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Investigation of pigeonpea (*Cajanus cajan* (L.) Millspaugh) germplasms for drought tolerance dynamics

A Thanga Hemavathy, P Anantharaju, S Kavitha and Dhanushkodi

A Thanga Hemavathy
Associate Professor, Department of Genetics and Plant Breeding, ADAC&RI, Trichy, Tamil Nadu, India

P Anantharaju
Assistant Professor, Cotton Research Station, Veppanthattai, Tamil Nadu, India

S Kavitha
Associate Professor, Department of SST, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Dhanushkodi
Assistant Professor, Department of SS&AC, ADAC&RI, Trichy, Tamil Nadu, India

Corresponding Author:
A Thanga Hemavathy
Associate Professor, Department of Genetics and Plant Breeding, ADAC&RI, Trichy, Tamil Nadu, India

Abstract

Experiments were performed to evaluate drought tolerance in hundred Pigeonpea genotypes. Drought stress was imposed through polyethylene glycol 6000 (osmotic potential -0.80 MPa and drought-tolerant genotypes were screened by parameters such as percentage germination, seedling growth, and seed vigor. The genotypes identified as drought tolerant by Poly Ethylene Glycol (PEG) analysis were further screened for drought tolerance under CRD design by pot culture study. Morphological, physiological, and biochemical parameters of the genotypes were investigated. Out of hundred genotypes screened, three were found to be drought tolerant *viz*., PLA 195, IC 15707/1, and PLA 217 based on all the morpho physiological and biochemical traits studied. Hence, recombination breeding programs involving crosses derived a high yielding genotype with these drought tolerant genotypes could help bring several allelic combinations that favor the solution of high yielding drought tolerant genotypes.

Keywords: Drought stress, pigeonpea, polyethylene glycol, pot culture study

Introduction

Pigeonpea or red gram, *Cajanus cajan* (L.) Millspaugh (2n=2x=22), is diploid with a genome size of 858 Mbp. It is the only cultivated food crop in the Cajaninae subtribe of the economically important leguminous tribe Phaseoleae, belongs to the family Fabaceae, and is the second most important protein-rich pulse crop in India. It is one of the major grain legume crops in tropical and subtropical regions of the world. Globally pigeonpea is cultivated on 7.03 Mha with an annual production of 4.89 mt. The average productivity of 695 kg/ha indicates further need for improving its genetic potential. India is the largest pigeonpea growing country in the world, accounting for 3.9 mha area with a of 4.6 mt and productivity of 847 kg/ha, and Tamil Nadu accounts for 0.08 mha with productivity of 1025 kg/ha.

Despite the larger area under pigeonpeas in India, the production levels are stagnant due to various biotic and abiotic stresses. Among abiotic stresses, yield loss due to drought in pigeonpea ranges from 20 to 50%. As seed germination and early seedling growth responses are critical phases in establishing stress tolerance genotypes, there is a need to study these responses to improve Pigeonpea yield.

Drought is a major constraint that reduces crop productivity. Pigeonpea thrives under drought prone conditions. Drought is a genetically complex quantitative trait involving high levels of genotype-by-environment interactions. The difficulty in establishing a stringent phenomics platform to dissect the genetic components of this complex trait has further put a risk on the breeding program of Pigeonpea. Evaluating and exploiting the gene pool for potential donor sources could help understand the knowledge gap involving the physiological, biochemical, and molecular complex aspects of drought tolerance mechanisms.

Polyethylene glycol (PEG) compounds were used to induce osmotic stress in petri dishes (*in vitro*) for plants to maintain uniform water potential during the experimental period. Polyethylene glycol (PEG) has often been used as an abiotic stress inducer in many studies to screen drought-tolerant germplasms. PEG-induced osmotic stress can decrease cell water potential. An increase in the concentration of PEG caused a decrease in germination percentage and seedling vigor in certain crop plants.

Important putative drought-resistance traits for pigeonpea include early vigor, leaf-area maintenance, root and shoot growth rates, and developmental plasticity (Johansen, 2003) [2].

The ability of plants to maintain turgor and related physiological processes under water stress has great practical significance, and is related to drought resistance in terms of osmoregulatory activities. Several quick methods have been employed for screening drought-tolerant varieties, such as the relative water content, membrane injury index, chlorophyll stability index, epicuticular wax content, chlorophyll content, osmotic potential, and plastochron index. Among these methods, the relative water content, osmotic potential, chlorophyll fluorescence, and chlorophyll content have been widely used to evaluate the drought tolerance capacity of pigeonpea genotypes.

