	46.85	44.34	1.17	8.30
0.3-14-1	7.40	50.14	34.51	0.97	7.59	6.44	36.25	40.09	0.96	7.47
0.3-15-1	8.60	52.75	31.33	0.97	11.31	8.71	42.01	36.48	1.12	9.04
0.3-22-2	8.76	52.20	35.66	1.02	16.44	8.24	35.61	29.03	0.92	12.39
0.3-24-1	8.04	60.40	31.68	0.99	11.98	8.14	48.11	36.90	1.14	9.57
0.4-1-1	7.72	69.00	29.28	2.09	14.36	7.27	47.07	23.84	1.90	10.82
0.4-2-1	8.12	66.57	31.87	2.14	19.37	7.32	60.41	25.06	2.33	15.78
0.4-20-1	7.78	60.80	41.88	2.20	23.91	8.87	49.24	44.06	1.94	21.05
0.5-3-1	8.99	65.83	30.12	2.73	19.87	9.97	60.43	29.65	3.16	24.24
0.5-16-3	9.16	58.75	36.63	2.86	16.10	9.81	58.59	32.03	3.37	15.76
0.5-16-5	8.83	64.00	35.34	2.74	12.14	9.02	45.68	26.67	2.91	10.12
0.5-33-1	8.82	63.80	42.10	2.85	23.90	10.24	63.84	62.14	1.69	24.20
CO 3	8.86	55.70	31.90	2.47	18.65	8.53	46.90	34.29	2.73	21.12

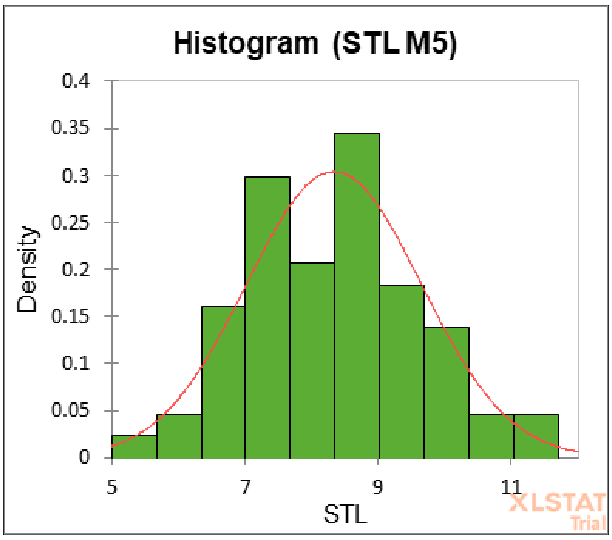
Table 2: Mean, Range, Skewness and Kurtosis of all the traits in M_4 and M_5 generations

Traits	Range		Mean		Skewness		Kurtosis	
	M_4	M_5	M_4	M_5	M_4	M_5	M_4	M_5
STL	6.32 - 9.41	5.38- 11.6	8.19	8.34	-0.331	0.198	-0.374	-0.464
STN	41.20 - 72.75	38.50 - 74.58	57.74	53.23	-0.302	-0.157	-0.479	-0.748
CI	20.99 - 50.01	16.95 - 62.14	31.45	32.28	0.971	0.954	1.737	0.685
CT	0.77 - 3.14	0.57 - 3.58	1.71	1.69	0.261	0.445	-1.234	-0.710
YLD	5.74 - 23.91	5.40 - 24.47	13.95	15.26	0.513	0.911	-0.647	0.784
STL	6.32 - 9.41	5.38- 11.6	8.19	8.34	-0.331	0.198	-0.374	-0.464

Table 3: Parent progeny regression, Intergenerational correlation and Narrow sense heritability of traits.

Traits	Correlation Coefficient (r)	Regression coefficient (b)	Narrow Sense Heritability h^2 (%)
STL	0.34	0.44	38.51
STN	0.54	0.57	47.03
CI	0.50	0.66	38.36
CT	0.97	0.92	52.84
YLD	0.80	0.88	45.02


