# Vikrant Tyagi\* and S. K. Dhillon Performance and Water-Use Efficiency of Wild Cytoplasmic Sources in Sunflower

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**Abstract:** Wild *Helianthus* species should have be a main interest sunflower breeding for the introgressing elite genes for biotic and abiotic stress. The future use of wild sunflower in development of drought resistance cytoplasmic sources should be expanded to the wide range of wild species as compared to conventional sources. The objective of this study was to evaluate different parental lines for their water-use efficiency and seed yield performance under two environments for two years. ARG-2A received from *H. argophyllus*, 234A and 38A from *H. petiolaris* were found to be the most susceptible genotypes, whereas, 42A, 42B from *H. petiolaris* and ARG-6A (*H. argophyllus*) were found to be tolerant to drought stress. CMS-XA, RCR-8297, E002-91A, NC-41B and P124R were observed to be well adapted to stress environments. ARG-3A from *H. argophyllus* had a stable performance over the environments, which may be useful to develop water use efficient hybrids having diversified cytoplasmic backgrounds in sunflower breeding.

**Keywords:** Cytoplamic male sterile lines, drought stress, sunflower, stress tolerance index and wild sources

# Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oil crops due to its high content of unsaturated fatty acids and a lack of cholesterol. It is the second largest hybrid crop, second only to maize, and the fifth largest oilseed crop, after soybean, rapeseed, cottonseed and groundnut. A major breakthrough in sunflower history was the discovery of cytoplasmic male sterility (CMS) in a wild sunflower, *Helianthus petiolaris* Nutt. (Leclercq, 1969), and restorer genes with recessive branching found in wild *H. annuus* populations (Kinman, 1970) and *H. petiolaris* (Leclercq, 1971) that led to the production of commercial sunflower hybrids.

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Hybrids had higher seed yield, high oil content and quality, uniformity and enhanced resistance to biotic and abiotic stresses compared to open-pollinated varieties. In general, most cytoplasm of wild annual *Helianthus* species can accommodate cultivated nuclear genes without significant unfavorable interactions and are potential sources of cytoplasmic diversity for sunflower breeding (Seiler et al., 2017). Cytoplasmic and nuclear genome interaction had a negative effects on chlorophyll, photosynthetic rate (Jan, 1990) and positive effects on oil content and quality (Serieys, 1992; Tyagi and Dhillon, 2014). The genus Helianthus is composed of 52 species and 19 subspecies, with 14 annual and 38 perennial (Schilling and Heiser, 1981; Schilling, 1993). The cultivated species is diploid (2n = 2x = 34; Schilling and Heiser, 1981), as well as all annual wild species. Tetraploid (2n = 4x = 68) and hexaploid (2n = 6x = 102) species are generally perennials. Abundant genetic variation exists in the sunflower crop wild relatives with details of the related species as potential sources of genetic improvement of sunflower reported by Warburton et al. (2017). Discovery of cytoplasmic male sterility (CMS) source and restorer genes from *H. petiolaris* paved the way for the development of single-cross hybrids, but also resulted in a genetic bottleneck in cultivated germplasm, by incorporating the same source of male sterility and restorer genes. Diversifying the sources of male sterility is one objective of modern sunflower breeders. Water availability for agriculture is becoming limited, and climate-change scenarios predict increased aridity in certain regions. Development of new hybrids and cultivars with more efficient water use and greater drought resistance is considered a sustainable and economically viable approach (Condon et al., 2004). Nine CMS analogues were developed from different wild sunflower spp. and evaluated under normal irrigation (Tyagi et al., 2013; 2015a; Tyagi and Dhillon, 2015), as well as under water stress conditions (Tyagi et al., 2015b; Tyagi and Dhillon, 2016a) at Punjab Agricultural University, Ludhiana. These sources were used for a hybrid breeding program to develop sunflower hybrids and study the effects of different wild sources on combining ability for seed yield and component traits under different irrigation environments (Dhillon and Tyagi, 2016; Tyagi and Dhillon, 2016b; Tyagi and Dhillon, 2017). The objectives of present research was to study the different drought stress indices, to help breeders design efficient breeding strategies to improve seed yield and under varied moisture environments and diversification of *cms* sources for modern sunflower hybrid breeding.

### Materials and methods

This study was carried out at the Department of Plant Breeding and Genetics, College of Agriculture, Punjab Agricultural University, Ludhiana, India.

