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Genotypic Variations in Stomatal Regulation and Root Traits and their Association with Yield Parameters in Moth Bean (*Vigna aconitifolia* Jacq.) under Rainfed Conditions of Arid Zone

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Abstract: The experiments were conducted during kharif 2003 and 2004 seasons to analyze the genotypic variations in stomatal regulation and root traits and their association with yield parameters in moth bean (*Vigna aconitifolia* Jacq.) under water scarcity conditions of arid zone. Wide range of variations in total dry matter (TDM), pod yield (PY) and seed yield (SY) were noticed among the 26 genotypes. Our results suggest that genotypes with thick leaves (low SLA) exhibit more drought tolerance by keeping their stomata open at lower relative water content (RWC) in leaves. Significant relationship of specific leaf area (SLA) with both seed yield ($r^2 = 0.57$) and relative water content (RWC) ($r^2 = 0.49$) suggested that low SLA could be an important leaf trait for selecting moth bean genotypes for higher yield under water-deficit conditions. The strong relationship of specific root weight (SRW, root weight per unit root length) with TDM ($r^2 = 0.73$) and PY ($r^2 = 0.56$) indicated that selection of genotypes with extensive root system would enhance the yield of moth bean under water scarcity conditions of arid zone. This study suggested that SLA, a cost-effective and easily measurable leaf trait, and/or SRW are the potential selection criteria to enhance the moth bean yield under rainfed conditions of the arid zone.

Key words: Arid zone, moth bean, pod yield, root length, root weight, seed yield, specific leaf area, specific root weight, total dry matter.

Moth bean, *Vigna aconitifolia* (Jacq.) Marechal), known as *Phaseolus aconitifolia* (Jacq.), is an important crop of the north-western arid zone of India. It is one of the most drought-tolerant legumes grown during kharif season on light sandy soil as a rainfed crop. The total area under this crop is approximately1.33 million hectare with production of 0.20 million tonnes of seed. Besides being a pulse crop, its green pods are consumed as vegetable and the plant, being highly palatable and rich in protein, forms an excellent fodder. On account of its mat-like growth habit, it is highly useful in retarding soil erosion, loss of soil moisture, crust formation and organic matter loss (Soni, 1992).

The cultivars of moth bean are inherently low-yielding and the productivity is only 250 kg ha^{-1} (Kumar *et al.*, 1998). Studies undertaken on growth, yield and various physiological attributes with selective moth bean genotypes under drought and good rainfall conditions indicated that early flowering genotypes (30-31 DAS to 50%

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flowering) provided significantly higher yield, especially in a drought year (Kumar *et al.*, 1998). Henry and Daulay (1983a, b) reported mutants, which exhibited combination of earliness in maturity, high degree of tolerance to drought condition and high yield potential could exploit favorable growing condition better than other varieties. Garg (2004) reported that significant genotypic differences existed for different physiological attributes and drought resistance traits in moth bean which need to be better understood for selection and development of drought-tolerant genotypes with higher yield potential under water-limited conditions. To improve the adaptation of moth bean to harsh climate of arid zone, identification of the genetic variability in physiological traits associated with drought tolerance and their association with the yield need to be established. The present study is aimed at identifying genotypic variations in stomatal regulation and root traits and examine their association with yield parameters in moth bean (*Vigna aconitifolia* Jacq.) grown under rainfed conditions of arid zone.

Material and Methods

The experiments were conducted during 2003 and 2004 kharif seasons at the research farm of Regional Research Station, Central Arid Zone Research Institute (CAZRI), Bikaner, India (latitude 28.4°N, longitude 74.3°E). The rainfall during 2003 and 2004 growing season were 227.9 mm and 127.7 mm, respectively, which were 17.0 and 53.6% less over the normal rainfall of 275 mm. The soil is alkaline (pH_2 8.5), non-saline (EC_2 0.22 dS m^{-1}), loamy sand in texture with organic carbon 0.10%. The water retention at field capacity was 8.1% (%, w/v). During kharif 2003, 26 genotypes of Moth bean were evaluated for yield components and drought resistant traits, while during kharif 2004, studies were conducted on contrasting nine genotypes selected on the basis of their performance and leaf traits expressed during kharif 2003. Both the experiments were sown in RBD design with three replications. Recommended packages and practices were followed. The required observations on phenology (days to emergence, flowering, pod initiation, maturity) were recorded during both the years.

Yield and yield components

At maturity, plant samples of 2.4 m^2 area were taken from each plot in both the experiments and separated into vegetative parts and pods. The samples were air-dried and dry weights were recorded to determine TDM, pod weight and harvest index.

