



Effect of Supplemental Irrigation on Growth, Physio-biochemical and Yield Responses of Mothbean (*Vigna aconitifolia* L.) Genotypes under Rainfed Condition in Semi-arid Regions of Rajasthan

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Abstract: Mothbean cultivation in arid and semi-arid regions is practiced mainly under rainfed condition but increasing temperature and irregular rainfall pattern lead to drought conditions and substantially decreasing the yield. Supplemental irrigation at critical crop growth stage can play crucial role under water deficit situations in such regions. The field experiment was conducted to assess growth, physio-biochemical and yield responses of seven contrasting mothbean genotypes, viz. RMO-257, RMO-40, RMB-25, RMO-2251, CZM-45, RMO-435 and RMO-225 under rainfed (RF, drought condition) and supplemental irrigation (SI, irrigation applied at flowering stage i.e. 30 DAS) conditions. SI significantly improved plant growth attributes and water status which ultimately enhanced yield up to 35% as compared to RF condition. The water stress conditions significantly increased activities of antioxidative enzymes i.e. ascorbate peroxidase (APOX) and guaiacol peroxidase (GPOX) especially in tolerant genotypes. The correlation matrix and principal component analysis emphasized that there is positive relationship between growth attributes, water indices and yield of the genotypes whereas antioxidative enzymes showed negative relationship with yield. Among the genotypes studied, RMO-257, RMO-40 and RMB-25 performed well, whereas RMO-435 is more susceptible to drought conditions, as also demonstrated by a heat map of drought tolerance indices.

Keywords: *Vigna aconitifolia*, Drought stress, Rainfed, Supplemental irrigation, Antioxidative enzymes

Mothbean [*Vigna aconitifolia* (Jacq.) Marechal] is an important pulse crop in Rajasthan's arid and semi-arid zones (cultivated on 1 million hectares, with 0.2 million tons produced and an average productivity of 228 kg/ha). It is a rainfed crop and requires less humidity, high temperature (24-32°C) and rainfall of around 50-75 cm. Climate change with increasing temperature, low average annual rainfall, erratic rainfall, and dry atmospheric conditions fulfills only 25-30% of the crop water requirements during the growing season thus water shortage is becoming a key challenge resulting in quantitative and qualitative yield losses in mothbean, especially in rainfed agriculture system (Kumar and Chander, 2020, Overpeck and Udall 2020). Although there is sufficient rainfall to enhance yield under rainfed farming system but it is not available during the critical stages of crop growth causing dry spells.

Drought stress has a negative impact on plant growth, reducing leaf area, other physio-biochemical processes such as membrane stability index (MSI), relative water content (RWC), water potential (WP), and photosynthesis, limiting development to achieve maximum yield (Vujanovic and Germida 2017). It also causes the production of reactive oxygen species (ROS) such as superoxide, peroxide, hydroxyl radical, singlet oxygen, and alpha oxygen, which

cause a variety of detrimental events such as lipid peroxidation of cell membranes, oxidation of proteins and nucleic acid, and ultimately cell death. Antioxidative enzymes such as ascorbate peroxidase (APOX), guaiacol peroxidase (GPOX), catalase (CAT) and superoxide dismutase (SOD) play an important role in regulation of these ROS under water stress conditions. Under drought stress, the level of normal metabolic enzymes such as nitrate reductase (NR) decreases while antioxidative enzymes increase to maintain the plant's overall (Hasanuzzaman et al 2020).

To maintain sustainable crop yields while dealing with the additional challenge of drought stress, there is an increasing need to replace rainfed cropping systems with irrigated systems, which has been shown to be an effective strategy for increasing up to 43% of global agricultural production (Okada et al 2018). Supplemental irrigation during the most sensitive stages of the crop growth, such as flowering and grain filling will help to increase survival and yield under rainfed conditions (Farooq et al 2017). Irrigation scheduling, which includes providing water that matches crop evapotranspiration and providing irrigation at critical growth stages, improves field crop water use efficiency. Water conservation by using a limited amount of water during critical crop growth stages result in a significant increase in

yield and an improvement in the livelihoods of smallholder farmers in dry rainfed areas (Molla et al 2021).

The information pertaining to adoption of supplemental irrigation under water stress conditions in mothbean genotypes under agro-climatic conditions of arid North-western India are meager. Therefore, the objective of the current study was to evaluate the effect of supplemental irrigation on physio-biochemical traits and yield of seven mothbean genotypes widely grown in semi arid region.

MATERIAL AND METHODS

Site description: The study was conducted on the experiment area of the Central Arid Zone Research Institute, Regional Research Station, Bikaner (28°4' N; 74°3' E; 238.3 m above mean sea level), Rajasthan. The experiment was executed from August to October, 2020 (Table 1). The soil of experimental site was loamy sand, having 8.5 pH, 0.15 % organic carbon, 85 kg ha⁻¹ available N, 96 kg ha⁻¹ available P and 256 kg ha⁻¹ available K.

Experimental design and treatment: Seven mothbean genotypes i.e. RMO-257, RMO-40, RMB-25, RMO-2251, CZM-45, RMO-435 and RMO-225 were sown in a randomized complete block design (RCBD) in lines with line to line distance of 40 cm and plant to plant distance of 15cm under two irrigation treatments of rainfed production (RF) and supplemental irrigation (SI). The main plots contained rainfed and supplemental irrigation condition and sub plots had seven mothbean genotypes with three replicates. Size of each plot was 4m x 2m with 2m gap in between and there were 5 rows in each replication. Seed rate was @10kg/ha and sowing was done on 8th August, 2020 after the rain. A basal dose of 10 kg N ha⁻¹ (as urea) and 20 kg P ha⁻¹ (as single superphosphate) was applied at sowing. For SI treatment, irrigation was applied before flowering (40% of soil available water was depleted at this stage). Soil available water was monitored using soil moisture probe (Profile Probe PR2), which monitors soil moisture content at 10, 20, 30, 40, 60, and 100 cm soil depth. The amount of supplemental irrigation (SI) was calculated by $I = 10 \cdot \gamma \cdot H \cdot (FC - \beta_j)$ (Shang et al 2020), where I is the amount of SI, γ (1.37 g.cm³) is the soil bulk density, H (7.35 cm) is the soil depth of the wetting layer, FC (30%) is the field capacity, and β_j (5%) is the soil water content before irrigation. Observations were recorded after 10 days of irrigation treatments in three replicates.

