



Evaluation of F₆ RILs for Drought Tolerance in Green Gram [*Vigna radiata* (L.)]

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ABSTRACT

An experiment was conducted at the experimental plot of College of Agriculture, Hassan, Karnataka, India during summer, i.e. January–April, 2018 with an objective to screen F₆ RILs for drought tolerance using Augmented Randomized Block Design with 152 RILs, two parental lines and 5 check entries under drought stress condition. The F₆ RILs are derived from cross between drought tolerant line VGG10-010 and drought susceptible AKL-225. Drought condition was imposed by withholding irrigation 25 days after sowing. The experiment was conducted in *summer* season, there were no unpredicted rains during the entire cropping period, hence the drought condition was effectively imposed. Analysis of variance (ANOVA) revealed highly significant mean squares attributable to F₆ RILs for all the traits under investigation. Mean squares attributable to 'RILs *vs* check entries' were significant for all the traits except for harvest index and relative water content. The results suggest significant differences among the F₆ RILs. The RILs as a group differed significantly for all of the traits under investigation, similarly, check entries as group differed significantly for all the traits except for harvest index which revealed high significant differences among 152 F₆ RILs for yield, yield component traits and also for drought tolerance traits. Assessment of genetic variability in RILs will help in identifying best transgressive RILs and will ultimately decide the success of plant breeding programmes. Selection among straight RILs is effective only if trait is controlled by higher heritability, hence assessment of genetic variability assumes lot of importance.

KEYWORDS: Drought tolerance, F₆ RILs, genotypic variance, phenotypic variance

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1. INTRODUCTION

Green gram is leguminous plant species belongs to the family Fabaceae with the chromosome number of $2n=22$. It is the self-fertilized species, originated from south Asia with the possible progenitor of *Vigna radiata* var. *sublobata*. Green gram is an important edible bean in the human diet worldwide (Goud et al., 2022). Since it is rich in protein, it can be considered as the meat for vegetarians (Sudhakaran and Bukkan, 2021). It is highly consumed legume and has the ability to withstand wide environmental conditions (Patil et al., 2021). Green gram is one of the principal legumes and is a very nutritive crop grown for its high protein seeds (Singh et al., 2017). The crop is considered to be potential crop because of its tolerance to drought and high temperature (Batzer et al., 2022). It is quite versatile crop can be grown for seeds, green manure and forage (Singh et al., 2023). The green gram crop can restore soil fertility by biological nitrogen fixation and so adds value in the rice-wheat rotation (Kaur et al., 2021). Essential amino acids especially lysine and tryptophan are mainly found in green gram along with other proteins (Chakraborty et al., 2021). Greater emphasis is now laid on increasing the productivity and thereby the total production of pulses (Sathyamoorthi et al., 2023). Despite holding such a great promise, mung bean is often grown in mostly rain-fed lands with limited inputs making it prone to a number of abiotic stresses. One of the most sensitive sectors to climate change is agriculture (Akbari et al., 2023). Among these stresses, drought is the major stress leading to heavy crop loss. Soil moisture deficit is a multidimensional stress affecting plants at various levels of their growth (Yordanov et al., 2000).

Pulses are more sensitive to high temperature stress at reproductive stage (Partheeban et al., 2017). Contemporary climate change is exposing plants drought stress and other abiotic conditions (Hamann et al., 2021). The presence of the genetic variability and suitable selection criteria is imperative for screening of genotypes for heat tolerance (Bhatti et al., 2023). During the reproductive stage, high temperatures cause flower drop, induce male sterility, impair anthesis, and shortens the grain-filling period (Basu et al., 2019). New improved crop varieties developed through breeding programmes can help up lift farmers economic status (Lenaerts et al., 2019). Yield is dependent on various factors, like morpho-physiological traits and response to various environmental factors (Saharia et al., 2023). The major constraints in achieving higher productivity are lack of exploitable genetic variability, absence of suitable ideotype for different cropping systems (Chippy et al., 2021). The molecular mechanisms driving capacity of plants to memorize a stress and generate stress resistant progenies are still unclear (Perrone et al., 2020).

