



Screening of three leguminous crops for drought stress tolerance at germination and seedling growth stage

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ABSTRACT

Responses of three leguminous crops (*Glycine max* L., *Macrotyloma uniflorum* L. and *Vigna mungo* L.) to drought stress imposed at germination and seedling growth stages were investigated in a laboratory experiment. Seeds of selected crops were surface sterilized and placed under five drought stress levels of 0 (control), -5, -10, -15, -20 bar using Mannitol solution. The results showed that with increasing drought stress, germination in all the species was delayed and decreased. The *G. max* showed maximum germination percentage while *M. uniflorum* showed minimum germination percentage. Decrease in dry mass of seedlings with increasing drought stress indicated that the stress not only affected germination but also the growth of seedlings. The proper understanding of interactive responses of different crops at different growth stages is a necessity for sustainable crop production under adverse environmental conditions.

Key words: Drought stress, Seed germination, Seedling growth, Seed vigour, Tolerance.

Seed germination is the beginning of the life-cycle of plants, seedling emergence is critical for establishment of plant populations (Khan and Gulzar 2003). It is the most sensitive stage to abiotic stresses (Patadu *et al.* 2011, Awasthi *et al.* 2016). In addition, abiotic stresses are the major cause of crop failure, decreasing average yield for major crops by more than 50% (Buchanan *et al.* 2000) and threatening the sustainability of the agricultural industry (Mahajan and Tuteja 2005). At sowing stage inadequate moisture results in irregular seed germination and unsynchronized seedling emergence affecting the establishment of a stand with negative effects on the yields (Okcu *et al.* 2005). For this reason, drought tolerance at the germination stage has specific importance, moreover in warm environments vulnerable to climate change (IPCC 2007). Drought imposes one of the commonest and most significant constraints to agricultural production, seriously affecting crop growth, yield and quality (Shi *et al.* 2009). It has been reported that drought stress can reduce or delay germination or completely prevent germination (Bargali and Bargali 2016).

Macrotyloma uniflorum Lam. (Horse gram), *Vigna mungo* L. (Mungbean) and *Glycine max* L. (Black soybean) are the common leguminous crops of Central Himalayan region (Pande *et al.* 2016). *M. uniflorum* is a popular pulse, locally known as *Gahet* that still remain an under exploited

legume crop. It is usually grown up to the area at 1 800 m asl. Its seeds are rich in protein iron, calcium and polyphenols and consumed in majority by poorest section of the society. *V. mungo* locally known as *urd* or *maas* is an important, environment friendly staple food legume with rich source of proteins, potassium, calcium, iron, vitamins and minerals for poor families where protein and micronutrient paucity are most omnipresent. It is third most important pulse crop of India. *G. max* locally known as *bhatt* is an important legume crop cultivated from near sea level to 2 000 m elevation and rich in protein, carbohydrates, calcium and other minerals (Shah 2006).

Germination and seedling development is very important for early establishment of plants under stress condition. Selection of plant species with a better drought tolerance is critical for early seedling establishment. Using natural field conditions is difficult because rainfall can eliminate water deficit. However, *in-vitro* drought screening methods are facilitating progress in our understanding of drought resistance traits and in selection of drought resistant species. The aim of present study was to compare the effects of induced drought stress on germination and early seedling growth stage of three leguminous crops to select best suitable species for sites with low moisture availability.

MATERIALS AND METHODS

In order to study the effects of drought stress on germination indices in selected leguminous crops, an experiment was conducted in factorial form, using a completely randomized design with three replications. In

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this experiment, three leguminous crops (*M. uniflorum*, *V. mungo* and *G. max*) were evaluated in five levels of drought treatment (distilled water as control, -5, -10, -15 and -20 bars). This experiment was carried out in the Department of Botany, DSB Campus, Kumaun University, Nainital. Mannitol was applied to induce stress and it was prepared by dissolving the required amount of Mannitol according to the formula given by Helmericks and Pfeifer (1954: see Vibhuti *et al.* 2015a and b, Shahi *et al.* 2015a and b):

$$\text{Water stress} = \frac{1}{-25} \times \frac{\text{Molality}}{\psi P}$$

where, ψP = Osmotic potential of mannitol solution (drought stress).