Plant growth characteristics: Ten plants were randomly selected from the central 2 × 2 m area of each plot to determine dry matter (DM) accumulation per plant. The plants were oven dried at 65 °C ± 5 °C till constant dry weight following which the shoots dry mass was recorded and expressed as g dwplant⁻¹. Chlorophyll and carotenoid

contents were extracted by the non-maceration method (Hiscox and Israelstam 1979). The total leaf area per plant was measured by portable leaf area meter model (Systronics leaf area meter 211) and expressed as cm².

Plant water status: Plant water relation parameters i.e. RWC, MSI and WP were observed by taking three replications of each genotype. RWC of leaf samples was determined as described by Barrs and Weatherley (1962). The MSI of leaf samples was determined following the procedure described by Sairam et al (2002). WP of fully expanded youngest leaves was measured during 9.30-11.30 h by WP 4 Dew-Point Potential Meter.

Enzymatic activity: *In vivo* leaf NR activity was assayed according to the procedure of Hageman and Hucklesby (1971) with slight modifications. NR was measured in 200 mg of finely chopped leaves that were incubated in a medium containing 5ml of 0.1 M phosphate buffer, 0.02M KNO₃, 5% propanol and two drops of chloramphenicol (0.5mg/ml). After incubation of samples for 2 h in the dark at 30C a mixture of 1 ml each of 1.0% sulphanilamide in 1N-HCl and 0.025% N-(1-Naphthyl)-ethylene diammonium dichloride (NEDD) were added. The absorbance was read at 540 nm, after 30 min using UV-Vis spectrophotometer (model Specord Bio-200, AnalytikJena, Germany). The calibration curve was prepared using sodium nitrite solution. The enzyme activity was expressed as $\mu\text{mol NO}_2 \text{ g}^{-1} \text{fw h}^{-1}$.

APOX activity was assayed by following the decrease in absorbance at 290 due to ascorbate oxidation ($\epsilon = 2.8 \text{ mM}^{-1} \text{ cm}^{-1}$) in a reaction mixture (1 ml) contained 530 μl of 50 mM phosphate buffer (pH 7.0), 200 μl of 0.5 mM ascorbic acid, 200 μl of 0.1 mM H₂O₂, 50 μl of 0.1 mM EDTA and 20 μl of enzyme extract for 1 min following the method of Nakano and Asada (1981). Protein content was determined by the method of Bradford (1976) using bovine serum albumin as the standard and used for the calculation of APOX enzyme. APOX enzyme specific activity is expressed as μmol (ascorbate oxidized) min⁻¹ (mg protein)⁻¹.

GPOX activity was assayed, was measured by following the increase in absorbance due to the oxidation of guaiacol at 470 nm ($\epsilon = 26.6 \text{ mM}^{-1} \text{ cm}^{-1}$) for 1 min. The assay mixture (1 ml) contained 530 μl of 50 mM phosphate buffer (pH 7.0), 50 μl of 0.1 mM EDTA, 200 μl of 10 mM guaiacol and 200 μl of 10 mM H₂O₂ and 20 μl of enzyme extract for 1 min as described by Chance and Maehly (1955). Protein content was determined by the method of Bradford (1976) using bovine serum albumin as the standard and used for the calculation of GPOX enzyme. GPOX activity is expressed as μmol (tetraguaiacol formed) min⁻¹ (mg protein)⁻¹.

Yield and yield attributes: Yield (Y) and its attributes i.e. branches plant⁻¹(BP), pod length (PL), number of pods plant⁻¹

(PP), number of seeds pod⁻¹ (SP) and test weight (TW) of seeds were recorded from each plot by manual harvesting of plants 3 cm above the ground and allowed to dry in the field.

Drought tolerance indices: Drought tolerance indices were calculated using the following equations (Fischer and Mourer 1978, Grzesiak et al 2019)

1. Stress Susceptibility Index (SSI) = $1 - [(Y_s) / (Y_p)] / [1 - (\bar{Y}_s) / (\bar{Y}_p)]$
2. Stress Tolerance (TOL) = $(Y_p - Y_s)$
3. Stress Tolerance Index (STI) = $[(Y_p) \times (Y_s)] / (\bar{Y}_p)^2$
4. Yield index (YI) = Y_s / \bar{Y}_s
5. Yield Stability Index (YSI) = Y_s / Y_p
6. Sensitivity drought index (SDI) = $(Y_p - Y_s) / Y_p$
7. Drought resistance index (DI) = $Y_s \times (Y_s / Y_p) / \bar{Y}_s$
8. Relative drought index (RDI) = $(Y_s / Y_p) / (\bar{Y}_s / \bar{Y}_p)$
9. Stress susceptibility percentage index (SSPI) = $(Y_p - Y_s) / (2 \times Y_p) \times 100$
10. Geometric Mean Productivity GMP = $[(Y_p) \times (Y_s)]^{0.5}$
11. Mean Productivity MP = $(Y_p + Y_s) / 2$

In the above formulas, Y_s , Y_p and \bar{Y}_s , \bar{Y}_p represent yield in stress (RF) and non-stress (SI) conditions for each genotypes, and yield mean in stress and non-stress conditions for all genotypes, respectively.

