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Screening techniques at seed, seedling and whole plant level for drought tolerance

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Abstract
The experiment was conducted under green house with two treatments, stress and non stress condition. In the present investigation, the pigeonpea genotypes were subjected to different screening techniques at seed, seedling and whole plant level for drought tolerance such as PEG induced moisture stress at seed level, gravimetric approach at seedling and whole plant level. Observations on root and shoot length, Root to Shoot ratio, Relative Water Content, rate of water loss, proline content, chlorophyll content and catalase activity were taken and finally from the present investigation which includes physiological screening of pigeonpea genotypes, it is evident that genotype ICPL-87 showed best performance under moisture stress condition and considered as a promising drought tolerant genotype and RVK-285 showed poor performance under moisture stress condition and considered as a drought sensitive genotype. However, the genotype TS-3R showed relatively better tolerance to moisture stress condition and considered as a moderately tolerant genotype.

Keywords: Screening techniques, seed, seedling, whole plant level, drought tolerance

Introduction
Plants have had to cope with periodic and unpredictable environmental stresses during growth and development because of their early migration from aquatic environments to the land. Surviving such stresses over a long evolutionary scale led them to acquire mechanisms by which they can sensitively perceive incoming stresses and regulate their physiology accordingly (Zhang *et al.*, 2006) [19].

In recent years, interest in crop response to environmental stresses has greatly increased because severe losses may result from heat, cold, drought and high concentrations of toxic mineral elements (Blum, 1996) [5]. Drought is one of the most damaging abiotic stresses affecting agriculture. Generally, different strategies have been proposed for the selection of relative drought tolerance and resistance, so some researchers have proposed selection under non stress conditions (Rathjen, 1994; Betran *et al.*, 2003) [15, 4], others have suggested selection in the target stress conditions (Ceccarelli and Grando, 2000; Rathjen, 1994) [6, 15] while, several of them have chosen the mid way and believe in selection under both non-stress and stress conditions (Fischer and Maurer, 1978; Clarke *et al.*, 1992; Fernandez, 1992; Rajaram and Van Ginkle, 2001) [9, 7, 8, 13, 14]. Another approach to identify tolerant genotypes to dry environment is that some drought stress indices or selection criteria like relative water content, rate of water loss, proline content and chlorophyll content have been suggested by different researches (Talebi *et al.*, 2009; Pireivatlou, 2010) [16, 12].

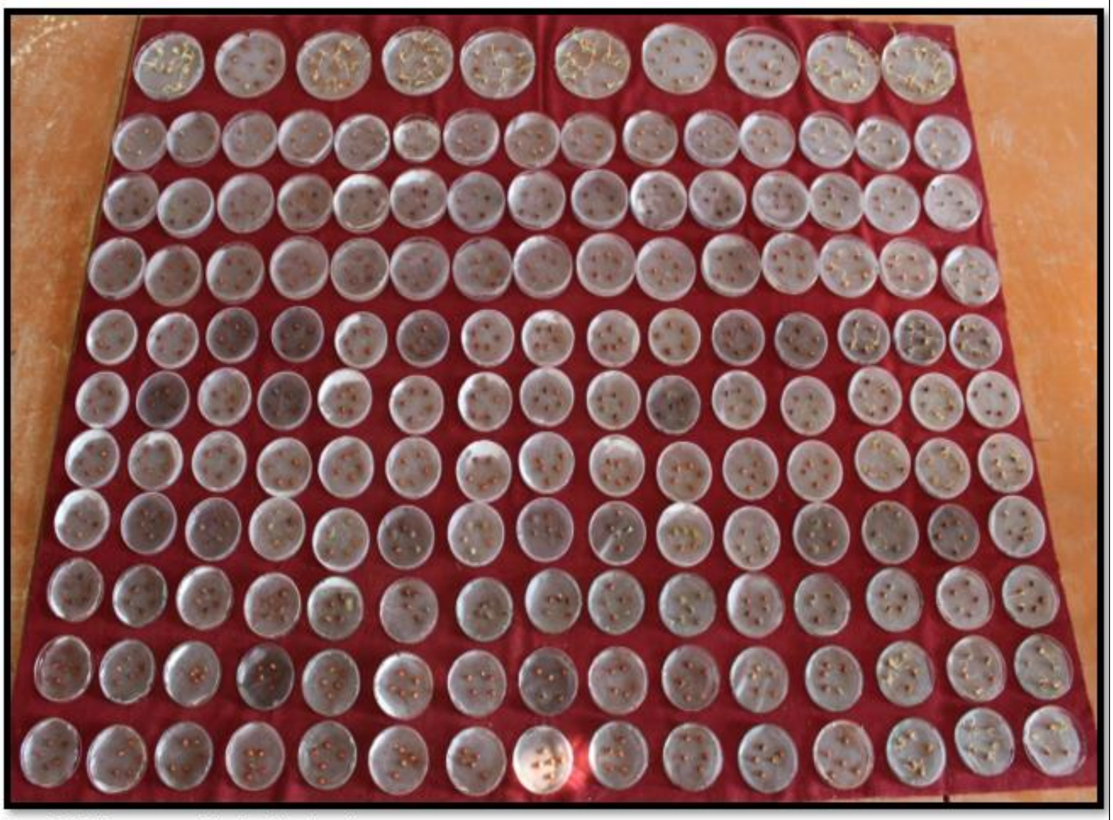
The objectives of the present investigation were to screen drought tolerant and susceptible genotypes of pigeonpea by subjecting them to different screening techniques at seed, seedling and whole plant level for drought tolerance