Ten healthy, uniform sized seeds of all selected crops were sterilized, washed with distilled water and were put in Petri dishes (with 9cm diameter) with moistened filter paper in 3 replications. The Petri dishes were covered to prevent the loss of moisture by evaporation. Germination test were conducted under condition of 12h light/dark cycle with 14°C minimum and 24°C maximum temperature. A seed was considered germinated when the emergent radicle reached 2 mm length. The germination percentage was determined by counting the number of germinated seeds every day. After 10 days, shoot length, root length, seedling length, and seed vigor of germinated seeds were measured. Early seedling dry weight was recorded after oven drying at 60°C for 48 h. Statistical analysis was performed using SPSS version 16. After final count the germination percentage was calculated by the following formula (Raun *et al.* 2002):

GP% = (Number of total germinated seeds/ Total number of seeds tested) × 100

where GP is germination percentage.

Seed vigour index was determined by following Abdul and Anderson (1970):

Seed vigor index = Germination percentage × seedling length (root + shoot)/ 100

Germination stress tolerance index (GSI), seedling length stress tolerance index (PHSI), root length stress tolerance index (RLSI) and seedling dry matter stress tolerance index (DMSI) were calculated using the following formulae given by Ashraf *et al.* (2006):

GSI= (Germination percentage in stressed seeds/ Germination percentage in control) × 100

SLSI= (Seedling length in stressed plants/ Seedling length in control) × 100

RLSI= (Root length in stressed plants/ Root length in control) × 100

SDSI= (Dry matter in stressed plants/ Dry matter in control) × 100

Sensitivity indexes for germination (SIG), seedling length (SIL) and seedling dry mass (SID) were calculated using following formulae

SIG = Germination percentage in control / Germination percentage water stress level.

SIL = Seedling length in control / Seedling length in drought stress level.

SID = Seedling dry mass in control / Seedling dry mass in drought stress level.

The Response Index (RI) was calculated as per the formula given by Williamson and Richardson (1988) for the magnitude of inhibition versus stimulation by environmental factor, i.e. drought stress on seed germination, seedling length and total seedling dry weight using following formula:

When germination/seedling length and dry mass of treatments (T) is lower than the control (C) :

$$RI = (T/C) - 1$$

When germination/seedling length and dry mass of treatments (T) is higher than the control (C) :

$$RI = 1 - (C/T)$$

If RI > 0 treatment stimulated germination/growth.

If RI = 0 no effect.

If RI < 0 treatment inhibited germination/growth.

RESULTS AND DISCUSSION

Statistical analysis showed significant effect of mannitol-induced drought stress on seed germination percentage and other observed growth parameters. However, difference among species in terms of germination and seedling growth parameters were insignificant (Table 1)

Effects on germination percentage

It was observed that, in all the species there was a reduction in germination percentage due to drought stress increment and germination was delayed (Fig 1). According to

Table 1 Analysis of Variance (ANOVA) for traits investigated for the selected leguminous crops in response to drought stress.

Parameter	Mean square					
	Df	SL (cm)	RL (cm)	GP %	SDW (g)	RDW (g)
Species	2	9.58 ^{ns}	7.01 ^{ns}	9.50 ^{ns}	0.02 ^{ns}	0.004 ^{ns}
Drought stress	3	51.15*	26.23*	30.88*	0.05*	0.02*

*Significant at 5%, and ns: not significant. Df: degree of freedom, SL: shoot length, RL: root length, GP%: germination percentage, SDW: shoot dry weight, RDW: root dry weight.

