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ORIGINAL ARTICLES

Genetic Role of Biochemical, Biophysical and Morpho-physiological Characters in Enhancing the Seed Yield in Mungbean (*Vigna Radiata* (L.) Wilczek)

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Abstract: Owing to mungbean's nutritional quality, short duration, high protein and suitability for multiple cropping systems, field experiments were planned to elicit information on biochemical, biophysical and morpho-physiological characters, their interactions, heterosis and genetics. Three crosses were evaluated for two generations *viz*., F_2 and F_3 at the Plant Breeding Farm, Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University. Observations were recorded on single plant basis. The observations were classified under different groups namely biochemical, biophysical and morpho-physiological. Ten characters were studied *viz*., total chlorophyll content, nitrate reductase activity, stem nitrogen content, leaf nitrogen content, seed protein content, photosynthetic rate, transpiration rate, stomatal conductance, dry matter production and leaf area index. The statistical tools namely, heterosis, and association analysis were worked out for all the ten characters. The crosses VRMGG 1/ VBN 1 and VRMGG Local / PMB 27 exhibited highest *per se* performance for almost all the characters studied. For heterosis studies, the biochemical, biophysical and morpho-physiological characters showed positive and significant heterosis for almost all the three crosses, except for transpiration rate and stomatal conductance. Association analysis studies showed that the biochemical, biophysical and morpho-physiological characters to be positive and significantly correlated with seed yield per plant, except for stem nitrogen and seed protein in both the F_2 and F_3 generations in all the three crosses studied.

Key words:

Introduction

Pulses contribute as a major source of dietary protein for the large portion of vegetarian population of the world. Pulses also play an important role by fixing biological nitrogen and thus enhance the soil fertility. It improves the nutrient status of soil through atmospheric nitrogen fixation and adds humus to the soil. It is also useful to cattle as it is a fodder and concentrate (Rahim *et al*, 2010). India is the largest producer and consumer of pulses in the world. Among the pulses, mungbean (*Vigna radiata* (L.) Wilczek) is a well-known crop among Asian countries. The dietary or nutritional value of mungbean has been very popular from the ancient times. Saleem *et al.* (1998) reported that the seed contains the following components namely total protein (22.88 -24.65 per cent), total amino acid (20.98-25.61 per cent), crude fibre (4.30 - 4.80 per cent) and lipids (1.53 - 2.63 per cent). Like other pulses, the protein of mungbean is rich in lysine, an essential amino acid that is absent in cereal grains.

The productivity of mungbean is very low and is often related to that of morpho-physiological constraints, besides its genetic makeup. Poor partitioning of assimilates to the reproductive sinks from the assimilatory source, indeterminate growth habit and poor pod setting are some of the important morpho-physiological barriers responsible for lower productivity. Hence to understand the check loop holes responsible for lower productivity, a complete knowledge of biochemical, biophysical and morpho-physiological characters is of

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utmost importance. An increase in yield potential of mungbean would undoubtedly assisted by increasing our understanding of the physiological processes namely photosynthesis, translocation and sink capacity which determines the yield potential (Kuo *et al.*, 1977). Physiological basis and understanding the relative impact of these limitations is very necessary because they vary with variety and growing conditions, especially with the sequence of events during flowering and seed filling stages. The source-sink relationship is found to be the equilibrium in high yielding varieties and it assumes greater importance in increasing productivity. Heterosis plays a predominant role in accelerating the agricultural production and heterosis breeding opens up tremendous potential among the crops for quantitative improvement. The magnitude of heterosis provides basis for determining genetic diversity and serves as a guide to the choice of desirable parents (Swindell and Poehlman, 1976). The presence of heterosis in mungbean have been strongly proved and demonstrated by many workers (Singh 1971; Shinde and Deshmukh, 1989). Heterosis occurring in hybrids at cellular level as compared to parents attains major importance. The study of characters like chlorophyll content, activities of different enzymes and other biochemical observations help a lot in evaluating the production potential of genotypes. Heterosis for chlorophyll content is known to increase the productivity by increased CO_2 fixation. Nitrate reductase activity has direct bearing on productivity by increasing nitrogen metabolism. Estimation of level of heterosis for nitrate reductase activity gives an idea about the response of hybrids to increased nitrogen assimilation.

Biochemical, biophysical and morpho-physiological characters play an important role in the process of development of high yielding genotypes. The rate of photosynthesis depends upon the carbon diffusion process and this in turn depends upon stomatal conductance (Wong *et al.*, 1978). In legumes, the photosynthetic rate during vegetative stage has more pronounced effect on the total dry matter production (Jain,1975). He also reported that the photosynthetic rate declined sharply as pod began to develop, which was further associated with fall in total nitrogen content. Similarly, Venkateswarlu and Balasubramanian (1993) noted that the various biophysical parameters like photosynthetic rate, transpiration rate and stomatal conductance showed a relatively higher rate during early pod growth and subsequently declined with the advancement of growth. Goswami *et al.* (2003), Wagh *et al.* (2003) and Singh and Dikshit (2003) also observed similar results.