Materials and Methods

One Hundred genotypes were examined to evaluate osmotic stress during germination and early seedling growth. PEG 6000 was used to induce the drought stress and diverse osmotic potentials: -0.30MPa, -0.45 MPa, -0.60 MPa, -0.80MPa, -1.00 MPa and -1.20MPa were arranged as described by Michel and Kauffman (1973) [4]. The seeds were initially treated with 0.1% $HgCl_2$and then with 70% ethanol, and finally washed with distilled water. The Petri dishes were thoroughly cleaned with a cotton swab.

The medium duration drought tolerance check CO-6 was tested for drought tolerance using polyethylene glycol (PEG) at osmotic potential control: -0.30MPa, -0.45 MPa, -0.60 MPa, -0.80MPa, -1.00 MPa and -1.20MPa. No significant difference was observed in the germination rate between the control and PEG-induced drought stress up to -0.30 a concentration. After this critical point, a reduction in germination rate was observed, and 50% germination was observed at – 0.80 MPa. One hundred pigeonpea genotypes were tested for drought tolerance using polyethylene glycol (PEG) solutions at an osmotic potential of -0.80 MPa

Two replicates of 10 seeds of each genotype were germinated in Petri plates on Whatman no. 1 filter paper, and10 ml of the respective test solution was added. Distilled water was used as the control. Germination tests were carried out under controlled environmental conditions at 24 ± 1 °C with 12 hour photoperiod. Out of the 100 pigeonpea accessions used to estimate genetic diversity, a subset of 15 accessions was screened for drought tolerance by PEG analysis. These included IC 201050, PLA 192-1, PLA 209, PLA 217, IC 26117, IC 15707, PLA 372-1, IC 47233, IC 139594, IC 139593, IC 22558, IC 16201-1, IC 15709, IC 52943 and PLA 195.

Medium-sized pots were collected and filled with farm soil up to the 3/4 level. Soil-borne pathogens were removed by complete drenching with copper oxychloride 50% WP in 3 g/L of water. Seed-borne pathogens were avoided by treating the chickpea seeds with 2% sodium hypochlorite for 20 min., followed by washing and imbibition in distilled water for 12 h before sowing. The seeds were sown in three replicate in a completely randomized design (CRD) on 16.11.2015. In each pot, six seeds were sown at a spacing of 50 mm and thinned to three plants one week after sowing. Six pots were used for each genotype. The experiment consisted of two water treatments and three replicates, with each pot serving as a replicate per treatment per genotype. All pots were watered regularly on alternate days until pre-flowering drought stress was imposed. The experiment comprised two watering treatments that were imposed on 65 DAS when all plants were in the pre-flowering stage: (i) three pots of each genotype were kept well-watered by daily watering (WW); and (ii) the other three pots were exposed to water stress (WS) by watering in alternate three days until 130 DAS, and the experiment was terminated. Three plants in each pot were used to record observations and destructive measurements.

Data for different morphological, physiological, and biochemical parameters were recorded after the 20th day of flowering drought stress.

Results and Discussion

A subset of the 15 genotypes screened by PEG analysis for drought tolerance was subjected to drought experiments by imposing pre-flowering water stress. Under pre-flowering drought stress conditions, changes in morphological, physiological, and biochemical parameters were observed between the drought-stressed and non-stressed plants.

In the present study, all genotypes showed an overall significant increase in root length under drought stress conditions compared to that under irrigated conditions. The genotype IC 15707/1 showed the highest percentage increase in root length compared the control, followed by PLA 372-1 and IC 47233 under drought stress. Root weight and volume showed a decreasing trend under drought stress compared to the control in all genotypes. The lowest percentage decrease in root weight was observed in IC 15709, followed by IC 22558. The lowest percent decrease in root volume was observed in PLA 217, followed by PLA 192-1 and IC 47233. Utilization of these lines in drought-tolerant breeding programs can help evolve genotypes with an improved root architecture to withstand drought.

In the present study, an overall reduction in the relative water content was observed in all genotypes exposed to pre-flowering drought stress. However, the reduction was the lowest in the genotypes PLA 195, PLA 217, IC 15707/1, and IC 201050 compared to the control. The genotypes PLA 217 and IC 26117 showed a drastic reduction in relative water content under drought stress, indicating their drought-susceptible nature.

