

# Patterns of osmotic adjustment in pigeonpea — its importance as a mechanism of drought resistance

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## Abstract

Osmotic adjustment (OA) is considered as an important physiological mechanism of drought adaptation in many crop plants. The present investigation was aimed at assessing the importance of OA in improving productivity under drought. Using two automated rain-out shelters, 26 extra-short-duration pigeonpea [*Cajanus cajan* (L.) Millsp.] genotypes were grown with irrigation during the growth period or with water deficit imposed from flowering until maturity. Mean leaf  $\Psi_{s100}$  (60–92 DAS) under drought correlated significantly ( $r^2 = 0.72^{**}$ ;  $n = 26$ ) to the mean OA (60–92 DAS) and contributed 72% of the genotypic variation in OA. Significant genotypic variation was observed in the initiation of OA, the duration of OA and the degree of OA. Based on the measured OA at 72, 82, and 92 days after sowing (DAS), genotypes were grouped into five different clusters. Genotypic differences in total dry matter production under drought were positively associated with OA at 72 DAS ( $r^2 = 0.36^{**}$ ,  $n = 26$ ). Significant positive relationship between OA at 72 DAS and grain yield under drought was found ( $r^2 = 0.16^*$ ;  $n = 26$ ). However, OA towards the end of pod filling phase, i.e. at 92 DAS, had a significant negative relationship with grain yield under drought ( $r^2 = 0.21^*$ ;  $n = 26$ ). Genotypic differences in grain yield under drought was best explained using stepwise multiple regression to account for differences in OA at 72, 82, and 92 DAS ( $r^2 = 0.41^{**}$ ;  $n = 78$ ). The degree of OA at 72 and 82 DAS contributed positively to the grain yield, whereas OA at 92 DAS contributed negatively to this relationship. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Osmotic; Adjustment; Drought resistance; Genotypic differences; Grain yield; Pigeonpea

*Abbreviations:* DAS, days after sowing; DSI, drought susceptibility index; GY, grain yield; HI, harvest index; OA, osmotic adjustment; OP, osmotic potential; ROS, rain out shelter; RWC, relative water content; TDM, total dry matter.

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## 1. Introduction

Osmotic adjustment (OA) has been reported to be an important mechanism for drought adaptation in many crop plants (Morgan, 1983; Ludlow, and Muchow, 1990; Subbarao et al., 1995; Hare et al., 1998). It was hypothesized that OA can lead to better water extraction from soil, by stim-

ulating root growth (Greacen and Oh, 1972; Morgan and Condon, 1986; Morgan, 1992). Also, OA has been reported to facilitate better translocation of pre-anthesis carbohydrate reserves to the grain during the filling process, and thus could help to stabilize yield under rain-fed environments (Morgan, 1980; Pierce and Raschke, 1980). A causal relationship between OA and grain yield in sorghum has been suggested (Ludlow et al., 1990; Santamaria et al., 1990). Correlations between high levels of OA and a series of processes linking OA to grain yield have been established (Santamaria et al., 1990). When water was limiting before anthesis, high OA lines produced up to 34% more grain than low OA lines (Santamaria et al., 1990).

Nevertheless, there are many conflicting reports as to the validity of OA as a causal mechanism controlling productivity under moisture-limited environments. Positive relationships between OA and dry matter production and/or grain yield under drought have been shown in grain sorghum (Ludlow et al., 1990; Santamaria et al., 1990; Tangpremsri et al., 1995), wheat (Morgan et al., 1986), and barley (Blum, 1989). Increases in grain yield of as much as 50–60% have been attributed to OA in wheat (Boyer, 1982; Morgan, 1983). However, other reports indicated a negative relationship between OA and grain yield under drought (Grumet et al., 1987; Kirkham, 1988; Tangpremsri et al., 1991). Also, there are reports indicating no relationship between OA and growth or grain yield under field conditions (Shackel and Hall, 1983; Munns, 1988; Blum, 1989; Flower et al., 1990; Krieg, 1993; Tangpremsri et al., 1995). Thus, the value of OA as a drought-resistance trait is somewhat debatable (Munns, 1988; Krieg, 1993). Also, it has been argued that OA could be a drain on the genotype's productivity under drought conditions as it leads to diversion of carbon and nitrogen reserves from grain filling and other growth related processes (Grumet et al., 1987; Kirkham, 1988; Tangpremsri et al., 1991). Other researchers propose that OA is a symptom of stress rather than a mechanism for maintaining metabolic activity during stress, and therefore, is more closely related to survival than productivity (Munns et al., 1979; Barlow et al., 1980; Krieg, 1993).

