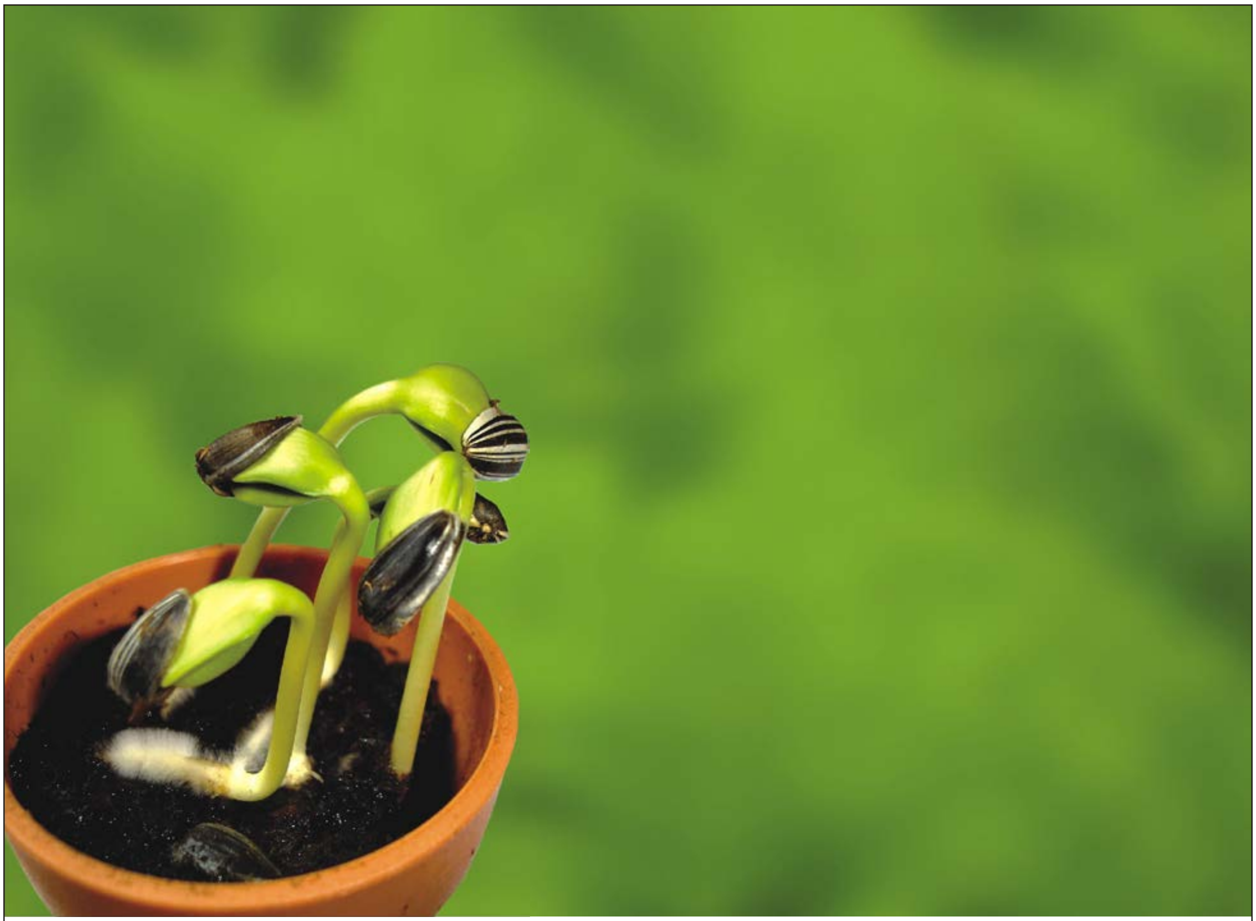

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Studies of Leaflet Mutants in Mungbean (*Vigna radiata* (L.) Wilczek)

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Abstract: The aim of the current study is investigation of multiple leaflets in mungbean and to study the effects of multiple leaflets to the desirable quantitative characters through correlation studies. Two multiple leaflet mutants were obtained from γ-irradiation and used to study correlation with the agronomic features. The higher doses of gamma ray irradiation in mungbean will provide enough scope to develop a wide range of variation in many desirable characteristics like increase in leaflets/leaf and variation in shape and size of leaf lamina. Increase in multi-foliation may increase the biomass production, which could make a positive impact on seed yield by increasing the transfer of assimilate from source to sink. Most of the variation in the lamina shape was observed in the trifoliate leaves of higher doses of gamma ray irradiation, which turned into tetrafoliate or pentafoliate with much variation in shape and size and lamina like crumpled, lanceolate, ovate, lobed were observed in both the varieties. Observation of mutants like multifoliate may be progressed in the M_2 generation through directed selection and these stable mutants might be used as donors for restructuring mungbean genotypes. Higher values for phenotypic and genotypic coefficient of variability for number of leaves suggest that there is a possibility for improving seed yield through direct selection for number of leaves with pods/plant and plant height.

