International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(2): 177-181

Received: 06-01-2019
Accepted: 10-02-2019

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Mutation studies in M_4 generation of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] for various quantitative traits

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Abstract
Mutation breeding is one of the best ways to induce genetic variability within a crop species in a short period of time. Cluster bean a leguminous crop where hybridization is a tedious and expecting less crossed seed. On other hand induction of mutation through physical irradiation is a potentially tool for its genetic improvement. The gamma radiation induced 190 M_4 mutant lines of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] obtained from Centre for Biotechnological Research (CBR), College of Horticulture, Bengaluru and which were used for the field experimentation on various quantitative traits (June - November) at College of Horticulture, Mysore. The knowledge of genetic variability and association of various characters are essential in planning of any breeding programmes. In the present study, 190 M 4 mutants of cluster bean were investigated for spectral variability for quantitative traits. The proportions of variability, broad sense heritability and genetic advance over mean were estimated. Analysis of variance revealed highly significant difference among the mutants for all the quantitative characters studied. Genetic variation as part of different component traits of yield was worked out, genotypic (GCV) and phenotypic (PCV) coefficient of variation, heritability and genetic advance over mean (GAM) were computed for all traits studied. Among all, High estimates of GCV and PCV were found for plant height (cm), branches per plant, pod length (cm), days to 50 per cent maturity, pod width (cm), Days for harvest, pods per cluster, pod clusters per plant, pods per plant, 10 pods weight (g), pod yield per plant (g), seeds per pod, seed yield per plant (g) and 100 seed weight (g). The performance of mutants C_{84}, C_{114} and E_{179} for various quantitative traits was found good.

Keywords: Mutation, M_4 generation, *Cyamopsis tetragonoloba*, quantitative

Introduction
Selection of effective and efficient mutagens is very essential to recover high frequency of desirable mutants. Mutation induced by chemical or physical mutagens has been a choice to induce variation. This mutation has been a most useful and vital technology for vegetable cluster bean and it is always a good practice to be followed for its improvement because of various hurdles of hybridization. India is the largest producer of cluster bean and contributes 75-82% of the total cluster bean production in the world. In North Indian states *viz.*, Rajasthan, Haryana, Gujarat and Punjab (Tawar *et al.*, 1988) [16]. In Karnataka, it is being grown in small patches and is cultivated mainly in northern districts like Dharwad, Belagavi, Vijayapura and Haveri *etc*. for tender vegetable pods and it is cultivated throughout the year, The economic traits of crop are being quantitative and governed by many genes, whose expression is influenced to a greater extent by environments, exhibit a wide spectrum of phenotype. Study of such quantitative traits in upcoming vegetable crop like cluster bean is important and need of the hour. Mutants of such crops obtained through irradiation were of great significance for improvement and understand the genetics of traits. The first successful attempt of mutation induced through physical mutagens in cluster bean was carried with gamma rays using ^{60}Co as source of radiation (Vig, 1965) [17]. Considering the importance as a vegetable crop and its adaptability to arid drought conditions, there is a prime need for its improvement. The creation of variability through hybridization is very difficult because of very small and delicate flower structures, which often result in very poor seeds setting in the manually hybridized buds and higher frequency of flower drop during and after crossing. Looking at this limitation, efforts were initiated to create variability in cluster bean by using the tool of induced mutations. The present investigation was therefore undertaken to study

performance of induced M4 to identify the variability created in different traits by morphological observations.

Material and Methods

The experiment was carried out at the PG research block, College of Horticulture, Mysuru, during the year 2017- 18 involving the 190 M_4 mutant lines and three checks which were field evaluated in 8 blocks in an Augmented Block Design with repeated checks in each block. These mutants obtained from Centre for Biotechnology Research (CBR) Department of BCI, COH, Bengaluru were planted at a spacing of 45 x 25 cm on 14th of June 2017. The experiment was laid out following the recommended package of practices of UHS, Bagalkot for cluster bean (Anonymous 2016) [3]. The data was recorded on number of days to 50 per cent flowering, number of days to 50 per cent maturity, number of days to harvest, plant height, number of branches per plant, pod breadth, pod length, number of pods per cluster, number of clusters per plant, number of pods per plant, ten pods weight (g), pod yield per plant (g), seeds per pod, seed yield per plant (g), 100-seed weight (g). All the data collected were subjected to analysis for drawing the conclusion.