Ludhiana represents the Indo-Gangetic plains and is situated at 36° – 54′ N latitude, 25° – 48'E longitude with a mean elevation of 247 meters above sea level. The 19 parental lines were composed of nine alloplasmic lines, four euplasmic lines, two maintainer lines and four restorer lines (Table 1) field evaluated for seed yield at two different environments (normal irrigated and water stress). Water stress was created by stopping water after anthesis for two seasons, spring of 2011 and 2012. A randomized complete block design with 3 replications was used at each location. The plot consisted of six rows 3 meter long with row to row and plant to plant distances of 0.60 m and 0.30 m, respectively. Sowing was done by dibbling three seeds per hill to ensure uniform stand which was later thinned to one plant per hill at the  $V_2$ stage (Schneiter and Miller, 1981). Seed yield data was recorded from five randomly selected plants in each replication for both environments over the years separately. The mean data was used to calculate the different stress indices for each genotype using the corresponding normal irrigated and water stressed plots in each block. Stress-tolerance indices were calculated using the following relationships (Table 2).

S. No.	Parental line designation	Genotype Type	Origin		
1	ARG-2A	CMS Line	Helianthus argophyllus		
2	ARG-3A	CMS Line	H. argophyllus		
3	ARG-6A	CMS Line	H. argophyllus		
4	PKU-2A	CMS Line	H. annuus		
5	E002-91A	CMS Line	H. annuus		
6	PRUN-29A	CMS Line	H. praecox ssp. runyonii		
7	DV-10A	CMS Line	H. debilis ssp. vestitus		
8	PHIR-27A	CMS Line	H. praecox ssp. hirtus		
9	CMS-XA	CMS Line	Unknown		
10	NC-41B (C)	Maintainer line of All 9 cms	H. petiolaris (conventional)		
11	40A	CMS Line	H. petiolaris		
12	42A	CMS Line	H. petiolaris		
13	42B	Maintainer line of 42A cms	H. petiolaris		
14	234A	CMS Line	H. petiolaris		
15	38A	CMS Line	H. petiolaris		
16	RCR-8297	Restorer lines	H. petiolaris		
17	P69R	Restorer lines	H. petiolaris		
18	P124R	Restorer lines	H. petiolaris		
19	P100R	Restorer lines	H. petiolaris		

Table 1: Sunflower Parental lines used in the current study.

#### Statistical analysis

The data obtained from a randomized complete block design was analyzed. Stress tolerance indices were calculated using the equations cited in Table 2. Also, correlations between grain yield and stress tolerance indices, principle component analysis (PCA), biplot display and cluster analysis were determined using SAS (SAS Institute, 1999).

S. No.	Drought tolerance indices	Formula	Reference
1	Drought susceptibility index	$DSI = \frac{1 - \left(\frac{Y_{S}}{Y_{P}}\right)}{1 - \left(\frac{\bar{Y}_{S}}{Y_{P}}\right)}$	Fischer and Maurer (1978)
2	Geometric mean productivity	$GMP = \sqrt{(Y_S)(Y_P)}$	Fernandez (1992)
3	Mean productivity	$MP = \frac{Y_S + Y_P}{2}$	Rosielle and Hamblin (1981)
4	Harmonic mean	$HM = \frac{2(Y_P, Y_S)}{Y_P + Y_S}$	Jafari <i>et al</i> . (2009)
5	Tolerance index	$TOL = Y_P - Y_S$	Rosielle and Hamblin (1981)
6	Stress tolerance index	$STI = \frac{(Y_S)(Y_P)}{(\bar{Y}_P)^2}$	Fernandez (1992)
7	Yield index	$YI = \frac{Y_S}{Y_S}$	Gavuzzi <i>et al</i> . (1997)
8	Yield stability index	$YSI = \frac{Y_S}{Y_P}$	Bouslama and Schapaugh (1984)

 Table 2: Drought tolerance indices used for evaluation of sunflower parental lines.

 $Y_S$  and  $Y_P$  are stress and normal irrigated environment yield of a given lines.  $\overline{Y}_S$  and  $\overline{Y}_P$  are average yield of all hybrids under stress and normal.