Key words: Induced mutation, γ-irradiation, mungbean

INTRODUCTION

Mutation breeding is a breeding technique that creates variability in the mutated population through heritable changes in the genotypic and phenotypic utilized for effective selection of a particular trait. The effective selection of a trait through mutagenesis is possible when the genotypic component (heritable) will be higher than the environmental (non heritable) component with high genetic advance of the trait to next generation (Ahloowalia *et al*., 2004; Jacobsen and Schouten 2007; Jain, 2005; Julio *et al*., 2008; Schouten *et al*., 2006). Mutagenesis could be possible through physical mutagens such as gamma rays, ultraviolet or x-rays and also through chemical mutagen (Chopra, 2005).

Several authors previously reported the use of morphological mutants in legumes in the commercial breeding programs that was considered as valuable genetic resources for crop improvement (Begum *et al*., 2008; Gaur *et al*., 2008; Gaur and Gour, 2003; Khattak *et al*., 2001, 2003; Sadiq *et al*., 2006; Sangsiri *et al*., 2005; Srinivasan *et al*., 2008), respectively. Induced genetic variability in Mungbean is considered as low compared to other crops and could enhance the genetic diversity and can create complimentary genetic resource of mungbean.

Only a few studies have been taken place that focus on influence of leaf in crop improvement (Soehendi *et al*., 2006 a, b; Steiner and Bañuelosb, 2003; Sung and Chen, 1989; Yeates *et al*., 2000), respectively. Trifoliate leaf with three leaflets in each leaf is a usual condition in mungbean. Though mungbean is trifoliate in nature, increase in multifoliation may increase the biomass production (including spreading trailing mutant type) and could make a positive impact on seed yield if the translocation activity in these genotypes were increased by genetic manipulation (Chopra, 2005; Khattak *et al*., 2001; Sharma-Natu and Ghildiyal, 2005). Previously Manchanda and Garg (2008) and Nandwal *et al*. (2000) also indicated the better functioning of the nodules of pentafoliate mutant than the trifoliate in the mungbean genotype K851. Sung and Chen (1989) indicated that mutant narrow leaflet canopies have increased the light penetration in Soybean. Whilst Soehendi *et al*. (2006a, b) reported that narrow leaf character in mungbean appears to be governed by two recessive genes symbolized by nl_1 and nl_2, respectively. It is well know that correlations between leaflets and quantitative characters represent co-ordinations of physiological process which is often achieved through linkage of genes controlling the different processes (Chen *et al*., 2007; Humphry *et al*., 2005; Lawn and Rebetzke, 2006), respectively.

The objectives of this experiment were to investigate the visible multiple leaflets in mungbean using gamma radiation and to study the effects of multiple leaflets to the desirable quantitative characters through correlation studies to more than one type of mutagen treated doses with a view to investigate any significant changes in the existing correlations between multiple leaflets and yield components.

MATERIALS AND METHODS

An experiment was conducted with an aim to develop novel mungbean mutants during the season 2000-2001 and 2001-2002 at the Department of Plant Breeding, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India. One hundred uniform and healthy seeds of mungbean K851 treated with gamma rays. About 400 treated and control (untreated) seeds were sown in the field and seeds of M_1 plants and control were harvested separately and planted in plant progeny rows in M_2 generation.

Uniform healthy and dry seeds of mungbean K851 were exposed to 10, 20, 30 and 40 Gy doses of gamma rays from Cobalt 60 at the Central Research Institute of Jute and Allied fibres (CRIJAF) Barrackpore, West Bengal, India. The variety K851 was selected from the cross of Amrit/Pusa Baisakhi for its early maturing property of 55-60 days after sowing. The seeds were shown during February 2001 for M_1 generation and February 2002 for M_2 generation. A spacing of 30 cm row to row and 15 cm plant to plant were maintained. A total of 6000 seeds were used for sowing in the M_1 generation (2001) of which plants 2468 for K851 were survived to produce seeds. Gamma ray treated seeds from all M_1 generation plants of each treatment were harvested separately and were bulked to grow a representative bulk M_2 generation.

Laminar variations in shape and size were found due to physical mutagenic treatment. Various macromutants on the lamina characteristics were studied and these were characterised on the basis of visual observations with respect to lobes and serration on lamina surface. Leaf multifoliation is also a type of morphological character and visible marcomutation were observed in the M_2 generation. The M_2 generation (planted in March 2002) consisted of 10000 plants consisting 80 rows of 10 m in length for K851.

Plants showing visible macro mutation such as multifoliate and variation in leaf lamina have been studied through 15 select mutants in the M_2 generation. Other morphological parameters such as variation in lamina shape, leaf orientation, variation in pod shape and size, petiole length were studied. Some other parameters that may have effect on both are plant height, internode length, number of leaves/plant was also studied in M_1 and M_2 generation. Sterility is another morphological parameter used to study the effect of mutation in M_2 generation. Variation in seed coat colour is studied on the basis of the pigmentation on seed coat. Variation in seed shape was also studied.