Results and Discussion

The experiment was undertaken to elucidate variability in 190 M_4 mutant lines and to document the "Spectral variability for quantitative traits in induced mutant population of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.]" The field studies were carried out at College of Horticulture Mysuru during the year 2017. The M_4 mutant lines of cluster bean field experimented in Augmented Block Design (ABD). The analysis of variance of the experiment indicated that mean sum of squares of different quantitative characters for 190 M_4 mutant lines was highly significant for all characters *viz.*, plant height (cm), branches per plant, pod length (cm), days to 50 per cent maturity, pod width (cm), days for harvest, pods per cluster, pod clusters per plant, pods per plant, 10 pods weight (g), pod yield per plant (g), seeds per pod, seed yield per plant (g), 100 seed weight (g), except for days to 50 per cent flowering. Significant mean sum of squares due to pod yield and attributing characters revealed existence of considerable variability in material studied for further improvement for various traits. The results of analysis of variance are given in Table 1.

Variability present in these characters was assessed through a simple approach of examining the range of variation. The study indicated presence of sufficient amount of variation among the accessions for all the characters studied. These results were in accordance with Dabas *et al.* (1982) [5], Anila and Balakrishnan (1990) [2], Hanchinamani (2004) [8], Saini *et al.* (2010) [15] and Girish *et al*. (2012) [7], in all these studies different sets of cluster bean genotypes were field evaluated. The amount of phenotypic variability, which is not reliable, is reflected by the range in the values, since it includes genotypic, environmental and genotype x environment interaction components and does not reveal as to which character is responsible for higher degree of variability. Further, the phenotype is influenced by additive gene effect (heritable), dominance (non-heritable) and epistasis (non-allelic interaction). Hence, it becomes necessary to split the observed variability into phenotypic coefficient of variation and genotypic coefficient of variation, which ultimately indicate the extent of variability existing for various traits. The heritability of a character can be relied upon, as it aids in deciding the extent of selection pressure to be applied. The estimation of heritability has a greater role to play in determining the effectiveness of selection of a character provided it is considered in conjunction with the predicted genetic advance as suggested by Johnson *et al.* (1955) [9]. Heritability is influenced by biometrical method, generation of accession, sample size of experimental material and environment.

The phenotypic co-efficient of variation (PCV) was higher than genotypic co-efficient of variation (GCV) for all the characters studied. Higher values of phenotypic and genotypic coefficient of variations were observed for days to 50 per cent flowering were observed by Saini *et al.* (2010) [15], plant height (cm) these results were in accordance with Anila and Balakrishnan (1990) [2], branches per plant, pod length (cm), pod yield per plant (g) were observed by Mital *et al.* (1968) [11], pod width (cm) was also reported by Hanchinamani (2004) [8], pod clusters per plant the similar variation was observed by Saindass *et al.* (1973) [14], pods per plant, pods per cluster were observed by Vijay (1988), days for harvest, 100 seed weight (g) were in accordance with Kumar and Ram (2015) [10] and Vir and Singh (2015) [18], Days to 50 per cent maturity, 10 pods weight (g), seeds per pod, seed yield per plant (g), were observed by Hanchinamani (2004) [8] and Dwivedi (2009) [6].

Differences between GCV and PCV were also found to be less for all the traits except pods per cluster indicating that these traits were less affected by environmental factors. Moderate to high values of PCV over GCV suggested also that there is a possibility of improvement through direct selection for these traits. Based on the above results, it is suggested that in different mutants, characters with high genetic variability like days to 50 per cent flowering, plant height (cm), branches per plant, pod length (cm), days to 50 per cent maturity, pod width (cm), days for harvest, pods per cluster, pod clusters per plant, pods per plant, 10 pods weight (g), pod yield per plant (g), seeds per pod, seed yield per plant (g) and 100 seed weight (g) would be responsive to selection in the positive direction. Thus, selection based on phenotypic is effective in the improvement of these traits. Similar in cluster bean possibilities were also reported by Saini *et al.,* (2010) [15] and Dwivedi (1990). Heritability estimates were high for all the characters studied in the working collections of cluster bean. Similar results were reported in previous study (Rai *et al*., 2012, Anandhi and Oommen, 2007) [13, 1] in cluster bean. According to Singh (2001), if heritability of a character is very high (70 per cent or more) selection for such traits could be fairly easy. This is because there would be a close correspondence between the genotype and the phenotype due to the relatively small contribution of the environment to the phenotype.

