

Implications of Genetic Heterogeneity Among Cultivated Genotypes for Global Food Security in Climate Change Era

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<https://doi.org/10.22207/JPAM.10.3.41>

(Received: 26 February 2016; accepted: 16 April 2016)

The experiment was undertaken in three water stress environments to assess the inherent diversity of the primary gene pool of sunflower (*Helianthus annuus* L.) following morphophysiological criteria and thereafter validation of these genotypes in the current trend of climate change. Plot achene yield was maximum in control and ranged from 10.3g to 54.7g in W₁, 7.2 to 52.2 in W₂, 9.7 to 49.8 in W₃ and 10.0 to 51.8 in W₄. The genotypes P-94-R, P-115-R and P-119-R were found to be severely affected by the treatments, however, 95-C-1-R and P-87-R resisted to water stress and showed minimum reduction in seed yield. D² statistics grouped the test genotypes into 6 clusters. Cluster I comprised of maximum number of genotypes (27 genotypes), followed by cluster II (5 genotypes), cluster III and V (3 genotypes in each), cluster IV (2 genotypes), and cluster VI (1 genotypes). Among the traits evaluated, leaf area index contributed the maximum (18.54%) towards the observed diversity, followed by early vigour (18.35%), oil content (12.35%), 100 seed weight (10.59), photosynthetic capacity (9.58%), and plant height (9.29%), and leaf water potential (6.16%), achene yield per plant (4.50%), head diameter (4.19%) and canopy temperature (3.16%). Genotypes P69R, P87R, P93R, P115R, NDLR2 (Cluster I), P107RP₁ (Cluster II), P121R (Cluster III), P111R (Cluster IV), 40B and 50B (Cluster V) and 7-1B (Cluster VI) identified as stress tolerant genotypes. The study concludes cytoplasmic restorer lines P111R, P112R and P110R and maintainer lines 71B, 40B and 50B as putative genetic material to comply threatening climatic phenomenon.

Keywords: Changing climate, genotypic diversity, water stress, food security.

Climate change is threatening the biodiversity

Anthropogenic environmental modifications, such as habitat loss and fragmentation, have already led to changes in the amounts and distribution of genetic diversity. In the UK, such changes are already apparent as species-level responses, including phenological changes and distributional shifts (Parmesan, 2006; Thackeray *et al.*, 2010; Pateman, 2013; Sparks 2013). Climate driven alterations in population size (Leimu *et al.*, 2006; Honnay and Jacquemyn, 2007), geographical connectivity (Aguilar *et al.*, 2008), distribution (Anderson *et al.*, 2008) and selection pressure (Phillimore *et al.*, 2010 and 2012) have been considered

as primary changes that influences the genetic diversity and gene flow. The loss of genetic diversity results in narrow mating, allowing the expression of deleterious genetic variants and leads to offspring with lower fitness (Reed and Frankham, 2003; Leimu *et al.*, 2006; Angeloni *et al.*, 2011). Further reduction in population size resulting from the loss of fitness may exacerbate the effects of inbreeding, and this positive feedback of reduced population size on fitness loss is known as an extinction vortex (Frankham *et al.*, 2010 and 2011). The fitness costs associated with inbreeding depression also limit responses to stressful environments (Ketola and Kotiaho, 2009; Fox and Reed, 2011; Bijlsma and Loeschcke, 2012; Dierks *et al.*, 2012).

Food security: The burning issue

The reduced precipitation, together with high evapotranspiration is expected to subject

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natural and agricultural vegetation at a great risk of severe and prolonged water stress with each passing year (Easterling *et al.*, 2007; Iqbal *et al.*, 2004 and 2013). In semi-arid areas, climate change may extend the dry season of no or very low flows, which particularly affects water users unable to rely on reservoirs or deep groundwater wells (Giertz *et al.*, 2006; Kundzewicz *et al.*, 2010). Agricultural irrigation demand in arid and semi-arid regions of Asia is estimated to increase by at least 10% for an increase in temperature of 1°C (Fischer *et al.* 2002; Liu 2002). According to a report by USDA Agriculture Weather Facility (2005), oilseed production in 2005 was down 2% from 2004 due to drier than normal growing season (Rauf and Sadaqat, 2007). In Spain in particular, the sunflower crop suffered substantially from drought, decreasing production by 41%. Similarly in the Americas, drought was a key factor responsible for yield losses of up to 20% (Reddy *et al.*, 2004). In Pakistan, sunflower acreage declined by 25% from 1998-99 to 2002-03, but the total sunflower production declined by 33% during the same period as a result of severe drought (GOP, 2003). Considering the present scenario, there is dire need of cultivars which can cope with aberrant climate, particularly reference to drought and moisture stress, which in turn has necessitated the development of more productive hybrids of diverse genetic background (Dhillon *et al.*, 2010; Shamshad *et al.*, 2014). The investigation considered sunflower, potential oilseed crop worldwide (Machikowa and Saetang, 2008; Ghaffari *et al.*, 2012; Shafi *et al.*, 2013; Kumar *et al.*, 2014; Shamshad *et al.*, 2014; Rao *et al.*, 2015), to tackle the food security issue rose by policy makers to combat climate change. The experiment was undertaken to assess the inherent diversity of the primary gene pool of sunflower (*Helianthus annuus* L.) following morphophysiological criteria and thereafter validation of these genotypes in the current trend of climate change.