Pigeonpea is well adapted to rain-fed environments in the semi-arid tropics (Nam et al., 1993). The traditional long-duration (240–270 days to mature) pigeonpea undergoes considerable degree of OA when exposed to moisture deficits (Flower and Ludlow, 1987). A wide range of genetic variation in OA has been observed in container-grown plants of long duration pigeonpea (Flower and Ludlow, 1987). The extent of OA in pigeonpea leaves is a function of both the degree of water deficits experienced and the rate at which these deficits develop (Flower and Ludlow, 1987). The recently developed extra-short duration (ESD) pigeonpea genetic stocks mature in less than 110 days and have yield potential similar to long-duration cultivars when grown under adequate moisture supply (Chauhan et al., 1992). But these ESD genetic stocks are poorly adapted to rainfed conditions because of their shallow rooting behavior (Chauhan, 1993). If drought stress occurs at flowering, these ESD types suffer severe yield losses (Nam et al., 1993). Earlier studies have shown that medium- and long-duration pigeonpea undergo substantial degree of OA when subjected to moisture deficits (Flower and Ludlow, 1986). Existence of genotypic variation in long-duration pigeonpea for OA has been reported under controlled conditions in container grown plants (Flower and Ludlow, 1987). For ESD pigeonpea, remobilization of non-structural carbohydrate reserves from stem may contribute to the degree of leaf OA (Subbarao et al., unpublished). Also, a significant relationship between the degree of OA, plant water status and leaf area duration under drought conditions was shown in pigeonpea using six genotypes (Subbarao et al., unpublished). The present investigation was aimed at determining the extent of genetic variation for OA, the patterns of OA during various growth stages, and the possible use of OA as a tool for improving yield of ESD pigeonpea under drought environments. Also understanding the relationship between osmotic adjustment and total dry matter and/or grain yield production under drought conditions is one of the primary objectives of this study.

## 2. Materials and methods

### 2.1. Location

This experiment was conducted on an Alfisol (a clayey-skeletal, mixed iso-hyperthermic Udic Rhodustalf) field at ICRISAT Asia Center, India (17° N, 78° E; 500 m elevation) in the rainy season of 1995 under an automatic rain-out shelter (ROS), which excluded rain from an experimental area 50 × 25 m<sup>2</sup>. Twenty six pigeonpea genotypes of extra-short-duration (ESD) maturity were used for this study (Table 1). A split-plot design with four replications was used. Two watering regimes (well-watered, and water-deficit imposed 50 days after sowing (DAS) by withholding irrigation) comprised the main plots while different pigeonpea genotypes comprised the sub-plots. Plants were in the vegetative stage at 50 DAS. Each sub-plot consisted of five 30-cm rows and were 1.5 m wide and 4.5 m long. Irrigated and un-irrigated plots were separated by 1 m to prevent seepage between them. All experimental plots were covered by the automatic ROS during rainfall events.

### 2.2. Field preparation and cultural details

The field was surface tilled incorporating 100 kg ha<sup>-1</sup> diammonium phosphate while establishing 60 cm wide ridges. Sowing was done in shallow furrows on both sides of the 60 cm ridges with 30 cm inter-row and 10 cm intra-row spacing. Two seeds per hill were sown which were thinned to one plant per hill at 20–25 DAS to give a density of 33 plants m<sup>-2</sup>. After sowing, the field was uniformly irrigated to field capacity

using perforated pipes to ensure good seed germination and plant establishment. Three hand weedings were done at 20 day intervals. The automated ROS was activated from 10 DAS until harvest and further water was applied to the main plots by a drip irrigation system at 3- to 5-day intervals, depending on the dryness of the soil surface. The amount of irrigation water applied was equal to pan-evaporation. For ESD pigeonpea, various growth phases are as follows: flowering phase is from 50 to 68 DAS; pod setting phase is from 70 to 80 DAS, and rapid pod-filling phase is from 80 to 100 DAS. Drought stress was imposed by withholding irrigation from the plots after 50 DAS. Plant protection measures and other experimental details regarding cultivation practices were described in our earlier paper (Nam et al., 1998). Plants were harvested at maturity and total dry matter was determined by drying in the oven for 3 days at 70°C. For determining grain yield, all pods of a plot were hand picked and seeds were separated by threshing after drying in sunlight. Total dry matter and grain yield were expressed as mg ha<sup>-1</sup>.