In the present study, high heritability coupled with high genetic advance as per cent over mean was recorded for all the characters *viz.,* days to 50 per cent flowering (h^2 = 96.70%, GAM = 36.74%), plant height (h^2 = 72.20, GAM = 43.63), number of branches per plant (h^2 = 62.70, GAM = 41.23), pod length (h^2 = 91.80, GAM = 63.74), days to 50 per cent maturity (h^2 = 99.10, GAM = 64.80), pod width (h^2 = 91.10, GAM = 62.15), days for harvest (h^2 = 99.30, GAM = 64.70), pods per cluster (h^2 = 99.60, GAM = 81.15), pod cluster per plant (h^2 = 99.60, GAM = 83.10), pods per plant (h^2 = 99.50, GAM = 96.76), 10 pods weight (h^2 = 90.40, GAM = 70.35), pod yield per plant (h^2 = 99.50, GAM = 99.10), seeds per pod (h^2 = 87.80, GAM = 65.33), seed yield per plant (h^2 = 99.30, GAM = 98.94) and 100 seed weight (h^2 = 99.00, GAM = 76.72). High heritability and high genetic advance as per cent over mean indicating predominance of additive gene component. Thus, there is ample scope for improving these characters through direct selection. days to 50 per cent maturity, 100 seed weight, days for harvest exhibited high heritability and high genetic advance as per cent over mean these were in confirmation with Vir and Singh (2015) [18]. Further, for plant height, branches per plant, pods per plant, pod yield per plant and days for 50 per cent flowering traits behaved similar findings of Rai *et al*. (2012) [13], Anandhi and Oommen (2007) [1] and Saini *et al.* (2010) [15]. Results of pod per cluster, pod cluster per plant are supported by Saini *et al.* (2010) [15], Backiyarani and Nandarajan (1996) [4]. With respect to yield and component parameters the characters like pods per plant, pod yield per plant and seed yield per plant recorded high heritability coupled with high genetic advance over mean in comparison with the all other characters. This indicates existence of additive components for these traits. Thus, there is ample scope for direct selection for these characters. The results were in accordance with Rai *et al*. (2012) [13], Hanchinamani (2004) [8], and Narayanankutty *et al.* (2003) [12]. Johnson *et al.* (1955) [9] suggested that high heritability combined with high genetic advance as per cent mean is indicative of additive gene action and selection based on these parameters would be more reliable. Studies revealed that both the additive and non-additive genes have important role in the expression of almost all the traits in clusterbean. High value of heritability coupled with genetic advance was observed for all the characters *viz.*, days to 50 per cent flowering, plant height (cm), number of branches per plant, pod length (cm), days to 50 per cent maturity, pod width (cm), days for harvest, pods per cluster, pod cluster per plant, pods per plant, 10 pods weight (g), pod yield per plant (g), seeds per pod, seed yield per plant (g) and 100 seed weight (g). But among all the characters pods per plant, pod yield per plant and seed yield per plant exhibited high heritability coupled with high genetic advance (the values are >99% for heritability and >95% for GAM). Similarly, results were obtained by Narayanankutty *et al.* (2003) [12] and Hanchinamani (2004) [8].

Table 1: Analysis of variance (ANOVA) for different quantitative traits in M_4 mutants of cluster bean