## **Results and discussion**

There were significant differences in yield between parental line under normal irrigated (yp) and water stressed (ys) conditions. Significant differences between sources were observed for all drought tolerance indices. The highest yield values were obtained in CMS-XA (37.48 g) followed by PHIR-27A (34.03), ARG-3A (32.17), E002-91A (32.07), RCR-8297 (30.98) and PRUN-29A (30.42) under stressed environments, and in 234A (46.43 g) followed by ARG-3A (41.42 g), DV-10A (36,38 g), ARG-2A (35.92 g) and 38A (35.05 g) under non-stressed conditions (Table 3). The lowest yield value was observed in 38A (10.00 g) followed by P69R (13.67 g) and ARG-2A (17.47 g) under stressed conditions, and CMS-XA (12.84 g), RCR-8297 (14.33 g), PKU-2A (16.33 g) and P124R (16.81 g) under non-stressed conditions. The inbred lines ARG-2A (2.51), 234A (2.72) and 38A (3.50)

S. No.	Genotypes	Үр	Ys	DSI	GMP	MP	нм	TOL	STI	YI	YSI
1	ARG-2A	35.92	17.47	2.51	25.05	26.7	23.51	18.45	0.29	0.47	0.49
2	ARG-3A	41.42	32.17	1.09	36.5	36.8	36.21	9.25	0.62	0.87	0.78
3	ARG-6A	25.92	24.2	0.32	25.05	25.06	25.03	1.72	0.29	0.65	0.93
4	PKU-2A	16.33	20.8	-1.34	18.43	18.57	18.3	-4.47	0.16	0.56	1.27
5	E002-91A	20.29	32.07	-2.84	25.51	26.18	24.85	-11.78	0.3	0.87	1.58
6	PRUN-29A	24.65	30.42	-1.15	27.38	27.54	27.23	-5.77	0.35	0.82	1.23
7	DV-10A	36.38	22.75	1.83	28.77	29.57	27.99	13.63	0.38	0.61	0.63
8	PHIR-27A	28.42	34.03	-0.97	31.1	31.23	30.97	-5.61	0.45	0.92	1.2
9	CMS-XA	12.85	37.48	-9.38	21.95	25.17	19.14	-24.63	0.22	1.01	2.92
10	NC-41B (C)	15.08	23.53	-2.74	18.84	19.31	18.38	-8.45	0.16	0.64	1.56
11	40A	27.57	18.58	1.6	22.63	23.08	22.2	8.99	0.24	0.5	0.67
12	42A	28.13	26.42	0.3	27.26	27.28	27.25	1.71	0.34	0.71	0.94
13	42B	27.33	28.37	-0.19	27.85	27.85	27.84	-1.04	0.36	0.77	1.04
14	234A	46.43	20.62	2.72	30.94	33.53	28.56	25.81	0.44	0.56	0.44
15	38A	35.05	10.00	3.5	18.72	22.53	15.56	25.05	0.16	0.27	0.29
16	RCR-8297	14.33	30.98	-5.69	21.07	22.66	19.6	-16.65	0.21	0.84	2.16
17	P69R	18.28	13.67	1.23	15.81	15.98	15.64	4.61	0.12	0.37	0.75
18	P124R	16.81	25.87	-2.64	20.85	21.34	20.38	-9.06	0.2	0.7	1.54
19	P100R	20.68	26.95	-1.48	23.61	23.82	23.4	-6.27	0.26	0.73	1.3

 Table 3: Mean seed yield under normal and stress environment and drought tolerance indices values of sunflower parental lines.

had the highest stress susceptibility index (SSI) values and were found to be the most susceptible genotypes, whereas inbred lines 42B (-0.19), 42A (0.30) and ARG-6A (0.32) had low values (near to zero) and were tolerant to drought stress. The inbred lines CMS-XA (-9.38), RCR-8297 (-5.69), E002-91A (-2.84), NC-41B (-2.74) and P124R (-2.64) with negative SSI values were observed to be well adapted (better performers under stress) to stress environments. The lower the numerical number of the SSI indicates the less stress susceptibility and more water stress tolerance the genotype. Stress tolerance index, considering (TOL index), a genotype would be more tolerant if it has lower TOL values. Based on TOL, the inbred lines ARG-6A (1.72) and 42A (1.71) with low values were considered as tolerant genotypes, whereas the inbred lines 234A (25.81), 38A (25.05) and ARG-2A (18.45) with the high TOL values were considered as susceptible. The inbred lines CMS-XA (-24.63), RCR-8297 (-16.65) and E002-91A (-11.78) with negative TOL values were considered good performing inbreds under stress. Yield stability index (YSI) was also calculated for a given inbred lines using grain yield under stressed and non-stressed conditions. The genotypes with high YSI are expected to have high yield under stressed and low yield under nonstressed conditions. The lowest YSI was observed for 38A (0.29), 234A (0.44) and ARG-2A (0.49) while the highest YSI was observed for CMS-XA (2.92), RCR-8297 (2.16) and E002-91A (1.58) inbred lines (Table 3). Drought tolerance indices appear to be useful for selection of drought tolerant sunflower genotypes with high yield performance under both water stressed and non-stressed states.