Diaz et al. (2018) reported that RILs evaluated under different abiotic stress condition in common bean showed superior performance for drought tolerance that combine stress and high cross-location productivity. Selection of high yielding genotypes may play a vital role to achieve sustainable high agricultural yield at farmer's field (Khatik et al., 2022). Identification of new germplasm resources with tolerance to both high and low extremes of precipitation is required to meet the impelling demand of climate resilient varieties (Roy et al., 2023). Proper selection of materials containing the desired gene(s) is a prerequisite to achieve the breeding objectives (Kumar et al., 2023). The higher magnitude of genotypic and phenotypic variability for seed yield per plant indicates the potential for selection of elite genotypes (Patel et al., 2023). To develop a new high yielding variety, it is important to generate utilitarian recombinants and devise an appropriate strategy for selection and advancement of those recombinants (Sharma et al., 2022).

2. MATERIALS AND METHODS

The experiment was conducted at experimental plot of College of Agriculture, Hassan, University of Agricultural Sciences, Bangalore, Karnataka, India during January–April, 2018. The experimental site is geographically located at Southern Transitional Zone (Zone-7) of Karnataka with an altitude of 827 m above Mean Sea Level (MSL) and at 12.9652°N latitude and 75°33' to 76°38' E longitude. The study material consisted of 152 recombinant inbred lines derived from cross between VGG10-010 and AKL-225. Two contrasting parents for drought tolerance and susceptible are used for generating crosses. Most drought tolerant line VGG10-010 and most drought susceptible line AKL-225 identified based on drought tolerant indices and also physiological traits governing drought tolerance were involved in generating crosses. The experiment was conducted in an Augmented Randomized Block Design with 152 straight RILs, two parental lines and 5 check entries. There were 4 blocks, each block had 4 plots of size 3×3 m² thus each block size was 12 m². The gross area of experimental plot was 48 m². The row spacing was 30 cm and inter plant distance was 10 cm. Recommended crop production practices were followed during the crop growth period to raise healthy crop.

2.1. Development of RILs

RILs were developed using single seed descent method (SSD) which is a modified form of bulk breeding method. In early *kharif* 2016, cross was generated among contrasting parents. In late *kharif* 2016, thirty F₁ seeds of cross were space planted to produce large quantity of F₂ seeds and seeds of the F₁ plants collected and bulked. During *rabi* 2016, advancing of F₂ generation of cross was made. One seed is randomly



picked from 200 plants of F₂ generation and bulked. Two hundred seeds are randomly collected. “Rapid Generation Advancement” method was followed for rapid advancement of generation (Tanaka et al., 2016). In Summer 2017, all the 200 plants were raised for advancing to F₃ generation. Because of poor germination, 190 plants survived. One seed randomly picked from all of these surviving plants and bulked. During *kharif* 2017, all 190 plants were raised for advancing to F₄ generation. Because of germination loss, 173 plants survived. One seed randomly picked from all of these surviving plants and bulked. In *rabi* 2017, all the 172 plants were raised for advancing to F₅ generation. Because of improper germination, 152 plants survived. One seed randomly picked from all of these surviving plants and bulked. During Summer 2018, all the 152 plants were raised for advancing to F₆ generation. Observations were recorded on recombinant inbred lines (RILs) for statistical analysis. Since we did not adopt background selection, it is only theoretical assumption that by the time progenies reach F₆ generation, they would have attained homozygosity to the tune of 98.43%. Another way of confirming homozygosity is that the RILs did not exhibit segregation.

2.2. Layout of the experiment

The experiment was conducted in an Augmented Randomized Block Design with 152 RILs, two parental lines and 5 check entries. There were 4 blocks, each block had 4 plots of size 3×3 m² thus each block size was 12 m². The gross area of experimental plot was 48 m². The row spacing was 30 cm and inter plant distance was 10 cm. The experiment was conducted during *summer* 2018. Recommended crop production practices were followed during the crop growth period to raise healthy crop.