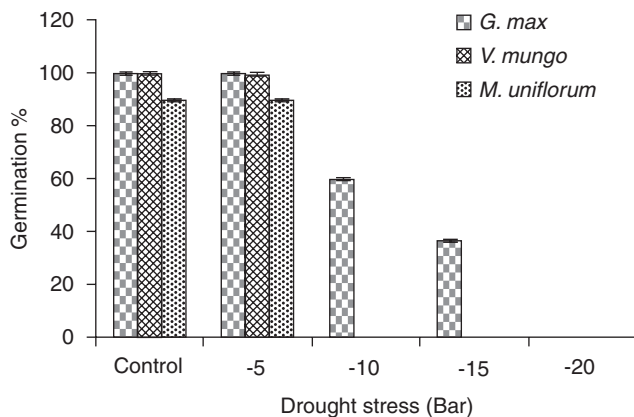


Fig 1 Effect of drought stress on seed germination (%) of three leguminous crops

Afzali *et al.* (2006), seed germination reduction under stress conditions is due to occurrence of some metabolic disorders. The germination was reduced under the stress levels with varying responses for each species. The germination percentage of *G. max* was higher than *M. uniflorum* and *V. mungo*. The latter two species were able to germinate only at the low (-5 bar) drought stress level and the germination was completely inhibited towards higher drought stress (-10 to -20 bar) levels. Seed germination is a process that is affected by both genetic and environmental factors and different species have evolved different mechanisms to adapt to adverse conditions.

Effect on seedling length

Plant height of selected leguminous crops was significantly affected by drought stress. For both root and shoot, the maximum length was observed in the controlled condition and minimum in the highest drought stress level (Fig 2). At control level, *G. max* (10.5 cm) and *V. mungo* (8 cm) produced significantly higher shoot length compared to *M. uniflorum* (4 cm) which produced lowest shoot length. Root length is one of the most important characters for drought stress because roots are in contact with soil and absorb water from soil. For this reason, root length provides

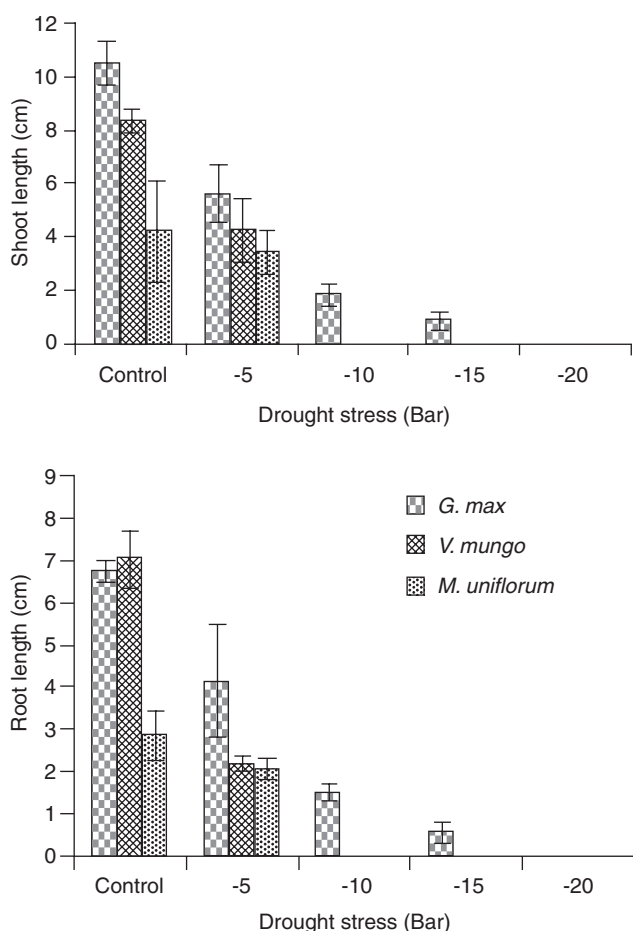


Fig 2 Effect of drought stress on shoot and root length of three leguminous crops

an important clue to the response of plants to drought stress. Among the species, the maximum root length was recorded in *V. mungo* (7 cm) while minimum was observed in *M. uniflorum* (2 cm). In the present study, root length was inhibited significantly by drought stress ($P < 0.05$) and it was shortest under the highest drought stress; moreover, increasing drought stress resulted in a decreasing root length (Fig 2).