### 2.3. Leaf water relations

Two sets of the five youngest fully expanded tri-foliolate leaves of pigeonpea were sampled at mid-day (between 12:00 and 13:00 h), quickly sealed in a humidified zipper locking polythene bags and kept on ice. One set was used to determine leaf relative water content (RWC) and the other was used to determine leaf osmotic potential ( $\Psi_s$ ). For  $\Psi_s$  measurements, leaf samples were stored at -40°C.

For relative water content (RWC) measurements, fresh weight of the sampled leaves was determined first, and turgid weight was obtained after floating the leaf lamina segments on deionized water for 5 h at 24°C and at near the compensation point for irradiance. The turgid tissue was then quickly blotted dry and turgid weight determined. Dry weight was determined after oven-drying to a constant weight. The RWC was calculated using the following formula:

Table 1

Days to flowering and maturity of extra-short duration pigeonpea genotypes used in this study

Characteristic	Mean	Range
Days to flowering	60	51–68
Days to maturity	95	89–99

Table 2

Leaf relative water content (%) of pigeonpea genotypes grown under contrasting moisture regimes

	Days after sowing (DAS)							
	60		72		82		92	
	Irrigation	Stress	Irrigation	Stress	Irrigation	Stress	Irrigation	Stress
Mean <sup>a</sup>	65	67	66	55	58	55	59	45
SE	2.4		5.1		1.9		2.2	
Range <sup>b</sup>	60–71	61–71	59–79	50–64	51–68	42–63	50–63	35–57

<sup>a</sup> Mean, mean of 26 genotypes used in this study.<sup>b</sup> Range, range of values among 26 genotypes.

$$\text{RWC (\%)} = 100 \times [(\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight})] \quad (1)$$

For leaf  $\Psi_s$  determinations, leaf samples were thawed, and centrifuged for 5 min at  $18\,000 \times g$ , and  $\Psi_s$  of the expressed sap was measured with a calibrated (with a range of sodium chloride solutions) Roebling Osmometer (Camlab, Cambridge, UK) (Flower et al., 1990). Osmotic potential ( $\Psi_s$ ) at full turgor (turgid  $\Psi_s$ ) ( $\Psi_{s100}$ ) was calculated using the formula of Wilson et al. (1979) assuming that apoplastic water content was negligible:

$$\Psi_{s100} = (\Psi_s \times \text{RWC})/100 \quad (2)$$

Osmotic adjustment was assumed to be the difference between  $\Psi_{s100}$  of well-watered and water-deficit pigeonpea leaves (Flower and Ludlow 1986).

Osmotic adjustment (OA)

$$= \text{droughted leaf } \Psi_{s100} - \text{irrigated leaf } \Psi_{s100} \quad (3)$$

#### 2.4. Clustering of genotypes based on osmotic adjustment responses at various growth stages

Cluster analysis for grouping of genotypes based on osmotic adjustment at 60, 72, 82, and 92 DAS was done using Ward's minimum variance method (SYSTAT, 1992). According to this method, at each analysis step, union of every possible pair of clusters is considered. Two clusters whose fusion results in the minimum increase in information loss, measured in terms of error sum of squares, are combined (SYSTAT, 1992).

### 3. Results

#### 3.1. Leaf water relations

Water stress significantly ( $P \geq 0.05$ ) decreased the leaf RWC in pigeonpea genotypes by 72 DAS. Genotypic differences in RWC were significant ( $P \leq 0.05$ ) at 72, 82, and 92 DAS (Table 2). Leaf RWC generally declined with time in both irrigated and drought treatments. However, RWC of many genotypes were significantly lower under drought than under irrigation; the differences grew larger with time. Under drought, the mean turgid osmotic potential (mean of 26 genotypes) of leaves ( $\Psi_{s100}$ ) reached a minimum ( $-0.93$  MPa) at 72 DAS, and became less negative by 92 DAS ( $-0.87$  MPa) (Table 3). In the fully irrigated plots, the mean  $\Psi_{s100}$  reached its minimum value by 72 DAS ( $-0.85$  MPa), and became less negative at 82 DAS ( $-0.60$  MPa). This is an indication of remobilization of solutes from the leaves to the reproductive growth. Genotypic differences in  $\Psi_{s100}$  were significant ( $P \leq 0.01$ ) at 72, 82, and 92 DAS. There was a significant positive correlation between mean  $\Psi_{s100}$  (60–92 DAS) of droughted plants and mean OA (60–92 DAS) ( $r^2 = 0.72^{**}$ ;  $n = 26$ ) (Fig. 1). Genotypic differences in OA were significant ( $P \leq 0.05$ ) from 72 DAS (Table 4). Genotypes varied widely in the onset of OA, the maximum degree of OA reached, and maintenance of OA until physiological maturity. The range for OA was 0.1 to 0.6 MPa and distribution was normal; with most genotypes having OA of 0.3–0.4 MPa (Table 4). The degree

