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Short communication

Genetic improvement of fodder cowpea through induced mutagenesis

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Abstract

The study was focused to develop superior fodder cowpea variety for fodder and seed yield through induced mutagenesis. For this, the seeds of fodder cowpea variety Aiswarya were taken and mutation was induced using ethyl methane sulphonate (EMS). Effective dosages were fixed based on lethal dose 50 (LD_{50}) and M_1 and M_2 populations were grown. There were nine treatments and one control. Both quantitative and qualitative traits were recorded and analyzed in M_2 generation. On analysis, green fodder yield and dry matter yield was found enhanced way better than control. All the quantitative traits like number of primary branches per plant, number of leaves per plant, days to first flowering, days to 50% flowering, green fodder yield per plant, dry matter yield per plant, leaf stem ratio, leaf dry weight per plant, stem dry weight per plant, leaf area index, seed yield and days to maturity except plant height showed significant variation in the mutated population. The results were comparable to control, for seed yield. From these observations, 0.43% EMS treatment was found to be the best treatment for developing dual purpose fodder cowpea. This treatment could be carried over for further generations and released as varieties or can be used as parents for future breeding programs.

Keywords: Dual purpose, EMS, Fodder cowpea, Green fodder, M_2 generation

Considering the land availability and climatic requirement of Kerala, fodder cowpea is one of the preferable choices for cultivation. Cowpea is primarily a food crop, but it can also be used as animal feed. Cowpea provides high quality protein rich fodder for livestock (Murdock *et al*., 2008). Generally, the green fodder yield of cowpea is around 30 to 32 tons/ha with crude protein 18-20%. The crop has good tolerance to shade, grows quickly and has fast ground covering ability (Anita *et al*., 2018). Besides, it is an excellent green manure crop and can be used for intercropping with maize (Gupta *et al*., 2019). Good palatability with more than 70 percentage digestibility, low fibre content and minimum wastage makes cowpea a good fodder crop. The genetic diversity of fodder cowpea is narrow because of self-pollination. So in order to meet the demands of the growing livestock population, research works for improving the desirable characters of fodder cowpea are required.

Mutation breeding is one of the common tools used for generating variability in the existing germplasm. Ethyl methane sulphonate (EMS) is a mono-functional alkylating agent, which can cause alteration in the DNA (Leitao, 2011). Mutants derived from these mutagens create variations and among them desirable lines can be selected for future breeding aspects. The present study was envisaged to induce mutation in seeds of fodder cowpea variety Aiswarya and to identify superior dual-purpose genotypes for higher fodder yield and seed yield from the mutants. Aiswarya is a fodder cowpea variety released in 2013 and developed by Kerala Agricultural University (Gopalakrishnan and Devdas, 2014). It has high fodder yield, better quality with crude protein content of 18.5%. The seeds were treated with different concentrations of EMS (0.1%, 0.2%, 0.3%, 0.4%, and 0.5%). Based on the LD_{50} value, effective doses were determined. Three hundred seeds per treatment were pre-soaked in distilled water for six hours and treated with effective doses of EMS (0.35%, 0.37%, 0.39%, 0.41%, 0.43% 0.45%, 0.47%, 0.49%, 0.51%) for another six hours. After thorough washing, seeds were sown in the field with three replications in randomized block design with the spacing of 30 cm between rows and 15 cm between plants. The field study was conducted during January to June 2021 in College of Agriculture, Vellayani, India. The seeds collected from the treatments of M_1 generation constituted M_2 generation. Five hundred plants were maintained in each treatment. The M_2 generation was evaluated for various quantitative and qualitative characters.

Quantitative traits like number of primary branches per plant, number of leaves per plant, days to first flowering, days to 50% flowering, green fodder yield per plant, dry matter yield per plant, leaf stem ratio, leaf dry weight per plant, stem dry weight per plant, leaf area index, plant height, seed yield per plant and days to maturity were recorded (Table 1 and 2). Qualitative characters were scored based on Roy *et al*. (2017). The characters studied were early plant vigour, plant growth habit, leaf texture, leaf colour and flower colour.

From germination and survival rate, LD_{50} value was estimated. M_1 generation was mainly observed for survival rates. Delayed germination was observed in mutated treatments. Among the M_1 population, the number of pods per plant was found higher in control, while seed yield was maximum in 0.51% EMS (Nair and Gayathri, 2022). Biometric characters were recorded and two-way ANOVA with more than one observation per cell (without interaction) was used for the analysis of M_2 generation. All the characters except plant height showed significant variation in treatment means. Induction of mutation affected many plant traits. The number of primary branches was found higher in 0.43% EMS treatment. This was on par with 0.47%, 0.45% and control. Wani and Khan (2006) also observed that the number of fertile branches varied in a positive direction in mung bean.