Singh *et al.* (1994) found that chlorophyll content to be in an increasing trend upto 45 days after sowing and declined significantly at 60 days after sowing. Nitrate reductase is a key enzyme in nitrogen metabolism in higher plants and is regulated by various environmental factors, apart from its own substrate, nitrate. The peak activity of nitrate reductase activity coincides with the maximum chlorophyll content and thereby complementing the carbon-nitrogen balance in plant (Li *et al.*, 1993; Dhumal and Laware, 2003). Grain legumes accumulate large quantities of nitrogen in seeds. It has been suggested that leaf nitrogen is remobilised for seed development and thus cause a decline in photosynthesis, enhanced leaf senescence and ultimately limits yield (Sinclair and Dewitt, 1976; Mitra *et al.*, 1987; Naidu *et al.*, 1993; Naidu and Ram, 1996). With greater awareness on the role of physiological characters determining yield, emphasis is being laid down to include some of these characters in hybrid development and to identify the physiological characters, which govern the productivity. However in mungbean, research efforts in this regard is scanty. Therefore, the present study was conducted.

Materials and methods

Five lines and four testers were crossed in Line x Tester analysis fashion at the Plant Breeding Farm, Department of Agricultural Botany, Faculty of Agriculture, Annamalai University. Of the twenty resulting hybrids, only three best hybrids *viz*., VRMGG 1/VBN 1, VRMGG Local /PMB 27 and EC 30072 /VBN 1 were selected based on per se performance and forwarded to the F_2 and F_3 generations. Observations were recorded on single plant basis. The observations were classified under different groups namely biochemical, biophysical and morpho-physiological. Ten characters were studied viz., total chlorophyll content, nitrate reductase activity, stem nitrogen content, leaf nitrogen content, seed protein content, photosynthetic rate, transpiration rate, stomatal conductance, dry matter production and leaf area index. The statistical tools namely, heterosis and association analysis were worked out for all the ten characters.

Total chlorophyll contents of leaf were determined by acetone extraction method of Arnon (1949). Fresh fully opened leaves from the top of the canopy were brought from the field in an icebox and cut into small pieces. 250 mg of leaf from each sample was weighed and homogenised with pure acetone using pestle and mortar. The extract was filtered through whatman No. 1 filter paper and washed 2-3 times with 80 per cent acetone. The final volume of the extract was made upto 25 ml with 80 per cent acetone. The absorbance of the extract was read at 645 and 663 nm in UV-VIS Spectrophotometer (ELICO Model SL-159) and for blank 80 per cent acetone was used. Chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents were calculated using the following formulae and expressed in mg g^{-1} fresh weight.

$$\text{Chlorophyll 'a'} = 12.7\ (A_{663}) - 2.69\ (A_{645})\ \text{x}\ \frac{V}{1000xWxa}$$

$$\text{Chlorophyll 'b'} = 22.9\ (A_{645}) - 4.68\ (A_{663})\ \text{x}\ \frac{V}{1000xWxa}$$

Total chlorophyll = Chlorophyll 'a' + Chlorophyll 'b'

Where,
A = Absorbance at specific wavelengths (645 and 663 nm)
V = Final volume of the chlorophyll extract
W = Fresh weight of the sample (g)
a = Path length of light (1 cm)

The nitrate reductase activity (NRA) in vivo was assayed by the method of Saradhamabal *et al*. (1978). Leaves were cut into small pieces, weighed and suspended in 25 ml flasks containing 0.1 M phosphate buffer (pH 7.6), 0.02 M KNO_3 and propanol (5 per cent). Two drops of chlroamphenicol (0.5 mg ml^{-1}) were added to it. The flasks were incubated at 30°C for 20 minutes and the reaction was stopped by adding 0.1 ml of 1.0 M zinc acetate and 1.9 ml of ethanol (70 per cent). The contents were centrifuged at 3000 rpm for 10 minutes. To the supernatant, 6 ml of sulphanilamide (1 per cent in 1 M HCl) and 1 ml of N^{-1} naphthyl ethylene diamine dihydrochloride (NNEDD) were added. The absorbance of the pink coloured solution was measured at 540 nm after 20 minutes with the help of a spectrophotometer (ELICO, Model SL-159). The activity of nitrate reductase was determined from a standard curve of KNO_2 and expressed in µmol of NO_2 formed g^{-1} fr. wt. hr^{-1}. Nitrogen content in leaf and stem were estimated at 25, 45 DAS and at harvest by Micro kjeldahl's method (Yoshida *et al*., 1972). Seed nitrogen content was also estimated at harvest by the same method. Nitrogen content in leaf, stem and seed were expressed in *per cent* on dry weight basis.