Table 3

Leaf turgid osmotic potential ( $\Psi_{s100}$ ) (MPa) of pigeonpea genotypes grown under contrasting moisture regimes

	Days after sowing (DAS)							
	60		72		82		92	
	Irrigation	Stress	Irrigation	Stress	Irrigation	Stress	Irrigation	Stress
Mean <sup>a</sup>	-0.81	-0.78	-0.85	-0.93	-0.60	-0.89	-0.74	-0.87
SE	-0.049		-0.066		-0.062		-0.045	
Range <sup>b</sup>	-0.75 to -0.90	-0.75 to -0.95	-0.68 to -0.99	-0.67 to -1.36	-0.46 to -0.81	-0.61 to -1.30	-0.62 to -0.91	-0.64 to -1.38

<sup>a</sup> Mean, mean of 26 genotypes used in this study.<sup>b</sup> Range, range of values among 26 genotypes.

of OA at 72 DAS predicted reasonably well the mean OA performance of a genotype from 60 to 92 DAS ( $r^2 = 0.42^{**}$ ;  $n = 26$ ). This indicates that early onset of OA can have a major effect on the average OA of a genotype during the life cycle. The degree of OA was mostly determined by  $\Psi_{s100}$  under drought conditions ( $r^2 = 0.72^{**}$ ;  $n = 26$ ), though  $\Psi_{s100}$  under irrigated conditions was taken into account while deriving OA (Fig. 1).

### 3.2. Osmotic adjustment and its relation to dry matter accumulation and grain yield

The genotypic differences in total dry matter (TDM) (at harvest) under drought (ranging from 2.6 to 5.0 t ha<sup>-1</sup>) (Table 5) was correlated with OA (ranging from -0.32 to 0.47 MPa) at 72 DAS ( $r^2 = 0.36^{**}$ ;  $n = 26$ ; Fig. 2); nevertheless, osmotic adjustment accounted for only 36% of the variation in dry matter production among genotypes. Genotypic differences in grain yield (varying from 1.0 to 1.77 mg ha<sup>-1</sup>) (Table 5), can best be attributed to the degree of OA at 72 DAS, i.e. positive correlation between the degree of OA and grain yield ( $r^2 = 0.16^*$ ;  $n = 26$ ) (Fig. 3a). Despite the significant positive relationship between OA at 72 DAS and grain yield under drought, OA at 72 DAS accounted for only 16% of the variation in grain yield under drought. Also, the degree of OA in leaves towards the end of pod filling phase (i.e. at 92 DAS) had a significantly negative effect on grain yield ( $r^2 = -0.21^*$ ;  $n = 26$ ) (Fig. 3b). This indicates that genotypes that continuously main-

tain OA until maturity will have a negative impact on grain growth (cost factor), as it is likely to inhibit remobilization of carbon, nitrogen and other nutrient sources from stem, root and leaves for pod filling.

### 3.3. Stepwise regression to establish the relationship between the degree of osmotic adjustment and productivity under drought

Genotypic differences in TDM under drought

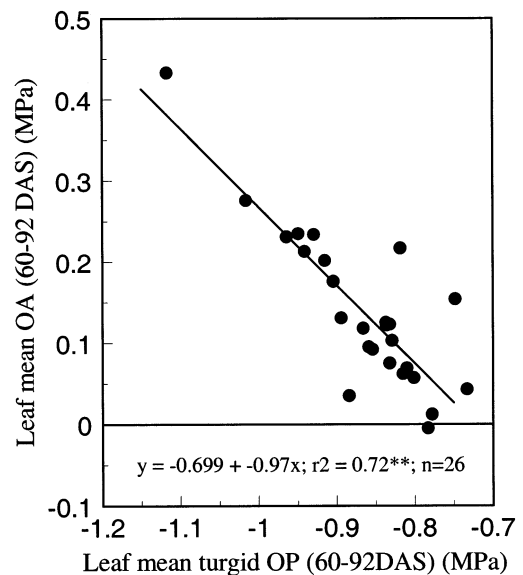


Fig. 1. Relationship between leaf mean  $\Psi_{s100}$  (60–92 DAS) under drought and leaf mean osmotic adjustment (60–92 DAS) in pigeonpea genotypes.