Materials and methods
Thirty genotypes of pigeonpea were used in the study which are the genotypes obtained from the research station, Kalaburgi. They are the genotypes obtained and collected from all over the country (Table 1). So they were selected to test their performance for drought tolerance at two different growth levels

i. Seed level
ii. Seedling level
iii. Whole plant level

Screening of pigeonpea genotypes at seed level by imposing water stress using Polyethylene glycol (PEG 6000)

The methodology followed in the present experiment was given by Turner (1997) [17]. Polyethylene glycol (PEG 6000) has been widely used to induce drought stress in plants. The seeds of selected genotypes were treated with PEG 6000 for imposing drought condition. Ten seeds of each genotype were placed in the moist petri dishes and 5ml each of -0.2 (T_1),-0.6 (T_2), -1.0 (T_3),-1.2 (T_4) and -1.6 (T_5) MPa PEG solution was dispensed once at the start of the experiment along with control (T_6) (Plate 1). For absolute control the sprinkling of distilled water was continued to maintain moisture in the petri plate. The incubation period was for 72 hours. After three days of treatment, the percentage of germination and morphology of the root and shoot was measured using measurement scale and noted down from each replication of absolute control and PEG treated seeds of all genotypes of pigeonpea seedlings.



T_1: –0.2 MPa
T_2: –0.6 MPa
T_3: –1.0 MPa
T_4: –1.2 MPa
T_5: –1.6 MPa
T_6: Control

R_1: Replication 1
R_2: Replication 2
R_3: Replication 3

Plate 1: Experimental setup to study germination in pigeonpea genotypes at different levels of moisture stress (–0.2, –0.6, –1.0, –1.2 and –1.6 MPa) using PEG 6000

Screening of pigeonpea genotypes at seedling level

Pigeonpea seeds were sown in polybags containing a soil mixture of 1:1:1 ratio of black soil: sand : FYM and were kept in green house to impose water stress (Plate 2). Gravimetric approach was followed to impose water stress (Uday *et al.*, 1998) [18]. Stress was imposed by withholding the irrigation to the seedlings when it attained the age of 30 days and continued till it lost 15% loss of weight of a plant depicts most severe stress to the plants and hence the experiment was carried out by weighing the polybags. After drought imposition at seedling level the samples were collected for further physiological screening. Relative water content (Barrs and Weatherly,1962) [2], rate of water loss (Gavuzzi *et al.*,1997) [10], proline content (Bates *et al.*,1973) [3] and chlorophyll content (Hiscox and Israelstam, 1979) [11] was estimated.