2.3. Imposing the drought condition

Drought condition was imposed by withholding irrigation 25 days after sowing (Baroowa and Gogoi, 2015). Since the experiment was conducted in *summer* season, there were no unpredicted rains during the entire cropping period hence the drought condition was effectively imposed. The rainfall data of experimental site during the cropping period is given in table 1.

Table 1: Meteorological data of experimental site for the year 2018

Year	Months	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
2018	January	22.72	77.72	Nil
	February	25.12	43.42	Nil
	March	25.32	52.10	Nil
	April	27.58	61.20	2.52
				(11.04.2018)

2.4. Plant sampling and data collection

Observations were recorded on five randomly chosen competitive plants from each of the RIL for all the characters except days to 50% flowering and days to maturity, which were recorded on plot basis. The values of five competitive plants were averaged and expressed as mean of the respective characters. The observations were taken on the traits like, Days to 50% flowering, Days to maturity, Plant height (cm), Clusters plant⁻¹, Pods cluster⁻¹, Pods plant⁻¹, Pod length (cm), Seeds pod⁻¹, test weight, Threshing percentage, Harvest index (%), SCMR (SPAD Chlorophyll meter reading), Leaf water potential (Mpa), Proline content (µg g⁻¹), Relative water content, Specific leaf area and Seed yield plant⁻¹.

2.4.1. Statistical analysis

The quantitative trait mean value of five randomly selected plants in each of the straight RILs and check entries were used for statistical analysis. ANOVA was performed to partition the total variation among genotypes and check entries into sources attributable to ‘RILs+Check entries’, RILs, Check entries’ and RILs vs check entries’, following the augmented design as suggested by Federer (1956, 1961) (Table 2) using statistical package for augmented design SAS version 9.3 and IndoStat. The adjusted trait mean of each of the genotype was estimated (Federer, 1956) and the same was used for all subsequent statistical analysis.

3. RESULTS AND DISCUSSION

3.1. Assessment of genetic variability for grain yield and its component traits

Assessment of genetic variability in RILs will help in identifying best transgressive RILs and will ultimately decide the success of plant breeding programmes. Selection among straight RILs is effective only if trait is controlled by higher heritability, hence assessment of genetic variability assumes lot of importance.

3.2. Analysis of variance

Analysis of variance revealed highly significant mean squares attributable to F₆ RILs for all traits under investigation (Table 2). Mean squares attributable to ‘RILs vs check entries’ were significant for all the traits except for harvest index and relative water content. The results suggest significant differences among the F₆ RILs. The RILs as a group differed significantly for all of the traits under investigation, similarly, check entries as group differed significantly for all the traits except for harvest index. Our research findings are on par with the report of Salman et al. (2021) who Analysis of variance revealed significant differences among the RILs, through analysis of variance indicating the presence of genetic variability for almost all



Table 2: Summary of Augmented ANOVA for grain yield and component traits of F₆ RILs developed from cross between VGG10-010×AKL-225 under drought condition

Sources of variations	DF	DFF	DM	PH	CPP	PPC	PPP	PL	SPP	TW
Blocks (b)	3	2.42	2.42	28.55**	2.00**	0.11**	32.11**	0.80**	6.07**	0.09**
Entries (e) (RILs+Checks)	158	18.82**	49.33**	63.71**	1.67**	0.47**	68.35**	0.83**	1.96**	0.15**
Checks	6	27.95**	30.90**	282.46**	7.22**	1.95**	210.31**	2.85**	8.14**	1.38**
RILs	151	17.44**	46.37**	51.75**	1.34**	0.37**	57.86**	0.66**	1.31**	0.10**
Checks vs RILs	1	171.55**	606.31**	558.07**	17.64**	6.58**	800.53**	14.77**	62.73**	0.001**
Error	18	0.87	0.87	5.05	0.14	0.05	3.57	0.14	0.30	0.009

Table 2: Continue...