Table 4

Grouping of pigeonpea genotypes based on osmotic adjustment (MPa) at various growth stages using cluster analysis (distance matrix is Euclidean distance and Ward minimum variance method)

Sl. no.	Genotype	Days after sowing (DAS)			
		60	72	82	92
		MPa			
<i>Group I</i>					
18	ICPL 85010	−0.09	−0.32	0.15	0.30
21	ICPL 89002	0.02	−0.08	0.00	0.22
17	ICPL 89021	0.00	−0.05	0.18	0.28
14	ICPL 83015	0.03	−0.10	0.24	0.33
19	ICPL 88032	0.03	0.02	0.39	0.37
Mean		−0.002	−0.11	0.19	0.30
<i>Group II</i>					
25	Manak	0.05	−0.02	0.65	0.42
2	ICPL 88009	0.00	0.13	0.62	0.19
12	ICPL 94020	0.15	0.21	0.45	0.11
6	ICPL 93081	0.14	0.40	0.57	0.63
Mean		0.09	0.18	0.57	0.34
<i>Group III</i>					
24	ICPL 84031	−0.02	0.47	0.20	0.29
20	ICPL 88039	−0.04	0.29	0.25	0.13
23	ICPL 91031	−0.01	0.29	0.20	−0.01
7	ICPL 93097	−0.03	0.23	0.15	0.02
22	ICPL 91024	−0.04	0.15	−0.04	−0.09
Mean		−0.03	0.29	0.15	0.07
<i>Group IV</i>					
4	ICPL 91011	−0.11	0.04	0.09	0.12
13	ICPL 94025	−0.16	0.08	0.23	0.13
15	ICPL 84023	−0.14	0.06	0.37	0.11
26	UPAS 120	−0.06	0.16	0.36	0.24
8	ICPL 94005	−0.12	0.24	0.41	0.32
Mean		−0.12	0.12	0.29	0.19
<i>Group V</i>					
10	ICPL 94008	−0.08	−0.03	0.48	0.12
16	ICPL 88007	−0.06	−0.12	0.43	−0.02
5	ICPL 91016	−0.03	−0.01	0.32	−0.02
9	ICPL 94006	−0.06	0.12	0.47	−0.15
1	ICPL 4	−0.07	0.16	0.43	0.00
3	ICPL 90008	−0.06	0.12	0.24	−0.12
11	ICPL 94009	0.00	0.24	0.28	−0.27
Mean		−0.04	0.07	0.38	−0.06
SE (for interaction)		0.04	0.08	0.05	0.04

were mostly attributed to the degree of OA at 72 DAS (Table 6), and the subsequent inclusion of OA at 60, 82, and 92 DAS, did not improve the

strength of this regression. Genotypic differences in grain yield (GY) under drought were best explained from a stepwise multiple regression by

Table 5

Total dry matter, and grain yield of pigeonpea genotypes (grouping of genotypes based on OA at various growth stages)