Reagents:

1. Boric acid (2%)
2. Mixed indicator: It was prepared by dissolving 0.3g bromocresol green and 0.2 g methyl red in 400 ml of 90 per cent ethanol.
3. Sodium hydroxide (40 per cent)
4. Methyl orange indicator (0.1 per cent in water)
5. Standard H_2SO_4 (0.01 N)
6. Salt mixture: 250 g of K_2SO_4 was mixed with 50 g $CuSO_4$ $5H_2O$ and 5g metallic selenium.

Digestion:

0.2 g of dried sample was placed in 100 ml of Kjeldahl flask, to which 3 ml of concentrated H_2SO_4 and a pinch of salt mixture was added. The contents were allowed to digest in the digestion chamber until a clear solution was obtained. Then, the contents were cooled and 10 ml of distilled water was added. After cooling, the contents were washed 3 to 4 times with distilled water. All the washings were transferred to the volumetric flask and the volume was made upto 25 ml. Blank digestion was also carried out. This was further used for distillation.

Distillation:

The contents of the kjeldahl flask were transferred into the Micro kjeldahl distillation apparatus. The flask was rinsed 2 to 3 times with distilled water. Then 10 ml of 40 per cent NaOH was added to the distillation apparatus. Simultaneously, a beaker containing 20 ml of 2 per cent boric acid was added with 3 drops of mixed indicator and placed at the outlet of the condenser. Steam from the boiler was allowed to pass through the sample and the ammonia released was captured in the boric acid mixed indicator. Distillation process was continued for seven minutes and then the contents of the beaker were titrated against standard H2SO4 until the bluish green colour turns into orange red. The nitrogen content in the sample was calculated using the following formula

$$\text{Nitrogen content (\%)} = \frac{\text{(Sample titre - Blank titre) X Normality of H2SO4 X 14 X 100}}{\text{Sample weight (g) X 1000}}$$

The protein content in seeds was computed by multiplying the nitrogen content (per cent) by the factor 6.25 (Tai and Young, 1974).

Photosynthesis was recorded on the abaxial surface of the 4th fully expanded leaf from the top at 45 DAS, between 9.00 AM and 11.00 AM with a portable Photosynthesis System (Model: LiCOR-6200) assembled with an Infra Red Gas Analyzer (IRGA) and data logger following the procedure described by Kubota and Hamid (1992). The size of clamp-on assimilation chamber was kept fixed at 6.25 cm^2. A metal halide lamp of 150 W was used to obtain a photosynthetic active radiation (PAR) of 1600 μmol m^{-2} s^{-1} during the measurement. Airflow through the chamber was 400 ml min^{-1}; relative humidity (RH) of air supplied in the chamber was adjusted to 50% and chamber temperature was maintained at 30ºC ± 1. Net photosynthesis rate (Pn), transpiration rate, stomatal conductance (Cs), and other related parameters were available at computer output attached to the system and expressed as following.

Parameters	**Units**
Rate of photosynthesis	μmole CO_2 m^{-2} s^{-1}
Rate of transpiration	mmole H_2O m^{-2} s^{-1}
Stomatal conductance	μmole CO_2 m^{-2} s^{-1}

The dry matter production of ten plants from each cross were randomly selected and dried separately at 80°C temperature in a hot air oven until constant weight was obtained. The dry weights of the different plant parts at harvest were recorded. Total dry matter production was calculated by adding the dry weights of different plant parts. The leaf area index was calculated by using the formula suggested by Watson (1952).

$$\text{Leaf Area Index (LAI)} = \frac{\text{Leaf area plant}^{-1}\ (\text{cm}^2)}{\text{Land area plant}^{-1}\ (\text{cm}^2)}$$

Results:

Per Se Performance:

The prime objective of hybridization between any two parents is to combine the desirable characters dispersed among them to compensate deficiencies found in one parent by the other. The progenies of these crosses will throw all possible combinations to promote yield. For a systematic breeding programme, it is necessary to identify the parents which can be exploited for genetic improvement through hybrid progenies. For this, the breeders are in absolute need of high mean value which is considered as a main criterion for effective selection forever. Among the three hybrids, VRMGG 1/VBN 1 showed higher mean values for total chlorophyll content, stem nitrogen content, seed protein content, photosynthetic rate, dry matter content and leaf area index whereas, VRMGG Local/PMB 27 showed higher mean values for nitrate reductase activity, leaf nitrogen content and stomatal conductance in both F_2 and F_3 generations (Table 1).

Heterosis:

Heterosis is defined as the deviation of F_1 hybrid over its mid parent (Relative heterosis), better parent (Heterobeltiosis) and standard parent (Standard heterosis), as the consequence of hybridization. The magnitude of heterosis provides a basis for the determination of genetic diversity and serves as a guide to the choice of desirable parents (Swindell and Poehlman, 1976). In legumes, heterosis is generally due to dominance gene effects but also sometimes due to epistatic interactions (Sethi, 1975). In self pollinated crops like mungbean, it is possible to exploit such genetic manifestations only with a potentially workable sterility mechanism, if available. A substantial component of epistatic interaction for agronomical and growth characters have been found in mungbean belonging to the fixable epistasis, *viz*., additive x additive (Khattak *et al.*, 2002).