Source of variation	DF	Mean Sum of Squares														
		X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}
Blocks	7	84.57	619.43	30.42	11.87	231.61	0.06	382.21	3.10	17.68	701.24	79.66	16827.24	4.92	55.34	2.41
Entries	192	25.47	252.23**	21.33**	10.72**	226.82**	0.08**	375.58**	5.84**	22.52**	1367.91**	181.02**	10157.84**	4.21**	47.55**	2.78**
(a) Checks	2	60.17**	187.81	564.80**	56.39**	628.87**	0.08**	1593.38**	22.55**	23.83**	926.84**	138.37**	46784.33**	20.00**	45.38**	1.20**
(b) Varieties	189	27.20	208.36	11.53**	10.69**	230.98**	0.09**	377.69**	5.75**	23.23**	1402.33**	180.69**	10319.21**	4.07**	42.68**	2.64**
(c) Checks vs. Varieties	1	-370.20	8672.34**	786.75**	-75.04**	-1364.44**	-0.30	-2458.11**	-12.11**	-114.04**	-4255.71**	329.48**	-93593.83**	0.61	973.56**	32.85**
Error	14	18.74	167.64	4.05	0.71	22.73	0.00	13.72	0.85	1.33	191.70	13.61	1447.93	0.86	1.30	0.14
CV (%)		15.13	23.61	16.39	8.44	9.93	7.23	6.01	15.20	9.69	17.43	10.04	18.42	16.09	8.54	8.55

* Significant at 5% level of significance ** Significant at 1% level of significance

X_1=Days for 50% flowering	X_6=Pod width (cm)	X_{11}=Ten pods weight (g)
X_2=Plant height (cm)	X_7=Days for harvest	X_{12}=Pod yield/plant (g)
X_3=Branches/plant	X_8=Pods/cluster	X_{13}=Seeds/pod
X_4=Pod length (cm)	X_9=Pod clusters/plant	X_{14}=Seed yield/plant (g)
X_5=Days for 50% maturity	X_{10}=Pods/plant	X_{15}=100 seed weight (g)

Table 2: Estimates of variability for various quantitative traits among the M_4 mutants of cluster bean

	Traits	Mean	Range		PCV (%)	GCV (%)	h^2	GA as % of mean
			Minimum	Maximum				
			26.00	38.00				
1	Plant height (cm)	54.85	33.80	96.60	29.57	25.13	72.20	43.63
2	Branches/plant	12.27	6.80	23.00	31.68	25.08	62.70	41.23
3	Pod length (cm)	9.96	0.00	13.85	33.76	32.36	91.80	63.74
4	Days for 50% maturity	48.00	0.00	58.00	31.75	31.61	99.10	64.80
5	Pod width (cm)	0.92	0.00	1.17	33.64	31.15	91.10	62.15
6	Days for harvest	61.58	0.00	75.00	31.66	31.54	99.30	64.70
7	Pods/cluster	6.07	0.00	11.50	39.59	39.52	99.60	81.15
8	Pod clusters/plant	11.91	0.00	20.10	40.49	40.41	99.60	83.10
9	Pods/plant	79.46	0.00	164.05	47.18	47.06	99.50	96.76
10	Ten pods weight (g)	36.76	0.00	62.70	37.72	35.87	90.40	70.35
11	Pod yield/plant (g)	206.62	0.00	368.72	49.17	49.05	99.50	99.10
12	Seeds/pod	5.77	0.00	8.40	36.72	34.40	87.80	65.33
13	Seed yield/plant (g)	13.33	0.00	29.16	48.68	48.50	99.30	98.94
14	100 seed weight (g)	4.33	0.00	7.92	37.45	37.27	99.00	76.72

h^2 - Broad sense heritability, GCV - Genotypic co-efficient of variation
PCV - Phenotypic co-efficient of variation, GAM - Genetic advance as per cent of mean

Summary and Conclusion

In the present study, 190 M 4 mutants of cluster bean were investigated for spectral variability for quantitative traits. Analysis of variance revealed highly significant difference among the mutants for all the quantitative characters studied. Genetic variation as part of different component traits of yield was worked out viz genotypic (GCV) and phenotypic (PCV) coefficient of variation, heritability and genetic advance over mean (GAM) were computed for all traits studied. High estimates of GCV and PCV were found for plant height (cm), branches per plant, pod length (cm), days to 50 per cent maturity, pod width (cm), Days for harvest, pods per cluster, pod clusters per plant, pods per plant, 10 pods weight (g), pod yield per plant (g), seeds per pod, seed yield per plant (g) and

100 seed weight (g). These characters also showed narrow difference between GCV and PCV, indicating that they are seldom affected by the environment. The performance of mutants $C_{84,}$ C_{114} and E_{179} for various quantitative traits was found good.