Relative water contents (RWC) and specific leaf area (SLA)

RWC and SLA were recorded at 40 and 60 DAS. Second fully expanded leaf, from the apex of main stem, from 10 randomly selected plants (40 leaflets) was plucked and their fresh weight was recorded immediately (W_f). The leaflets were floated on water in a petridish for 4 hours and saturated weight were recorded after wiping off the free water from the surface of leaflets (W_s). The leaf areas of these leaflets were measured using leaf area meter (Systronics made model 211). The leaflets were oven dried at 80°C for 48 hours and weights were recorded (W_d). RWC and SLA were calculated as follow:

RWC = $[(W_f-W_d)/(W_s-W_d)]*100$

SLA = leaf area/W_d

Critical value of RWC for stomatal closure

The critical value of RWC, at which stomata almost closed, was recorded by determining the RWC in two contrasting genotypes chosen on basis of specific leaf area (Clavel and Annerose, 1995). Second, fully expanded leaves from the top of main stem from 5 plants of each plot were collected and immersed in distilled water for 4 hours under natural full light at room temperature so as to maintain the complete turgor of cells and stomatal aperture. The leaves were then quickly wiped and weighed to obtain their turgid weight. Thirteen successive weighings of leaves were made under ambient conditions during a period of dehydration lasting 180 minutes. The weights were taken after 2, 4, 6, 8, 10, 12, 15, 20, 30, 60, 90, 120, and 180 minutes of drying. Leaf dry weight was obtained after oven-drying for 24 h at 80°C. The RWC of the leaves at each time was calculated.

Measurement of root traits

To study the root traits, three monoliths (each of 50 x 50 cm) were exposed under each genotype. The depth of monolith varied till the end of root tips. Three plants of each genotype with intact roots were carefully freed from soil at 60 days after planting. Roots were thoroughly washed free of soil with tap water, followed by deionized water. Root length under each monolith was measured using a modified line intercept method (Tennant, 1975). Roots were dried in oven at 80°C for 48 hours to a constant weight and root weight was recorded on a digital balance. TDM and pod weight were determined on per plant basis by oven-drying of the samples. Specific root weight (SRW) was calculated as follow:

SRW = Root weight (mg)/Root length (cm)

Results and Discussions

Yield components

Significant genotypic variations for total dry matter, pod and seed yield were noticed among the moth bean genotypes evaluated (Table 1). Range of total biomass accumulated among the 26 genotypes was 52.8 to 101.2 g m^{-2}. The genotypes like RMM 225, CGM 79 produced double total dry matter than local check RMO 40. Large variability was observed in pod yield (3.9 to 49.0 g m^{-2}) and seed yield (1.2 to 36.1 g m^{-2}). The data revealed that in spite of sufficient amount of dry matter production, the crop was a poor seed yielder (Srivastava and Soni, 1995). The low yield in this crop is owing to low net assimilation rate (NAR) during the reproductive phase and poor migration coefficient of assimilates towards seeds (Srivastava and Soni, 1995).

Relative water content (RWC) and specific leaf area (SLA)

Significant genotypic differences exist for different physiological attributes and drought tolerance in moth bean as reported by Garg (2004). Many traits are associated with drought resistance and these include relative leaf water contents, NAR, nitrate reductase activity and free proline (Garg *et al.*, 2001), specific leaf area (Nageswara Rao *et al.*, 2001), and crop growth and partitioning (Nageswara Rao *et al.*, 1993). The relationship between water-use efficiency and leaf thickness (SLA) has been well

Table 1. Variation in total dry mater (TDM), pod yield (PY) and seed yield (SY) of 26 moth bean genotypes grown during kharif 2003 under rainfed conditions

Genotype	TDM	PY	SY
	------------ g	m^{-2}	------------
RMO 40	52.8	30.6	17.6
RMO 257	64.9	14.6	8.5
RMO 225	100.4	24.8	10.1
RMM 50	61.7	17.7	12.2
RMM 47-2	48.4	10.5	6.2
RMM 44	78.8	31.0	21.5
RMM 4	75.6	38.8	26.2
RMM 35	62.6	36.3	27.1
RMM 24	56.1	25.8	18.4
RMM 21	55.5	8.6	4.8
RMM 2	88.5	15.6	9.7
RMM 12	66.1	17.6	11.7
RMM 102	66.1	29.3	21.1
RMM 101	85.5	49.0	36.1
RMB 54	64.3	17.7	11.3
RMB 50	78.8	26.4	19.0
RMB 25	69.8	16.9	9.9
JAWALA	97.0	3.9	1.2
F_4-58	86.7	4.9	1.6
Marumoth	74.5	26.6	17.2
F_4-57	73.4	31.6	23.3
F_4-1	62.8	11.3	6.5
EXP 221	79.5	24.2	15.1
CGM 79	101.7	22.5	16.2
EXP 38	63.5	18.1	11.0
EXP 213	82.9	16.3	9.5
Mean	73.0	21.9	14.3
SEm±	5.8	2.8	0.8