MATERIALS AND METHODS

Variable stress simulation model and Breeding Material

Along with future temperature shifts, current climate models also predict an increase in the frequency of extreme precipitation fluctuations,

including flooding and drought throughout the globe (Allan and Soden 2008, Min *et al.*, 2011). Understanding and predicting the consequences of climate change for natural populations is of critical importance. Therefore it was attempted to test the parental stock in stress simulation model by withholding the water at critical crop growth stage *viz* W₁: (control); W₂: water stress before button stage and after soft dough stage; W₃: stress at 50 percent flowering stage and soft dough stage thereafter and after hard dough stage; W₄: stress at anthesis completion stage and after soft dough stage. Apart from secondary yield contributor traits, participation of physiological parameters like canopy temperature (CT) (°C), leaf area index (LAI), photosynthetic capacity (PS) and leaf water potential (LWP) (mpa) were also assessed. 41 Cytoplasmic and restorer lines were taken for study having sufficient geographical and parental diversity (Table 01).

Statistical background of study

Mahalanobis D² statistics between two populations estimated on the basis of the 'p' characters is:

$$Dp^2 = \sum_1^p \sum_1^p W^{ij} (X_{i1} - X_{i2}) (X_{j1} - X_{j2})$$

Where,

W_{ij} = Variance – covariance matrix W^{ij} is the reciprocal of (W_{ij}), (i,j=1,2,.....p)

X_{i1} = Sample mean for ith character for first sample

X_{i2} = Sample mean for ith character for 2nd sample. In

the present study characters (P=1-10) were used to perform the above analysis. For conducting the D² analysis, the computer programme, WINDOSTAT 8.0 cluster analysis was used. The distance from D² values was calculated for each pair of parents (Mahalanobis, 1934). The D₂ values of all the combinations were arranged in descending order. Treating D₂ as generalized statistical distance, all the genotypes were clustered into six groups. The intra and inter-cluster distances and contribution of individual characters towards divergence were computed (Mohan and Seetharam, 2005).

RESULTS AND DISCUSSION

Morphometric Variability and water stress

In the study, pooled analysis of variance (Table 2a; 2b) revealed that environment differed

significantly in respect of all the characters and showed significant interaction with the genotypes. Days to 50 percent flowering were found to be higher in control i.e. W_1 followed by W_4 , W_2 and W_3 in continuation with earlier reporting of Ghani *et al.* (2000). In W_1 the genotype 48-B was the earliest (57 days) and P-111-R was the latest (81 days) to flower (**Table 3**). Whereas, in W_2 genotypes P-61-R, P-75-R, P-94-R, P-119-R, 11-B, 50-B and RHA-297 recorded earliest flowering (60 days) and P-112-R registered the (76 days) latest. In W_3 48-B recorded to be earliest (56 days) while P-111-R and P-112-R took maximum days (74 days) to flowering. In W_4 , 48-B was observed as earliest (57 days) and P-111-R latest (79 days). Overall, the genotype P-111-R followed by P-112-R took maximum number days to 50 percent flowering and 48-B minimum. Seed yield was maximum in control (Ramchander *et al.*, 2014) and ranged from 10.3g to 54.7g in W_1 , 7.2 to 52.2 in W_2 , 9.7 to 49.8 in W_3 and 10.0 to 51.8 in W_4 . The genotypes P-94-R, P-115-R and P-119-R were found to be severely affected by the treatments, however, 95-C-1-R and P-87-R resisted to water stress and showed minimum reduction in seed yield. Water Stress during the flowering stage causes abortion of ovaries and embryo, sterility of pollen and decrease in leaf area index. This reduces the fertile achene per head and 100 achene weight (Reddy *et al.* 2004). The average 100-achene weight was significantly high in W_1 (7.1g) as compared to W_2 (5.5 g), W_3 (5.7 g) and W_4 (6.1g) Genotype 7-1-B recorded the highest 100-seed weight followed by P-75-R and the genotype 95-C-1-R exhibited minimum 100-achene weight followed by and RCR-8297 over the entire respective environment. For the same parameter genotypes P-107-R-P₁, P-75-R, 10-B and 45-B were least effected in all environments indicating tolerance. However, the effect of water stress was highly significant in 95-C-1-R, NDLR-1, 7-1-B and RCR-8297. Reduction of head size, plant height, achene weight and achene yield were reported previously (Hossain *et al.* 2010; Vanaja *et al.* 2011).

Associations of Cytoplasmic restorer and maintainers

Species diversity has been shown to stimulate productivity, stability, ecosystem services, and resilience in natural (Cadotte *et al.*, 2012; Gamfeldt *et al.*, 2013; Zhang *et al.*, 2012; Cabell and Oelofse, 2012) and in agricultural

ecosystems (Kremen and Miles, 2012; Davis *et al.*, 2012; Bonin and Tracy, 2012; Mijatovic *et al.*, 2013). The genetic diversity in these resources allows crops and varieties to adapt to ever-changing conditions and to overcome the constraints caused by pests, diseases and abiotic stresses (Chandirakala *et al.*, 2015). D^2 statistics grouped

Table 1. Geographical and parental diversity among test genotypes

S. No	Genotypes/Source
1	P61R/RHA-61
2	R273/DOR, Hyderabad
3	P93R/GP ₂ -378
4	95C1R/Bangalore
5	P91R/GP ₄ -357
6	P107RP ₁ /OPH
7	P107RP ₂ /OPH
8	P69R/IL-50-1
9	3376R/DOR, Hyderabad
10	P100R/LIPO-8-1
11	P110R/RHA 855
12	P87R/OPH-15-1
13	P89R/GP ₄ -280
14	P75R/OPH-34-1-1
15	P94R/GP ₂ -661
16	P111R/GP2-2861
17	P112R/DRS1-414
18	P115R/GP ₂ -237
19	P119R/1538-1
20	P121R/GP ₆ -35
21	P124R/OPH-29-4-1
22	NDLR2/Nandyal
23	NDLR1/Nandyal
24	44B/—
25	40B/PAU, Ludhiana
26	10B/PAU, Ludhiana
27	234B/Bangalore
28	11B/PAU, Ludhiana
29	304B/Bangalore
30	395B/Perendovic 301
31	7-1-B/Andhra Pradesh
32	45B/PAU, Ludhiana
33	47B/PAU, Ludhiana
34	48B/PAU, Ludhiana
35	49B/PAU, Ludhiana
36	50B/PAU, Ludhiana
37	52B/PAU, Ludhiana
38	53B/PAU, Ludhiana
39	36B/PAU, Ludhiana
40	RCR8297/DOR, Hyderabad
41	RHA297/DOR, Hyderabad

