Agro-morphological performance of moth bean (*Vigna aconitifolia*) lines in severe drought stressed and rain-fed condition

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Research Paper

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Germplasm comprising of 32 entries collected from the main core collection was selected and evaluated at National Bureau of Plant Genetic Resources (NBPGR), Pusa campus in New Delhi under terminal drought (no moisture) and rain-fed (control) conditions. The results show highly significant differences for genotype, treatment (Stress) and GxS effect. Agronomic morphological characters were observed under both the severe drought and rain-fed conditions. Water stress had an effect in plant height and roots of plant: plants in control condition were taller in height of the upper vegetative part but the roots showed a different response as the roots of plants under drought stress where longer than those in control samples. Chlorophyll content showed highly significant difference for genotype and combination effect of genotype by Stress treatment, the treatment effect showed significant difference. Even though the chlorophyll was high in both the treatments, had almost similar chlorophyll content. Five accession, (IC 129177, IC 103016, IC 415139, IC 415155 and IC 36157) and two varieties (Maru moth and Jadia) were drought tolerant as they managed to reach flowering stage, had pod set and reached maturity even though the yield was significantly lower than in plants under rain-fed conditions.

Key words: *Vigna aconitifolia*, severe drought stress, drought tolerance, chlorophyll, relative water content

INTRODUCTION

Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agricultural production and the natural status of the environment. Plant growth and productivity are greatly affected by environmental stresses such as drought, high salinity, and low temperature with water crisis being a severe threat to sustainable agriculture, particularly in most of the African countries where lower and erratic rainfall amounts are experienced and also in Asian countries where irrigated agriculture accounts for 90% of total diverted fresh water (Huaqi *et al*. 2002). Drought stress has been identified as one of the major limitations to crop productivity worldwide due to its multi-gene nature, making the production of transgenic crops a challenging prospect. A basic understanding of morphological, physiological, biochemical and gene regulatory networks is very essential in order to be able to develop crop plant with enhanced tolerance to drought stress.

Drought-tolerant plants constitute a good source of genetic traits to improve agronomical important plants as such moth bean and because of its drought tolerance qualities, the crop possess a bi-functional enzyme which catalyses the proline biosynthases in plants (Hu *et al*.1992). Water deficit is one of the major abiotic stresses, which adversely affects the crop growth and yield (Jaleel *et al*. 2009). A plant that is able to withstand these stresses while maintaining good productive health may only be a solution to continued resource limiting agriculture. The moth bean, a crop of hot desert region shows a great deal of tolerance to drought stress. Such crops investigated systematically could also be a source of efficient alleles for various stress tolerant pathways or mechanisms (Soni *et al*. 2011). Moth bean [*Vigna aconitifolia*], is an annual plant with a spreading and prostate growth habit. It grows to about 15-30 cm in height, it is somewhat angular and hairy with short internodes, and has large internodes for primary branches. Because of its small statue it gives small seeds of about 2.05-5.0cm long mostly yellow brown to mottled black in color. The crop is a native crop to hot and dry habitats of northern and western parts of India. It is also known by other common names such as Kidney gram, Aconite bean, Mat bean, Matki bean, Mout bean, or Dew gram is an important legume in India. The crop is one of the very drought tolerant crops which can survive with minimum to no irrigation at all and produce average yield, it can survive up to 40-50 days in open fields without water with temperatures up to 40°C. It has a deep and fast penetrating root system as one of its drought avoidance mechanism or capability. *Vignaaconitifolia* species have a great potential to provide useful genes for crops in a world where breeders require genes to address altered environments due to climate change. The ability of plants to tolerate drought conditions is crucial for agricultural production worldwide. The most tolerant genotypes has to be selected and studied further for the agronomic traits and documented for further breeding purposes.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the National Bureau of Plant Genetic Resources (NBPGR), Pusa campus in New Delhi which is located at 28.080°N 77.120°E. The average summer temperature ranged from 25º C on normal day to 46º C on extreme hot days. May and June are considered to be the hottest months of the year which at times experiences the heat waves which are immensely hot. Summer in Delhi stays till October before chill of winter starts to sets in.