It has been established that drought stress is a very important limiting factor at the initial phase of plant growth and establishment. It affects both elongation and expansion growth due to low turgor pressure (Shao *et al.* 2008). The reduction in plant height was associated with a decline in the cell enlargement in *Abelmoschus esculentus* under water stress (Bhatt and Srinivasa Rao 2005). Maximum seedling height was reported in *G. max* followed by *V. mungo* and *M. uniflorum* in controlled as well as stressed (-5 bar) condition (Fig 3).

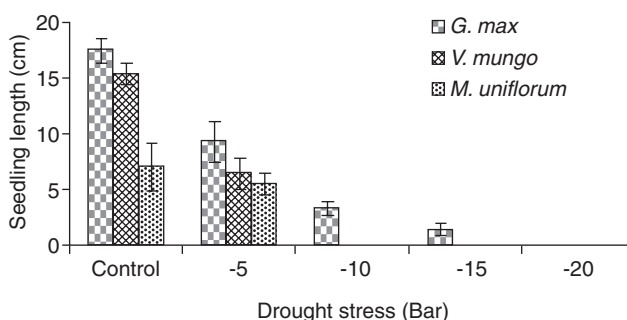


Fig 3 Effect of drought stress on total seedling length of three leguminous crops

Effects on seed vigour index

Seed vigour index decreased with increasing water stress and showed 52% reduction in *G. maxi*, 45% in *M. uniflorum* and 63% in *V. mungo* (Table 2). Among the species, *M. uniflorum* was affected the least by drought stress because it gave the lowest reduction rate for seed vigor. Although the species showed different responses to drought levels, the highest value in control was observed in *G. max*. Dhanda *et al.* (2004) indicated that seed vigor index and seedling length are among the germination traits that are most sensitive to drought stress.

Table 2 Seed vigour index of selected leguminous crops as affected by drought stress.

Species	Drought stress level				
	Control	-5 Bar	-10 Bar	-15 Bar	-20 Bar
<i>G. max</i>	17.52	8.44	2.05	0.54	
<i>M. uniflorum</i>	6.44	3.52			
<i>V. mungo</i>	15.41	5.65			

Effects on dry weight of seedlings

Shoot dry weight was inversely related to drought stress levels. At control, total seedling dry weight was maximum for *V. mungo* (0.56 g) while in stressed condition it was

maximum in *G. max*. Farooq *et al.* (2009) also reported that common adverse effect of water stress on crop plants is the reduction in fresh and dry biomass production.

Effects on initiation and completion of germination:

At control level, the minimum time taken by all the selected leguminous crops was 2 days and for completion was 6 days (Table 3). However, with increasing drought stress levels emergence time also increased. *G. max* showed better initiation and completion at lower drought stress levels (-5, -10 bar) while in *M. uniflorum* initiation of seed germination was inhibited at higher drought stress levels (-10, -15 bar).

Table 3 Effect of drought stress on initiation and completion of seed germination in selected leguminous crops (I = initiation in days, C = completion in days)

Drought stress Level (Bar)	Selected leguminous crops					
	<i>G. max</i>		<i>V. mungo</i>		<i>M. uniflorum</i>	
	I	C	I	C	I	C
0	2	6	2	6	2	6
-5	3	8	3	8	6	9
-10	3	8				
-15	4	9				
-20						

Effects on root: shoot ratio

In *G. max*, the root to shoot length ratio (R: S) increased with increasing drought stress while in other two species it decreased with increasing the stress (Table 4). Bajji *et al.* (2000) also reported increase in R: S ratio with increasing drought stress level. The ability to develop extensive root systems contributes to differences among species for drought tolerance and root length is considered an important trait

Table 4 Root: shoot length ratio as affected by drought stress level

Species	Root: shoot length ratio				
	Control	-5 Bar	-10 Bar	-15 Bar	-20 Bar
<i>G. max</i>	0.64	0.74	0.84	0.67	
<i>M. uniflorum</i>	0.68	0.62			
<i>V. mungo</i>	0.84	0.52			

in selection of drought resistant species (Abd Allah *et al.* 2010). In the present study, total root length decreased with increasing drought stress more in *V. mungo* than in the other two species, indicating a higher sensitivity to osmotic stress.