Plate 2: Response of pigeonpea plants to stress (15% loss in weight) at seedling level

Selection of promising genotypes for drought tolerance through gravimetric approach

The contrasting drought tolerant and sensitive genotypes were selected based on their response and performance pertaining to physiological screening experiments.

Three drought tolerant genotype was selected on the basis of their physiological performance, two moderately tolerant genotypes were selected as a second line of support to the study if in case the best performing or tolerant lines show poor performance in the further investigations done with regards to yield and yield attributing parameters and three sensitive genotypes were selected on the basis of their physiological performance. Seeds of the drought tolerant, moderately tolerant and sensitive genotypes were sown in pots containing soil mixture in the 1:1:1 ratio of black soil: sand : FYM and grown for 30 days (Plate 3). After 30 days, two levels of water stress (40% FC and 60% FC) was imposed to selected genotypes and an absolute control (the maximum water holding capacity of the pot was considered as 100% field capacity) was maintained. The water stress was imposed for 15 days and the corresponding leaf samples from all the treatments were collected. RWC and RWL were calculated. Proline content, chlorophyll content and catalase activity (Barber, 1980) [1] was estimated.

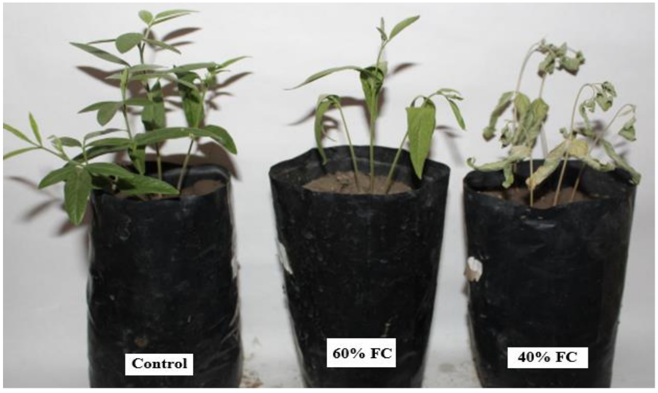


Plate 3: Growth response of pigeonpea plants to moisture stress conditions (60% FC and 40% FC)

Results and discussion

Selection of contrasting genotypes for further stringent physiological screening

Selection of contrasting genotypes (varying in germination percentage, relative water content**,** rate of water loss and proline content subjected to stress and non stress condition) for further stringent physiological screening.

The selection of contrasting genotypes *viz.*, drought tolerant and drought sensitive was necessary and were selected based on their performance related to germination percentage, relative water content, rate of water loss and proline content under stress and non stress conditions. The genotypes showing better performance were selected as drought tolerant and the poor performance showing genotypes were selected as drought sensitive genotypes

Among the 30 genotypes subjected for physiological screening, genotypes GC-11-39, ICPL-87 and JKM-7 were selected as drought tolerant genotypes. GC-11-39, ICPL-87, JKM-7 could germinate under reduced water potential (-0.2 MPa) (Plate 4) and showed high root to shoot ratio (Plate 5) compared to other genotypes. They also showed high values for the relative water content and proline content under stressed conditions. Relatively lower rate of water loss was observed in these genotypes under stressed conditions. These are some of the parameters supported to select drought tolerant genotypes by comparing their performance under stressed situations for the above mentioned physiological parameters.

In the same manner, NDA-1, RVK-285 and TJT-501 were selected as drought sensitive genotypes, considering their poor performance in terms of germination under reduced water potential treatment, low relative water content, high rate of water loss and low proline content under stressed conditions. NDA-1, RVK-285 and TJT-501 showed low germination percentage at -0.2 MPa which is relatively a high water potential. These three genotypes showed less relative water content and also less proline content under stressed condition. They proved sensitive by showing high rate of water loss under stressed conditions. Therefore NDA-1, RVK-285 and TJT-501 genotypes were selected as the sensitive genotypes in further screening experiments.