Sl. no.	Genotype	Total dry matter (mg ha <sup>-1</sup> )		Grain yield (mg ha <sup>-1</sup> )	
		Irrigated	Drought	Irrigated	Drought
<i>Group I</i>					
18	ICPL 85010	5.40	3.20	2.10	1.34
21	ICPL 89002	5.60	3.00	1.91	1.08
17	ICPL 89021	3.60	2.61	1.56	1.19
14	ICPL 83015	5.37	3.59	2.10	1.31
19	ICPL 88032	5.71	3.34	2.03	1.22
Mean		5.14	3.15	1.94	1.23
<i>Group II</i>					
25	Manak	5.22	3.73	1.91	1.39
2	ICPL 88009	5.70	3.75	2.37	1.37
12	ICPL 94020	6.56	4.35	2.11	1.43
6	ICPL 93081	6.71	4.56	1.97	1.42
Mean		6.05	4.10	2.09	1.40
<i>Group III</i>					
24	ICPL 84031	6.99	4.12	1.93	1.28
20	ICPL 88039	5.46	4.04	2.09	1.60
23	ICPL 91031	7.89	4.34	2.09	1.39
7	ICPL 93097	4.77	3.70	1.95	1.54
22	ICPL 91024	7.34	3.89	2.29	1.33
Mean		6.49	4.02	2.07	1.43
<i>Group IV</i>					
4	ICPL 91011	6.52	3.93	2.13	1.36
13	ICPL 94025	6.25	3.87	2.39	1.43
15	ICPL 84023	5.31	3.22	2.07	1.37
26	UPAS 120	5.91	4.23	1.86	1.42
8	ICPL 94005	8.39	3.68	2.99	1.33
Mean		6.48	3.79	2.29	1.38
<i>Group V</i>					
10	ICPL 94008	7.36	4.09	2.43	1.32
16	ICPL 88007	5.67	3.21	1.94	1.26
5	ICPL 91016	8.70	4.28	2.57	1.52
9	ICPL 94006	5.84	4.32	1.96	1.47
1	ICPL 4	5.62	3.44	2.01	1.37
3	ICPL 90008	5.41	3.32	2.11	1.41
11	ICPL 94009	6.76	4.96	2.08	1.77
Mean		6.48	3.95	2.16	1.45
SE		0.119 (for treatment)		0.056 (for treatment)	
SE		0.336 (for genotypes)		0.125 (for genotypes)	
SE		0.483 (for interaction)		0.183 (for interaction)	

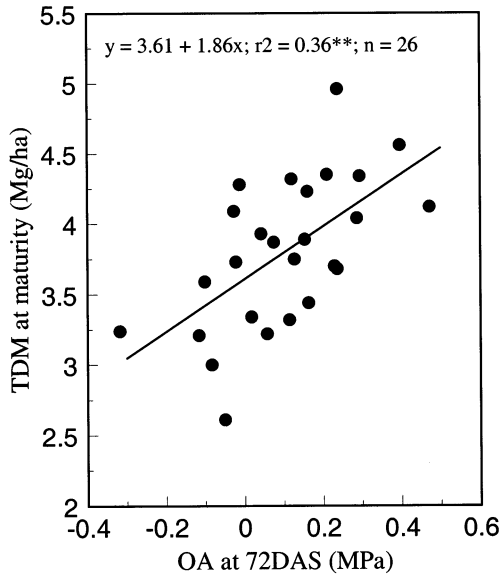


Fig. 2. Relation between osmotic adjustment (OA) at 72 DAS and total drymatter (TDM) at maturity in ESD-pigeonpea genotypes under drought (Water was withheld from 50 DAS).

taking into account OA at 72, 82 and 92 DAS (Table 6). The degree of OA at 72, and 82 DAS had a positive effect on GY, whereas the degree of OA at 92 DAS had a significant negative effect on GY (Table 6).

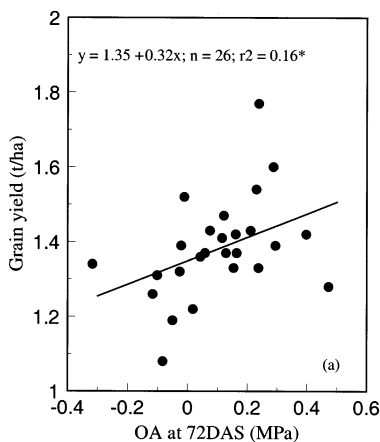


Table 6

Forward stepwise multiple regressions of osmotic adjustment (OA) at various growth periods to total dry matter and grain yield of pigeonpea genotypes under drought ( $n = 26$ )<sup>a</sup>

Variable added	Model $r^2$
<i>Total dry matter at harvest</i>	
OA 72	0.362**
<i>Grain yield at maturity</i>	
OA 72	0.161*
OA 82	0.068
OA 92	-0.210*
OA 72+OA 82+OA 92	0.407**

<sup>a</sup> Contributions of added variable (partial  $r^2$ ) significant at \*  $P < 0.05$  or \*\*  $P < 0.01$ .