Statistical Analysis

Only 2 varieties with 5 treatments were used for statistical analysis. Statistical analysis (2/5 treatments) were performed through factorial random block design in M_1 generation. In M_2 generation the statistical analysis was performed simply through randomised block design. Statistical software SPSS (version 15) was used for Pearson multivariate and Spearman non-parametric test for correlation studies. Heritability of the traits was calculated as per (Tefera *et al*., 2003), where as Expected genetic advance (GA) was estimated using the formula of (Allard, 1960) as: GA = (k)(h^2) (square root of σ^2p), where h^2 and σ^2p are heritability and phenotypic variance, respectively and k is a selection differential that varies depending on the selection intensity. In the present analysis 2.06 was considered for k, which is the value for 5% selection intensity. The estimation of genotypic and phenotypic coefficient of variation was performed as per (Pliura *et al*., 2007).

RESULTS

Significant ($p<0.05$) variations due to variety and different treatments were evident in number of leaves in M_1 generation. All the treatments consistently brought improvement for this character over control. The 30 Gy proved most efficient with 14 leafs or 42 leaflets then the control with only 33 leaflets. A multifoliate variant with normal shape was isolated from one of the M_2 progenies of mungbean K851. The control plants of K851 have uneven lamina shape. Some important macro mutation like bushy, trailing, multifoliate, cluster pods, synchronous maturity and some sterile mutants were isolated in the M_2 generation. The multifoliate variant has tetrafoliate (four leaflets in a leaf) and pentafoliate (five leaflets in a leaf) leaves with the fourth and fifth apical leaflet is slightly smaller than the other leaflets. Twelve multifoliate plants were identified from 30 Gy and 40 Gy treated K851. Of the 12 multifoliate, 4 were isolated from 30 Gy treated population and 8 were isolated from 40 Gy treatment. Five plants in K851 were appeared to be sterile which were isolated from 40 Gy treated population. These sterile mutants had thick, leathery, dark green leaves and uneven lamina surface. Flower buds dropped before blooming and these plants with multifoliation were very short in height with small leaves. The difference in the range of fertility level was found wide at the highest level of treatments in both K851. Pollen fertility in the M_2 generation was higher in control than the treatments in both the varieties and fertility percentage tended to reduce to every increase in the treatment dose and pollen fertility level becomes lowest at 40 Gy in both varieties.

Variation in lamina shape was also very much found prevalent in the leaves of K851 of mungbean. Most of the variation in the lamina shape was observed in the trifoliate leaves of 40 Gy of K851 turned into tetrafoliate and pentafoliate with variation in shape and size and lamina like crumpled, lanceolate, ovate and lobed in K851. Variation in leaflets in the leaf has also resulted variation in lamina shape of leaves. Induced variation for the seed coat colour was also observed in some plants in the M_2 generation. The control plants of the K851 produced seeds of dull green colour but some plants of 10 and 20 Gy produced dark greenish with greenish black seeds. However, the seed size was normal in mutants of 30 Gy produced shiny green seed and smaller in size, while mutants of 40 Gy produced brownish green and black seeds and larger in size. Despite most morphological mutants are undesirable, some may be beneficial and could be beneficial in yield attributing characters of mungbean. Correlation studies of such morphological attiributes could be useful and visible macromutants such as multiple leaflets could be beneficial in higher yield of mungbean.

Table 1: Range, mean, standard deviation, variance and Coefficient of variation (CV) for number of leaflets and some of its attributing traits in 15 selected K851 mutants in 30 treated seeds in M_2 generation

					Mean								
	Range	Min	Max	Sum			SD	PCV (%)	Var (P)	Var (G)	GCV (%)	$h^{2(\%)}$	GA
Traits	Stat	Stat	Stat	Stat	Stat	SE	Stat	Stat	Stat	Stat	Stat	Stat	Stat
Plant height	34.19	23.35	57.54	638.43	42.561	2.374	9.195	21.6	84.559	20.872	20.872	21.821	413.371
Internode length	1.22	2.38	3.60	43.46	2.897	0.094	0.367	12.6	0.135	12.246	12.246	0.0348	0.026
No. of leaf	9.86	9.44	19.29	223.39	14.892	0.774	2.999	20.1	8.994	19.455	19.455	2.321	14.340
Secondary branch	5.68	11.16	16.84	212.10	14.139	0.450	1.744	12.3	3.044	11.921	11.921	0.785	2.823
Primary branch	1.50	2.10	3.60	42.70	2.846	0.102	0.396	13.9	0.157	13.445	13.445	0.040	0.033
Cluster/pod	10.30	8.80	19.10	180.50	12.033	0.773	2.995	24.8	8.971	24.046	24.046	2.315	14.284
Pod/cluster	0.59	4.40	4.99	69.93	4.661	0.045	0.176	3.7	0.031	3.657	3.657	0.008	0.002
Pods/plant	31.00	38.80	69.80	758.50	50.566	2.371	9.185	18.1	84.377	17.549	17.549	21.774	412.036
Pod length	0.99	5.34	6.32	88.80	5.920	0.075	0.290	4.9	0.085	4.747	4.747	0.021	0.013
Petiole length	1.22	13.43	14.65	208.95	13.929	0.069	0.269	1.9	0.072	1.866	1.866	0.018	0.010
Pod width	0.04	0.44	0.48	6.92	0.461	0.003	0.012	2.6	0.000	2.605	2.605	3.99564E-05	1.02E-06
100 seed weight	0.01	4.20	4.21	63.02	4.201	0.0007	0.002	0.0	0.000	0.064	0.064	2.01458E-06	1.16E-08
Seed yield/plant	0.29	11.40	11.69	173.24	11.549	0.019	0.076	0.6	0.006	0.641	0.641	0.001	0.000239