Sources of variations	DF	TP	HI	SCMR	LWP	PC	RWC	SLA	SYPP
Blocks (b)	3	122.66**	236.04**	62.69**	1.05**	253.88**	571.75**	352.98*	3.34**
Entries (e) (RILs+Checks)	158	26.88*	47.81*	51.06**	5.39**	2076.33**	393.57**	1771.89**	18.75**
Checks	6	37.70*	29.77	155.13**	14.83**	7384.28**	275.85*	8880.16**	43.87**
RILs	151	23.86*	48.67**	32.00**	4.23**	1394.26**	400.10**	1236.55**	16.34**
Checks vs RILs	1	417.60**	25.68	2303.89**	122.57**	73221.49**	114.22	39957.45**	232.60**
Error	18	11.54	18.22	8.03	0.11	39.16	73.43	-	0.52

DFF: Days to 50% flowering; Pods plant⁻¹; HI: Harvest index (%); SLA: Specific leaf area; DM: Days to maturity; PL: Pod length (cm); SCMR: SPAD Chlorophyll meter reading; SYPP: Seed yield plant⁻¹; PH: Plant height (cm); SPP: Seeds pod⁻¹; LWP: Leaf water potential (Mpa); CPP: Cluster plant⁻¹; TW: test weight (g); PC: Proline content (µg g⁻¹); PPC: Pods cluster⁻¹; TP: Threshing %; RWC: Relative water content (%); *: Significant at p=0.05; **: Significant at p=0.01

the traits studied in Green gram. Allahmoradi et al. (2011) has also reported similar findings in his research where there was no significant difference between control and drought stress during reproductive growth stage about yield and yield components, but drought stress during vegetative growth stage decreased significantly yield and yield components.

3.3. Descriptive statistics for yield parameters

Descriptive statistical parameters such as, mean, range, standardized range, PCV, GCV, expected genetic advance as per cent mean (GAM), skewness and kurtosis will help to understand relative mean performance and nature of distribution of traits (Table 3). Days to 50% flowering varied from 34.60 days to 53.70 days with a mean of 41.49 days. Days to maturity varied from 59.20 days to 91.10 days with a mean of 70.62 days. Plant height ranged from 21.77 cm to 57.27 cm with mean value of 42.26 cm. Cluster plant⁻¹ varied from 2.30 to 7.83 with mean value of 5.61. Minimum value of 2.05 and maximum value of 4.97 with mean value of 3.71 was observed for the trait pods cluster⁻¹. Pods plant⁻¹ had range from 4.71 to 38.93 with mean value of 21.54. Pod length varied from 5.38 to 9.28 with mean value of 7.38.

Seeds pod⁻¹ ranged from 5.04 to 11.41 with a mean of

8.76. Minimum and maximum values for the trait test weight were 3.64 g and 6.85 g respectively with mean value of 5.27 g. Threshing percentage varied from 45.27% to 79.38% with mean value of 63.12%. Minimum value of 21.88 and maximum value of 50.40 with mean value of 36.08 was observed for the trait harvest index. Spad chlorophyll meter reading had range of values from 44.19 to 80.59 with mean value of 64.55. Leaf water potential varied from -9.89 Mpa to -2.15 Mpa with mean value of -4.71. Proline content values ranged from 80.16 (µg g⁻¹) to 244.29 (µg g⁻¹) with mean value of 170.99 (µg g⁻¹). Relative water content recorded lowest value of 34.32 and highest value of 94.84 with a mean of 67.47 Specific leaf area had a minimum value of 118.95 and maximum of 266.50 with mean value of 205.55. Seed yield plant⁻¹ ranged from 0.90 g to 20.10 g with a mean value of 7.84 g.