However, genotypes TS-3R and WRP-R-29-4 were selected as moderately tolerant genotypes. Considering the same criteria of selection in terms of moderately good germination percentage, relative water content and also proline content. They also performed better than the selected sensitive genotypes in terms of maintaining comparatively lower rate of water loss.

Overall three drought tolerant, three drought sensitive and two moderately tolerant genotypes were selected for subjecting them to stringent physiological screening.

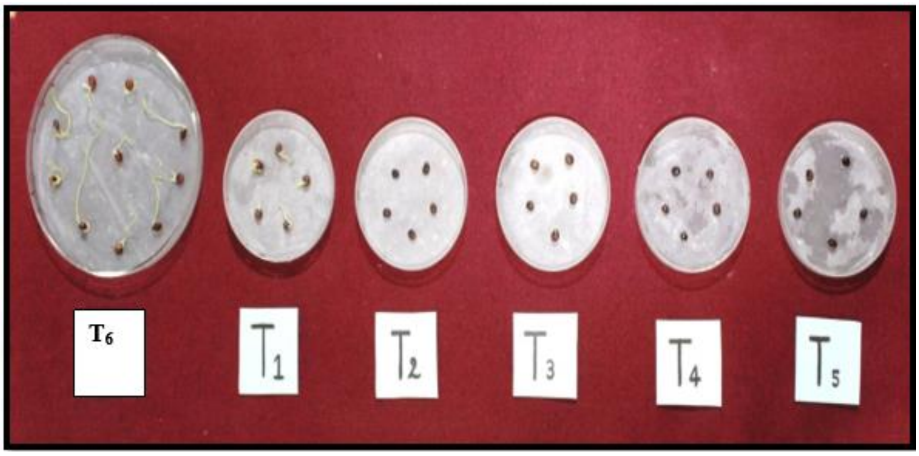


Plate 4: Response of pigeonpea seedlings to different levels (–0.2, –0.6, –1.0, –1.2 and –1.6 MPa) of moisture stress using PEG 6000 T_1: –0.2 MPa, T_2: –0.6 MPa, T_3: –1.0 MPa, T_4: –1.2 MPa, T_5: –1.6 MPa, T_6: Control

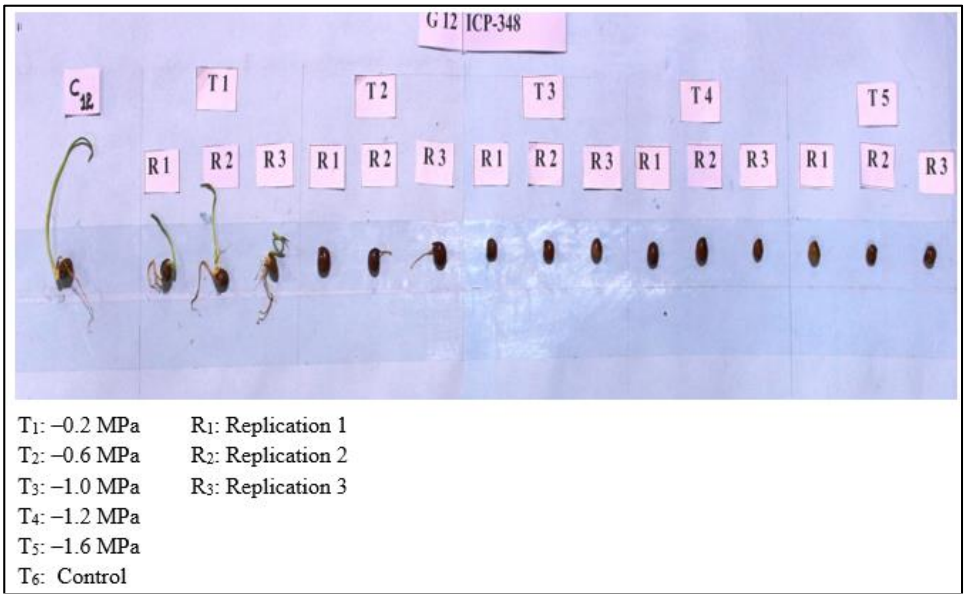


Plate 5: Comparative growth of pigeonpea seedlings subjected to different levels (–0.2, –0.6, –1.0, –1.2 and –1.6 MPa) of moisture stress using PEG 6000