Mutation affected the number of leaves per plant significantly. Maximum number of leaves per plant was recorded in 0.43% EMS treatment, followed by 0.45% and control (Table 1). Green fodder yield was reported to be highly correlated with number of leaves per plant (Ahmed *et al*., 2013; Kapoor, 2014). Kham *et al*. (2015) also noted variation in the number of leaves of plants in a mutated population. Early flowering was observed in control and low concentration treatments. High concentration treatments like 0.49%, 0.47% and 0.51% showed delayed onset of flowering. Similar results were observed for days to 50% flowering. Early flowering among mutant lines of butterfly pea were reported by Zulchi *et al*. (2022).

Green fodder per plant (g) was higher in 0.43% and 0.45% EMS treatments than in control. In the untreated population, the treatment mean was about 151g plant^{-1}. But in the treated population, some plants weighed about 440g, 260g, etc. Almost double the fodder yield than control was recorded in 0.43% of EMS. According to the estimation, it gave about 40 t/ha green fodder against 29.92 t/ha of control. The variation of green fodder yield per plant in mutated population was noted in various crops like berseem and rice bean (Usha, 2006; Dash, 2012).

Table 1. Range values of characters in M_2 generation progenies

Treatment	NPB	NLP	DFF	DFif	GFY (g)	DMY (g)
Control	0-5	33-117	34-38	37-41	120-200	11-32
0.35% EMS	0-5	18-87	35-38	37-41	60-40	5-16
0.37% EMS	0-5	24-78	35-37	38-40	50-120	3-13
0.39% EMS	0-4	18-51	34-38	37-41	60-125	5-13
0.41% EMS	0-4	30-72	34-39	38-42	50-180	5-15
0.43% EMS	0-6	58-144	35-39	38-43	80-260	14.5-33
0.45% EMS	0-6	21-183	35-39	38-42	60-440	4-51
0.47% EMS	2-5	30-138	37-42	42-46	100-200	11-21
0.49% EMS	0-5	27-84	34-42	39-46	60-160	4-15
0.51% EMS	0-3	24-81	35-43	38-45	40-160	2-18
F stat	6.02**	11.74**	9.55**	23.20**	19.03**	21.38**
P value	0.00	0.00	0.00	0.00	0.00	0.00
C D	1.04	15.85	1.04 1.05	1.05	27.74	3.54
MSE	2.08	481.91	2.09	2.14	1476.4	24.03
SE(m)	0.37	5.67	0.37 0.38	0.38	9.92	1.27
SE(d)	0.53	8.02	0.53	0.53	14.03	1.79
CV(%)	53.86	35.97	3.91	3.6	29.24	35

NPB: Number of primary branches plant^{-1}; NLP: Number of leaves plant^{-1}; DFF: Days to first flowering; DFif: Days to fifty percent flowering; GFY: Green fodder yield plant^{-1}; DMY: Dry matter yield plant^{-1}

Yadav (2016) recorded a stimulatory effect at smaller doses of mutagen, while higher doses were inhibitory. Similar pattern of results was observed in dry matter yield per plant.

Leaf dry weight per plant was found highest in 0.43% EMS treatment, followed by control and 0.45% EMS treatment (Table 2). Comparable results were obtained in stem dry weight per plant. Both 0.43% and 0.45% EMS treatments recorded higher mean than control. Leaf area index (LAI) was found higher in 0.45% and 0.43% EMS treatments and followed by control. LAI is related directly to gross photosynthesis, evapo-transpiration, canopy interception (Fang and Liang, 2014), carbon and nutrient use (Breda, 2008). Abou El-Yazied (2011) recorded increased leaf area in mutated snapbean population. Mutation did not create significant variation in the plant height of the population. Regardless of bushy control plants, the mutagenized plants showed trailing and semi-trailing plants.

Among the treatments, seed yield was found higher in control and 0.51% EMS. This was on par with 0.43% EMS. In the study, seed yield decreased in M_2 compared to M_1. Basu *et al*. (2008) reported that the variation might be caused by the detrimental effects of EMS, which might pass on to the next generations. Nguyen *et al*. (2021) reported direct and positive effect of biological yield per plant on seed yield of cowpea. All the mutated treatments showed late maturing plants. The plants that showed stunted growth and reduced flowers recorded delayed maturation. Mishra *et al.* (2018) also recorded the presence of late maturing plants due to mutation in rice bean.