The estimates of standardized range across traits provide clues about the occurrence of RILs with extreme expression. The standardized range were relatively higher for all the quantitative traits, plant height (0.84), cluster plant⁻¹ (0.99), pods cluster⁻¹ (0.79), pods plant⁻¹ (1.59), pod length (0.53), seeds pod⁻¹ (0.73) test weight (0.61), threshing percentage (0.54), harvest index (0.79), spad chlorophyll meter reading

Table 3: Descriptive statistics for grain yield and its component traits of F₆ straight RILs under drought condition

Descriptive	DFF	DM	PH	CPP	PPC	PPP	PL	SPP	TW
Mean	41.49	70.62	42.26	5.61	3.71	21.54	7.38	8.76	5.27
Std. Error	0.30	0.49	0.56	0.09	0.04	0.58	0.06	0.10	0.04
Variance	16.65	43.55	57.23	1.52	0.42	61.23	0.79	1.88	0.31
Minimum	34.60	59.20	21.77	2.30	2.05	4.71	5.38	5.04	3.64
Maximum	53.70	91.10	57.27	7.83	4.97	38.93	9.28	11.41	6.85
Standardized Range	0.46	0.45	0.84	0.99	0.79	1.59	0.53	0.73	0.61
Skewness	0.70	0.76	-0.51	-0.52	-0.45	0.02	-0.27	-0.56	-0.02
Kurtosis	-1.19	0.09	0.18	-0.53	-0.26	-0.72	-0.62	-0.29	0.18
GCV (%)	8.99	8.74	14.59	17.68	13.69	30.39	8.87	10.31	7.68
PCV (%)	9.27	8.84	15.48	18.89	15.13	31.53	10.25	12.01	7.88
h ² (BS) (%)	94.21	97.81	88.79	87.63	81.82	92.87	74.81	73.72	96.15
GAM (5%)	17.99	17.82	12.28	34.10	25.51	60.33	15.80	18.24	16.20

Table 3: Continue...

Descriptive	TP	HI	SCMR	LWP	PC	RWC	SLA	SYPP
Mean	63.12	36.08	64.55	-4.71	170.09	67.47	205.55	7.84
Std. Error	0.38	0.51	0.51	0.16	3.19	1.42	2.96	0.30
Variance	26.95	47.99	46.93	0.16	1840.95	364.37	1577.10	16.66
Minimum	45.27	21.88	44.19	-9.89	80.16	34.32	118.95	0.90
Maximum	79.38	50.40	80.59	-2.15	244.29	94.84	266.50	20.10
Standardized Range	0.54	0.79	0.56	-1.64	0.96	0.90	0.72	2.45
Skewness	-0.49	-0.01	0.18	-0.24	-0.35	-0.55	-0.37	0.58
Kurtosis	1.27	0.18	0.03	0.18	0.18	-1.52	-0.93	-0.08
GCV (%)	5.20	14.22	6.86	43.09	19.07	24.93	14.91	44.21
PCV (%)	7.52	18.53	8.09	43.80	19.39	28.01	15.44	45.06
h ² (BS)(%)	47.80	58.91	71.90	96.78	96.74	79.24	93.35	96.28
GAM (5%)	7.41	22.49	11.98	87.33	38.64	45.72	29.69	89.37

DFF: Days to 50% flowering; PL: Pod length (cm) LWP: Leaf water potential (Mpa); DM: Days to maturity SPP: Seeds pod⁻¹ SCMR: SPAD Chlorophyll meter reading; PH: Plant height (cm) TW: test weight (g); RWC: Relative water content (%); CPP: Cluster plant⁻¹; TP: Threshing percentage; SLA: Specific leaf area; PPC: Pods cluster⁻¹; HI: Harvest index (%); SYPP: Seed yield plant⁻¹; PPP: Pods plant⁻¹; PC: Proline content (%)

(0.56), leaf water potential (-1.64), proline content (0.96), relative water content (0.90), specific leaf area (0.72) and seed yield plant⁻¹(2.45) except days to 50% flowering (0.46) and days to maturity (0.45)

The magnitude of variation revealed by GCV and PCV in F₆ RILs were low for days to 50% flowering (8.99% and 9.27% respectively) days to maturity (8.74% and 8.84% respectively) threshing percentage (5.20% and 7.52% respectively), spad chlorophyll meter reading (6.86% and 8.09% respectively), and pod length (8.87% and 10.25% respectively). Low GCV and PCV values indicate presence of limited variability for these traits. Moderate