Experimental design

The experiment was conducted in a RCBD split plot design with three replications for each accession and with rain-fed and drought conditions as the main treatment factors. The experiment was conducted during the rainy/monsoon season between the months of June - October 2013. The drought condition (treatment) was maintained by pulling a permanent overhead cover to keep the rain out (moisture). The cover roofed with clear polyethylene to allow sunlight to pass through to the plants, the sides of the cover were open to allow normal ventilation to the plants.

Plant material and planting

Thirty two Moth bean accession selected from the main core collection were used as plant material for the experiment. The land was allowed to be soaked by rainfall to a near field capacity before planting and pulling over the rain-out cover. The soil was double ploughed and fine tilth was made before plots were demarcated. Cow manure was applied to the plots and mixed with the soil during double ploughing and fine tilth preparation as basal application before planting. No further inorganic fertilizer were added either as basal dressing or top dressing to the plants. After demarcating plots, rows were manually cut open at 5cm deep for planting. Planting was done by hand where three seeds per hill were planted at 30 cm between rows and 20cm between plants.

Crop management

Two weeks after seedling emergence, plants were thinned to maintain one plant per hill/ hole. Weeding was done at two growth stages, the initial weeding was done four weeks after seedling emergence and the second weeding was done at flowering stage. Weeding is normally done to avoid the weeds from out-competing the plants for nutrients, moisture, space and light.

Irrigation or moisture maintenance

Moisture was the main treatment for this experiment, drought experiment was grown in a motorized moveable rain-out shelter and exposed severe drought stress by switching on the rain-sensor (Schneider, 2003). The other treatment received its moisture mainly from rainfall and no supplementary irrigation was supplied because rainfall was frequent enough for the moisture requirements of the crops.

Data collection

Number of days from seedling emergence to the point when the plants in a plot reached 50% flowering were recorded as number days to flowering. Number of secondary branches were recorded when the plants reached 50% flowering. Plant height of plants was collected from three randomly selected healthy looking plants. The height was collected from the soil surface to

Table 1. Analysis of variance with mean squares for agronomic characteristics of 31Moth bean accessions.

SOV	Df	Plant height (cm)	Root length (cm)	Branches	Plant dry mass	Root dry mass	chlorophyll	Relative water content
Genotype (G)	31	89.39**	13.54**	11.59**	12.55**	0.17**	79.54**	343.25**
Stress (S)	1	12977.52**	52.61**	884.08**	459.05**	2.04**	194.41*	1487.97
G x S	31	119.62**	15.21**	13.14**	28.17**	0.02**	90.54**	250.35**
C.V. (%)		16.19	13.14	18.00	10.70	19.98	17.05	4.44

*,**indicates significant at 0.05 and 0.01percent probability level respectively.DF- Degrees of Freedom

the last tip of the plant when held up. Root length was measured from the base of the plant to the tip of the root on three randomly selected plants which were dug out so as not to disturb their roots, pulling out was avoided as it could have damaged the roots with the tip getting cut and remaining in the soil. A ruler was used to measure the length from the very last tip to the point where the root meets the stems. After collecting fresh weight of the plant and root, then plants were the oven dried at 60˚C for 72hrs, the plants were then taken out and weighed and the mass was recorded as the plants dry mass. When measuring the plant dry mass the quickest time was practiced from oven to weighing to avoid plant accumulations of atmospheric moisture again.

The three healthiest whole leaves were randomly collected from three different plants and used for the calculate Relative Water Content. Initial mass of the leaves was taken, the leaves were then immersed in water and kept at room temperature with sufficient light for 4hours, after which immersed leaves were taken out, blow dried to dry out the water and then weighed to get the turgid mass of the leaves. The leaves were then kept in the oven at 60˚C for 48hours after which they were taken out and weighed to get the dry mass of the leaves. Relative water content was calculated as follows:

$$((Fm-dm)/(tm-dm))*100$$

Fm- fresh mass
Dm – dry mass
Tm – turgid mass

Soil moisture: a tensiometer was used to measure the soil moisture content up to 30cm below the soil surface which was regarded as the root zone.