#### Correlation between grain yield and drought tolerance indices

A correlation coefficient matrix (Table 4) revealed that TOL, MP, GMP, STI and YI indices could effectively be used for screening drought-resistant sunflower cultivars. Correlation coefficients were used to identify the best criterion for selecting drought tolerant genotypes. Stress indices and seed yield were highly significantly and positively associated with DSI (0.774\*\*), GMP (0.691\*\*), MP (0.746\*\*), HM  $(0.621^{**})$ , TOL  $(0.863^{**})$  and STI  $(0.697^{**})$ , while a highly significant negative correlation was observed with YSI (-0.773\*\*) under the normal environment. But, under a stress environment, significant positive correlations were observed between seed yield and GMP (0.470\*), HM (0.484\*), and highly significant positive with YI (1.000\*\*) and YSI (0.712\*\*), while highly significant negative association with DSI  $(-0.712^{**})$  and TI  $(-0.728^{**})$ . All the indices were positive and negative relative to each other. According to the literature, a suitable index must have a significant relationship with yield in both stressed and non-stressed states. Indices DSI, TOL, STI and YI were highly and significantly correlated with each other, as well as with seed yield under stress and normal environment. There were significant positive correlations between Yp and stress-tolerance indices (DSI, GMP, MP, HM, TOL and STI). Likewise, there were positive significant correlations between Ys and the stress-tolerance indices. The positive correlations among STI, GMP and MP also exhibited the same trends. The study revealed that STI, GMP, MP and HM were better predictors of Yp and Ys than TOL and SSI. Our

	Үр	Ys	DSI	GMP	MP	нм	TOL	STI	YI	YSI
Yp										
Ys	-0.281									
DSI	0.774**	-0.712**								
GMP	0.691**	0.470*	0.25							
MP	0.746**	0.430	0.234	0.975**						
HM	0.621**	0.484*	0.255	0.984**	0.920**					
TOL	0.863**	-0.728**	0.929**	0.246	0.306	0.189				
STI	0.697**	0.444	0.271	0.991**	0.964**	0.978**	0.264			
YI	-0.282	1.000**	-0.713**	0.468*	0.428	0.482*	-0.729**	0.443		
YSI	-0.773**	0.712**	-1.000**	-0.249	-0.234	-0.255	-0.928**	-0.271	0.713**	

**Table 4:** Association between different drought tolerance indices and mean yield of sunflower parental lines under normal and stress environment.

\*, \*\* significant at 5 and 1% probability level, respectively.

results are consistent with those reported by Fernandez (1992) in mungbean; Shirinzadeh *et al.* (2010) in corn; Mahajan *et al.* (2012) in Rice; Darvishzadeh *et al.* (2010) and Safavi *et al.* (2015) in Sunflower. The present study confirmed that stress tolerance index (STI), mean productivity (MP), Geometric mean productivity (GMP), harmonic mean (HM) and yield index (YI) exhibited a high correlation with seed yield in either environment. These indices were recognized as the best for selecting cultivars with high yield potential in either of the stress or non-stress environments.

#### Multivariate analysis

Principal Component Analysis (PCA) is one way to compress data sets of high dimensional vectors into lower dimensional ones. PCA revealed that the first PC (PCA1) explained 52.76 % of the total data variation and was positively correlated with performance under both stressed and non-stressed environments (Table 5). Thus, the first dimension represents yield potential and drought tolerance. In other words, this component was able to separate the cultivars with higher yield under both stressed and non-stressed conditions. The second PC (PCA2), which explained 44.60 % of total data variation, had a positive and high correlation with SSI but a negative correlation with yield under stressed conditions (Table 5). Therefore, the stress susceptibility dimension was able to separate the drought-

S. No.	Parameters	Principal component 1	Principal component 2
1	Eigen value	5.27	4.46
2	Percentage of variance	52.76	44.60
3	Cumulative percentage	52.76	97.36
4	Үр	0.41	-0.07
5	Ys	-0.05	0.46
6	DSI	0.32	-0.30
7	GMP	0.35	0.26
8	MP	0.35	0.25
9	HM	0.34	0.27
10	TOL	0.32	-0.30
11	STI	0.36	0.25
12	YI	-0.05	0.46
13	YSI	-0.32	0.30

**Table 5:** Eigen value and vectors of principal component analysis for potential yield (Yp), stress yield (Ys) and drought tolerance indices.