Acknowledgements
Authors are thankful to Dean Dr. G. Janardhan and Farm superintendent Dr. Yathindra at College of Horticulture, Mysuru, Karnataka, Dr. Mukesh L. Chavan, Profesor and Head Department of Biotchnology and Crop Improvement, Dr. V. Nachegowada, Director of Research UHS Bagalkot, Dr. N. Basavaraja Dean (Post Graduate Studies) at UHS Bagalkot for their support during the time of this investigation, Authors are also thankful to all the III B.Sc.(Horti) students of College of Horticulture, Mysore for extending timely help, The first author dedicated all her success to her beloved parents. Her journey in this world paves through the path curved by her lovable father Sri. Manjappa, B. The first author also found immeasurable amount of sacrifices, love, affection, support and concern from her mother Smt. Kamala, O., The first authors gratitude is beyond words to her lovely sister Monika, N. M. and dearest brother Siddarth, N. M. (ricky) for their unselfish struggle towards her wellbeing and attainment of perfection in carrier goals.

Appendix: The details of various M4 cluster bean mutants used in the investigation

S. No.	Reference code	Mutant name	Sl. No.	Reference code	Mutant name	Sl. No.	Reference code	Mutant name
1.	A_1	80-17-1	2.	B_{63}	100-ST80-17	3.	D_{125}	100-16-6
4.	A_2	80-3-2	5.	B_{64}	100-10-17	6.	D_{126}	100-5-12
7.	A_3	80-24-3	8.	B_{65}	100-5-7	9.	D_{127}	100-5-15
10.	A_4	80-20-3	11.	B_{66}	100-10-4	12.	D_{128}	100-5-8
13.	A_5S	80-28-3	14.	B_{67}	100-5-14	15.	D_{129}	100-5-10
16.	A_6	80-6-6	17.	B_{68}	100-1-2	18.	D_{130}	100-22-7
19.	A_7	80-9-5	20.	B_{69}	100-ST80-23	21.	D_{131}	100-25-2
22.	A_8	80-6-5	23.	B_{70}	100-1-10	24.	D_{132}	100-10-3
25.	A_9	80-3-4	26.	B_{71}	100-1-5	27.	D_{133}	100-25-3
28.	A_{10}	80-17-5	29.	B_{72}	100-22-1	30.	D_{134}	100-2-3
31.	A_{11}	80-19-2	32.	B_{73}	100-21-2	33.	D_{135}	100-16-5
34.	A_{12}	80-17-2	35.	B_{74}	100-2-4	36.	D_{136} S	100-26-6
37.	A_{13}	80-18-17	38.	B_{75}	100-ST80-27	39.	D_{137}	100-26-1
40.	A_{14}	80-27-1	41.	B_{76}	100-ST80-3	42.	D_{138}	100-5-9
43.	A_{15}	80-17-3	44.	B_{77}	100-22-3	45.	D_{139}	100-25-5
46.	A_{16}S	80-18-3	47.	B_{78}	100-5-1	48.	D_{140}	100-5-2
49.	A_{17}	80-9-2	50.	B_{79}	100-75-7	51.	D_{141}S	100-26-4
52.	A_{18}	80-18-7	53.	C_{80}	100-5-13	54.	D_{142}	100-10-6
55.	A_{19}	80-3-1	56.	C_{81}	100-2-5	57.	D_{143}	100-21-1
58.	A_{20}	80-18-2	59.	C_{82}	100-2-7	60.	D_{144}	100-22-8
61.	A_{21}	80-3-3	62.	C_{83}	100-21-5	63.	D_{145}	100-10-1
64.	A_{22}	80-6-2	65.	C_{84}	100-22-6	66.	D_{146}	100-1-8
67.	A_{23}S	80-9-4	68.	C_{85}	100-2-6	69.	D_{147}	100-25-9
70.	A_{24}	80-28-7	71.	C_{86}	100-10-8	72.	D_{148}	100-2-1
73.	A_{25}	80-28-6	74.	C_{87}	100-26-5	75.	D_{149}	100-1-9
76.	A_{26}	80-23-3	77.	C_{88}	100-1-4	78.	D_{150}	100-5-3
79.	A_{27}	80-19-1	80.	C_{89}	100-16-7	81.	D_{151}	100-5-16
82.	A_{28}	80-20-6	83.	C_{90}	100-26-8	84.	D_{152}	100-25-1
85.	A_{29}	80-28-1	86.	C_{91}	100-16-3	87.	D_{153}	100-5-17
88.	A_{30}	80-24-5	89.	C_{92}	80-24-1	90.	D_{154}	100-10-2
91.	A_{31}	80-28-2	92.	C_{93}S	80-9-3	93.	D_{155}	100-2-2
94.	A_{32}	80-20-4	95.	C_{94}S	80-6-4	96.	D_{156}	100-ST80-22
97.	A_{33}NP	80-27-5	98.	C_{95}NP	80-18-5	99.	D_{157}	100-ST80-1
100.	A_{34}	80-20-2	101.	C_{96}	80-6-3	102.	D_{158}	100-ST80-4
103.	A_{35}	80-20-1	104.	C_{97}	80-17-4	105.	D_{159}NP	100-1-7
106.	A_{36}	80-27-2	107.	C_{98}	80-9-1	108.	D_{160}	100-25-9
109.	A_{37}	80-23-2	110.	C_{99}	80-3-5	111.	D_{161}	100-10-5
112.	A_{38}	80-27-3	113.	C_{100}	80-P58-7	114.	D_{162}	100-25-4
115.	A_{39}	80-27-4	116.	C_{101}	80-P58-9	117.	D_{163}	100-1-1
118.	B_{40}	100-21-3	119.	C_{102}S	80-P58-8	120.	D_{164}	100-16-1
121.	B_{41}	100-ST80-14	122.	C_{103}	100-P3-80-1	123.	D_{165}	100-16-2
124.	B_{42}	100-21-4	125.	C_{104}	100-P3-80-4	126.	E_{166}	100-MS2-5
127.	B_{43}	100-ST80-8	128.	C_{105}	100-P3-80-2	129.	E_{167}	100-MS2-3
130.	B_{44}	100-ST80-28	131.	C_{106}	100-P3-80-3	132.	E_{168}	100-MS2-1
133.	B_{45}	100-ST80-12	134.	C_{107}	100-A80-4	135.	E_{169}	100-MS2-2
136.	B_{46}	100-ST80-20	137.	C_{108}	100-A80-2	138.	E_{170}	100-MS2-4
139.	B_{47}	100-ST80-16	140.	C_{109}NP	100-A80-1	141.	E_{171}	100-MS2-6
142.	B_{48}	100-ST80-24	143.	C_{110}	100-A80-5	144.	E_{172}	80-P58-11
145.	B_{49}	100-ST80-7	146.	C_{111}	100-A80-3	147.	E_{173}	80-P58-3
148.	B_{50}	100-ST80-13	149.	C_{112}	100-PNB-1	150.	E_{174}	80-P58-4
151.	B_{51}	100-25-8	152.	C_{113}	100-PNB-2	153.	E_{175}	80-P58-10
154.	B_{52} S	100-5-19	155.	C_{114}	100-PNB-3	156.	E_{176}	80-P58-1