Genetic information regarding heterosis provides a clue for selecting the most suitable parents for hybridization. Singh (1974) has demonstrated the presence of heterosis in legumes. The presence of heterosis can only be utilized in pulse crop for the development of high yielding pure line varieties (Singh, 1971). Little information is available about heterosis in mungbean. The results on heterosis are presented in figures 1 to 10. Among the three crosses, the cross VRMGG 1 / VBN 1 recorded the maximum relative heterosis percentage for the traits leaf nitrogen content, stem nitrogen content and transpiration rate; VRMGG Local / PMB 27 for total chlorophyll content, nitrate reductase activity, photosynthetic rate, dry matter production and leaf area index; EC 30072 / VBN 1 for stomatal conductance. For heterobeltiosis, VRMGG 1 / VBN 1 recorded the maximum percentage for the traits total chlorophyll content, stem nitrogen content, photosynthetic rate, dry matter production and leaf area index; VRMGG Local / PMB 27 for nitrate reductase activity, leaf nitrogen content and stomatal conductance; EC 30072 / VBN 1 for transpiration rate. The standard heterosis was maximum in the traits, total chlorophyll content, stem nitrogen content, seed protein, photosynthetic rate, dry matter production and leaf area index in the cross VRMGG 1 / VBN 1; nitrate reductase activity and stomatal content in the cross VRMGG Local / PMB 27; leaf nitrogen content and transpiration rate in EC 30072 / VBN 1.

Association Analysis:

Correlation among the traits may result from physiological association among the characters. From breeder's point of view, type of association of seed yield with agronomic characters is of paramount importance. The correlation coefficients were calculated for biochemical, biophysical and morpho-physiological characters with seed yield per plant. The correlation co-efficients in three crosses between seed yield per plant and and biochemical characters, seed yield per plant and biophysical and morpho-physiological characters are presented in the table 2.

VRMGG 1 / VBN 1:

Among the biochemical characters, stem nitrogen content showed negatively significant association with seed yield per plant in both F_2 and F_3 generations, whereas seed protein showed significant and negative correlation with seed yield per plant only in the F_2 generation.
All the biophysical and morpho-physiological characters showed positive and significant correlation with seed yield per plant in both F_2 and F_3 generations.

VRMGG Local / PMB 27:

For biochemical characters, total chlorophyll content, nitrate reductase activity and leaf nitrogen content showed positive and significant association with seed yield per plant, whereas stem nitrogen content and seed protein showed negative correlation with seed yield per plant in both F_2 and F_3 generations.
For this cross, the biophysical and morpho-physiological characters showed positively significant association with seed yield per plant in both F_2 and F_3 generations.

EC 30072 / VBN 1:

Seed yield per plant was negatively correlated with total chlorophyll content in both F_2 and F_3 generations, whereas it showed positively significant association with nitrate reductase activity and seed protein content in both the generations. Seed yield per plant was negatively correlated with leaf nitrogen content. The seed yield per plant and its correlation with stem nitrogen content was positive and low. In this cross, seed yield per plant recorded positively significant correlation with photosynthetic rate, stomatal conductance (F_2), dry matter production and leaf area index and was negatively correlated with transpiration rate in F_2 and F_3 generations.

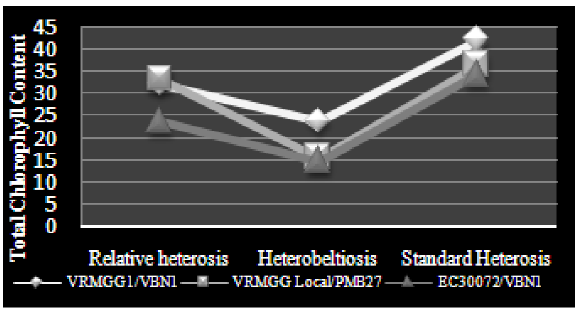

Fig. 1: Total Chlorophyll Content

Discussion:

Per Se Performance:

Accumulation of more photosynthates during growth phases and their partitioning at later stages to storage organs could be responsible for increased hundred seed weight and seed yield per plant. Borah and Hazarika (1995) reported similar relationship. Among the hybrids, VRMGG1 / VBN 1 recorded higher values for total chlorophyll and seed protein, whereas for stem nitrogen, LGG 460/ PMB 27 recorded the highest value.