Statistical analysis: Before analysis, the Shapiro Wilk test at 0.05 was conducted using R software (v. 4.1.0) to test the normality of the data and fitting data transformation was performed for any data that was not normally distributed. Analysis of variance (ANOVA) was also performed using the same software. Correlation (Pearson) analysis, Principal component analysis and box plot were performed using "R v. 4.1.0" in Rstudio 1.3.1039. "Pheatmap" package of R program was used to create heat map and hierarchical clustering for various stress indices.

RESULTS AND DISCUSSION

Plant growth characteristics: Plant growth parameters showed significant increase under SI condition as compared to RF condition in all the genotypes (Table 2). Irrespective of treatments, plant biomass was estimated maximum in RMO-40 followed by RMO-257 and minimum in CZM-45 and maximum under SI (6.7 g plant⁻¹) condition which was almost

double than biomass under RF (3.1 g plant⁻¹) condition. The interaction between genotypes and treatments revealed that plant biomass was maximum in RMO-40 followed by RMO-257 under SI condition and minimum in RMO-225 under RF condition. Chen et al (2018) also showed that supplemental irrigation increased the above ground biomass in sorghum and cotton plants. Irrespective of treatments, no significant variation was observed in plant height among the genotypes. Plant height was maximum in SI condition (26.6 cm) which was 49 % higher than RF (17.9 cm) condition. The interaction between genotypes and treatments was non significant for plant height (Table 5). Souza et al (2020) observed similar results in which varied irrigation regimes had no significant effect on plant height.

Total chlorophyll was estimated maximum in RMB-25 followed by RMO-40 irrespective of treatments (Table 2). Total chlorophyll under SI (2.4 mg g⁻¹ fw) condition was 24.4% higher than RF (1.9 mg g⁻¹ fw) condition. The total chlorophyll was maximum in RMO-40 in SI condition and minimum in CZM-45 in RF condition. Similarly carotenoid content was maximum in SI (0.67 mg g⁻¹ fw) condition as compared to RF (0.55 mg g⁻¹ fw) condition (Table 2). Carotenoid content was maximum in RMO-40 with par value in RMB-25 under SI condition while minimum in CZM-45 under RF condition. The main reason for decrease in chlorophyll and carotenoids content as affected by water stress is that during drought stress the plant tends to produce reactive oxygen species which can lead to lipid peroxidation and chlorophyll and carotenoid destruction (Mafakheri et al 2010, Hu et al 2023).

The leaf area was maximum in SI (470.9 cm²) condition which was almost double than RF (229.9 cm²) one (Table 2). Irrespective of treatments, was highest in RMO-40 followed by RMO -2251 and minimum in RMO-25. Perusal of data pertaining to the interaction between genotypes and treatments revealed that leaf area was maximum in RMO-40 under SI condition while minimum in RMO-225 under RF condition. The genotypes, treatments difference and interaction were significant for chlorophyll, carotenoids and leaf area (Table 5). Supplemental irrigation increased the chlorophyll content that in turn increased the photosynthetic activity and leaf area of the plants (Liao et al 2022), while

Table 1. Weather data for the growing period of mothbean at the CAZRI (RRS), Bikaner

Months	Relative humidity (%)	Temperature		Total rainfall (mm)
		Minimum (°C)	Maximum (°C)	
August	44.4	37.0	27.5	99.2
September	34.9	38.4	25.4	16.4
October	28.0	35.7	18.6	0.0

Source: Meteorological station, CAZRI (RRS), Bikaner

water limiting conditions caused reduction in leaf area due to inhibition of cell expansion by declining rate of cell division and loss of cell turgidity to minimize transpiration losses (Bangar et al 2019). Furthermore, the variation in the growth attributes among the genotypes might be due to the genotypic variations associated with the different genotypes under the present investigation.

Plant water status: Plant water status is the basic criteria for drought tolerance measurement. RWC, MSI and WP were significantly improved under SI condition as compared to RF condition (Table 3). Irrespective of treatments, RWC was maximum in RMO-257 with non-significant variation with RMO-40 while was minimum in RMO-435 with par value in RMO-225. Regardless of genotypes, SI (84%) treatment improved RWC of plant which was 26.12% higher than plants

under RF (66.6%) condition. The interactive effect of genotypes and treatments showed that RWC was maximum for CZM-45 under SI condition followed by RMO-257 whereas minimum RWC was recorded in RMO-435 under RF condition.

Similarly, regardless of treatments, MSI and WP were estimated maximum in RMO-40 were at par with RMO 225 and minimum in RMO-225. MSI and WP were significantly improved under irrigated condition i.e. 22.9% and 39.5% higher than respective RF genotypes. Irrigated RMO-40 genotype had maximum MSI and WP values with minimum in rainfed RMO-225 genotype. Genotypic variability, treatment difference and the interaction of genotypes and treatments was found to be statistically significant for RWC, MSI and WP) (Table 5). Chowdhury et al

Table 2. Plant growth parameters of mothbean genotypes grown under rainfed and supplemental irrigation conditions