157.	B_{53}	100-ST80-21	158.	C_{115}	100-PNB-4	159.	E_{177}	80-P58-5
160.	B_{54}	100-ST80-15	161.	C_{116}	P58-7	162.	E_{178}	80-P58-2
163.	B_{55}	100-ST80-10	164.	C_{117}	100-ST80-5	165.	E_{179}	80-P58-6
166.	B_{56}	100-22-2	167.	C_{118}	100-ST80-9	168.	E_{180}	80-MN2-2
169.	B_{57}	100-ST80-19	170.	C_{119}	100-ST80-3	171.	E_{181}	80-MN2-6
172.	B_{58}	100-10-11	173.	C_{120}NP	100-ST80-2	174.	E_{182}	80-MN2-7
175.	B_{59}	100-ST80-25	176.	C_{121}	80-MN3-6	177.	E_{183}	80-MN2-1
178.	B_{60}S	100-ST80-18	179.	C_{122}	80-MN3-3	180.	E_{184}	80-MN2-4
181.	B_{61} S	100-ST80-6	182.	D_{123}	100-1-6	183.	E_{185}	80-MN2-5
184.	B_{62}	100-ST80-11	185.	D_{124}S	100-26-7	186.	E_{186}	80-MN3-5
187.	E_{188}	80-MN3-2	188.	E_{189}	80-MN3-1	189.	E_{187}	80-MN3-7
190.	E_{190}	80-MN3-3	191.			192.		

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