SD: Standard deviation, Var (P): Phenotypic variance, Var(G): Genotypic variance, PCV: Phenotypic coefficient of variation, GCV: Genotypic coefficient of variation, h^2: Broad sense heritability, GA: Genetic advance

Table 2: Range, mean, standard deviation, variance and Coefficient of variation (CV) for number of leaflets and some of its attributing traits in 15 selected K851 mutants in 40 Gy treated seeds in M_2 generation

					Mean								
	Range	Min	Max	Sum			SD	PCV (%)	Var (P)	Var (G)	GCV (%)	$h^{2(\%)}$	GA
Traits	Stat	Stat	Stat	Stat	Stat	SE	Stat	Stat	Stat	Stat	Stat	Stat	Stat
Plant height	16.94	28.50	45.44	526.18	35.078	1.484	33.057	33.057	16.39	30.853	15.834	9.595	113.646
Internode length	2.04	1.09	3.130	31.11	2.073	0.162	0.396	0.395	30.341	0.369	29.312	0.114	0.148
No. of leaf	11.85	8.70	20.55	209.49	13.965	0.821	10.123	10.122	22.781	9.447	22.009	2.938	19.257
Secondary branch	6.16	9.61	15.77	186.11	12.407	0.452	3.065	3.065	14.110	2.860	13.632	0.889	3.208
Primary branch	1.00	2.00	3.00	36.20	2.413	0.070	0.076	0.075	11.387	0.070	11.001	0.021	0.012
Cluster/pod	5.20	11.30	16.50	189.10	12.606	0.341	1.749	1.749	10.491	1.632	10.135	0.507	1.383
Pod/cluster	0.24	4.43	4.66	68.47	4.564	0.018	0.005	0.005	1.566	0.004	1.513	0.001	0.0002
Pods/plant	20.50	49.20	69.70	808.80	53.920	1.205	21.780	21.780	8.655	20.328	8.361	6.321	60.778
Pod length	1.19	5.96	7.16	100.10	6.673	0.067	0.069	0.0692	3.942	0.064	3.808	0.02	0.010
Petiole length	2.09	10.51	12.60	171.97	11.464	0.159	0.384	0.3830	5.402	0.358	5.219	0.111	0.142
Pod width	0.07	0.44	0.51	7.27	0.484	0.004	0.00	0.0002	3.392	0.0002	3.277	7.85E-05	2.66E-06
100 seed weight	0.14	4.33	4.47	65.53	4.368	0.007	0.001	0.0008	0.683	0.0008	0.66	0.0002	1.59E-05
Seed yield/plant	0.14	11.81	11.95	178.26	11.884	0.011	0.002	0.0018	0.364	0.0017	0.351	0.0005	4.85E-05

SD: Standard deviation, Var (P): Phenotypic variance, Var(G): Genotypic variance, PCV: Phenotypic coefficient of variation, GCV: Genotypic coefficient of variation, h^2: Broad sense heritability, GA: Genetic advance

The mean, range phenotypic (PCV) and genetic coefficient of variation (GCV), estimates of components of variance and heritability (h^2) and Genetic Advance (GA) are presented in Table 1 and 2. Generally only slightly higher PCV values than GCV were obtained for all the traits indicating that genetic factor exerted the major effect in estimating the variation. Both PCV and GCV values were moderate for plant height, number of leaves, pods/plant and lower for 100 seed weight and seed yield. Heritability and genetic advance for number of leaves were 2.321 and 14.340 in 30 Gy, where as 2.938 and 61.269 for 40 Gy treated seeds. Higher genetic advance in 40 Gy compared to 30 Gy for number of leaves suggests that there is a possibility of improving seed yield through direct selection of these traits.

Of the mutagen doses, 30 and 40 Gy treated K851 could induce significant changes in the undesirable negative correlations existing in the control towards desirable (positive) correlations for various quantitative characters. Analysis of variance (ANOVA) from the regression studies indicated highly significant association ($p<0.0001$) of number of leaves, seed yield, number branches, pods/plant, petiole length, pods/cluster and pod length in mutants to the constant or control seeds (Table 3). Further correlation studies were undertaken to investigate the positive and negative correlation of multiple leaflets with the yield attributing traits (Table 2, 4).