Treatment		Plant biomass (g)	Plant height (cm)	Total chlorophyll (mg g ⁻¹ fw)	Carotenoids (mg g ⁻¹ fw)	Leaf area (cm ²)
Genotypes						
RMO-257		5.7a	22.6a	2.0d	0.56c	336.6cd
RMO-40		5.8a	21.8a	2.3ab	0.64ab	396.7b
RMB-25		4.8bc	22.2a	2.4a	0.66a	326.2d
RMO-2251		4.9b	22.1a	2.0cd	0.63ab	362.6b
CZM-45		4.4c	22.1a	2.0d	0.55c	351.1bc
RMO-435		4.6bc	22.0a	2.3ab	0.64ab	350.7bc
RMO-225		4.4c	22.9a	2.2bc	0.60bc	328.9d
Treatments						
Rainfed (RF)		3.1b	17.9b	1.9b	0.55a	229.9b
Supplemental irrigation (SI)		6.7a	26.6a	2.4a	0.67b	470.9a
Genotypes x Treatments						
RMO-257	RF	4.3d	18.6bc	1.7hi	0.51ef	253.0ef
	SI	7.1b	26.7a	2.2cd	0.61bcd	420.2d
RMO-40	RF	3.9d	18.4bc	2.0fgh	0.56de	262.4e
	SI	7.7a	25.1a	2.7a	0.72a	531.1a
RMB-25	RF	3.1e	16.4c	2.2cdef	0.61cd	220.5ghi
	SI	6.5bc	28.0a	2.6a	0.72a	431.9d
RMO-2251	RF	3.1e	16.1c	2.0efg	0.58cd	237.7efg
	SI	6.7bc	28.1a	2.1def	0.68ab	487.4bc
CZM-45	RF	2.6ef	17.6bc	1.7i	0.49ef	231.0fgh
	SI	6.1c	26.6a	2.3cd	0.62bcd	471.1bc
RMO-435	RF	2.6ef	18.0bc	2.2cde	0.63bc	207.80hi
	SI	6.6bc	25.9a	2.4bc	0.64bc	493.5b
RMO-225	RF	2.3f	20.1b	1.8ghi	0.48f	196.5i
	SI	6.5bc	25.7a	2.6ab	0.72a	461.2c
CV%		7.8	9.1	6.4	6.6	4.7

Data with different alphabet, are significantly different ($p < 0.05$) as analyzed by Duncan's multiple comparison tests for post hoc analysis
CV: coefficient of variation

(2017) also showed that water stress condition significantly decreased the RWC, MSI and WP of plants. Loss of water from plant tissues under drought conditions decreases WP and RWC in plants and also impairs the membrane structure and function thus more membrane electrolyte leakage (Buchanan et al 2015). Different genotypes showed different RWC and WP which may be due to differences in ability of genotypes to absorb water from soil or the ability to accumulate osmolytes to maintain tissue turgor. Based on RWC, WP and MSI, the genotypes RMO-257 and RMO-40 were showing higher values and thus more tolerance to drought conditions and RMO-435 and RMO-225 were showing lesser values thus more susceptible to drought.

Enzymatic activities: Irrespective of treatment, NR activity

was estimated maximum in RMB- 25 with at par value in RMO -40 and RMO-257, however, was minimum in RMO-435 and was at par with RMO-225 (Table 3). NR activity was calculated significantly higher in irrigated mothbean plants as compared to rainfed one (53.6 %). The interaction between genotypes and treatments revealed that NR activity was maximum in irrigated RMB-25 and minimum in rainfed RMO-225. This might be due to increased total chlorophyll content which had a positive relationship with the NR activity. In previous studies with mothbean, the NR activity of mothbean decreased by around 80% at 10 days after drought stress treatment and resulted in altered nitrogen metabolism (Garg et al 2001).

The activity of antioxidative enzymes APOX and GPOX

Table 3. Plant water status and enzymatic activities of mothbean genotypes grown under rainfed and supplemental irrigation conditions

Treatment	RWC (%)	MSI (%)	WP (-MPa)	NR ($\mu\text{moles g}^{-1} \text{fw h}^{-1}$)	APOX ($\mu\text{moles min}^{-1} \text{mg protein}^{-1}$)	GPOX ($\mu\text{moles min}^{-1} \text{mg protein}^{-1}$)	
Genotypes							
RMO-257	80.0a	81.7a	-3.6ab	1.3a	228.9a	1280.1a	
RMO-40	79.4a	82.6a	-3.4a	1.3a	210.4b	1176.8b	
RMB-25	74.3bc	70.4b	-3.9b	1.4a	185.2c	912.1c	
RMO-2251	73.3bc	71.1b	-3.8b	1.2bc	173.9c	821.2d	
CZM-45	77.2ab	70.8b	-4.3c	1.2b	201.6b	757.4e	
RMO-435	71.3c	73.7b	-4.6cd	1.1c	143.3d	715.4e	
RMO-225	71.7c	66.3c	-4.9d	1.1c	141.3d	764.7de	
Treatments							
Rainfed (RF)	66.6b	66.2b	-5.1b	1.0b	233.5a	1058.9a	
Supplemental irrigation (SI)	84.0a	81.4a	-3.1a	1.5a	133.6b	777.6b	
Genotypes x Treatments							
RMO-257	RF	72.7c	76.9cd	-4.0de	1.2fg	282.8a	1517.2a
	SI	87.4ab	86.6a	-3.3bc	1.5cd	175.2gh	1043c
RMO-40	RF	74.8c	78.0cd	-4.3ef	1.1gh	264.1ab	1325.9b
	SI	84.6ab	87.1a	-2.5a	1.6abc	156.7h	1027.7c
RMB-25	RF	66.1de	58.0f	-4.9g	1.1gh	234.2cd	1068.3c
	SI	82.5ab	82.9ab	-2.8ab	1.7a	136.2i	755.9e
RMO-2251	RF	64.3de	62.3ef	-4.6fg	1.0h	248.1bc	897.2d
	SI	82.2ab	79.8bc	-3.0bc	1.3ef	99.9j	745.2e
CZM-45	RF	66.6d	66.4e	-5.5h	1.0h	218.1de	882.2d
	SI	87.8a	75.2cd	-3.1bc	1.4de	184.9fg	632.7f
RMO-435	RF	60.5e	63.6e	-5.9hi	0.7i	182.9g	850.3d
	SI	82.0b	83.8ab	-3.2bc	1.5bcd	103.7j	580.6f
RMO-225	RF	61.6de	57.9f	-6.2i	0.6i	204.ef	871.3d
	SI	81.7b	74.6d	-3.6cd	1.6ab	78.5k	658.1f
CV%	4.5	3.9	-7.6	6.6	6.3	5.3	

Data with different alphabet, are significantly different ($p < 0.05$) as analyzed by Duncan's multiple comparison tests for post hoc analysis
CV: coefficient of variation

were estimated maximum in RMO-257 followed by RMO-40 (Table 3). APOX and GPOX in rainfed plants were 42.8 and 26.6% higher than respective irrigated genotypes. Furthermore, it was observed that rainfed RMO-257 had maximum APOX and GPOX activity were minimum for APOX in irrigated RMO-225 followed by RMO-435 and for GPOX in irrigated RMO-435 which was at par with irrigated RMO-225. The difference between genotypes, treatments and the interaction effect was statistically significant for NR, APOX and GPOX (Table 5). Antioxidative enzyme activity was increased more under drought condition in tolerant genotype as compared to susceptible genotype (Sarker and Oba 2018). This increased in antioxidative activity is a defense response against the generated ROS to cope up with the stress.