Similar to quantitative traits, qualitative characters also showed variation in the mutagenized population. The EMS concentration 0.35%, 0.43% and 0.47% treated population recorded better early plant vigour than higher concentration mutants. Olasupo *et al*. (2016) observed the reduction of seedling vigour of mutants, but Khan and Tyagi (2013) found an increase in plant vigour among mutants. Plant growth habits varied from bushy to semi-erect and semi-trailing in M_2, whereas the untreated plant population appeared as luxuriously bushy. Arulbalachandran and Mullainathan (2009) reported similar results in black gram. On the contrary, the presence of a lesser number of dwarf and bushy statured plants in the EMS treated population of green gram was recorded by Vairam and Ibrahim (2014). Leaf texture was smooth for untreated plants, but both rough and smooth textured leaves were found in mutated populations. Presence of thick leaves was noticed among cowpea mutants by Preethi and Muthuswamy (2020).

Control showed green colour leaves whereas in the treated population, there were variations regardless of concentration. Variations in leaf colour due to mutation were reported earlier by Premnath *et al*. (2010), Yadav (2016) and Taziun *et al*. (2018). Flower colour did not show any variation among the control and mutated population. Chlorophyll mutants appeared in both M_1 and M_2, but the frequency of chlorophyll mutants was lesser in M_2

Table 2. Range values of characters in M_2 generation progenies

Treatment	LDW (g)	SDW (g)	L:S	LAI	PH (cm)	SY (g)	DM
Control	7-17	2.5-13	1-3.1	4.64-9.81	40-82	14.1-18.9	72-76
0.35% EMS	3-10	1-7	1-4	4.01-9.20	33-124	9.7-16.2	73-78
0.37% EMS	2-8	1-5	1-3.5	2.04-13.10	36-110	6.5-10.4	72-79
0.39% EMS	4-8	1-5	1-3	2.05-6.24	33-108	2.7-5.2	75-79
0.41% EMS	4-9	1-9	0.667-4	2.26-7.62	40-131.5	4.8-6.9	73-79
0.43% EMS	11-18	5.2-13.2	1.121-3.135	2.03-15.82	49-105	10.8-19.8	76-84
0.45% EMS	3-26	2-26	1-3	6.28-15.12	39.5-112	2.2-5.2	73-82
0.47% EMS	6-13	3-10	1-2.667	2.04-7.08	38-138	2.4-5.6	73-86
0.49% EMS	3-10	1-7	1.3-3	2.16-6.52	49-130	3.9-6.5	76-84
0.51% EMS	1-11	1-8	0.875-2.5	3.70-10.40	24-128	9.8-19.6	75-92
F stat	19.03**	21.38**	35.91**	14.43**	1.58^{NS}	13.71**	175.048**
P value	0	0	0	0	0.13	0	0
CD	27.74	3.54	1.69	1.74	-	1.87	1.142
MSE	1476.4	24.03	5.47	5.78	0.35	6.68	2.5
SE(m)	9.92	1.27	0.6	0.62	0.15	0.67	0.408
SE(d)	14.03	1.79	0.85	0.88	0.22	0.94	0.577
CV(%)	29.24	35	27.65	42.49	37.04	3.32	17.252

LDW: Leaf dry weight; SDW: Stem dry weight; L:S: Leaf to stem ratio; LAI; Leaf area index; PH: Plant height; SY: Seed yield $plant^{-1}$; DM: Days to maturity than M_1 and the majority of the mutants were viable. Chlorophyll mutants are considered as an indicator for mutation. Adekola and Oluleye (2007) reported that mutation resulted in the elevation in the protein content and dry matter content of cowpea. Similarly, variations in seed coat colour (Gaafar *et al*., 2016), seed yield (Raina *et al*., 2022), flower colour (Girija and Dhanavel, 2013), number of pods per plant, pod length (Badr *et al*., 2014), early maturity (Dorvlo *et al*., 2022) of cowpea were recorded.

It was concluded that induced mutation using EMS was effective to enhance both fodder quantity and seed yield in fodder cowpea variety Aiswarya. In the present study, 0.43% and 0.45% EMS treatments provided extraordinarily high fodder yield. The estimated yield was nearly 10 tonnes per hectare more than the control. Considering the seed yield, 0.51% EMS and 0.43% showed better seed yield near to control. So for developing higher fodder yield varieties of fodder cowpea, 0.43% and 0.45% EMS treatments could be used. Similarly, for seed purpose 0.51% EMS treatment could perform well, or higher concentrations of EMS treatments could be further studied. Thus for a dual-purpose variety, 0.43% EMS treatment was the promising one. This mutant population could be carried over for further generations and released as varieties or they could be used as parents for future breeding programs.

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