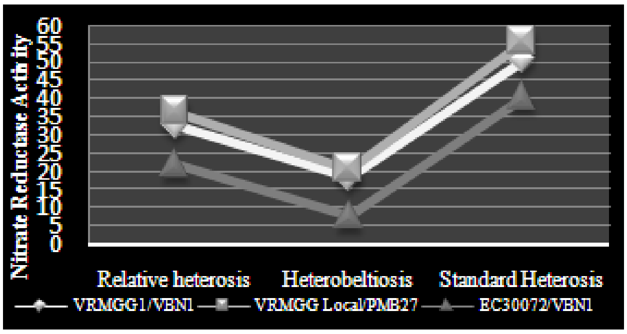

Fig. 2: Nitrate Reductace Activity.

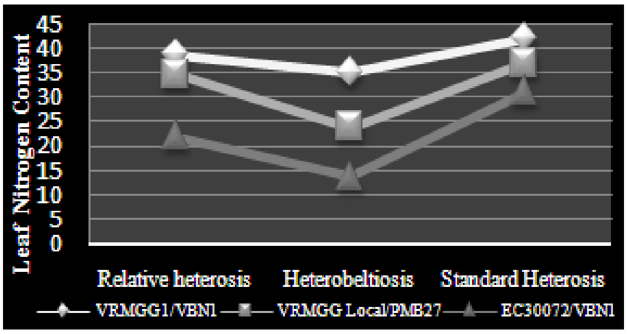

Fig. 3: Leaf Nitrogen Content.

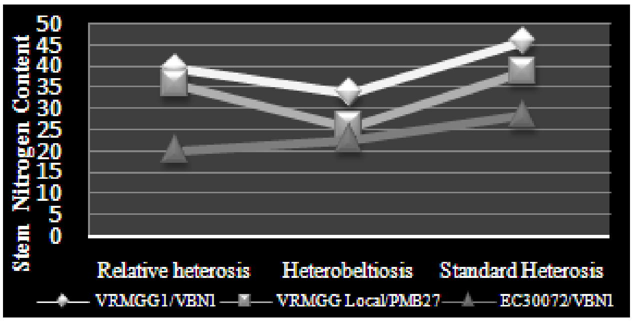

Fig. 4: Stem Nitrogen Content.

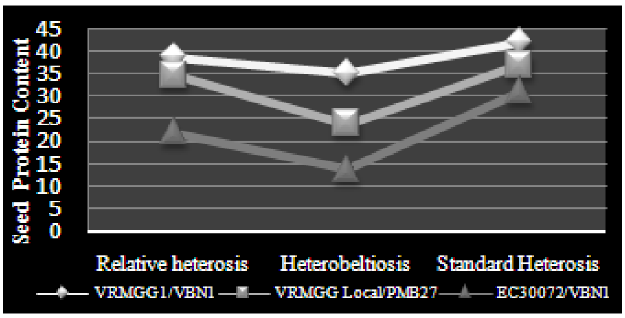

Fig. 5: Seed Protein Content.

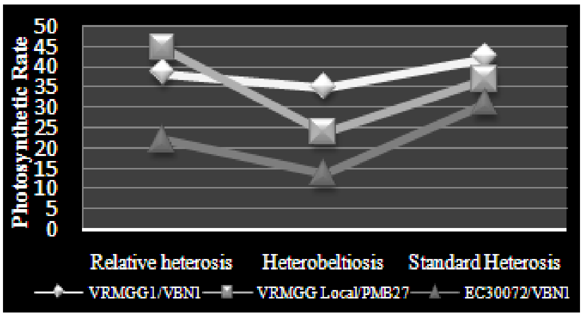

Fig. 6: Photosynthetic Rate.

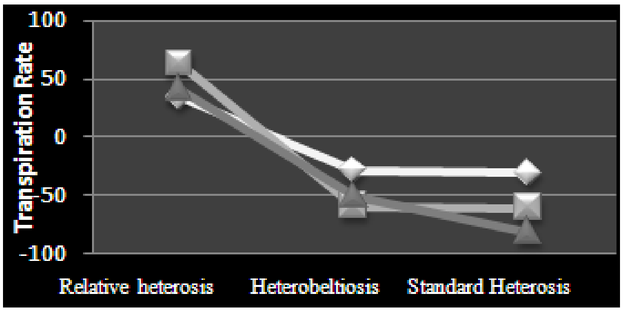

Fig. 7: Leaf Nitrogen Content.

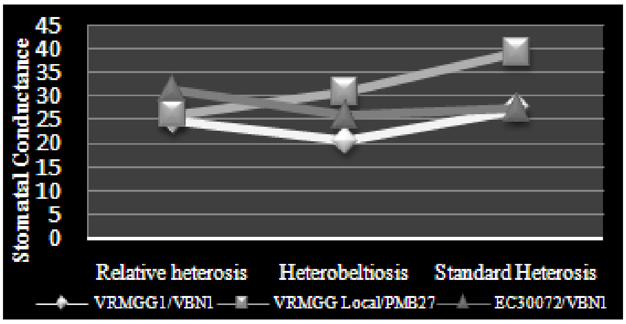

Fig. 8: Stem Nitrogen Content.