Yield and yield attributes: The results showed that irrespective of treatment, yield and its all attributes were estimated maximum in RMO-257 and BP, PP and SP were recorded minimum in CZM-45, while PL and seed yield were recorded minimum in RMO-435 and RMO-225, respectively (Table 4). SI improved BP, PL, PP, SP, TW and yield which is 30.5, 14.2, 82.9, 13.3, 7.6 and 34.3%, higher than RF one respectively. The genotypes and treatments interaction revealed that all the yield parameters except BP were maximum in RMO-257 under RF condition. The values for BP, PP and SP were minimum in RMO-435 genotype and TW and Y were observed minimum in RMO-225. The effect of genotype and treatment was statistically significant for almost all the yield components but the interaction between genotype and treatment was non-significant (Table 5). The

Table 4. Yield attributes of mothbean genotypes grown under rainfed and supplemental irrigation conditions

Treatment		Branches per plant	Pod length (cm)	Pods per plant	Seeds per pod	Test weight (g)	Seed yield (kg/ha)
Genotypes							
RMO-257		4.5a	4.1a	40.1a	5.7a	36.3a	757.1a
RMO-40		4.2b	4.0ab	33.8b	5.4abc	35.9ab	648.7cd
RMB-25		4.3ab	4.09ab	38.0a	5.6ab	34.1bcd	649.5cd
RMO-2251		4.3ab	4.0ab	31.9bc	5.1c	32.3d	696.1abc
CZM-45		3.7c	4.0ab	29.8c	5.1c	34.5abc	729.3ab
RMO-435		3.8c	3.9b	32.9bc	5.3bc	34.0bcd	690.2bc
RMO-225		3.8c	4.0ab	31.1bc	5.4abc	33.3cd	613.4d
Treatments							
Rainfed (RF)		3.5b	3.7b	24.0b	5.0b	33.1b	583.4b
Supplemental irrigation (SI)		4.6a	4.3a	43.9a	5.7a	35.6a	783.6a
Genotypes x Treatments							
RMO-257	RF	4.1ef	3.9b	29.5e	5.3bcde	34.0bcd	649.1cd
	SI	4.9ab	4.3a	50.8a	6.1a	38.5a	865.1a
RMO-40	RF	3.9f	3.7bc	24.4fg	5.0ef	35.1bc	599.6de
	SI	4.4cde	4.3a	43.2bcd	5.7abcd	36.8ab	697.8c
RMB-25	RF	3.4gh	3.7c	28.3ef	5.2def	33.1cd	593.2de
	SI	5.2a	4.3a	47.8ab	6.0a	35.1bc	705.8c
RMO-2251	RF	3.8fg	3.8bc	23.4fg	4.9ef	31.9d	586.9de
	SI	4.8abc	4.3a	40.5d	5.4bcde	32.7cd	805.3ab
CZM-45	RF	3.3h	3.8bc	21.3g	5.0ef	32.8cd	606.7de
	SI	4.2def	4.3a	38.3d	5.3cdef	36.3ab	852.0a
RMO-435	RF	3.0h	3.6c	19.9g	4.8f	33.0cd	552.3e
	SI	4.6bcd	4.2a	45.8abc	5.9ab	35.0bc	828.2a
RMO-225	RF	3.4gh	3.7bc	21.2g	5.0ef	31.6d	496.2f
	SI	4.3cde	4.2a	41.1cd	5.7abc	34.9bc	730.7bc
CV%		6.2	3.7	9.0	5.9	5.2	7.8

Data with different alphabet, are significantly different ($p < 0.05$) as analyzed by Duncan's multiple comparison tests for post hoc analysis
CV: coefficient of variation

reduction in seed yield and test weight under RF condition is thought to be caused by a decrease in photosynthate assimilation and poor carbohydrate partitioning to the developing grain because of drought stress (Nathawat et al 2018). Improving the status of water through irrigation at the reproductive stage helps to sustain reproductive success and the partition of assimilates for optimum yields in water-limited conditions (Molla et al 2021).

Pearson correlation matrix plot, principal component analysis and box plot: The results of the correlation analysis for the yield and different traits in mothbean

genotypes under rainfed and irrigated condition revealed the variegated strengths and directions of the relationships (Fig. 1). Positive correlations were found between the Y and BM, PH, LA, RWC, BP, PL, PP, WP, MSI and NR. Low significant positive correlation was found between the Y and TC, CT, SP and TW. Similarly low significant negative correlation between the Y and APOX and insignificant negative correlation between Y and GPOX was observed.