Correlation Between Multifoliate Leaflets on Yield and Other Morphological Traits

In K851, 30 Gy showed highly significant correlation ($p<0.01$) was found between plant height/internode length ($r = 0.841$), plant height/secondary branch ($r = 0.651$), number of leaves and

Table 3: Analysis of variation (ANOVA) through linear regression analysis of 30 and 40 Gy mutagen treated seeds of K851

Dose	Parameters	Sum of squares	df	Mean square	F	Sig.
30 Gy	Regression	2893.291	8	361.661	1950.091	0.000[a]
	Residual	0.742	4	0.185		
	Total	2894.032	12			
40 Gy	Regression	2893.953	9	321.550	12078.781	0.000[a]
	Residual	0.080	3	0.027		
	Total	2894.032	12			

[a] Predictors: Control, plant height seed yield, No. of leaves, pod length, number of branches, pods/cluster, petiole length, pods/plant, df: Degree of freedom; F: F distribution: Sig: Level of Significance at $p<0.05$ and $p<0.0001$

Table 4: Correlations among seed yield and its contributing traits of 15 selected mutant lines in M_2 generation of 30 Gy treated K851 parent

Traits	Coefficient	Plant height	Internode length	No. of leaves	Secondary branch	Primary branch	Cluster/ plant	Pod/ cluster	Pods/ plant	Pod/ length	Petiole length	Pod width	100 seed weight	Seed yield/ plant
Plant height	Cor.	1.00												
	Sig.													
Internode length	Cor.	0.841**												
	Sig.	0.00												
No. of leaves	Cor.	0.588*	0.604*											
	Sig.	0.021	0.017											
Secondary branch	Cor.	0.651**	0.561*	0.616*										
	Sig.	0.009	0.03	0.014										
Primary branch	Cor.	0.63*	0.401	0.526*	0.845**									
	Sig.	0.012	0.139	0.043	0.00									
Clusters/plant	Cor.	0.436	0.412	0.622*	0.253	0.221								
	Sig.	0.10	0.127	0.013	0.362	0.427								
Pod/clusters	Cor.	0.29	0.221	0.111	0.089	0.261	-0.20							
	Sig.	0.30	0.428	0.693	0.751	0.346	0.42							
Pods/plant	Cor.	0.49	0.418	0.644**	0.265	0.243	0.97**	-0.177						
	Sig.	0.06	0.121	0.009	0.339	0.381	0.00	0.527						
Pod length	Cor.	0.18	0.012	-0.095	0.046	0.263	-0.10	0.028	0.03					
	Sig.	0.52	0.967	0.736	0.869	0.342	0.70	0.918	0.916					
Petiole length	Cor.	0.15	0.426	0.213	0.386	0.058	0.40	-0.203	0.236	-0.53				
	Sig.	0.60	0.113	0.444	0.154	0.836	0.14	0.467	0.398	0.041				
Pod width	Cor.	0.09	0.259	0.243	-0.026	-0.201	0.15	-0.038	0.205	-0.33	0.14			
	Sig.	0.76	0.352	0.382	0.926	0.472	0.59	0.891	0.463	0.234	0.619			
100 seed weight	Cor.	-0.40	-0.340	-0.209	-0.194	-0.209	-0.70*	0.303	-0.64*	0.06	-0.33	-0.0		
	Sig.	0.18	0.218	0.454	0.487	0.453	0.01	0.272	0.01	0.831	0.236	1.0		
Seed yield/plant	Cor.	0.42	0.247	0.622*	0.357	0.398	0.91**	-0.149	0.929**	0.034	0.18	0.05	-0.6*	
	Sig.	0.12	0.376	0.013	0.191	0.141	0.00	0.595	0.00	0.904	0.521	0.87	0.01	

*$p<0.05$, **$p<0.01$

pods/plant (r = 0.644), secondary branch and primary branch (r = 0.845), cluster/plant and pods/plant (r = 0.97), cluster/plant and seed yield/plant (r = 0.91), pod length and seed yield/plant (r = 0.929) (Table 4). Significant correlations ($p<0.05$) was found between plant height and number of leaves (r = 0.588), plant height and primary branch (r = 0.63), internode length and number of leaf (r = 0.604), Internode length and secondary branch (r = 0.561), number of leaf and secondary branch (r = 0.616), number of leaf and primary branch (r = 0.526), number of leaf and cluster/plant (r = 0.622), Number of leaf and seed yield /plant (r = 0.622), cluster/plant and 100 seed weight (r = -0.7), pods/plant and 100 seed weight (r = 0.64), 100 seed weight and seed yield/plant (r = -0.6).

Multiple leaflets in mungbean tended to give shorter (r = -0.093) but more pods/plant (r = 0.644) and higher seed yield (r = 0.622). Higher seed yield due to multiple leaflets is also due to higher number of primary (r = 0.526) and secondary (r = 0.616) branches and higher number of pod clusters (r = 0.622) in plant. Multiple leaflets in mungbean also tended to give higher yield with reduced 100 seed weight (r = -0.209), higher plant height (r = 0.604) and higher internode length (r = 0.604). As number of pods per plant was positively correlated with seed yield, both the traits are also positively correlated with multiple leaflets in 30 Gy treated K851 mutant lines. This gave higher yield advantage in selection of S7 that set more profuse pods (69 pods/plant) compared to S12 (52 pods/plant) as multiple leaflets set less number of pod clusters with reduced petiole length in later (Table 5).