documented in groundnut (Nageswara Rao *et al.,* 2001). A wide range of variation in SLA (119-339 $cm^2 g^{-1}$) was noticed among the genotypes. These variations in SLA (leaf thickness) might be due to the variations in photosynthetic machinery per unit leaf area e.g., Rubisco, soluble total nitrogen and chlorophyll content in groundnut (Nageswara Rao *et al.,* 1995). Therefore, maintenance of higher photosynthetic rates and better metabolic efficiency in genotypes with low SLA (thicker leaves) under water stress at either growth stages led to significantly less reduction in their seed yield and dry matter production as compared to genotypes with higher SLA (thinner leaves) (Garg *et al.,* 2004). Variations in RWC were also significant among genotypes tested (74.7-87.8%) and a strong negative relationship of SLA with RWC (Fig. 1a) and seed yield (Fig. 1b) indicates that selection for lower SLA (thicker leaves) should result in the identification of the genotypes with improved drought tolerance as well as improved seed yield.

Stomatal regulation

The stomatal control of water losses has been identified as an early event in plant response to water deficit under field conditions, which limits carbon uptake by the leaves (Chaves *et al.*, 2002). The curves shown in Fig. 2 were established by calculation of the RWC after determination of leaf weight loss during a drying period of 180 minutes. The point where a strong decrease of the slope of the curves is observed corresponds to the RWC (and time) at which stomatal closure occurs. The genotypes which has thinner leaves (higher SLA) cv. EXP 38 showed an early stomatal closure which was obtained at RWC of 54.7±1% (Fig. 2a) while the genotype with thicker leaves cv. RMO 40 showed a late stomatal closure when leaf RWC reached 48.5±2% (Fig. 4b). Previously, numerous experiments in other crops have shown that stomata closure in response to hydraulic

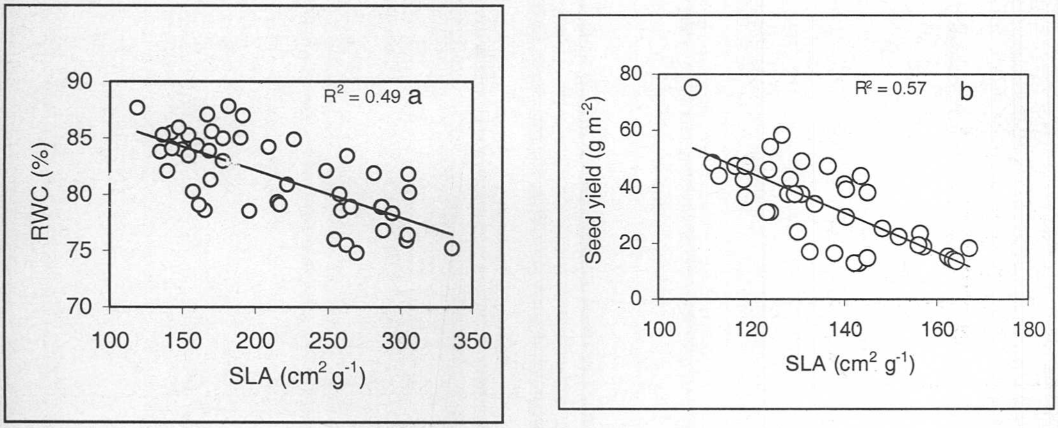

Fig. 1. Relationship of specific leaf area (SLA) with (a) relative water content (RWC) and (b) seed yield in selected set of moth bean genotypes.

and chemical signals, mainly abscisic acid (ABA), produced as a result of soil moisture decrease allowing the leaf water status to be maintained (Chaves *et al.*, 2002). It is hypothesized that genotype with thicker leaves (low SLA) exhibit more drought tolerance by keeping their stomata open under severe water deficits as reported earlier in *Phaseolus vulgaris* (Costa França *et al.*, 2000).

Root traits with yield components in moth bean

The genotypes EXP 213 and F_4 -58 performed best in terms of PY and TDM (Table 3). Our study clearly indicated that