Table 2(a): Analysis of variance under differential water stress

Parameter	Mean sum of squares															
	W ₁				W ₂				W ₃				W ₄			
	Source of variation		Error		Source of variation		Error		Source of variation		Error		Source of variation		Error	
	Rep	Genotype	Error	Rep	Genotype	Error	Rep	Genotype	Error	Rep	Genotype	Error	Rep	Genotype	Error	
	df=2	df=40	df=80	df=2	df=40	df=80	df=2	df=40	df=80	df=2	df=40	df=80	df=2	df=40	df=80	
EV	1.02	1.65**	0.43	0.58	1.35**	0.35	1.93	1.46**	0.39	0.1	1.85**	0.44				
CT	0.97	3.64**	0.98	8.61	3.5**	0.9	1.37	2.65**	0.58	3.87	2.45**	0.55				
PS	0.25	2.75**	0.2	0.35	1.25**	0.02	0.29	1.75**	0.3	0.38	2.23**	0.38				
LAI	0.04	0.26**	0.01	0.01	0.06**	0.01	0.03	0.10**	0.01	0.05	0.15**	0.01				
LWP	0.01	0.14**	0.01	0.49	0.47**	0.18	0.61	0.23**	0.2	0.29	0.15**	0.12				
DF	4.84	17.09**	3.5	25.58	29.34**	5.2	4.3	16.47**	4.67	28.19	48.97**	3.41				
DM	5.51	14.79**	0.93	0.62	9.66**	0.85	2.08	26.18**	1.01	0.03	8.94**	0.85				
PH	16.95	1263.92**	9.39	21.98	1203.6**	11.33	4.77	1016.95**	9.11	20.45	1219.89**	9.23				
HD	0.16	11.00**	0.4	0.2	10.03**	0.27	0.28	8.39**	0.34	0.11	13.64**	0.38				
SY	0.38	287.02**	1.37	1.87	247.46**	2.77	4.83	256.38**	1.75	4.05	246.45**	1.65				
SW	0.08	4.57**	0.1	0.06	4.39**	0.09	0.09	3.34**	0.09	0.15	6.09**	0.09				
OC	4.84	17.09**	3.5	25.58	29.34**	5.2	4.3	16.47**	4.67	28.19	48.97**	3.41				

*, ** - significant at 5 % and 1 % level respectively

W1 – control; W2 – first water stress environment; W3 – second water stress environment; W4 – third water stress environment

EV: Early Vigour ; CT : Canopy Temperature ; PS : Photosynthetic capacity ; LAI : Leaf Area Index ; LWP : Leaf Water Potential ; DF: Days to 50% Flowering; DM: Days to Maturity; PH: Plant Height ; HD : Head Diameter ; SY : Seed Yield per plant ; SW : 100 Seed Weight ; OC : Oil content

Table 2(b). Analysis of variance for various morphophysiological traits pooled over environments

	df	Mean Squares											
		EV	CT	PS	LAI	WP	DF	DM	PH	HD	SY	SW	OC
Replication (in env)	8	2.46**	3.7**	0.07	0.01	0.35*	3.16**	2.06**	16.04*	0.19	2.78*	0.1**	15.73**
Env.	3	8.55**	1422.11**	0.80**	0.57**	0.84**	37.28**	68.11**	2763.73**	47.22**	469.41**	20.41**	243.22**
Genotypes	40	5.35**	3.74**	0.08	0.46**	0.44**	174.7**	40.51**	4432.31**	38.53**	960.95**	15.01**	52.61**
Geno. X env	120	2.66**	2.83**	0.90**	0.03**	1.11**	5.27**	6.35**	90.68**	1.51**	25.45**	1.12**	19.75**
Pooled error	320	0.41	0.76	0.08	0.01	0.2	1.23	0.91	9.77	0.34	1.88	0.09	4.2