Table 5: Effect of number of leaves on yield components and some morphological characters of 15 mungbean lines derived from 30 and 40 Gy treated K851 parent

Dose	Plant height (cm)	Internode length (cm)	No. of leaf	Secondary branch	Primary branch	Clusters pod	Pod cluster	Pods/ plant	Pod length (cm)	Petiole length (cm)	Pod width (cm)	100 seed weight (g)	Seed yield/ plant (g)
Control	57.599	3.422	12.001	10.055	2.276	10.091	3.808	30.296	6.635	10.755	0.369	3.627	10.101
30 Gy	42.562	2.897	14.892	14.139	2.846	12.033	4.661	50.566	5.920	13.929	0.461	4.201	11.549
40 Gy	35.078	2.073	13.965	12.407	2.413	12.606	4.564	53.920	6.673	11.464	0.484	4.368	11.884

Table 6: Correlations among seed yield and its contributing traits of 15 selected mutant lines in M_2 generation of 40 Gy treated K851 parent

Traits	Coefficient	Plant height	Internode length	No. of leaf	Secondary branch	Primary branch	Cluster/ plant	Pod/ cluster	Pods/ plant	Pod length	Petiole length	Pod width	100 seed weight	Seed yield/ plant
Plant height	Cor.													
	Sig.													
Internode length	Cor.	0.34												
	Sig.	0.21												
No. of leaves	Cor.	0.72**	0.265											
	Sig.	0.00	0.34											
Secondary branch	Cor.	0.53*	-0.00	0.540*										
	Sig.	0.04	0.998	0.037										
Primary branch	Cor.	0.48	-0.270	0.463	0.712*									
	Sig.	0.07	0.324	0.081	0.002									
Clusters/plant	Cor.	0.17	0.029	0.172	0.633*	0.684*								
	Sig.	0.54	0.919	0.539	0.011	0.004								
Pod/clusters	Cor.	0.24	0.153	0.468	0.297	0.486	0.401							
	Sig.	0.39	0.587	0.078	0.281	0.065	0.138							
Pods/plant	Cor.	0.12	0.033	0.126	0.601*	0.536*	0.915**	0.193						
	Sig.	0.66	0.908	0.653	0.017	0.039	0.00	0.490						
Pod length	Cor.	-0.10	0.017	-0.038	-0.094	-0.220	-0.242	-0.393	-0.14					
	Sig.	0.66	0.953	0.892	0.737	0.430	0.383	0.146	0.621					
Petiole length	Cor.	-0.20	-0.250	-0.201	0.125	0.184	0.076	0.144	0.009	-0.380				
	Sig.	0.43	0.365	0.471	0.656	0.511	0.786	0.606	0.973	0.157				
Pod width	Cor.	-0.10	-0.080	-0.295	0.167	0.139	0.163	0.232	0.074	-0.450	0.293			
	Sig.	0.61	0.784	0.284	0.551	0.620	0.560	0.404	0.792	0.091	0.289			
100 seed weight	Cor.	0.38	-0.370	0.227	0.213	0.274	0.029	0.068	-0.070	0.243	-0.070	-0.4		
	Sig.	0.16	0.178	0.414	0.444	0.322	0.917	0.809	0.807	0.383	0.796	0.16		
Seed yield/ plant	Cor.	0.22	0.349	0.180	0.356	0.046	0.567*	0.066	0.618*	-0.300	-0.110	-0.10	-0.00	
	Sig.	0.42	0.203	0.520	0.192	0.868	0.027	0.813	0.014	0.276	0.694	0.63	0.99	

*p<0.05, **p<0.01

Highly significant correlation ($p<0.01$) was found in 40 Gy between the plant height and number of leaves, cluster/plant and pods/plant of K851 (Table 6). The highest correlation was found between plant height and number of leaves ($r = 0.72$) and cluster/plant and pods/plant ($r = 0.915$). Significant correlation ($p<0.05$) was found between number of leaves and secondary branch ($r = 0.540$), secondary branch and plant height ($r = 0.53$), primary branch and secondary branch ($r = 0.712$), secondary branch and clusters/plant ($r = 0.633$), secondary branch and pods/plant ($r = 0.601$), primary branch and cluster/plant ($r = 0.684$), primary branch/pods/p ($r = 0.536$), cluster/p/seed yield/p ($r = 0.567$), pods/p/seed yield/p ($r = 0.618$) of cv K851.

The results indicated number of branches/plant was correlated with multiple leaflets. However, none of the traits were correlated with seed yield. This further illustrates that reduction in number of pods and seed yield in S_2 mutant line is due to the reduced number of branches, pod clusters, 100 seed weight and petiole length than S_3 mutant line, which had the highest seed yield.

Direct and Indirect Correlation of Effects of Multifoliate Leaflets to Yield Components in Selected Lines

Given the highly significant ($p<0.0001$) correlation between number of leaves and number of pods/plant in 30 Gy treated seeds, effects of multifoliate leaflets on yield are other morphological traits were studied through 15 selected lines in the M_2 generation in 30 Gy only (Table 2). Total number of leaflets per plant differed among multifoliate lines tested which was highest in S_{12} (19 leafs or 57 leaflets) in 30 Gy. However, the number of pods/plant in S_{12} was only 52 compared to 67 in S_6 and 69 in S_7. The average number of leaflets per plant was 17 leafs (51 leaflets) in S_6 and 18 leafs (54 leaflets) in S_7 at 30 Gy (Fig. 1, Table 5).