### 3.4. Clustering of genotypes based on osmotic adjustment at different growth stages

Based on OA at 72, 82, and 92 DAS, genotypes were grouped using the cluster analysis (distance metric is Euclidean distance and Ward minimum variance method). The clustering divided genotypes into five groups (Table 4). The first group consisted of five genotypes, in which OA peaked towards the rapid grain filling phase, i.e. 92 DAS (Fig. 4a, Table 4). The second group consisted of four genotypes in which OA peaked at 82 DAS, then declined slightly thereafter, but still remained substantial at 92 DAS (Fig. 4a, Table 4). The

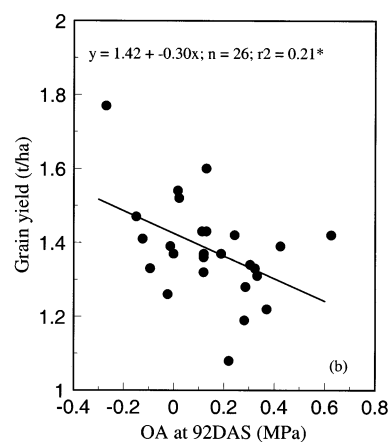


Fig. 3. (a) Relationship between osmotic adjustment at 72 DAS and grain yield under drought in pigeonpea genotypes. (b) Relationship between osmotic adjustment at 92 DAS and grain yield under drought in pigeonpea genotypes.



third group consisted of five genotypes, in which OA peaked at 72 DAS, then declined (Fig. 4b, Table 4). The fourth group consisted of five genotypes in which OA peaked at 82 DAS, and then declined (Fig. 4b, Table 4). The fifth group consisted of seven genotypes on which OA peaked 82 DAS, and then declined rapidly (Fig. 2b, Table 4). In most genotypes of this group, OA became negative by 92 DAS. This result suggests that these genotypes are very efficient in remobilizing solutes previously used for OA for grain filling (rapid grain filling occurs at 80–95 DAS in ESD pigeonpea), thus resulting in a negative OA at 92 DAS. Genotypes differed in their degree of OA in each cluster group at various growth stages, though all genotypes in a cluster group showed a similar pattern of OA between 60 and 92 DAS.

Among five clusters of genotypes, the first cluster where the degree of OA continued to rise even at 92 DAS had the lowest average grain yield ( $1.23 \text{ mg ha}^{-1}$ ) under drought (Fig. 4a, Table 5). The fifth cluster of genotypes which has a pattern of rising OA until 82 DAS, which was coupled with a sudden fall in OA by 92 DAS, had the highest mean grain yield of  $1.45 \text{ mg ha}^{-1}$  under drought (Fig. 4b, Table 5). Genotypes in each cluster varied widely in their grain yield and in total dry matter under drought (Table 5). Under

drought, grain yield of cluster I genotypes ranged from  $1.08$  to  $1.34 \text{ mg ha}^{-1}$ ; in cluster II,  $1.37$ – $1.43 \text{ mg ha}^{-1}$ ; in cluster III,  $1.28$ – $1.60 \text{ mg ha}^{-1}$ ; in cluster IV,  $1.36$ – $1.43 \text{ mg ha}^{-1}$ ; in cluster V,  $1.26$ – $1.77 \text{ mg ha}^{-1}$  (Table 5). In cluster V, the mean pod number under drought was highest ( $856 \text{ m}^{-2}$ ) compared to other clusters, whereas it was lowest in the cluster I ( $753 \text{ m}^{-2}$ ). However, the pod number ranged from 581 to  $1094 \text{ m}^{-2}$  among genotypes with cluster I; in cluster V, the pod number ranged from 478 to  $1150 \text{ m}^{-2}$ . (Table 5).

#### 4. Discussion

The proportion of genotypes having some degree of OA under water stress increased from 35% at 60 DAS to 95% by 82 DAS. This indicates that OA in pigeonpea is a widespread phenomenon. Our results suggested that ESD pigeonpea genotypes can be broadly grouped into two categories; early adjusters (OA at 72 DAS), and late adjusters (OA at 82 DAS). The early or late adjusters do not belong to any particular maturity group as each group have genotypes with days to flowering ranging from 52 to 68 DAS. Thus, phenology is not directly influencing the onset of OA. Also, our results suggests that OA should be ‘turned on’ as