Table 3. Morphometric diversity among test genotypes in four treatments

Inbred line	Canopy/Temperature (°C)				Photosynthetic Capacity				Leaf/Area Index				Leaf Water Potential (mpa)			
	W ₁	W ₂	W ₃	W ₄	W ₁	W ₂	W ₃	W ₄	W ₁	W ₂	W ₃	W ₄	W ₁	W ₂	W ₃	W ₄
P-61-R	27.56	28.11	29.28	33.95	0.74	0.66	0.68	0.64	0.5	0.3	0.4	0.4	-2.35	-2.77	-2.96	-2.56
R-273	26.87	29.09	28.58	35.24	0.71	0.62	0.69	0.65	0.5	0.3	0.3	0.5	-2.77	-3.29	-3.17	-2.96
P-93-R	27.01	27.44	29.95	32.65	0.76	0.63	0.68	0.72	0.5	0.3	0.3	0.4	-2.71	-3.96	-3.28	-3.15
95-C-1-R	26.98	27.67	28.62	34.47	0.76	0.71	0.64	0.72	0.4	0.3	0.3	0.4	-2.30	-3.83	-3.32	-2.55
P-91-R	27.74	27.94	29.20	35.43	0.77	0.70	0.70	0.71	0.5	0.2	0.4	0.4	-2.33	-3.21	-3.56	-2.88
P-107-R-P ₂	27.73	28.21	29.55	35.23	0.69	0.64	0.64	0.67	0.6	0.3	0.4	0.5	-2.33	-2.71	-3.25	-2.70
P-107-R-P ₁	26.48	27.40	29.23	34.04	0.70	0.57	0.68	0.62	0.5	0.4	0.4	0.5	-2.26	-2.55	-2.40	-2.50
P-69-R	26.93	28.67	28.33	34.80	0.76	0.73	0.71	0.73	0.5	0.3	0.4	0.4	-2.74	-3.07	-3.13	-2.98
3376-R	26.82	26.97	29.55	35.27	0.73	0.72	0.65	0.71	0.7	0.4	0.3	0.6	-2.86	-2.96	-3.31	-2.64
P-100-R	26.07	28.48	28.87	35.35	0.74	0.69	0.66	0.73	1.2	0.7	0.7	1.1	-2.57	-3.01	-2.92	-2.60
P-110-R	27.05	27.82	29.27	34.10	0.73	0.65	0.63	0.72	1.0	0.4	0.6	0.8	-2.79	-2.86	-2.98	-2.81
P-87-R	25.88	27.53	29.22	35.50	0.75	0.69	0.63	0.67	0.7	0.4	0.4	0.6	-2.59	-2.91	-2.77	-2.82
P-89-R	25.94	28.85	27.72	35.11	0.75	0.69	0.62	0.70	0.5	0.3	0.4	0.5	-2.35	-2.84	-2.98	-2.81
P-75-R	27.52	27.81	29.22	33.50	0.74	0.70	0.67	0.71	0.6	0.3	0.3	0.6	-2.67	-2.99	-2.94	-2.81
P-94-R	27.28	26.80	30.40	34.07	0.74	0.67	0.70	0.73	0.7	0.5	0.5	0.6	-2.74	-3.20	-2.93	-3.64
P-111-R	26.58	27.41	29.02	35.03	0.78	0.67	0.65	0.73	0.6	0.3	0.3	0.6	-2.80	-2.85	-3.21	-3.02
P-112-R	26.90	27.57	28.28	34.20	0.76	0.67	0.68	0.70	1.3	0.4	1.0	1.1	-2.38	-2.76	-2.98	-3.09
P-115-R	26.84	27.86	29.55	34.23	0.74	0.72	0.65	0.68	0.4	0.3	0.2	0.4	-2.26	-3.73	-2.43	-2.78
P-119-R	26.60	28.41	29.20	34.05	0.75	0.67	0.68	0.69	0.5	0.2	0.2	0.3	-2.59	-3.35	-3.34	-3.31
P-121-R	26.23	27.73	28.93	34.05	0.75	0.65	0.62	0.67	0.7	0.3	0.6	0.6	-2.64	-2.73	-3.31	-3.30
P-124-R	26.74	26.06	29.07	33.50	0.69	0.63	0.65	0.66	0.6	0.4	0.3	0.5	-2.71	-3.34	-3.41	-3.44
NDLR-2	26.23	26.82	29.68	34.43	0.73	0.67	0.65	0.69	0.6	0.5	0.4	0.5	-2.13	-3.26	-3.69	-3.45
NDLR-1	26.05	28.31	28.13	33.53	0.69	0.63	0.64	0.67	0.5	0.3	0.3	0.4	-2.76	-3.16	-3.19	-3.25
44-B	26.44	28.24	28.85	34.38	0.75	0.62	0.60	0.69	0.6	0.5	0.5	0.5	-2.53	-2.80	-2.83	-2.68
40-B	26.42	27.22	29.58	34.83	0.77	0.70	0.67	0.71	1.5	0.7	0.6	1.3	-2.53	-3.48	-3.33	-3.50
10-B	26.89	28.09	28.72	34.75	0.74	0.57	0.63	0.65	0.7	0.3	0.5	0.6	-2.67	-3.27	-3.03	-3.48
234-B	25.96	28.62	28.52	33.34	0.72	0.65	0.63	0.66	0.6	0.4	0.4	0.4	-2.33	-2.74	-2.56	-3.01
11-B	26.73	27.73	28.02	33.67	0.77	0.65	0.62	0.72	0.5	0.2	0.2	0.4	-2.68	-3.33	-3.73	-3.44
304-B	27.21	27.66	28.93	34.57	0.76	0.66	0.63	0.71	0.7	0.3	0.5	0.6	-2.83	-2.77	-3.04	-3.01
395-B	26.68	27.83	29.38	33.33	0.76	0.69	0.68	0.74	0.6	0.3	0.4	0.5	-2.72	-3.59	-3.57	-3.44
7-1-B	26.31	27.54	27.88	33.82	0.72	0.60	0.66	0.69	0.4	0.3	0.3	0.4	-2.46	-2.70	-3.21	-3.26
45-B	26.92	29.90	29.18	34.78	0.69	0.59	0.58	0.65	0.4	0.4	0.3	0.3	-2.50	-3.47	-3.34	-2.57

