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EXAMINATION OF ROOT CHARACTERS, ISOTOPE DISCRIMINATION, PHYSIOLOGICAL AND MORPHOLOGICAL TRAITS AND THEIR RELATIONSHIP USED TO IDENTIFY THE DROUGHT TOLERANT SUNFLOWER (*Helianthus annuus* L.) GENOTYPES

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SUMMARY

In order to study the drought tolerance in sunflower an experiment was conducted with 65 sunflower entries including *cms*-lines, R-lines, inbred, hybrids and varieties grown in temporary cement root structures. During the active vegetative growth, several physiological parameters were recorded. As flowering plants were harvested, the observations on root traits were recorded. Results showed a wide and significant genetic variability for leaf area, specific leaf area, plant height, chlorophyll content, root length, root dry weight, root volume and total dry matter (TDM). $\Delta^{13}C$, a surrogate approach to quantify water use efficiency also showed a significant variability. Since our major objective was to look for genetic variability for drought tolerant traits and to select lines with superior drought tolerant lines, the entries were grouped into high and low root, TDM and $\Delta^{13}C$ types. Promising sunflower entries were selected based on high TDM with better root system and low $\Delta^{13}C$ to be used for heterosis breeding to develop drought tolerant hybrids. In all screened entries, it was examined how the TDM and its components are related to each other by correlating many growth parameters with TDM. The results revealed that a positive and significant relationship between total leaf area and TDM was found. Similarly root dry weight also showed a significant positive relationship with TDM. However, $\Delta^{13}C$ values neither related to TDM nor to root dry weight.

Key words: **drought tolerance, isotope discrimination, root traits, sunflower, water use efficiency**

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INTRODUCTION

Sunflower is one of the major oil crops in the world. The relative thermo and photo-insensitivity of sunflower allows its cultivation throughout the year in different seasons. From the agro-eco analysis of growing seasons, it is evident that drought stress is an important factor limiting the productivity in the major sunflower growing states. In addition, certain biotic stresses, especially diseases, cause significant recurring yield losses. Some of these diseases have remained the major issues for many years demanding close attention. Sunflower production in India continues to face challenges, since the crops are often grown on marginal land which is inherently deficient in moisture and nutrients (Connor and Sadras, 1992).

Among abiotic stresses, water stress is one of the major limitations to crop yields worldwide and possible global climate change scenarios suggest a future increase in the risk of drought. Drought alone reduces 15-50% of the yield. Hence, it is necessary to protect the yield loss caused by drought, in order to realize the full potential. Breeding crop varieties for improved water use efficiency is, therefore, of great importance (Condon *et al.*, 2004).

Genetic improvements through enhanced drought tolerance, which are believed to be very rewarding, have not received the significant attention of crop breeders. This is mainly due to the physiological complexities involved in the development of adequate screening techniques to precisely document the available variability. Some selection gains have been reported, but these have mostly been due to empirical screening using absolute yield under stress, in comparison to that obtained under conditions as a selection criterion. These yield gains possibly resulted from indirect selection for traits conferring some adaptive advantage under water stress conditions. Evolving varieties/hybrids with a high level of drought tolerance is the effective and cheapest strategy to overcome bad effects.

Variability for drought and related traits was first demonstrated and documented by Fereres *et al.* (1986) and it is evident from the earlier reports that enormous existence of variability among crop plants can be exploited. The success rate of the breeding entirely depends on the efficient screening technique for the desirable trait (Udaya Kumar *et al.*, 1998).

Enhanced drought tolerance is achieved by incorporating some traits that help the plant to cope with drought stress effectively (Chaves *et al.,* 1991). The application of Water Use Efficiency (WUE) as an important screening technique has helped in finding out the significant variation among the genotypes (Condon *et al.,* 2004). With this background, a research was carried out for screening the sunflower genotypes for better root system and high water use efficiency.