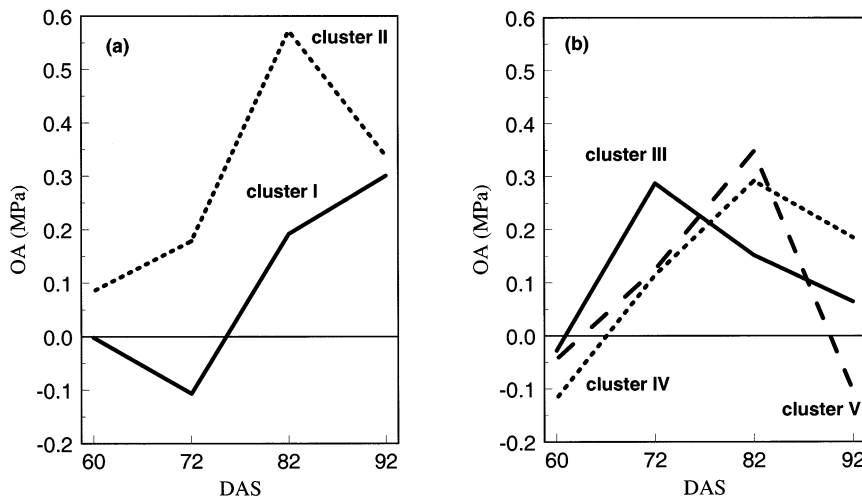


Fig. 4. (a, b) Developmental patterns of osmotic adjustment (OA) among 26 ESD-pigeonpea genotypes based on cluster analysis (Water was withheld from 50 DAS).

the soil moisture decreases to direct carbon partitioning towards reserve building rather than other growth related processes. This would improve the degree of OA during the crucial pod setting phase (70–80 DAS) by maintaining a more favorable water status thus improving podset. Our results are consistent with this argument as evidenced by the significant positive relationship between the degree of OA at 72 DAS and grain yield of genotypes. Given the range of possible morphological and physiological mechanisms that can influence productivity under water deficit environments (Ludlow and Muchow, 1990; Subbarao et al., 1995), the significant positive relationship of dry matter production and grain yield with the degree of OA reflects the importance of this mechanism for improving the drought resistance in pigeonpea. However, it must be mentioned that only 16% of the variation in grain yield differences among genotypes under drought was explained by OA at 72 DAS. Also, the strong negative effect of the degree of OA at 92 DAS on grain yield contributed to 21% of the variation. By taking into account OA at 72, 82, and 92 DAS using stepwise regression approach, the contribution of OA to grain yield differences under drought could be explained up to 40%. The weak positive effect of OA on grain yield is as important as the weak negative effect of OA on grain yield at 92 DAS in pigeonpea. However, for determining the dry matter production under drought, OA at 72 DAS has significant positive effect, and explained 36% of the variation among genotypes. This lends credibility to earlier fears that OA could be metabolically expensive, and thus could reduce grain yield under drought because of blockage of the remobilization of carbon and nitrogen reserves for the reproductive growth.

Thus, 'turning on' of the OA early in the stress development phase has its maximum adaptive value only if it 'turns off' during active reproductive growth phase (i.e. pod filling; 80–100 DAS), as this would facilitate retranslocation of carbon and nitrogen reserves (i.e. the solutes used for OA) from various plant parts including leaves to reproductive tissue such as grain. The ability to sense moisture stress and trigger OA sufficiently early so that it could have a role both in pod

setting and de-osmotic adjustment for pod filling (which may be linked with their ability to remobilize carbon, nitrogen reserves out of leaves and stems), would enhance the importance of OA as a mechanism for improving grain yield of pigeonpea under drought environments. Thus, the degree of OA, the timing of adjustment, the duration of OA, and the ability to remobilize carbon and nitrogen that were used for OA to reproductive growth are some of the factors determining the relative importance of this mechanism for the adaptation to drought-prone environments. Thus, selecting for OA based on sampling at one growth stage is insufficient to assess the multi-faceted ramifications of OA to improve drought adaptation. It may be necessary to evaluate genetic stocks for OA at various growth stages, in order to understand and characterize the patterns, be able to select or design through breeding the optimum pattern of OA. Although, the pattern of osmotic adjustment for cluster V seems to be advantageous in improving productivity under drought, it would be difficult to attribute a particular pattern in improving the productivity, given the wide range of variation among genotypes within a cluster. Nevertheless, this pattern of OA (i.e. pattern in cluster V) coupled with early onset of higher degree of OA might be the best combination that was shown in the genotype ICPL 94009, where the grain yield was greatest under drought.

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