47-B	26.46	27.37	28.28	33.00	0.72	0.69	0.67	0.69	0.5	0.2	0.3	0.4	-2.60	-2.64	-2.56	-3.04
48-B	27.05	27.80	28.45	35.27	0.72	0.65	0.62	0.68	0.4	0.3	0.3	0.4	-2.47	-3.61	-3.72	-3.44
49-B	26.33	29.38	28.30	33.73	0.75	0.62	0.63	0.74	0.7	0.3	0.4	0.5	-2.49	-3.11	-3.28	-2.67
50-B	25.61	27.29	28.20	34.51	0.71	0.65	0.64	0.69	0.7	0.5	0.4	0.6	-2.80	-3.77	-3.55	-2.92
52-B	27.04	27.33	28.73	33.08	0.75	0.62	0.59	0.70	0.6	0.2	0.4	0.5	-2.73	-3.27	-3.13	-3.05
53-B	26.91	27.48	28.57	34.70	0.78	0.68	0.66	0.70	0.4	0.2	0.3	0.4	-2.71	-3.36	-3.26	-2.86
36-B	27.10	28.22	28.07	34.64	0.71	0.61	0.59	0.62	1.2	0.8	0.9	0.9	-2.34	-3.73	-3.41	-3.50
RCR-8297	26.88	27.67	28.53	35.09	0.74	0.69	0.68	0.70	0.5	0.3	0.3	0.4	-2.51	-3.29	-3.34	-3.15
RHA-297	26.74	27.09	27.83	35.03	0.72	0.63	0.65	0.68	1.3	0.7	0.8	1.0	-2.55	-3.39	-3.60	-2.85
Exp Mean	26.72	27.84	28.85	34.35	0.74	0.66	0.65	0.69	0.66	0.37	0.42	0.56	-2.56	-3.16	-3.17	-3.02
P-61-R	63	60	59	63	93	88	85	89	127	116	112	125	11.7	9.6	9.4	10.1
R-273	67	62	63	66	93	90	92	91	127	102	125	120	10.5	9.3	9.3	10.2
P-93-R	68	63	64	68	95	92	90	93	131	118	124	125	16.5	13.0	12.9	16.1
95-C-1-R	65	63	60	64	95	88	89	94	148	132	134	143	10.4	9.7	9.2	8.5
P-91-R	65	62	62	65	95	87	87	93	135	131	126	132	15.4	14.7	14.4	15.1
P-107-R-P ₂	64	62	65	64	99	93	92	96	120	109	115	118	14.4	12.5	12.1	13.5
P-107-R-P ₁	68	63	64	65	92	89	90	91	132	125	121	128	14.5	13.2	13.2	13.7
P-69-R	68	63	59	66	93	91	92	92	98	90	94	98	16.4	14.0	13.4	14.9
3376-R	64	64	63	64	90	86	85	89	130	121	119	128	9.7	8.4	9.1	7.5
P-100-R	63	63	61	62	89	85	86	89	122	115	115	121	12.9	11.5	11.6	9.8
P-110-R	61	61	60	61	94	90	92	91	123	85	110	119	13.0	11.5	10.4	12.5
P-87-R	62	62	59	63	93	89	88	91	140	127	110	132	17.1	13.9	14.1	15.2
P-89-R	64	68	63	65	93	88	87	91	146	131	134	140	14.5	12.9	12.7	14.1
P-75-R	62	60	60	62	94	86	87	92	150	122	119	140	15.5	13.5	13.3	15.1
P-94-R	63	60	59	63	95	90	91	92	128	87	118	123	14.6	10.9	12.9	14.3
P-111-R	81	74	74	79	96	91	93	92	168	144	131	163	16.0	13.7	13.1	14.1
P-112-R	79	76	74	78	94	90	90	92	207	182	192	203	15.3	15.0	13.6	15.7
P-115-R	66	63	64	64	93	92	89	92	124	114	117	121	16.4	12.8	13.5	15.4
P-119-R	62	60	59	60	89	83	85	88	135	125	122	127	14.9	11.7	10.8	13.0
P-121-R	61	61	62	61	94	91	90	92	96	87	84	90	15.4	12.7	13.2	15.0
P-124-R	69	65	67	67	94	88	89	92	110	98	99	105	13.9	13.2	13.0	13.0
NDLR-2	62	61	62	62	93	91	92	92	131	121	125	125	15.5	14.4	13.9	14.2
NDLR-1	76	72	73	75	94	91	92	92	144	126	125	134	15.9	14.4	14.0	14.8
44-B	64	63	62	63	95	92	89	93	142	123	118	138	15.3	13.2	13.2	13.7
40-B	65	63	61	63	90	87	86	89	127	108	108	120	14.5	11.9	11.9	13.3
10-B	67	66	66	66	95	91	88	92	130	98	94	120	13.4	10.8	11.0	11.7
234-B	68	63	65	67	94	88	89	93	130	111	117	126	14.6	12.8	12.7	13.9
11-B	63	60	59	63	95	91	93	92	107	95	94	102	14.3	12.4	10.4	13.4

Inbred line	Seed Yield per plant (g)				100 Achene Weight (g)				Oil Content (%)					
	W ₁	W ₂	W ₃	W ₄	W ₁	W ₂	W ₃	W ₄	W ₁	W ₂	W ₃	W ₄		
	304-B	63	61	60	64	94	92	93	92	81	89	13.1	10.7	10.3
395-B	68	64	66	66	90	85	86	90	107	113	14.9	10.8	11.4	14.2
7-1-B	69	65	66	66	93	90	91	92	123	131	17.9	15.6	15.5	16.3
45-B	67	64	64	65	90	87	89	88	136	156	13.6	11.6	11.6	12.8
47-B	64	59	58	62	92	98	86	91	121	138	15.8	13.9	13.7	15.7
48-B	57	56	57	57	92	91	91	91	105	111	14.6	13.4	12.3	13.7
49-B	62	59	58	60	95	92	92	93	91	99	15.3	12.7	12.3	12.9
50-B	63	60	62	62	90	88	89	88	115	123	16.5	14.6	10.6	15.5
52-B	65	65	62	64	96	93	92	94	115	124	13.8	12.0	12.4	13.3
53-B	67	64	64	65	97	90	91	95	121	131	16.2	14.2	14.5	15.5
36-B	67	66	64	66	94	85	88	91	87	94	16.3	12.3	12.2	15.4
RCR-8297	67	65	66	66	98	93	90	94	123	128	10.0	8.3	8.8	9.3
RHA-297	66	60	62	66	92	89	91	90	110	120	13.1	9.1	10.2	11.5
Exp Mean	65	63	62	64	93	91	92	91	115	124	14.4	12.3	12.1	13.4