RESULTS AND DISCUSSION

Morphological data

The results showed highly significant differences for genotype, treatment (Stress) and GxS effect (Table 1).The plants in control conditions performed well as they were taller in height of the upper vegetative part but the roots showed a different response as the roots of plants under drought stress where longer than those in control samples. This meant that the roots grew deeper into the soil in trying to reach out to the water table so as to access water to meet the requirement needed for plant growth and assimilation of nutrients. The deep growth of roots was explained by Gesimba *et al.*, (2004) that many plants success in the dry conditions have no specific adaptation for controlling water loss but can mainly be attributed to the development of the extensive and deep systems that can obtain water from a large volume of deeper soil in the water table.

Leaf Pigmentation

Drought in many plants as stress has a tendency of causing the plant leaves to lose the green color (Lessani and Mojtahedi, 2002),The results in this study shows little or no difference between the moth bean subjected to stress condition and well rain-fed condition during the seedling and vegetative growth stages of the crop. The only difference spotted between the two treatments was the shrinking of the leaves in the drought treatment but the leaves did not lose their color during the shrinking process. The leaf shrinking can well be attributed to the drought avoidance mechanism of the crop.Leaf pigmentation which is also highly related to chlorophyll content has been found to be highly reduced by water deficit, resulting in lower chlorophyll content under stress conditions (Montagu and Woo, 1990; Nilsen and Orcutt, 1996).

Chlorophyll content

Chlorophyll content showed highly significant

Table 3. Mean comparison of the studied traits under the drought stress condition of the selected tolerant and susceptible genotypes.

	Accession	Plant height	Root length	Branches	Plant dry mass	Root dry mass	Chlorophyll	Relative water content
	IC16218	30.67 E	10.90D	10.67ABC	5.28E	0.31BC	36.60 CDE	63.18E
	IC 129177	42.07 B	15.23AB	12.00A	9.39B	0.34B	43.70B	66.56D
Drought stress	IC 103016	41.97B	9.87DE	12.00A	12.67A	0.31BC	39.50BCD	76.26A
	IC 140622	39.23BC	15.53A	10.67ABC	8.38C	0.43A	44.63B	71.71B
	IC 140663	53.37A	13.57C	9.67CD	7.02D	0.34B	34.50DE	67.91C
	IC 415139	42.37B	9.93DE	9.00DE	3.43F	0.16DE	37.10CDE	76.87A
	IC 39800	38.73BC	13.30C	11.33AB	7.91C	0.34B	39.47BCD	62.41E
Normal rainfed	IC 415155	38.80BC	13.93BC	12.00A	2.49G	0.35AB	41.03BC	55.47F
	IC 39754	30.03E	10.50D	11.67A	5.213E	0.25CD	30.87E	52.68G
	IC 36157	42.57B	8.56E	10.00BCD	7.92C	0.19DE	42.03BC	66.57D
Varieties	MARU MOTH	31.37DE	12.97C	9.67E	3.46F	0.15E	32.93E	66.88CD
	JADIA	35.93CD	10.90D	10.00BCD	3.60F	0.31BC	52.23A	67.99C
LSD $_{0.05}$		5.05	1.49	1.52	0.59	0.09	6.44	1.21

difference for genotype and combination effect of genotype by Stress treatment (Table 1), the treatment effect showed significant difference. Even though the chlorophyll was high in both the treatments, the plants in the in both treatments had almost similar chlorophyll content (Table 3). Vurayai *et al.* (2010) observed that water stress does not significantly reduce the leaf chlorophyll in Bambara groundnuts, with Cornellisen (2005) concluding that drought tolerant plants do maintain high amounts of chlorophyll content despite the development of moisture deficit stress and this trait can be considered to be a line of difference against drought which can result in drought resistance. Mafakheri *et al.* (2010) found out that drought stress imposed at vegetative stage, significantly decreased all forms of chlorophyll content both at vegetative and flowering stages. In contradiction Mensah *et al.* (2006) found that subjecting Sesame to drought stress caused leaf chlorophyll to increase and then remain unchanged. Beeflink *et al.* (1985) also reported increase in chlorophyll in onion crops under stress. The main reason for decrease in chlorophyll content as affected by water stress is that during drought or heat stress the plant tends to produce reactive oxygen species such as O_2 and H_2O_2 which can lead to lipid peroxidation and consequently chlorophyll destruction (Mirnoff, 1993; Foyer *et al.* 1994).