Sensitivity Index

G. max maintained sensitivity index for germination between 1.11–2.73, for plant height between 1.86 –11.84 and for plant weight between 1.15–7.29, indicating that seedling length was most affected by drought stress as compared to seedling dry weight and germination, though all the three indexes increased with increasing water stress (Table 5). Among species, *M. uniflorum* was most sensitive

Table 5 Drought sensitivity index of selected leguminous crops.

Drought stress level (Bar)	<i>G. max</i>			<i>M. uniflorum</i>			<i>V. mungo</i>		
	GP	SL	SD	GP	SL	SD	GP	SL	SD
-5	1.11	1.86	1.15	1.42	1.29	1.60	1.15	2.36	1.74
-10	1.67	5.11	2.26	-	-	-	-	-	-
-15	2.73	11.84	7.29	-	-	-	-	-	-
-20	-	-	-	-	-	-	-	-	-

GP= Germination percentage; SL=Seedling length (cm); SD=Seedling dry weight (g).

Table 6 Response Index of selected leguminous crops.

Drought stress level(Bar)	<i>G. max</i>			<i>M. uniflorum</i>			<i>V. mungo</i>		
	GP	SL	SD	GP	SL	SD	GP	SL	SD
-5	-0.13	-0.46	-0.13	-0.38	-0.22	-0.38	-0.42	-0.53	-0.42
-10	-0.56	-0.80	-0.56						
-15	-0.86	-0.92	-0.86						
-20									

GP= Germination percentage; SL=Seedling length (cm); SD=Seedling dry weight (g).

Table 7 Drought stress tolerance index of selected leguminous crops.

Drought stress level (Bar)	<i>G. max</i>				<i>M. uniflorum</i>				<i>V. mungo</i>			
	GSI	SLSI	RLSI	SDSI	GSI	SLSI	RLSI	SDSI	GSI	SLSI	RLSI	SDSI
-5	90	53	62	87	70	78	32	62	87	78	32	57
-10	60	46	23	44								
-15	37	20	9	14								
-20												

GSI= Germination stress tolerance; SLSI=Seedling length stress tolerance; RLSI= Root length stress tolerance; SDSI=Seedling dry weight stress tolerance.

in terms of germination, while *V. mungo* was sensitive in terms of seedling length and dry weight. *G. max* was the least sensitive species.

Response Index

The response index values were negative for all the three species indicating that most of the investigated traits were inhibited due to drought stress (Table 6). Thus, with the increasing drought stress the germination and seedling growth in all the species were decreased.

Stress tolerance index

In all the species, germination, seedling height, root length and seedling dry mass stress tolerance index decreased with increasing in drought stress levels (Table 7). When compared between control and -5 bar stress level, *G. max* performed better in terms of germination, root length and seedling dry mass, while *M. uniflorum* performed better in terms of seedling length. Drought stress tolerance is seen in almost all plants but its extent varies from species to species and even within species (Muscolo *et al.* 2014). In the present study, *G. max* emerged as most tolerant species while *V. mungo* was the least tolerant species.

Taken together, the findings of the present study suggest that seed germination, seedling height and dry mass can be used as traits for selection of drought resistant species. Among the three species investigated, *G. max* can be considered as valuable drought tolerant species. With the aim to increase the probability of successful legume establishment in environments where water scarcity is a frequent constraint, *G. max* can be sown in fields with low moisture availability while *M. uniflorum* and *V. mungo* can be grown in fields with comparatively high moisture availability.

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