Inbred line	Seed Yield per plant (g)				100 Achene Weight (g)				Oil Content (%)			
	W ₁	W ₂	W ₃	W ₄	W ₁	W ₂	W ₃	W ₄	W ₁	W ₂	W ₃	W ₄
P-61-R	26.5	20.5	19.2	21.4	7.7	6.0	5.8	6.2	42.6	38.3	40.7	35.1
R-273	19.5	16.0	12.4	16.2	6.3	4.7	4.7	5.8	45.2	37.3	34.6	31.1
P-93-R	39.3	32.8	32.7	37.0	7.7	5.3	8.0	6.2	42.7	37.3	36.7	42.0
95-C-1-R	10.3	7.2	9.7	10.0	5.0	2.2	4.0	3.2	44.8	38.3	38.1	40.6
P-91-R	23.4	21.3	23.3	21.5	6.5	5.2	5.3	3.6	43.9	38.8	40.5	41.6
P-107-R-P ₂	34.1	26.0	26.6	28.0	7.1	5.6	5.8	6.7	44.1	36.4	36.5	39.9
P-107-R-P ₁	47.1	42.0	38.8	42.4	6.3	5.4	5.1	5.8	40.4	32.9	33.4	36.6
P-69-R	34.1	24.8	29.6	32.5	7.3	5.7	6.0	6.8	44.1	39.0	38.0	41.9
3376-R	20.9	17.1	13.4	17.1	5.2	3.2	4.7	2.7	40.6	35.3	38.1	30.7
P-100-R	26.0	23.6	23.8	25.0	6.8	5.2	5.6	6.2	44.5	37.6	40.7	42.4
P-110-R	26.6	22.1	23.7	26.8	7.1	6.2	5.5	4.4	43.3	38.6	38.4	41.9
P-87-R	54.7	52.2	49.8	51.8	6.4	4.7	5.5	4.4	45.6	38.8	38.8	42.4
P-89-R	44.2	37.1	40.8	31.6	8.4	6.8	6.7	6.8	45.3	41.2	37.7	41.9
P-75-R	46.2	36.5	35.8	36.5	7.4	5.4	5.7	6.1	41.8	40.4	40.4	36.9
P-94-R	34.3	21.0	31.3	33.1	8.3	5.1	7.2	6.7	39.1	35.4	34.9	36.9
P-111-R	46.7	40.3	44.2	45.5	7.6	6.9	6.3	5.0	45.0	39.8	38.3	42.2
P-112-R	35.6	22.1	15.0	30.0	7.2	5.2	4.7	6.2	42.2	36.3	38.2	38.3
P-115-R	39.0	32.6	32.7	36.8	6.9	5.8	6.4	6.7	43.7	40.4	39.6	42.4
P-119-R	39.3	19.7	30.5	37.5	6.4	4.9	4.7	5.7	44.3	36.5	39.3	38.9

P-121-R	46.1	32.7	36.8	41.6	6.5	5.7	5.3	6.1	40.7	35.4	36.3	39.6
P-124-R	37.1	31.9	23.7	34.2	7.8	6.4	6.7	7.0	45.1	40.8	39.4	35.3
NDLR-2	46.4	37.7	27.0	41.0	6.6	6.3	6.2	6.4	43.2	38.7	38.7	40.9
NDLR-1	31.3	21.4	18.2	28.8	7.2	4.1	5.1	6.4	39.7	35.7	35.1	38.3
44-B	33.7	30.4	26.4	27.4	7.1	5.4	6.4	7.0	44.3	36.7	36.9	35.9
40-B	38.4	32.5	33.4	34.3	8.5	5.7	5.7	6.2	44.7	39.5	42.1	39.0
10-B	32.4	23.2	27.6	29.1	6.9	6.7	6.1	6.5	41.6	37.3	35.8	29.6
234-B	34.3	23.4	24.9	30.0	7.2	5.0	6.7	6.6	45.3	38.1	40.8	41.6
11-B	33.5	28.2	29.3	31.2	7.7	6.1	6.4	7.2	43.2	30.9	35.8	29.4
304-B	15.6	12.7	13.2	13.5	7.4	5.9	4.6	5.9	43.8	40.8	40.8	41.9
395-B	30.6	16.7	20.5	26.3	7.1	5.4	4.8	3.9	42.4	40.1	37.8	41.0
7-1-B	28.8	18.7	21.0	23.9	12.8	10.5	9.6	10.9	44.9	37.7	38.5	31.0
45-B	32.9	27.4	24.2	28.6	6.9	6.2	6.1	5.9	41.5	36.9	37.8	40.2
47-B	33.7	28.4	28.0	30.4	7.0	5.2	5.4	6.7	42.6	42.1	31.8	39.0
48-B	33.5	23.9	26.9	31.2	6.3	5.7	5.3	5.3	47.6	41.6	40.4	36.0
49-B	36.3	27.9	27.0	34.2	7.6	5.7	6.2	5.1	42.2	45.0	40.2	38.8
50-B	28.2	23.1	24.5	26.2	6.7	5.8	5.3	5.6	44.0	40.0	38.7	42.7
52-B	29.1	18.5	17.6	25.5	7.3	5.2	6.3	6.9	46.7	40.0	41.8	41.7
53-B	39.4	34.2	35.5	29.0	7.9	6.3	5.8	6.7	34.1	35.5	40.2	32.0
36-B	30.4	23.6	15.5	24.9	7.2	6.4	5.7	4.8	44.3	37.9	42.4	34.1
RCR-8297	14.9	12.5	12.9	12.7	5.1	3.9	4.2	4.0	42.7	49.9	38.4	38.8
RHA-297	15.2	12.8	13.0	13.3	4.9	4.8	4.1	3.5	46.4	39.2	38.4	43.6
Exp Mean	32.9	25.7	25.8	29.2	7.1	5.5	5.7	5.8	43.2	38.5	38.3	38.3