The chlorophyll fluorescence and chlorophyll content values also declined in all genotypes under drought stress conditions compared to those under irrigated conditions. However, by recording the least reduction in the F_v/F_m values compared to the control, genotypes IC 16201-1, PLA 209, IC 47233, and PLA 195 exhibited better photosynthetic efficiency under drought conditions. Genotypes IC 47233 and PLA195 recorded the lowest percent decrease in chlorophyll a, chlorophyll b, and total chlorophyll content compared to the control, indicating their potential for osmotic adjustment to withstand drought.

In the present study, all 15 genotypes showed a significant increase in proline content. The genotypes PLA 195, IC 15707/1, and PLA 217 showed higher expression of proline, whereas IC 139594 and IC 139593 exhibited lower expression of proline under drought stress compared to the control.

Moreover, all 15 genotypes showed significant increases in catalase and peroxidase content. Among the 15 pigeonpea genotypes evaluated, catalase activity was higher in PLA 195, IC 15707/1, and PLA 217, and peroxidase activity was higher in IC 22558, IC 15707/1, and PLA 195.

From this study, it has been identified that genetic diversity exists for drought tolerance in the subset of pigeonpea genotypes evaluated for morpho-physiological and biochemical traits. This study identified three drought-tolerant genotypes PLA 195, IC 15707/1, and PLA 217, based on the morpho-physiological and biochemical traits studied.

Table 1: Effect of drought on root length, root volume and root weight in Pigeonpea accession under study

SI. No	Genotypes	Root length (cm)			Root weight (g)			Root volume (cc)		
		Control	Drought	% Increase over control	Control	Drought	% Decrease over control	Control	Drought	% Decrease over control
1	IC 201050	18.5	40.5	118.92	5.1	3.8	25.49	11	8	27.27
2	PLA 192-1	20.2	48.6	140.59	4.2	2.9	30.95	12	10	16.67
3	PLA 209	14.9	32.1	115.44	3.9	2.6	33.33	13	9	30.77
4	PLA 217	15.6	30.6	96.15	4.6	3.6	21.74	10	9	10.00
5	IC 26117	22.6	42.9	89.82	3.2	1.9	40.63	8	6	25.00
6	IC 15707/1	48.6	138.2	184.36	4.8	3.2	33.33	16	12	25.00
7	PLA 372-1	12.1	33.8	179.34	4.4	3.1	29.55	8	6	25.00
8	IC 47233	19.3	48.9	153.37	4.5	3.3	26.67	6	5	16.67
9	IC 139594	20.8	36.8	76.92	5.3	3.8	28.30	7	5	28.57
10	IC 139593	14.8	27.9	88.51	3.9	2.4	38.46	7	4	42.86
11	IC 22558	26.9	55.7	107.06	2.4	1.9	20.83	11	8	27.27
12	IC 16201-1	13.9	28.6	105.76	2.7	2.1	22.22	5	3	40.00
13	IC 15709	33.9	62.8	85.25	4.7	3.8	19.15	9	6	33.33
14	IC 52943	24.8	38.4	54.84	2.1	1.6	23.81	12	9	25.00
15	PLA 195	36.8	79.5	116.03	4.6	3.3	28.26	14	9	35.71
	Mean	22.91	49.68	114.15	4.02	2.88	28.18	9.93	7.26	27.27
		G	T	G x T	G	T	G x T	G	T	G x T
	S.Ed	0.18	0.06	0.25	0.01	0.004	0.02	0.006	0.003	0.01
	CD (P=0.05)	0.35**	0.13**	0.49**	0.02**	0.01**	0.03**	0.01**	0.005**	0.019**

Table 2: Effect of drought on relative water content, chlorophyll fluorescence and proline content in Pigeonpea accession under study