Histogram (STL M4)
Density
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
6 7 8 9 10
STL
XLSTAT Trial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Density
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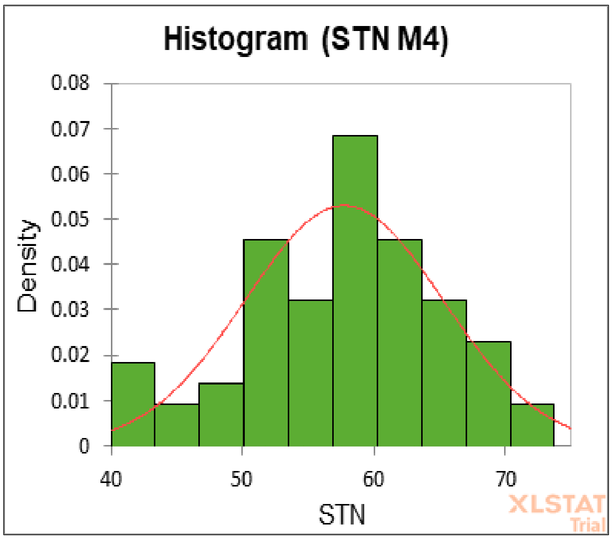
5 7 9 11
STL
XLSTAT Trial


Histogram (STN M4)
Density
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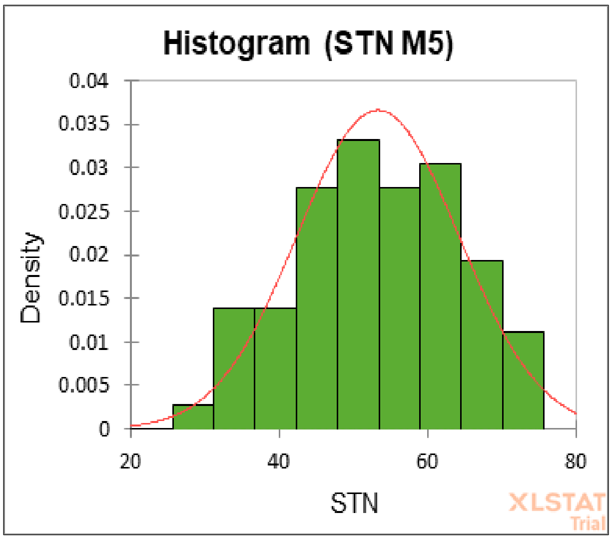
40 50 60 70
STN
XLSTAT Trial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Density
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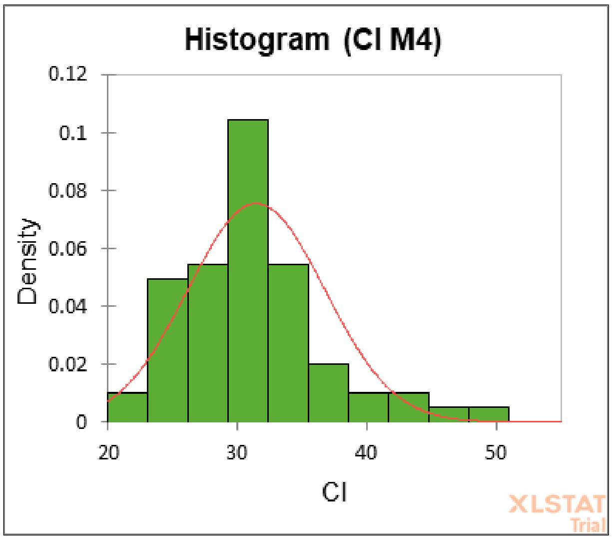
20 40 60 80
STN
XLSTAT Trial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Density
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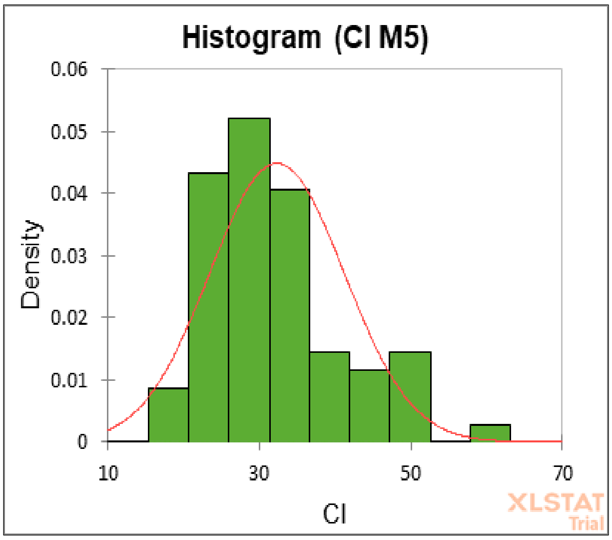
20 30 40 50
CI
XLSTAT Trial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CI
XLSTAT Trial