The total contribution to the first two components of variation was 78.6% (Fig. 2). The first principal component (PC1) contributed to the variations by 68% and similar to the

Table 5. Mean squares and degree of freedom of all parameters of mothbean plants

Variation source	df	BM	PH	TC	CT	LA	RWC
Genotypes (G)	6	2.16 *** (0.000)	0.95 ns (0.962)	0.18*** (0.000)	0.01*** (0.000)	3535*** (0.000)	76.80*** (0.000)
Treatments (T)	1	137.03*** (0.000)	793.01*** (0.000)	2.37*** (0.000)	0.15*** (0.000)	610043*** (0.000)	3205.10*** (0.000)
G X T	6	0.30 ns (0.090)	8.56 ns (0.089)	0.11*** (0.000)	0.01** (0.004)	2433*** (0.000)	24.12 ns (0.090)
Error	26	0.15	4.09	0.02	0.00	266	11.61
Variation source	df	MSI	WP	NR	APOX	GPOX	BP
Genotypes (G)	6	224.72*** (0.000)	1.68*** (0.000)	0.08*** (0.000)	6615*** (0.000)	297812*** (0.000)	0.55*** (0.000)
Treatments (T)	1	2450.42*** (0.000)	41.66*** (0.000)	2.83*** (0.000)	104724*** (0.000)	831031*** (0.000)	12.26*** (0.000)
G X T	6	58.52*** (0.000)	0.70*** (0.000)	0.11*** (0.000)	1998*** (0.000)	15237*** (0.000)	0.280** (0.003)
Error	26	8.29	0.09	0.01	135	2406	0.06
Variation source	df	PL	PP	SP	TW	Y	
Genotypes (G)	6	0.03 ns (0.233)	85.40*** (0.000)	0.28* (0.027)	11.76** (0.008)	15007** (0.001)	
Treatments (T)	1	2.95*** (0.000)	4159.13*** (0.000)	4.83*** (0.000)	67.30*** (0.000)	420538*** (0.000)	
G X T	6	0.01 (0.842)	14.01 ns (0.214)	0.09 ns (0.475)	2.44 ns (0.603)	6905 (0.051)	
Error	26	0.02	9.30	0.09	3.19	2811	

****, and *** are significance codes at 0.001 and 0.001, respectively and "ns" is not significant

BM (biomass), PH (plant height), TC (total chlorophyll), CT (carotenoids), LA (leaf area), RWC (relative water content), WP (water potential), MSI (membrane stability index), NR (nitrate reductase), APOX (ascorbate peroxidase), GPOX (guaiacol peroxidase), BP (branches per plant), PL (pod length), PP (pods per plant), SP (seeds per pod), TW (test weight) and Y (yield).

Table 6. Drought tolerance indices calculated for seed yield of seven mothbean genotypes under rainfed (RF) and supplemental irrigation (SI) condition

Genotypes	Drought indices (Seed yield)												
	Ys	Yp	SSI	TOL	STI	YI	YSI	SDI	DI	RDI	SSPI	GMP	MP
RMO-257	649.1	865.1	0.98	216.0	0.915	1.11	0.75	0.25	0.83	1.01	13.8	749.4	757.1
RMO-40	599.6	697.8	0.55	98.1	0.682	1.03	0.86	0.14	0.88	1.15	6.3	646.9	648.7
RMB-25	593.2	705.8	0.62	112.6	0.682	1.02	0.84	0.16	0.85	1.13	7.2	647.1	649.5
RMO-2251	586.9	805.3	1.06	218.4	0.770	1.01	0.73	0.27	0.73	0.98	13.9	687.5	696.1
CZM-45	606.7	852	1.13	245.3	0.842	1.04	0.71	0.29	0.74	0.96	15.7	719.0	729.4
RMO-435	552.3	828.2	1.30	275.9	0.745	0.95	0.67	0.33	0.63	0.90	17.6	676.3	690.3
RMO-225	496.2	730.7	1.26	234.5	0.591	0.85	0.68	0.32	0.58	0.91	15.0	602.1	613.5

Ys (Yield under stress condition), Yp (Yield under irrigated condition), SSI (Stress susceptibility index), TOL (Stress Tolerance), STI (Stress tolerance index), YI (Yield index), YSI (Yield stability index), SDI (Sensitivity drought index), DI (Drought resistance index), RDI (Relative drought index), SSPI (Stress susceptibility percentage index), GMP (Geometric mean productivity) and MP (Mean productivity)

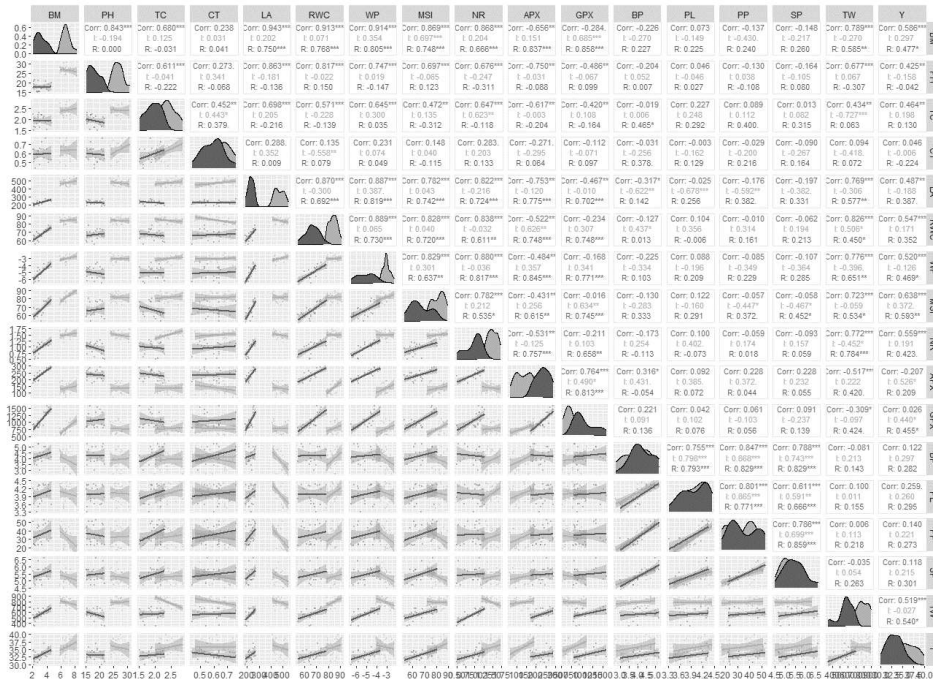


Fig. 1. Correlation matrix plot among all evaluated traits in seven mothbean genotypes studied under rainfed (R) and supplemental irrigation (I) conditions. The tested variables included. BM (biomass), PH (plant height), TC (total chlorophyll), CT (carotenoids), LA (leaf area), RWC (relative water content), WP (water potential), MSI (membrane stability index), NR (nitrate reductase), APX (ascorbate peroxidase), GPX (guaiacol peroxidase), BP (branches per plant), PL (pod length), PP(pods per plant), SP (seeds per pod), TW (test weight) and Y (yield).