Relative water content

Analysis of variance for drought tolerant and susceptible plants grown under stress condition showed highly significant difference for the relative water content (Table 2). Relative water content (RWC) was lower for plants grown under stress condition as compared to plants grown in control conditions which recorded a little higher RWC. Rahimi *et al.* (2010) concluded that as water stress intensified relative water content is usually significantly affected. Leaf RWC can be best be used as growth/biochemical indices revealing the stress intensity in crops (Alizade, 2002) and the rate of RWC in plant with high resistance against drought is higher than in others.

Flowering and pods set

Most of the crops in stressed condition failed to reach the flowering point or some had a delayed flowering onset as compared to the crops in the normal rain-fed condition. Only five accession (IC 129177, IC 103016, IC 415139, IC 415155 and IC 36157) and two varieties (Maru moth and Jadia) did manage to reach flowering and pod set and maturity. Most of the flowers wilted and dropped off after opening because of the drought stress, similar pattern was then observed after fruit set for the flowers that did not wilt off after fertilization. Four genotypes being Maru moth, Jadia, IC 415155 and IC 415139 produced few pods that reached maturity and also produced health and viable seeds that were recorded as yield. The earliest to reach flowering and pods sets among

Table 2. Analysis of variance with mean squares agronomic characteristics of 5 drought resistant, 5 drought susceptible accessions and 2 (Maru moth and Jadia) varieties of Moth Bean.

SOV	**Df**	**Plant height (cm)**	**Root length (cm)**	**Branches**	**Plant dry mass**	**Root dry mass**	**chlorophyll**	**Relative water content**
Reps	2	3.05	5.43	0.78	0.23	0.01	29.71	0.19
Genotype	11	127.36**	15.22**	5.54**	27.43**	0.02**	101.37ns	156.53**
C.V. (%)		14.44	13.68	15.96	10.27	11.96	18.11	2.03

*,**indicates significant at 0.05 and 0.01percent probability level respectively.

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Table 4. A simple correlation coefficients for agronomic characters under stress condition.

	PH	**RL**	**BR**	**PDM**	**RDM**	**CHLO**
RL	0.03					
BR	0.29**	0.21*				
PDM	0.54**	0.07	0.50**			
RDM	0.39**	0.24*	0.37**	0.52**		
CHLO	-0.04	0.13	-0.02	-0.03	0.19	
RWC	-0.06	-0.13	-0.21*	-0.06	-0.32**	-0.08

PH- Plant height,**RL** – Root Length,**BR** – Number of branches,**PDM** – Plant Dry Mass,**RDM** – Root Dry Mass,**CHLO** – Chlorophyll Content, **RWC** – Relative Water Content.

the crop in the stressed condition was the IC415139 and IC 415155 which produced the first flowers after 35 and 40 days after planting respectively. The two accessions produced a few healthy pods which reached maturity at 20days after pods set for each accession.

Correlation analysis

The results of the correlation coefficients showed that root length had no significant correlation with most of the studied agronomic characters except with number of branches were it had a positive significant difference (Table 4). Relative water content showed a negative highly significant correlation (-32**) with the root biomass. The roots under the drought stress developed quickly and deeper thus have more biomass while the RWC was lower in those plants that were highly tolerant to the water stress than in the normal rain-fed plants. Most of the agronomic characters had no significant correlation with the other traits studied.

Conclusion

Results of this study indicated that drought stress had the significant effect on all the studied traits; therefore, in areas that usually experience severe drought stress using genotypes or accessions which are drought tolerant can be very valuable. Even though drought had significant effect on most of the studied traits, most of the moth bean genotypes and landraces showed high tolerance to drought which is very critical in the plant breeding not only of moth bean but on most field crops.

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