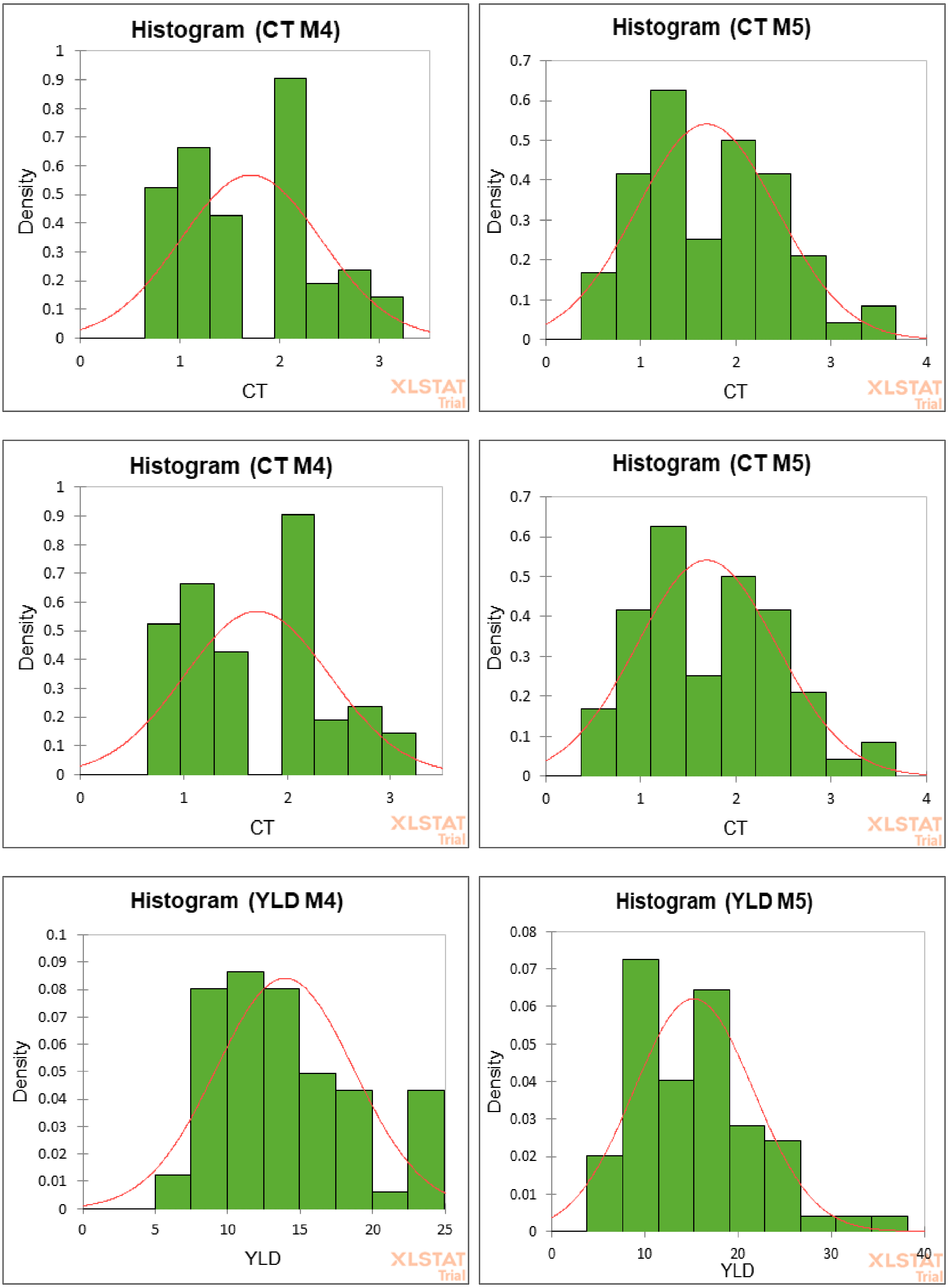

Fig 1: Frequency distribution of all the traits in M_4 and M_5 generations

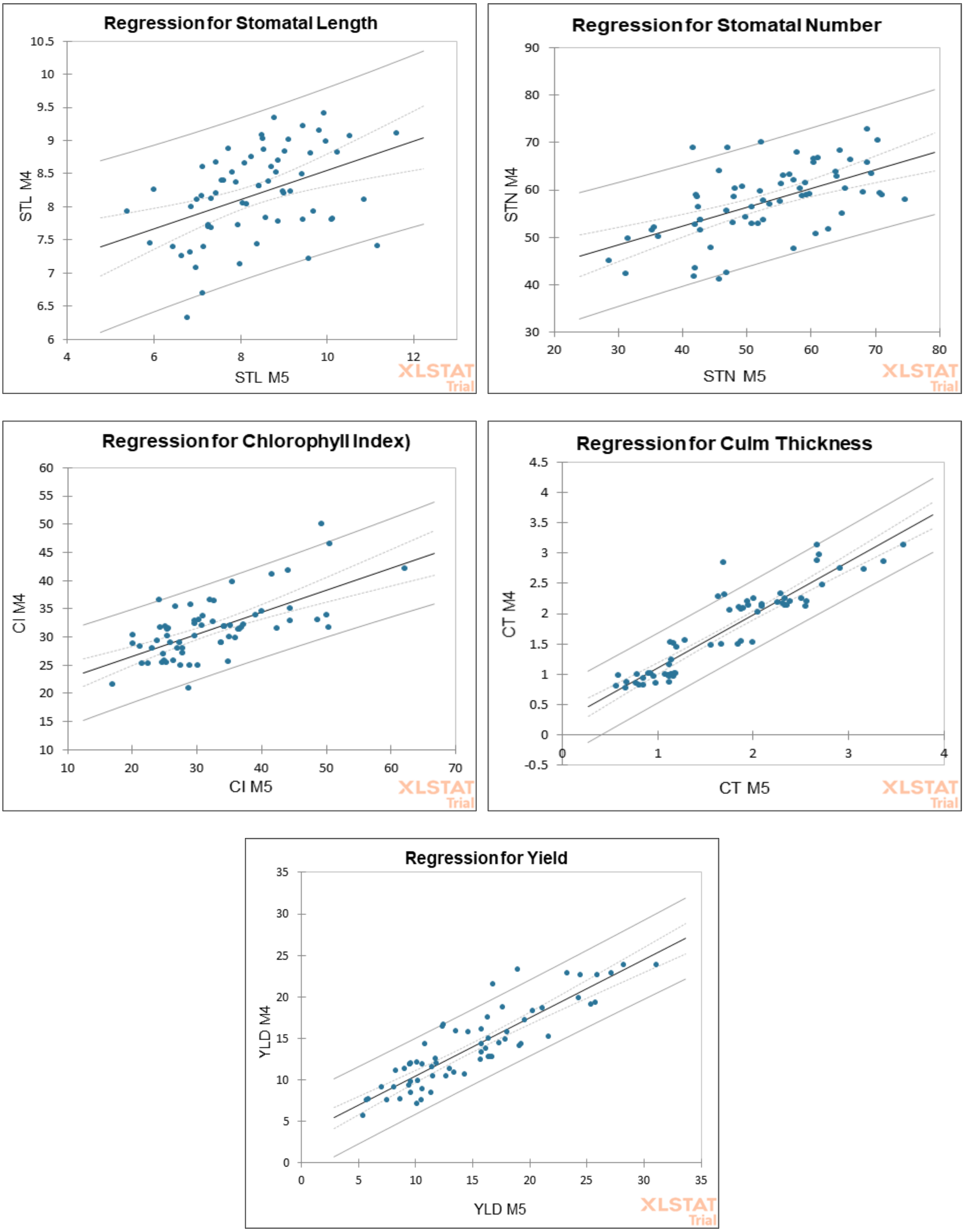

Fig 2: Parent progeny regression for Traits under study

Conclusion

Selection is the primary role of plant breeders in the improvement of crops. In this study the selection carried out to obtained genetic gain for yield, photosynthetic efficiency (Stomatal number, stomatal length and Chlorophyll index) and non-lodging (Culm Thickness). Parent progeny regression, Intergenerational correlation, narrow sense heritability, skewness, kurtosis infers the selection based on these trait in M_4 generation is rewarding in the improvement and attaining genetic gain for these traits in M_5 mutant generations. The selection will be effective in the further generation to attain homozygosity for the genes.

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