Table 4. Association of inbreds as per genetic closeness and utility under stress

Cluster	Inherent genetic diversity among inbreds	Stress tolerant heterogeneous inbreds
I	27 P61R, R273, P93R, 95C1R, P91R, P107RP ₂ , P69R, 3376R, P110R, P87R, P89R, P75R, P94R, P115R, P119R, P124R, NDLR2, 44B, 234B, 11B, 47B, 48B, 49B, 52B, 36B, RCR8297, RHA297	P93R, P69R, P87R, P115R, NDLR2
II	5 P107RP ₁ , NDLR1, 395B, 45B, 53B	P107RP ₁
III	3 P121R, 10B, 304B	P121R
IV	2 P111R, P112R	P111R
V	3 P100R, 40B, 50B	40B, 50B
VI	1 7-1B	7-1B

Table 5. Association divergence between and among clusters

5a. Inter and intra cluster distance		5b. Variability of morphophysiological parameters (mean value)															
I	II	III	IV	V	VI	EV	CT	PS	LAI	LWP	DF	DM	PH	HD	SY	SW	OC
I	2.77	3.05	3.01	5.19	4.17	5.50	2.30	22.40	0.78	-2.35	63.22	91.19	127.15	11.89	22.40	7.31	43.90
II		2.59	4.18	4.41	5.05	6.16	4.00	21.53	0.65	-2.71	69.23	95.46	146.15	13.00	26.50	10.92	40.80
III			1.96	5.58	5.29	6.13	2.70	18.47	0.81	-2.67	63.45	90.15	128.26	14.20	44.22	6.04	37.60
IV				2.15	7.16	6.97	2.30	19.53	0.77	-2.80	75.89	92.44	207.45	12.63	32.11	7.83	32.90
V					2.18	7.04	3.00	21.57	0.78	-2.83	61.49	98.16	113.19	15.31	26.89	12.80	44.90
VI						0.00	4.30	26.96	0.64	-2.61	66.58	91.58	127.92	16.33	23.10	0.95	38.04

the test genotypes into 6 clusters (**Table 4 & fig. 1**), on the basis of aggregate differences in characters taken, with variable number of entries in each cluster indicating the presence of genetic diversity in the material. Cluster I comprised of maximum number of genotypes (27 genotypes), followed by cluster II (5 genotypes), cluster III and V (3 genotypes in each), cluster IV (2 genotypes), and cluster VI (1 genotypes). Taklewold *et al.*, (2000), Mohan and Seetharam (2005), Parameshwarappa *et al.*, (2009) and Kumari and Singh (2015) also observed similar clustering pattern of genotypes among clusters, as some clusters were unique having only single genotype. The genotypes included in the same cluster are considered genetically similar in respect to the aggregate effect of the characters examined; the hybridization attempted between these is not expected to yield desirable recombinants (Bandila *et al.*, 2011; Zala *et al.*, 2014). Therefore, putative parents for crossing programme should belong to different clusters characterized by large inter-cluster distance. The further choice of genotype should be made considering the mean performance of genotype in respect of various characters.

Morphometric traits in divergence

Among the traits evaluated, leaf area index contributed the maximum (18.54%) towards the observed diversity (**Fig. 2**), followed by early vigour (18.35%), oil content (12.35%), 100 seed weight (10.59), photosynthetic capacity (9.58%), and plant height (9.29%), and leaf water potential (6.16%), achene yield per plant (4.50%), head diameter (4.19%) and canopy temperature (3.16%). Days to 50 per cent flowering and days to maturity contributed very little (1.58 and 0.96 % respectively) towards the divergence. However in previous studies, achene yield per plant (Sasikala, 2000; Loganathan, 2002; Loganathan *et al.*, 2006 and Punitha *et al.*, 2010) plant height, days to 50 percent flowering and days to maturity (Sreedhar *et al.*, 2006; Parameshwarappa *et al.*, 2009) contributed substantially towards genetic divergence.

Association distance of inbreds

Plant genetic resources serves as a source of novel alleles for ongoing plant breeding efforts in a variety of species (Acquaah, 2006; Mandel *et al.*, 2011). Unlocking the full potential of crop germplasm collections, however, requires an

Table 6. Promising genotypes for various levels of water stress

Stress Levels	Stress Resistant Genotypes	
	<i>Cytoplasmic Male Sterile/maintainer line</i>	<i>Restorer Line</i>
At normal rainfall	304 A/B	P87R, P89R, P107RP2, P61R, P69R
Stress at button and soft dough stage	234 A/B, 40A/B	P75R, P107RP2, P93R
Stress at flowering and hard dough stage	11A/B	P89R, 3376R, P91R, P94R
Stress at anthesis completion stage	40A/B, 304A/B	P69R, P93R

understanding of the amount and distribution of genetic variation contained within them. To this end, we analyzed the association nature of inbreds with respect to their genetic closeness (Clustering). The intra cluster distances ranged from 0 (cluster VI) to 2.77 (cluster I) indicating that the single genotype in cluster VI whereas, genotypes in cluster I were more dissimilar in morphological features and performance than other clusters (**Table 5a**). The members of cluster IV and V exhibited maximum divergence (inter-cluster distance 7.16) followed by the members of cluster V and VI (inter-cluster distance 7.04), cluster IV and VI (inter-cluster distance 6.97), cluster II and VI (inter-

cluster distance 6.16), cluster III and VI (inter-cluster distance 6.13), cluster III and IV (inter-cluster distance 5.58) and cluster III and V (inter-cluster distance 5.29). The members of cluster I and III were least divergent (inter-cluster distance 3.01). The inter-cluster distances were larger than the intra-cluster distances indicating wider genetic diversity between genotypes of the clusters with respect to the traits considered. Therefore, combination with high heterotic response and superior combination may be obtained through hybridization between genotypes across the clusters (Subrahmanyam *et al.*, 2003; Amorim *et al.*, 2007). Gohil and Pandya (2006) have also

pointed out in *Salicornia brachiata* Roxb (a nontraditional Oilseeds) that selection of parents for hybridization should be done from two clusters having wider inter-cluster distance to get maximum variability.