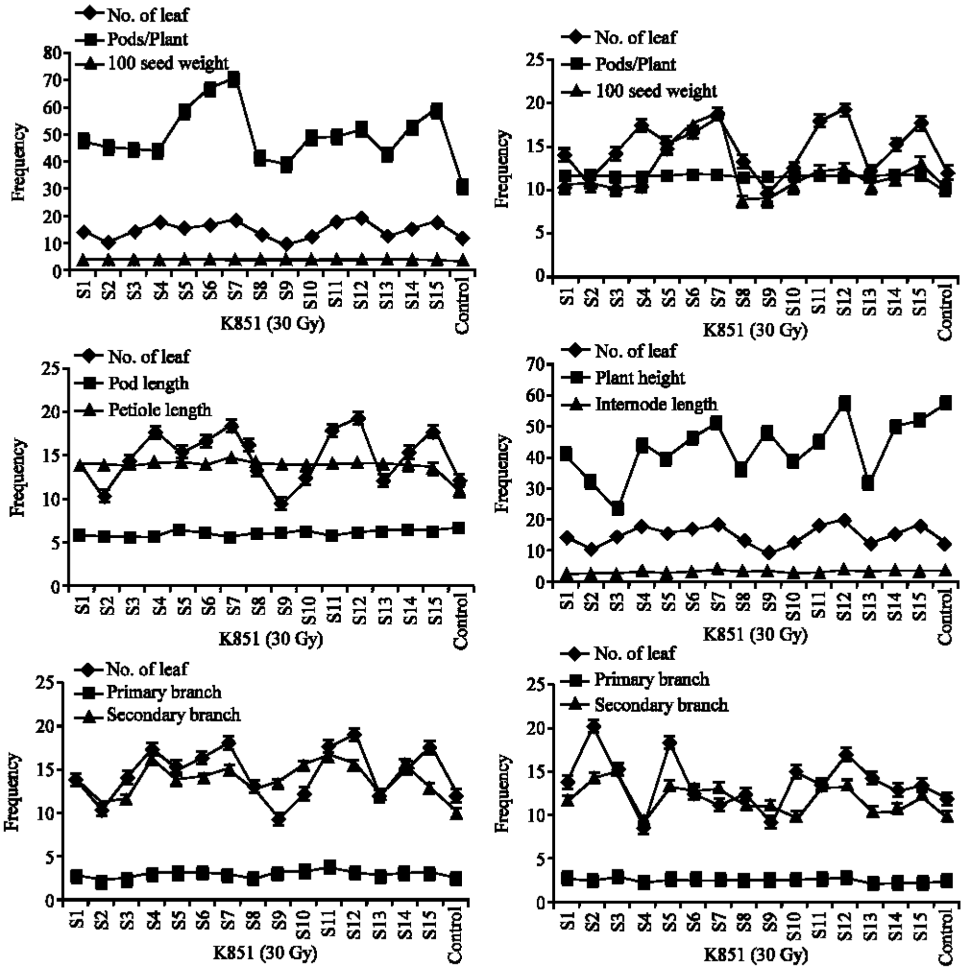

Fig. 1: Effect of number leaves (Mean±SE) on yield and some morphological components in 15 selected mutant lines of K851 based on Pearson correlation studies. Standard error of mean of each leaflet type is represented by a vertical bar

The 100 seed weight of S_6 and S_7 were lower compared to other mutant lines in 30 Gy. Further in correlation studies indicated an inverse correlation between number and leaves and 100 seed weight in 30 Gy (Table 4). A similar inverse correlation was also observed in the 40 Gy treated seeds. The 100 seed weight of S_6 and $S_7$was close to average seed weight of 4.19 g. Reduction in 100 seed weight in 30 Gy could be due to smaller size of the seeds as noted.

Significant ($p<0.05$) correlation between number of leaves to pod clusters and seed yield indicated S_6 and S_7 had the higher yield of 12 g compared to 10 g in control. A maximum of 17-19 pod clusters was observed in S_6 and S_7 compared to only 10 pods in the control. Higher pod clusters in S_6 and S_7 did not increase seed yield and may reflect a low number of pods in the clusters. However, moderate PCV and GCV percentage in number of leaves and pod clusters in 30 Gy could indicate both the traits may improve the seed yield through directed selection.

An inverse correlation between pod length and number of leaves could be a reason of lowest pod length (5.3 cm) in S_7 in 30 Gy treated seeds (Fig. 1, Table 5). Similarly an inverse correlation between number of leaves to pod length, pod width and petiole length was noted in S_2 and S_3 that had higher number of leaves and yield components than control in 40 Gy treated seeds. Further a low level of PCV and GCV in pod length, petiole length and pod width could minimise its contribution in improvement in seed yield through direct selection of these traits.