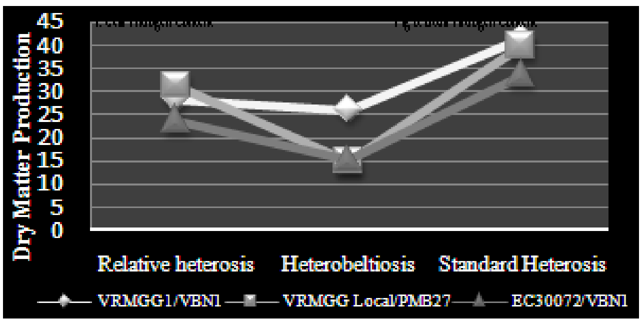

Fig. 9: Dry Matter Pr

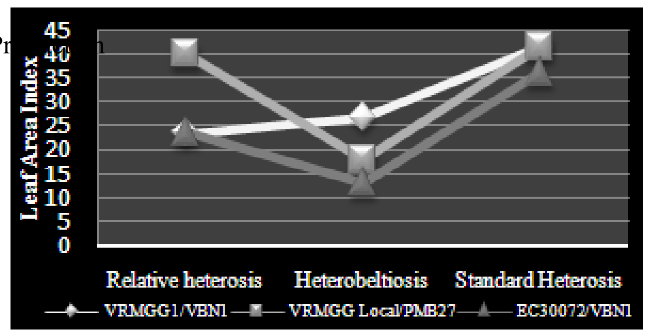

Fig. 10: Leaf Area Index.

Table 1: Mean of biochemical and biophysical characters in mungbean

Characters	Generations	Hybrids VRMGG 1 / VBN 1	VRMGG Local / PMB 27	EC 30072 / VBN 1
Total chlorophyll content	F_2	1.63	1.53	1.58
	F_3	1.59	1.50	1.45
Nitrate reductase activity	F_2	12.16	12.35	10.12
	F_3	11.92	12.11	10.89
Leaf nitrogen content	F_2	2.55	2.55	2.38
	F_3	2.41	2.47	2.32
Stem nitrogen content	F_2	2.24	2.03	2.12
	F_3	2.20	1.99	2.15
Seed protein content	F_2	26.09	21.72	21.11
	F_3	25.59	21.29	21.42
Photosynthetic rate	F_2	34.68	32.49	32.29
	F_3	34.02	32.81	32.61
Transpiration rate	F_2	4.20	4.82	4.68
	F_3	4.24	4.87	4.73
Stomatal conductance	F_2	0.42	0.50	0.45
	F_3	0.42	0.51	0.45
Dry matter production	F_2	21.24	21.17	21.21
	F_3	21.26	21.15	21.19
Leaf area index	F_2	1.39	1.36	1.38
	F_3	1.38	1.35	1.37

Table 2: Correlation between seed yield per plant and its biochemical and biophysical characters in mungbean

Characters	Generations	Hybrids VRMGG 1 / VBN 1	VRMGG Local / PMB 27	EC 30072 / VBN 1
Total chlorophyll content	F_2	0.77**	0.63**	0.53**
	F_3	0.55**	0.43**	0.48**
Nitrate reductase activity	F_2	0.75**	0.92**	0.62**
	F_3	0.69**	0.79**	0.59**
Leaf nitrogen content	F_2	0.03	0.24**	-0.13
	F_3	0.11	0.24**	-0.17*
Stem nitrogen content	F_2	-0.39**	-0.22**	0.09
	F_3	-0.41**	-0.13**	0.01
Seed protein content	F_2	-0.79**	-0.71**	-0.55**
	F_3	-0.01	-0.46**	-0.60**
Photosynthetic rate	F_2	0.84**	0.89**	0.57**
	F_3	0.83**	0.81**	0.66**
Transpiration rate	F_2	-0.27**	-0.61**	-0.54**
	F_3	-0.36**	-0.59**	-0.31**
Stomatal conductance	F_2	0.41**	0.72**	0.61**
	F_3	0.36**	0.71**	-0.70**
Dry matter production	F_2	0.744**	0.61**	0.82**
	F_3	0.526**	0.63**	0.96**
Leaf area index	F_2	0.747**	0.35**	0.88**
	F_3	0.812**	0.04**	0.89**