GCV and PCV were observed for plant height (14.59% and 15.48% respectively), cluster plant⁻¹ (17.68% and 18.89% respectively), pods cluster⁻¹ (13.69% and 15.13% respectively), seeds pod⁻¹ (10.31% and 12.01% respectively), harvest index (14.22% and 18.53% respectively), proline content (19.07% and 19.39% respectively) and specific leaf area (14.91% and 15.44% respectively). Higher standardized range resulted in higher GCV and PCV values for pods plant⁻¹ (30.39% and 31.53% respectively), leaf water potential (43.09% and 43.80% respectively), relative water content (24.93% and 28.01% respectively) and seed yield plant⁻¹ (44.21% and 45.06% respectively).



Our results are on par with results of Esimu et al. (2020) who evaluated 64 RILs in green gram and observed significant variability among RILs for the characters; Days to 50% flowering, Plant height, Branches plant⁻¹, clusters plant⁻¹, pods plant⁻¹, pod length, seeds pod⁻¹, Pod yield, Seed yield, Threshing % and Test weight. Our research findings are in line with Sineka et al. (2021) who reports high heritability for single plant yield, plant height and hundred seed weight and also higher genetic advance was for the number of pods plant⁻¹. Our results are also in agreement with Abdus Subhan Salman et al. (2021) who reports high PCV and GCV estimates for number of pods plant⁻¹, seed yield plant⁻¹, number of cluster plant⁻¹ and number of pods cluster⁻¹ in green gram. He also reports high heritability along with high genetic advance as per cent of mean observed for number of pods plant⁻¹ seed yield plant⁻¹ number of cluster plant⁻¹, number of pods cluster⁻¹, number of branches plant⁻¹ and number of seeds pod⁻¹

RILs exhibited relatively higher heritability for all the quantitative traits, days to 50% flowering (94.21%), days to maturity (97.81%), plant height (88.79%), cluster plant⁻¹ (87.63%), pods cluster⁻¹ (81.82%), pods plant⁻¹ (92.87%), pod length (74.81%), seeds pod⁻¹ (73.72%) test weight (96.15%), spad chlorophyll meter reading (71.90%), leaf water potential (96.78%), proline content (96.74%), relative water content (79.24%), specific leaf area (93.35%) and seed yield plant⁻¹ (96.28%). Lower heritability was observed for the traits threshing percentage (47.80%) and harvest index (58.91%),

Expected GAM in F₆ RILs were low for threshing percentage (7.41%). Moderate GAM were observed for days to 50% flowering (17.99%) days to maturity (17.82%), plant height (12.28%), pod length (15.80%) and spad chlorophyll meter reading (11.98%) and test weight (16.20%). Higher GAM values were recorded for cluster plant⁻¹ (34.10%), pods cluster⁻¹ (25.10%), pods plant⁻¹ (60.33%) seeds pod⁻¹ (18.24%), harvest index (22.49%), leaf water potential (87.33%), proline content (36.84%), relative water content (45.72%), specific leaf area (29.69%) and seed yield plant⁻¹ (89.37%). Since varied sources of tolerance may have different mechanisms of plant response, we will have to identify different methods of phenotyping to recognize the relevant traits. These findings and their exploitation will hold promise for adopting suitable breeding methods to protect crop plants from certain abiotic stresses. Similar report has been made by Ahmad et al. (2014) and Beebe et al. (2013)

4. CONCLUSION

Descriptive statistical parameters such as; mean, range, standardized range, PCV, GCV differed significantly

among F₆ RILs. High significant differences among the F₆ RILs for drought tolerance was revealed by ANOVA. The RILs as a group differed significantly for all of the traits under investigation, similarly, check entries as group differed significantly for all the traits except for harvest index. RILs exhibited high broad sense heritability coupled with high expected GAM for drought tolerance.

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