Phenotypic plasticity

Each cluster has its own uniqueness that separated it from other clusters (Table 5b). For example, Cluster I with the largest number of lines was characterized by the lowest mean value for early vigour, seed yield per plant (g), leaf water potential and leaf area index and highest mean value for oil content and leaf water potential. The lowest average for days to maturity and 100 seed weight and highest average for seed yield per plant and photosynthetic capacity among six clusters were characterized by cluster III. Cluster IV included the two genotypes viz. P111R and P112R, which was identical in performance to P107RP₁ of cluster

II and P107RP₁ of cluster I respectively. However it was distinct for high mean days to 50 percent flowering, plant height and leaf area index. Cluster V harbored three genotypes (P100R, 40B and 50B) with highest number of days to maturity and 100 seed weight. The lowest number of days to flowering, plant height and leaf water potential was also recorded in this cluster. Cluster VI had only one genotype (71B) characterized by highest mean value for early vigour, canopy temperature and head diameter and high mean value for most of the characters. Therefore rather than selecting lines from the cluster which have high inter cluster distance for hybridization, parents should be selected based on the extent of divergence in respect to a character of interest i.e. if breeders intention is to improve achene yield, he should go for selecting parents which are highly divergent with respect to this trait (Parameshwarappa *et al.*,

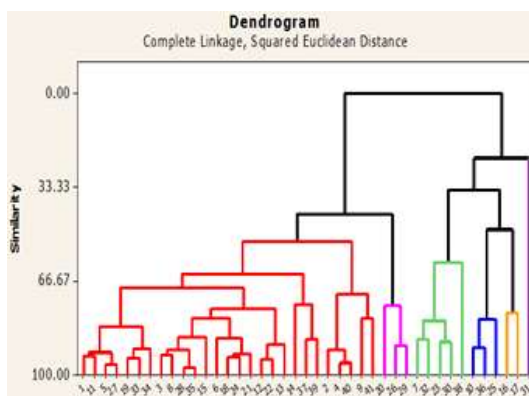


Fig. 1. Genetic distance of genotypes

2009). Cluster mean analysis indicated the extent of genetic diversity among different clusters and that is of real value in plant breeding (Arshad *et al.*, 2007; Camarano *et al.*, 2010). The genotypes grouped into same cluster displayed the lowest degree of divergence from one another and in case crosses are made between genotypes belonging to the same cluster, no transgressive segregants are expected from such combinations (Tripathi *et al.*, 2013). Therefore, hybridization programmes should always be formulated in such a way that the parents belonging to different clusters with maximum divergence could be utilized to get desirable transgressive segregants (Shekhawat *et*

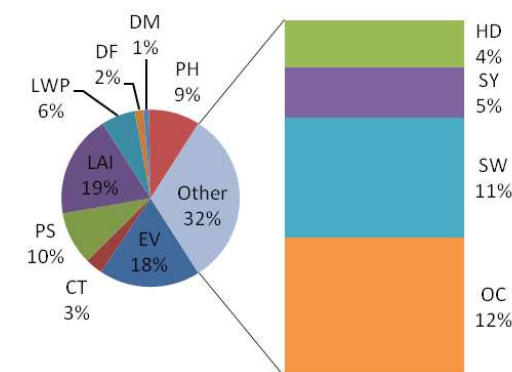


Fig. 2. Contribution of parameters towards total divergence

al., 2014). The genotypes with high values of any cluster can be used either for direct adoption or for hybridization, followed by selection.

Validation of heterogeneous populations under water stress

As most of the cultivated hybrids evolved under optimum conditions, breeding for drought tolerance is required. This indeed would depend on the presence of diverse germplasm so that potential sources of drought tolerance might be identified and subsequently used to assure high yield. However, high yield and drought tolerance are two different mechanisms that are often found to oppose each other. Traits, such as small plant

size, reduced leaf area, and prolonged stomatal closure, limits the water losses, but also leads to reduced dry matter production and, therefore, reduced final yield. To this end, the heterogeneous population so obtained were subjected to differential levels of water stress to find out whether this heterogeneity is practically applicable to water stress ecology or not. Among observations recorded, optimum plant height and crop duration, higher leaf area index and water potential and lower canopy temperature were found to be critical selection criteria. The results were so surprising that of forty one inbred only eleven were found to be suitable to water stress. Genotypes P69R, P87R, P93R, P115R, NDLR2 (Cluster I), P107RP₁ (Cluster II), P121R (Cluster III), P111R (Cluster IV), 40B and 50B (Cluster V) and 7-1B (Cluster VI) identified as stress tolerant genotypes (**Table 6**). Hence it is suggested crosses should be attempted among these cytoplasmic and restorers to when drought is expected to occur at respective growth stages. It is also concluded that merely presence of genetic variation is not going to serve in present scenario of challenging food security; researchers need to validate the utility of divergence for stress environments particularly water stress.

Agriculture and climate change are inextricably linked – crop yield, biodiversity and water use as well as soil health are directly affected by changing climate. Development of resilient crop varieties that tolerate temperature and precipitation stress will greatly rely upon crop genetic resource and available heterogeneity among them. Moreover this heterogeneity required subjection to periods of water shortage to evaluate their stress applicability. Our study concludes significance of genetic divergence towards climate change and methodology to validate divergence for water stress. This will be useful for implication of genetic resource towards climate resilient crop breeding.

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