The cross VRMGG Local/ PMB 27 recorded maximum values for nitrate reductase activity and leaf nitrogen. The importance of these biochemical characters in growth, development and yield potential of crop plants is an established fact. The superior performance of VRMGG 1 / VBN 1 and VRMGG Local/ PMB 27 with enhanced biochemical parameters might have contributed for increased seed yield and yield parameters. The influence of chlorophyll combined with higher photosynthetic rate, dry matter production and seed yield per plant has also been reported by Goswami *et al.* (2003) and Wagh *et al.* (2003). Li *et al.* (1993) reported the importance of nitrate reductase activity in reducing nitrate to ammonia. In the present study also, the peak activity of nitrate reductase activity was in coincidence with the maximum chlorophyll, which was also supported by Dhumal and Laware (2003) and Beevers and Hageman (1969). Photosynthesis is the primary process, which forms the basis for yield determination. The hybrid VRMGG 1/VBN 1 recorded highest photosynthetic rate, dry matter production and leaf area index, whereas, comparatively low values for transpiration rate and stomatal conductance. High photosynthetic rate coupled with lower transpiration rate and stomatal conductance would improve the seed yield per plant by reduced water loss. However, very low transpiration rate and stomatal conductance will reduce the yield potential due to the imbalance in the CO_2 and H_2O interactions. Similar observations were recorded by Venkateswarlu and Balasubramanian (1993), Dhumal and Laware (2003), Ashwathama and Patil (2003) and Goswami *et al.* (2003). Total dry matter production is an index for over all efficiency of resources and better light interception. The reproductive organs are the important yield determinant characters for total dry matter production and partitioning in mungbean. Leaf area index, photosynthetic rate and leaf angle also seems to be the major determinant of total dry matter production (Yoshida *et al.* 1972). The hybrids with higher seed yield possessed significantly higher dry matter production compared to the low yielding hybrids. This may be attributed to higher number of pods, clusters and better partitioning efficiency. Chowdhary (2000) reported that dry matter production after flowering significantly influenced the seed yield and observed that most of the photosynthates produced at this stage were used for pod and seed development in mungbean. Similarly, all the high yielding hybrids recorded higher leaf area index than their parents. Nijhawan and Chandra (1979) and Panwar *et al.* (1986) reported that leaf area index was important and desirable traits for higher yield potential. Among the hybrids VRMGG 1/VBN 1 recorded the maximum mean values for most of the characters and moderate values for transpiration rate and stomatal conductance.

Estimation of Heterosis:

Mungbean is one of the important pulse crops and an excellent source of easily digestible proteins. The magnitude of D^2 is a basis for expression of heterosis. D^2 serves the breeder as a guide in the choice of diverse parents and is a pre-requisite in the development of hybrids. Relative heterosis is of limited importance, because it is only the deviation of F_1 from mid-parental value. Heterobeltiosis is the measure of hybrid vigour over the better parent. Reddi Sekhar *et al.* (1994) stressed the importance of computing heterosis for exploitation of hybrid vigour.

Hence, the evaluation of standard heterosis i.e., heterosis over the standard variety should be more emphasized than other types (Swindell and Poehlman, 1976). The study of biochemical characters like chlorophyll content, nitrate reductase activity, nitrogen content and seed protein help a lot in evaluating the production potential of genotypes. Estimation of heterosis for nitrate reductase activity gives an idea about the response of hybrids to nitrogen assimilation. Almost all the hybrids recorded positive heterosis for total chlorophyll over standard variety. Among hybrids, VRMGG 1/ VBN 1 and LGG 460/PMB 27 recorded the highest standard heterosis.

As the chlorophyll content is the main component of the photosynthetic system, it implies that the more the chlorophyll content, more will be the photosynthesis and higher the photosynthesis, the higher will be the dry matter production. These results therefore corroborate the conclusion of Goswamy *et al.* (2003), who stated that heterosis of dry matter production and seed yield in hybrids was the result of the interaction of heterotic photosynthetic rate and chlorophyll content. Further, it was noted that high yielding hybrids were associated with high levels of chlorophyll and photosynthetic activity compared to low yielding hybrids in mungbean.

Similarly, Dumal and Laware (2003) and Ashwathama and Patil (2003) observed the same findings. For nitrate reductase activity, all the hybrids recorded positively significant standard heterosis. VRMGG 1/VBN 1 recorded highest percentage of standard heterosis for stem nitrogen and seed protein, whereas LGG 460/ PMB 27 recorded highest standard heterosis for leaf nitrogen. Similar significant heterosis for protein content was reported by Aher *et al.* (1998) and Ashwathama and Patil (2003).

Estimation of heterosis for biophysical and morpho-physiological characters is of utmost importance as they give an idea about the stability of these hybrids to a particular environmental condition and can be used as a selection tool for increasing seed yield. All the hybrids, which possessed high heterosis for seed yield, recorded high percentage of heterosis for photosynthetic rate over mid parent and standard variety. Koti (1997), Jirali *et al.* (2003) and Ashwathama and Patil (2003) also observed similar results. The high yielding hybrid VRMGG 1/VBN 1 recorded high percentage of standard heterosis for photosynthetic rate, in contrast recorded negative heterosis for the transpiration rate and stomatal conductance. Similar type of finding was observed by Chimmad and Kamatar (2003). For dry matter production, the crosses VRMGG1/VBN 1 and LGG 460/ PMB 27 recorded high percentage of standard heterosis.