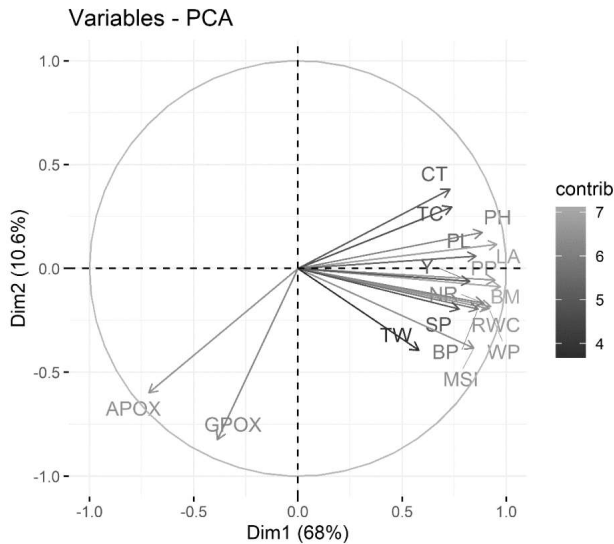


Fig. 2. Principal component analysis of all evaluated traits in seven mothbean genotypes studied under rainfed and supplemental irrigation conditions. The tested variables included. BM (biomass), PH (plant height), TC (total chlorophyll), CT (carotenoids), LA (leaf area), RWC (relative water content), WP (water potential), MSI (membrane stability index), NR (nitrate reductase), APOX (ascorbate peroxidase), GPOX (guaiacol peroxidase), BP (branches per plant), PL (pod length), PP(pods per plant), SP (seeds per pod), TW (test weight) and Y (yield)

results of correlation matrix plot indicated a strong correlation between Y and BM, PH, LA, MSI, RWC, WP, NR, BP, PL, PP while weak correlation with SP, TC, CT and TW. The second principal component (PC2) contributed to the variations by 10.6%, and it had a strong correlation with the APOX and GPOX.

Box plots for descriptive statistic parameters were also constructed (Figure 3). From the box plot representation it was observed that the values for almost all the traits increased under SI condition except APOX and GPOX, which showed a decrease under SI condition.

Drought tolerance indices: Drought tolerance indices indicate the ability of the genotypes to survive in drought stress conditions. Drought tolerance indices for individual genotypes were estimated based on seed yield and were varied significantly indicating genotypic variability (Table 4). STI, YI, DI, GMP and MP were calculated highest in RMO-257 followed by RMO-40 and RMB-25 whereas SSI, TOL, SDI and SSPI were observed highest in RMO-435 genotype. RMO-2251, CZM 45 and RMO-225 were showing intermediate range of drought tolerance indices. The selection of genotypes based on drought tolerance indices were done by many researchers in which GMP and STI were found to be suitable genotype tolerance indices under non stresses and stressed conditions (Kumar et al 2008, Raman et al 2012).