Significant ($p<0.05$) correlation between plant height and number of leaves was noted in S_{12} mutant in 30 Gy which had a highest height and number of leaves of 58 cm and 19, respectively. However, the higher height in S_{12} than S_6 and S_7 was due to higher internode length, which is also significantly correlated with the number of leaves (Fig. 1, Table 5). The internode length in S_6 mutant was only 3 cm compared to 4 cm in S_{12}.

Highly significant ($p<0.01$) correlation between plant height and number of leaves was also noticed in S_2 that had a plant height of 42.14 cm. Hence improvement in seed yield could be possible through direct selection of S_6 and S_7in 30 Gy and S_2 mutant line in 40 Gy, respectively. Number of primary is significantly ($p<0.05$) correlated in 30 Gy. Number of secondary branches is also significant correlated in 30 and 40 Gy treatment. A higher increase in number of leaves in S_4 and S_{11} was noted due to both increases in primary and secondary branches at 30 Gy. While in 40 Gy both S_2 and S_3 had higher increase in number of secondary branches. A moderate to low level of PCV and GCV could indicate number of leaves with number branches could improve the seed yield through directed selection.

Higher heritability of 22% with considerable higher amount of genetic variability and very high genetic advance in plant height and pods/plant at 30 and 40 Gy could indicate both the traits with number of leaves could improve the seed yield to a maximum extend through their direct selection. Given no correlation between number of leaves and pods/plant in 40 Gy selection of S_6 and S_7 for pods/plant and plant height in 30 Gy and S_2 only for plant height at 40 Gy could be performed.

DISCUSSION

The major objective of the current study was to evaluate the extent of multiple leaflets appearing in the M_2 generation and to estimate the effects of multiple leaflets to the desirable quantitative characters through correlation studies. Though not all, but some leaf mutants appeared to be useful in the current study for breeding as it will increase the net photosynthetic area by increasing the concentration of sink to the developing seeds with an increase in transfer of assimilates to the grain responsible for a positive effect in seed yield.

Despite (Soehendi *et al*., 2006a, b) indicated that multiple leaflet have lesser seed yield, plant height and number of pods per plant than the trifoliate (control) leaflets, in the current study the 30 Gy treatment offered maximum potentiality to increase number of leaves per plant in M_1 generation and this treatment also could be useful in isolation of plants showing promising yield with improvement in yield and a number of contributing traits in M_2 generation. The tetrafoliate and pentafoliate lines were higher in seed yield at 30 Gy only.

A moderate heritability and very high genetic advance in pods/plant and plant height would indicate the predominance of additive gene action and as such, both the traits is likely to respond effectively on number of leaves for phenotypic selection of S_6 and S_7 in 30 Gy and S_2 and 40 Gy for increase in seed yield. Thus moderate heritability with high genetic advance combined with high genetic variability realized in the present study for the number of leaves revealed the scope of improving the yield effective selection based on number of leaves.

Results of the present study and earlier reports also showed that different leaflet types correlated with mungbean yield in 30 Gy treatments. Even though S_6 and S_7 had numerous leafs distributing evenly in the canopy, each leaf was comparatively smaller in size and in area than those in control in 30 Gy. Thus they absorb less sunlight and could be a reason for not significant difference in seed yield in S_6 and S_7than average seed yield of all 15 selected lines. The number of leaflets was also previously identified as negatively correlated with leaf area by (Soehendi *et al*., 2006b). Although the mutant S_6 and S_7 lines had more number of pods in 30 Gy, it also had smaller seeds per pod and thus it served as a source to increase the pod number (highest clusters per plant in S_7) to increase the seed yield. Hence the less difference in seed yield and 100 seed weight could be due to less number of seeds in the pod. Further aim could be to break the negative relationship between seed size and seeds per pod for plant breeders.

Variation in seed weight and seed yield between 30 and 40 Gy could be due to variation in seed size. Increase in seed yield in 40 Gy could be due to increase in size of the seed that had no correlation with multiple leaflets and number of pods/plant. Increase in the yield in 40 Gy could also be due to differently leaflet types or uneven lamina surface in higher dose. Small multifoliate leaflets and sterility could be another reason of no high increase in yield. It expected the genetic background of all lines will be homozygous within the same family of K851, any existing qualitative genes controlling the leaflet types may result in the difference between lines within each family of K851 treated with 30 and 40 Gy, respectively. Begum *et al.* (2008) indicated that visible macro mutants were not isolated in the lower doses showing lesser variation indicates the potential of higher doses to induce more alternations in genomic DNA. This prior research justifies higher variability and effective selection of mutant lines in the current study in higher dose. Further opportunity will be to investigate if the multifoliate mutant breeds true in M_3 generation.

CONCLUSION

This study has indicated the direct and indirect effects of multiple leaflets with agronomic characters. Results indicated number of leaflets in K851 has increased more in 30 Gy than in 40 Gy. Significant correlation of multiple leaflets with seed yield was observed in 30 Gy only. However, at higher dose level (40 Gy) no correlation with seed yield was observed. Multiple leaflets were significant correlated with number pods and branches in K851. Only two mutant lines (S_6 and S_7) in 30 Gy and only one mutant line (S_2) in 40 Gy could be selected for high yielding variety in K851.

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