The same crosses also recorded high heterosis for seed yield per plant. This could be due to increased translocation of assimilates to the reproductive parts. Thandapani (1985) and Dodwad *et al.* (1998) also observed similar results. All the hybrids recorded positive standard heterosis for dry matter production and leaf area index. The highest standard heterosis for leaf area index was recorded in the crosses VRMGG 1/ VBN1 and VRMGG Local / PMB 27, which also recorded highest standard heterosis for total dry matter production and seed yield per plant. Wallace *et al.* (1972) found that high yielding hybrids possessed 10 per cent higher leaf number and area in mungbean.

Association Analysis:

Association of yield components with seed yield assumes special importance for indirect selection. In a crop like mungbean, the association analysis has been reported mostly on biometric characters. But with the recent advances in physiology and biochemistry, the role of biochemical, biophysical and morpho-physiological characters in effecting seed yield per plant have been much stressed. Moreover, it is desirable to elicit the information of their association with seed yield in order to have a clear picture on the seed yield factor.

The biochemical character, nitrate reductase activity recorded significant positive association with seed yield per plant for each of the F_2 and F_3 generations for all the three crosses *viz.*, VRMGG 1/VBN 1, VRMGG Local / PMB 27 and EC 30072 / VBN 1. Similarly, the crosses VRMGG 1/VBN 1 and VRMGG Local/PMB 27 recorded positive and significant association of total chlorophyll with seed yield per plant in both F_2 and F_3 generations. From the results, it is evident that total chlorophyll content is one of the important biochemical characters for enhanced seed yield determination in mungbean. High chlorophyll content especially in the later stages of growth helps in maintaining the leaves to remain active for longer period to supply photosynthates to the developing pods. Hence there is a need to identify high yielding genotypes having high chlorophyll content for getting higher productivity (Bharud and Sagare, 2003). Nitrate reductase showed positive and significant correlation with total chlorophyll content and these two play a vital role in determining the productive potential of mungbean. Similar results were also reported by Awasthi, (1988) in peas; Pandey and Singh, (1991) in lentil and Koti, (1997) in soyabean. Stem nitrogen recorded negative significant association with seed yield per plant for the crosses, VRMGG 1/VBN 1 and VRMGG Local /PMB 27 in both F_2 and F_3 generations, whereas the cross EC 30072/VBN 1 revealed negative association between leaf nitrogen and seed yield per plant in both the generations. It was found that seed protein content was inversely associated with seed yield per plant in VRMGG 1/VBN 1 and VRMGG Local /PMB 27, whereas Yohe and Poehlman (1972) reported similar negative association of seed yield per plant and seed protein content in mungbean and Koti (1997) in soyabean.

Correlation studies indicated that seed yield per plant possessed significant positive correlation with photosynthetic rate, transpiration rate, stomatal conductance, dry matter production and leaf area index. These results were in conformity with Buttery *et al.* (1993). It was also found that decline in photosynthetic rate was closely associated with stomatal conductance and transpiration rate and were instrumental in the increase or decrease of seed yield per plant. This was in support with Dhumal and Laware (2003) and Ashwathama and Patil (2003). It was noted that the high yielding hybrids were associated with higher dry matter production. Similar findings were reported by Wilson (1984), Lampang *et al.* (1987), Dodwad *et al.* (1998) and Hossain *et al.* (2009) . Gill *et al.* (1992) emphasised maintenance of optimum leaf area index, particularly at the flowering and maturity stages in order to get higher productivity in mungbean.

Conclusion

In this investigation, much emphasis was laid on the biochemical, biophysical and morpho-physiological characters and their impact on the seed yield per plant. It was also assumed to elicit information on various aspects relating to the various characters of mungbean having high yield potentials and their relationship towards productivity.

To achieve the above facts, experiments were conducted to study the heterosis, association analysis and genetic architecture of characters viz., seed yield per plant, total chlorophyll content, nitrate reductase activity, stem nitrogen content, leaf nitrogen content, seed protein content, photosynthetic rate, transpiration rate, stomatal conductance, dry matter production and leaf area index. The parent, VRMGG 1 recorded the maximum per se performance for photosynthetic rate, dry matter production and leaf area index, whereas LGG 450 and EC 30072 revealed high mean values for transpiration rate and stomatal conductance, respectively. Among the biochemical characters, the cross VRMGG 1/ VBN 1 recorded the maximum mean values for total chlorophyll content, seed protein, photosynthetic rate, dry matter production and leaf area index, whereas LGG 460/ PMB 27 showed maximum means for stem nitrogen and VRMGG Local / PMB 27 for nitrate reductase activity and leaf nitrogen.

All the biochemical, biophysical and morpho-physiological characters showed positive and significant association with seed yield per plant, except for transpiration rate and seed protein. The crosses VRMGG 1/VBN1, VRMGG Local/ PMB 27 and EC 30072/ VBN 1 recorded high percentage of heterosis for characters like Photosynthetic rate, chlorophyll content and dry matter production and also recorded positively significant associastion of these characters with seed yield per plant. Hence, these hybrid combinations can be utilized for commercial exploitation.

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