Comparative performance of selected contrasting pigeonpea genotypes varying in different physiological parameters after stringent stress imposition

Among the 8 genotypes subjected for physiological screening, genotypes GC-11-39, ICPL-87 and JKM-7 were selected as drought tolerant genotypes based on their performance under stress. In the same manner, NDA-1, RVK-285 and TJT-501 were selected as drought sensitive genotypes, considering their poor performance under stress. However, genotypes TS-3R and WRP-R-29-4 were selected as moderately tolerant genotypes based on their average performance under stress.

In the present investigation, among the genotypes selected as drought tolerant, ICPL-87 showed the better performance under both stressed (60% and 40% FC) and non stressed conditions. This genotype recorded high relative water content, proline, total chlorophyll content and catalase activity. This proved drought tolerant by maintaining low rate of water loss in both the conditions. Among the selected drought tolerant genotypes, JKM-7 stood second followed by GC-11-39 with relatively poor performance in all the physiological parameters used for the drought studies.

Among the moderately tolerant pigeonpea genotypes, TS-3R showed good performance under both stressed (60% and 40% FC) and non stressed conditions. This genotype recorded high relative water content, proline, chlorophyll 'a', chlorophyll 'b', total chlorophyll content and catalase activity under both stressed and non stressed condition. The genotype also maintained low rate of water loss in both the conditions. However, the genotype WRP-R-29-4 showed relatively poor performance in all the physiological parameters used for the drought studies

In the same manner, among the drought sensitive genotypes, RVK-285 showed poor performance under both stressed (60% and 40% FC) and non stressed condition. The genotype recorded low relative water content, proline, chlorophyll 'a', total chlorophyll content and catalase activity under both stressed and non stressed condition comparing to other genotypes. This genotype showed relatively high rate of water loss compared to other two sensitive genotypes. Among the selected drought sensitive genotypes, NDA-1 and TJT-501 showed relatively better performance in all the physiological parameters used for the drought studies.

Table 1: List of pigeonpea genotypes and their salient features

Sl No.	Genotypes	Remarks
1	ASHA	Wilt and sterility mosaic disease (SMD) disease resistant, high yielding variety matures in 185-190 days
2	AKT-9913	High yielding, medium duration variety from Akola
3	BDN-2008-01	High yielding, medium duration variety from Badnapur
4	BAHAR	Long duration variety, Resistant donar for SMD
5	BSMR-736	Sterility mosaic disease (SMD) disease resistant, high yielding variety matures in 185-190 days, suitable for transplanting
6	Bennur Local	Local land race, matures in about 150 days, high yielding but susceptible to *Fusarium* wilt disease
7	BDN-2008-12	High yielding, medium duration variety from Badnapur
8	GRG-152	High yielding, medium duration variety, resistant to wilt
9	GRG-811	Wilt resistant and Sterility mosaic disease (SMD) disease tolerant, high yielding variety, matures in about 165 days
10	GRG-333	High yielding, white seeded, mid early duration variety, resistant to wilt
11	GC-11-39	Early maturing variety, determinate type, matures in about 125 days, suitable for double cropping
12	ICP-348	Medium duration germplasm line from ICRISAT
13	ICP-6668	Medium duration germplasm line from ICRISAT
14	ICP-13270	Medium duration germplasm line from ICRISAT
15	ICPL-87	Early maturing variety, matures in about 135 days, suitable for double cropping
16	ICP-7366	Medium duration germplasm line from ICRISAT
17	JKM-7	High yielding, medium duration variety, resistant to wilt
18	MARUTI	Wilt resistant, high yielding variety suited to deep soils, matures in about 165 days. Not suitable for SMD infested area