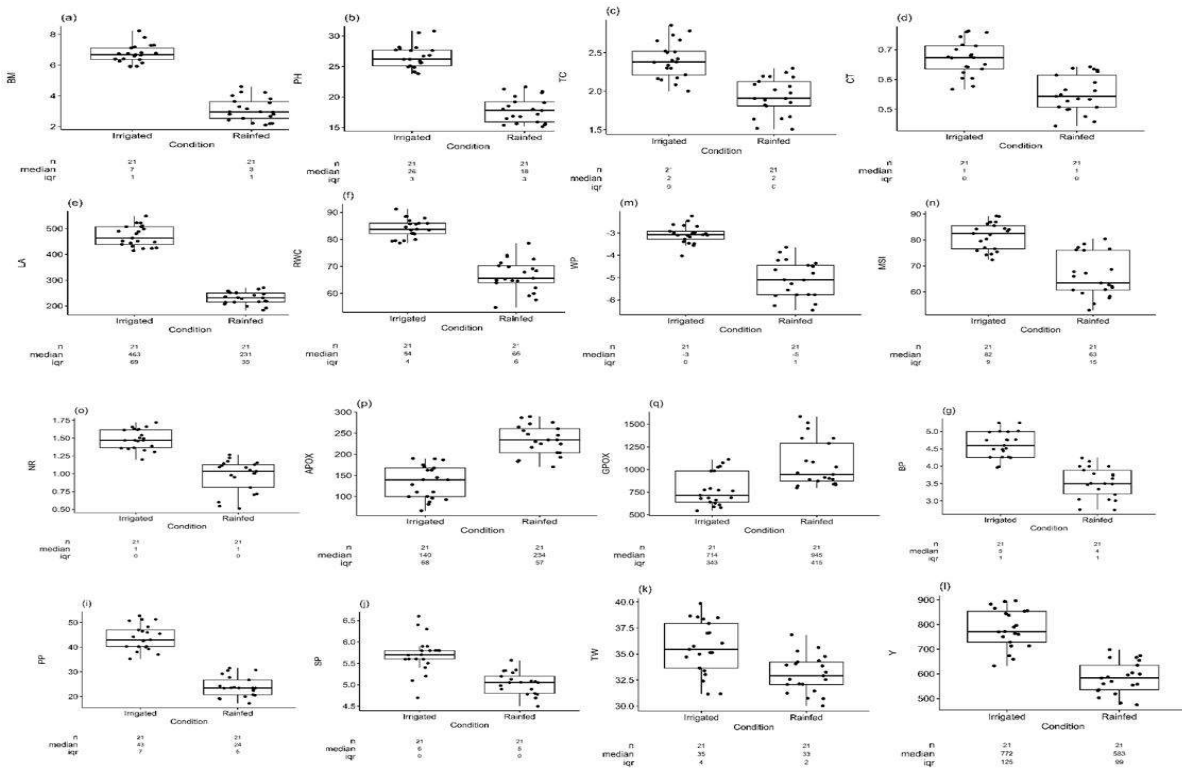


Fig. 3. Box plot of all evaluated traits in seven mothbean genotypes studied under rainfed and supplemental irrigation conditions. The tested variables included. BM (biomass), PH (plant height), TC (total chlorophyll), CT (carotenoids), LA (leaf area), RWC (relative water content), WP (water potential), MSI (membrane stability index), NR (nitrate reductase), APOX (ascorbate peroxidase), GPOX (guaiacol peroxidase), BP (branches per plant), PP (pods per plant), SP (seeds per pod), TW (test weight) and Y (yield)

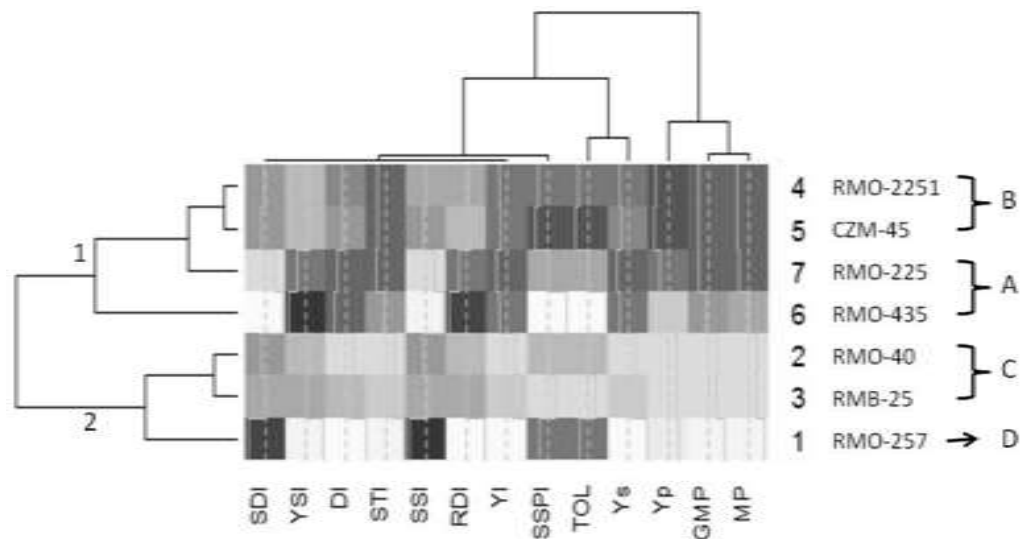


Fig. 4. Heatmap of stress indices among seven mothbean genotypes. Group 1 refers to stress susceptible genotypes, whereas group 2 refers to stress tolerant genotypes. Subgroup A is stress susceptible; subgroup B is moderately stress tolerant; subgroup C is stress tolerant; whereas D is highly stress tolerant. Black to white range is according to lower to higher correlation among genotypes and indices. SDI (Sensitivity drought index), YSI (Yield stability index), DI (Drought resistance index), STI (Stress tolerance index), SSI (Stress susceptibility index), RDI (Relative drought index), YI (Yield index), SSPI (Stress susceptibility percentage index), TOL (Stress Tolerance), Ys (Yield under stress condition), Yp (Yield under irrigated condition), GMP (Geometric mean productivity) and MP (Mean productivity)

Genotypic classification based on stress tolerance

indices: Drought stress tolerance indices were studied for hierarchical clustering using a heat map (Fig. 4) and seven mothbean genotypes were classified in two major groups and four subgroups based on their stress responses. Group 1 was categorized as stress susceptible and group 2 as stress tolerant. Subgroup A consisted of two genotypes i.e. RMO-225 and RMO-435 with lowest grain yield and stress tolerance indices under RF condition, so these were considered as stress susceptible genotypes. Subgroup B consisted of two genotypes i.e. RMO-2251 and CZM-45 with intermediate values of yield and stress indices so considered as moderately stress tolerant. Subgroup C consisted of two genotypes i.e. RMO-40 and RMB-25 with higher yield and stress indices values so considered as stress tolerant. Genotype D i.e. RMO-257 had highest values for yield and stress indices so it was highly stress tolerant. Similar relationship between the drought tolerance indices and genotypes were also reported by Hussain et al (2021). Therefore, the drought tolerance indices were rapid selection criteria for selection of suitable genotypes under water stress conditions.

CONCLUSIONS

SI before flowering in areas where water availability is low under RF condition is beneficial to overcome the water stress conditions in mothbean genotypes. This helps the crop to improve its physiological and biochemical processes such as increase in photosynthetic pigments, water relation parameters such as WP, RWC and MSI, enzymes such as NR and growth traits which ultimately favors high yield as compared to the RF condition. Genotypes such as RMO-257, RMO-40 and RMB-25 having higher drought tolerance capacity can also be useful to compensate the yield losses under water limiting conditions. This study may help farmers to give sustainable yield of mothbean under anticipated water deficit conditions and improve the livelihood of farming community of hot arid region. The knowledge will also help in the identification of suitable genotypes of mothbean for achieving sustainable yield under water-deficit conditions.

AUTHORS CONTRIBUTIONS

VS and NSN initiated and designed the experiment. VS performed the experiments, collected the data and wrote the manuscript. MKB and CKJ analyzed the data. NSN revised the manuscript.

ACKNOWLEDGEMENTS

Authors thank ICAR-CAZRI (RRS), Bikaner and ICAR-NRCSS, Ajmer for providing funds and facilities for conducting this study.

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