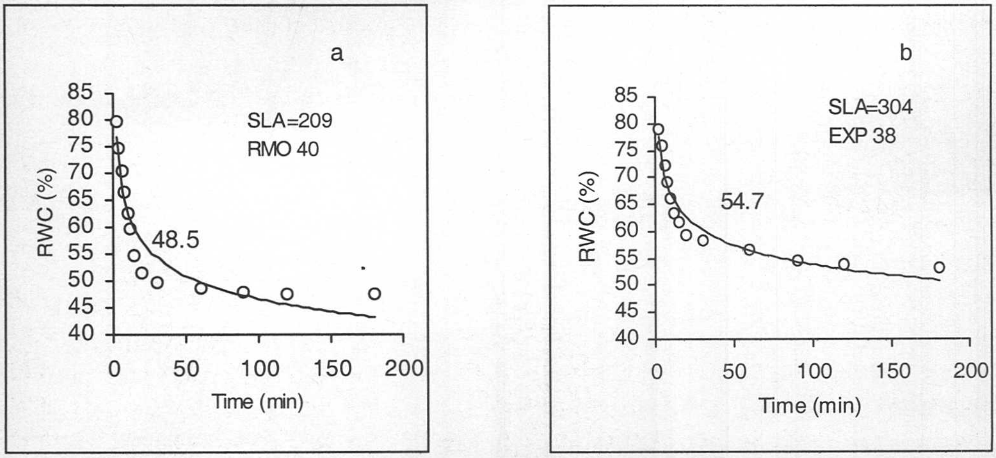

Fig. 2. Relationship between changes in relative water content with time in two contrasting genotypes (variable in leaf thickness, SLA) of moth bean (stomata closes at 48.5% and 54.7% relative leaf water content in RMO 40 (a) and EXP-38 (b), respectively).

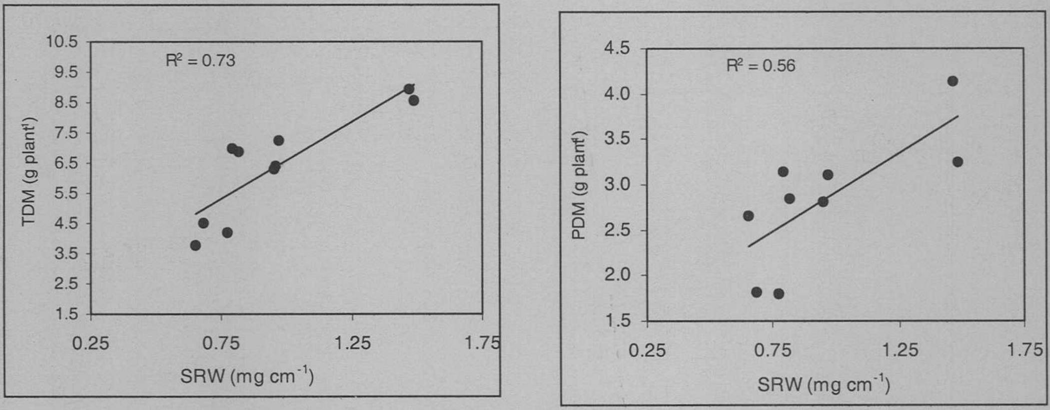

Fig. 3. Relationship of specific root weight (SRW) with total dry matter (TDM) and pod dry matter (PDM) in nine genotypes grown during 2004 rainy season.

these two genotypes had more extensive root system either in term of root length and/or root weight. This suggested that these genotypes display a mechanism of dehydration avoidance by maximization of water uptake through deep and extensive root systems (Garg, 2004). The deeper and extensive root system of these genotypes rendered them more drought-tolerant and productive than the other genotypes (Kumar, 2002). Association between deep root system and drought tolerance has been demonstrated in soybean (Kaspar *et al.*, 1978) and common beans (Sponchiado *et al.*, 1989). The strong relationship of specific root weight (root weight per unit root length) with TDM (Fig. 3a; r^2= 0.73) and pod yield (Fig. 3b; r^2= 0.56) indicated that selection of genotypes with extensive root system would enhance the yield of clusterbean under water scarcity conditions of arid zone. Recently, significant correlations between root length density and

Table 2. Variations in root length (RL), root dry weight (RDW), specific root weight (SRW), total dry matter (TDM), pod dry matter (PDM) and shoot dry weight (SDW)

Genotype	RL (cm plant^{-1})	RDW (g plant^{-1})	SRW (mg cm^{-1} RL)	PDM (g plant^{-1})	TDM (g plant^{-1})	SDW (g plant^{-1})
F_4-57	552.2	0.43	0.77	1.80	4.20	2.40
RMO-40	669.3	0.43	0.65	2.65	3.78	1.13
F_4-1	536.5	0.51	0.95	2.80	6.30	3.50
RMO257	669.7	0.53	0.79	3.13	7.00	3.87
Jawala	521.3	0.51	0.97	3.10	7.23	4.13
Maru moth	595.7	0.49	0.81	2.85	6.87	4.02
F_4-58	575.7	0.86	1.49	3.25	8.57	5.32
Exp-38	532.2	0.36	0.68	1.82	4.52	2.70
EXP 213	666.7	0.97	1.46	4.13	8.92	4.70
Mean	591.0	0.48	0.95	2.84	6.38	3.54
SE	18.2	0.052	0.029	0.186	0.392	0.248

seed yield have been demonstrated in chickpea (Kashiwagi *et al.*, 2006). This study suggested that SLA and/or SRW can be potential selection criteria for improving yields of moth bean under rainfed conditions of arid zone.

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