Yp: yield in normal environment, Ys: yield in stress environment and drought stress indicators.

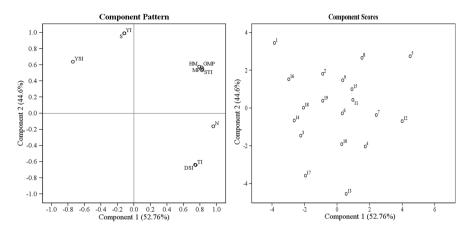
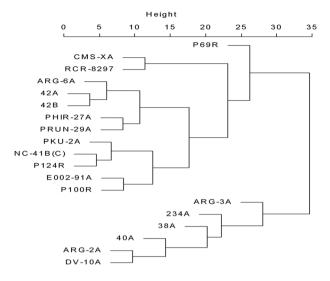


Figure 1: Screening drought tolerance indicators using biplot analysis (a and b).

susceptible genotypes. Hence, selection of genotypes that have high PCA1 and low PCA2 are suitable for both stressed and non-stressed conditions. Khodarahmpour *et al.* (2011) revealed that genotypes with larger PCA1 and lower PCA2 scores gave high yields (stable genotypes), and genotypes with lower PCA1 and larger PCA2 scores had low yields (unstable genotypes). The use of a biplot show in selecting drought-tolerant genotypes has already been used by Khodarahmpour *et al.* (2011) in maize and Mohammadi *et al.* (2010) in wheat.

Biplot indicated that the genotypes E002-91A, PRUN-29A, PHIR-27A and CMS-XA are very close in the vicinity of the vectors of drought tolerance indices, namely GMP, STI, MP and HM very close. These genotypes had the highest seed yield in drought stress conditions, and the highest yield stress of genotypes CMS-XA, respectively. On the other hand, there was genetic variability among the parental lines based on their drought resistance indices. Using important resistance indices comprising MP, GMP, TOL, YI and STI, genotypes were clustered using the unweighted pair group method with arithmetic mean classification and four clusters were established that paralleled the results of the biplot analysis (Figure 1a to 1b). It might not be possible to introduce a genotype which has high seed yield into a non-stress environment while maintaining high tolerance to drought stress. However, it is possible to introduce relatively ideal genotypes using these criteria. Following this line of thought, cluster analysis grouped the sunflower cultivars into groups based on their important resistance indices comprising MP, GMP, TOL, YI and STI (Figure 2).

Dendrogram of genotypes based on ranks of drought resistance indices and seed yield of stress and non-stress conditions according to a hierarchical



**Figure 2:** Dendrogram from cluster analysis based on drought tolerance indices and seed yield of sunflower parental lines in both normal and stress environment.

clustering method (Figure 2), grouped the 19 sunflower parental lines within 4 clusters, each of which having 12, 5, 1 and 1 of inbred lines, respectively. Group I was comprised of genotypes that had low yield in normal state and had high seed yield under stress environments. Hence, genotypes in to these groups could be stable in stressed state and considered as group C (Table 3). Group II comprised genotypes that had low yield in stressed states. Hence, genotypes in to these groups could be stable in non-stressed state and considered as group B. Clustering results revealed that the Group IV genotype P69R locate in group D (low Ys and Yp) among all studied genotypes. Group III was included genotype ARG-3A from *H. argophyllus* that had highest value of STI, HM and GMP indices accompany by higher seed yield under both the environments respectively (Table 2) and located in Group A of Fernandez's (1992). The classification based on cluster analysis and the similarity with biplot analysis was consistent with the findings of Darvishzadeh *et al.* (2010).

## Conclusion

Conventional cms source *H. petiolaris* (PET-1) of sunflower has a narrow genetic background and is deficient in genes for water use efficiency and other abiotic stress. The diversity of wild sunflower species should be utilized to develop new

elite cms sources, which offers breeders a diverse cytoplasmic gene pool from which to discover unique genes for resistance and tolerance to drought stress and water use efficiency. It can be further use in hybrid breeding programmes to develop a high yielding water use efficient hybrids.

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