SI. No	Genotypes	Relative Water Content (%)			Chlorophyll fluorescence (F_v/F_m)			Proline content(µg g^{-1})		
		Control	Drought	% Decrease over control	Control	Drought	% Decrease over control	Control	Drought	% Increase over control
1	IC 201050	52.81	49.72	5.85	0.721	0.703	2.50	356	621	74.44
2	PLA 192-1	61.24	57.31	6.42	0.681	0.649	4.70	321	519	61.68
3	PLA 209	57.91	52.53	9.29	0.723	0.711	1.66	405	551	36.05
4	PLA 217	56.39	55.66	1.29	0.778	0.723	7.07	369	651	76.42
5	IC 26117	62.33	56.91	8.70	0.698	0.654	6.30	335	548	63.58
6	IC 15707/1	58.91	55.91	5.09	0.701	0.678	3.28	376	685	82.18
7	PLA 372-1	54.78	47.68	12.96	0.748	0.732	2.14	493	624	26.57
8	IC 47233	58.91	52.67	10.59	0.692	0.679	1.88	563	724	28.60
9	IC 139594	65.81	58.31	11.40	0.765	0.749	2.09	597	712	19.26
10	IC 139593	53.84	45.62	15.27	0.666	0.632	5.11	634	763	20.35
11	IC 22558	60.14	56.12	6.68	0.724	0.706	2.49	391	561	43.48
12	IC 16201-1	65.12	57.87	11.13	0.782	0.769	1.66	127	176	38.58
13	IC 15709	57.23	49.62	13.30	0.693	0.681	1.73	432	579	34.03
14	IC 52943	63.86	58.12	8.99	0.746	0.712	4.56	342	529	54.68
15	PLA 195	59.71	59.02	1.16	0.705	0.691	1.99	371	962	159.30
	Mean	59.26	54.20	8.541	0.72	0.69	3.276	407.46	613.66	54.61
		G	T	G X T	G	T	G X T	G	T	G X T
	S.Ed	0.84	0.33	1.20	0.012	0.004	0.018	9.13	3.45	12.91
	CD(P=0.05)	1.69**	0.66**	2.41**	0.025**	0.009**	0.036**	18.29**	6.91**	25.86**

Table 3: Effect of drought on chlorophyll a, chlorophyll b and total chlorophyll content in Pigeonpea accession under study

SI No	Genotypes	Chlorophyll a (mg g^{-1})			Chlorophyll b (mg g^{-1})			Total chlorophyll (mg g^{-1})		
		Control	Drought	% Decrease over control	Control	Drought	% Decrease over control	Control	Drought	% Decrease over control
1	IC 201050	1.51	1.38	8.61	0.64	0.63	1.56	1.8	1.71	5.00
2	PLA 192-1	1.49	1.35	9.40	0.55	0.48	12.73	2.2	2.11	4.09
3	PLA 209	1.76	1.49	15.34	0.59	0.4	32.20	2.52	2.22	11.90
4	PLA 217	1.55	1.51	2.58	0.62	0.61	1.61	1.82	1.76	3.30
5	IC 26117	1.47	1.43	2.72	0.51	0.48	5.88	1.89	1.6	15.34
6	IC 15707/1	1.15	1.13	1.74	0.57	0.54	5.26	1.75	1.65	5.71
7	PLA 372-1	1.42	1.28	9.86	0.48	0.38	20.83	2.21	2.15	2.71
8	IC 47233	1.56	1.37	12.18	0.63	0.62	1.59	2.26	2.23	1.33
9	IC 139594	1.53	1.49	2.61	0.48	0.45	6.25	2.19	2.13	2.74
10	IC 139593	1.66	1.49	10.24	0.55	0.54	1.82	2.1	2.01	4.29
11	IC 22558	1.49	1.45	2.68	0.61	0.58	4.92	2.31	2.07	10.39
12	IC 16201-1	1.45	1.33	8.28	0.51	0.48	5.88	2.56	2.26	11.72
13	IC 15709	1.49	1.47	1.34	0.51	0.45	11.76	2.09	1.97	5.74

14	IC 52943	1.58	1.53	3.16	0.62	0.55	11.29	2.31	2.23	3.46
15	PLA 195	1.53	1.52	0.65	0.52	0.49	5.77	2.45	2.4	2.04
	Mean	1.50	1.41	6.09	0.55	0.51	8.62	2.16	2.03	5.98
		G	T	G X T	G	T	G X T	G	T	G X T
	S.Ed	0.02	0.01	0.03	0.009	0.004	0.016	0.031	0.01	0.04
	CD(P=0.05)	0.04**	0.02**	0.06**	0.02**	0.011**	0.03**	0.06**	0.02**	0.09**

Conclusion

In India, pigeonpeas have more than a supporting role. Indian farmers produce the majority of the world’s crops, and are widely used in Indian households. With the recognition of its role in food security, pigeonpea has grown from an orphan crop to a high-priority research crop. The need for high-yielding genotypes with drought tolerance is a prerequisite for high pigeonpea productivity. The drought-tolerant genotypes identified in this study, such as PLA 195, IC 15707/1, and PLA 217, can be used for recombination breeding with high-yielding genotypes, which could help in bringing several allelic combinations and solving the low productivity issues of the pigeonpea crop.

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