19	NDA-1	Long duration variety, high yielding variety
20	PT-221	High yielding, medium duration variety from Parbhani
21	PUSA-2001	High yielding, medium duration variety from PUSA, IARI
22	RVK-275	High yielding, medium duration variety
23	RVK-285	High yielding, medium duration variety
24	RVK-284	High yielding, medium duration variety
25	TJT-501	High yielding, medium duration variety from Trombay,BARC
26	TTB-7	High yielding, SMD resistant, medium duration variety
27	TS-3	High yielding, white seeded, medium duration variety with spreading plant habit
28	TS-3R	Presently ruling, high yielding, red and bold seeded, mid early duration variety matures in about 155 days. Resistant to wilt
29	WRP-1	High yielding, white seeded, medium duration variety with resistant to wilt
30	WRP-R-29-4	High yielding, red seeded, medium duration variety with resistant to wilt

Conclusion

The present investigation envisages the importance of physiological screening of pigeonpea genotypes under moisture stress conditions. The genotypes were subjected to stringent screening at seedling level as well as whole plant level. ICPL-87 proved to be drought tolerant by showing high relative water content, proline content, total chlorophyll, with relatively low rate of water loss and high catalase activity. Further, from this study, RVK-285 proved to be sensitive genotype by showing less relative water content, proline content, chlorophyll ‘a’, total chlorophyll, catalase activity with relatively high rate of water loss.

References

1. Barber JM. Catalase and peroxidase in primary leaves during development and senescence. Z. Pflanzen physiol. 1980; 97:135-144.
2. Barrs HD, Weatherly PE. A reexamination of relative turgidity for estimating water deficit in leaves. Australian J of Biol. Sci. 1962; 15:413-428.
3. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water stress studies. Plant Soil. 1973; 39:205-207.
4. Betran FJ, Beck D, Banziger M, Edmeades GO. Crop Sci. 2003; 43:807-817.
5. Blum A. Plant Growth Regul. 1996; 20:135- 148.
6. Ceccarelli S, Grando S. Euphytica. 2000; 57:157-167.
7. Clarke JM, DePauw RM, Townley-Smith TF. Crop Sci. 1992; 32:723-728.
8. Fernandez GCJ. Effective selection criteria for assessing stress tolerance. In: Kuo CG (ed), Proceedings of the international symposium on adaptation of vegetables and other food crops in temperature and water stress. Public Tainan Taiwan, 1992, 257-270.
9. Fischer RA, Maurer R. Aust J Agric Res. 1978; 29:897-912.
10. Gavuzzi P, Rizza F, Palumbo M, Campanile RG, Ricciardi GL, Borghi B. Evaluation of field and laboratory predictors of drought and heat tolerance in winter cereals. Canadian Journal of Plant Science. 1997; 77:52-531.
11. Hiscox JD, Israelstam GF. A method of extraction of chlorophyll from leaf tissue without maceration. Canadian J Bot. 1979; 57:1332-1334.
12. Pireivatlou AS, Masjedlou BD, Aliyev RT. Afric J Agric Res. 2010; 5:2829-2836.
13. Rajaram S, Van Ginkle M. Mexico, 50 years of international wheat breeding, Bonjean AP, WJ Angus, 2001.
14. Rajaram S, Van Ginkle M. Mexico. 50 years of international wheat breeding, Bonjean AP, WJ Angus, 2001.
15. Rathjen AJ. The biological basis of genotype-environment interaction: its definition and management, In ‘Proceedings of the Seventh Assembly of the Wheat Breeding Society of Australia, Adelaide, Australia, 1994, 13-17.
16. Talebi R, Fayaz F, Naji AM. General and Appl Plant Physiol. 2009; 35:64-74.
17. Turner NC. Further progress in crop water relations. Adv Agron. 1997; 58:293-338.
18. Uday Kumar M, Rao RCN, Wright GC, Ramaswamy GC, Ashok, Roystephan, *et al*. Measurement of transpiration efficiency in field condition. J Plant Physiol Biochem. 1998; 1:69-75
19. Zhang J, Wensuo J, Jianchang Y, Abdelbagi MI. Field Crop Res. 2006; 97:111-119.