MATERIALS AND METHODS

To study the root traits, it is extremely difficult or sometimes impossible to extract the complete roots from the land grown plants and hence the plants were grown in root structures. Temporary root structures of the dimension of 4 ft height, 10 ft wide and 60 ft long were built using cement blocks on the ground surface and they were completely filled with red soil up to a height of 3.5 ft and compacted to imitate the natural conditions that prevail in the actual field. Required amounts of farm yard manure and chemical fertilizers were applied and seeds were sown according to the recommendations from the package. Like in the field condition, watering was done immediately after sowing and once the seedlings have emerged, thinning was carried out and the plant populations maintained, as recommended. As and when required, watering was done and after 30 days of sowing half the dosage of nitrogen was given. At regular intervals of time during the experimental period, several physiological parameters were studied. These parameters include measurement of total leaf area, specific leaf area (which is an indication of leaf thickness) and SCMR (SPAD chlorophyll meter reading, which is an indication of leaf chlorophyll and in turn the leaf nitrogen status).

When the plants were in flowering stage, the walls of temporary root structures were dismantled and with a high water pressure, the soil surrounding the root system of each of the plants was washed and the plants with entire root system were taken out. Once the plants were harvested from the root structures, roots, leaves and stem were separated and measured separately. Accordingly, several root traits, like root length (cm/pl), root volume (cc/pl) *etc.*, were measured in the roots. Similarly, all the leaves were harvested from the plant and the total leaf area (cm^2/pl) was measured using leaf area meter. Once the primary observations were made, all plant parts (root, stem, leaves and flower heads) were put in an oven to be completely dried. Having done that, the dry weight of individual plant parts, such as root dry weight (g/pl), stem dry weight (g/pl) including the weight of flower head and leaf dry weight (g/pl) were measured and finally included in the total dry matter (g/pl) of an individual plant.

A small quantity of dried leaf sample which was ground into a fine powder which was used for $\Delta^{13}C$ analyses using Isotope Ratio Mass Spectrometer (IRMS). Since the main objective of the study was to look for genetic variability for drought tolerant traits and to select lines with superior drought tolerance traits, the entries were grouped into high and low root types, high TDM & low TDM types and high & low $\Delta^{13}C$.

RESULTS AND DISCUSSION

The results of the experiment indicated a significant and wide genetic variability for several traits. The total leaf area (cm^2/pl) ranged from 862.96 cm^2/pl (RHA-418) to 6070.62 cm^2/pl (R-45) with a mean value of 2482.60 in R-lines. *Cms*-lines showed more leaf area and their range was from 1999.08-7266.85 cm^2/pl (*cms*-103A) to 7266.85cm^2/pl (ARM-246-A) with a mean value of 3787.31. The minimum leaf area was bigger in inbreds *i.e.*, 3013.14 cm^2/pl and its maximum was 5976.01 with a mean of 4224.37 (Table 1) whereas, it was 11064.96 cm^2/pl in check, hybrid KBSH-44.

Table 1: Genetic variability for physiological traits in *cms*, R-lines & inbreds of sunflower

Traits	R-lines		*Cms*-lines		Inbreds	
	Range	Mean	Range	Mean	Range	Mean
No. of leaves/plant	13.50-26.00	19.62	16.67-28.33	22.11	18.50-26.83	21.66
Plant height (cm/pl)	55.50-142.50	94.07	27.50-184.00	104.62	103.58-164.33	134.70
Leaf area (cm^2/pl)	862-6070.62	2482.60	1999.08-7266.85	3787.31	3016.14-5976.01	4224.37
SLA (cm^2/g)	30.19-132.25	70.36	31.53-195.50	81.62	39.64-195.23	102.97
Root volume (cc)	33.33-77.08	54.06	18.75-83.33	51.94	45.83-75.00	60.63
Root length (cm/pl)	20.25-51.00	31.17	21.08-46.00	32.74	23.25-43.00	35.84
Root dry weight (g/pl)	1.26-33.03	13.24	3.46-72.58	22.45	13.86-46.54	22.72
Total dry matter (g/pl)	36.46-150.40	71.76	47.05-280.88	109.70	51.04-175.71	110.11
$\Delta^{13}C$ (‰)	20.23-23.90	22.87	21.55-25.34	23.23	22.12-24.12	23.05

A specific leaf area (cm^2/g), an indication of leaf thickness, also showed a significant genetic variability where its value ranged from 30.19 (RHA-418) to 132.25 (P-107-RP2) with a mean value of 70.36 for R-lines. The range is 31.53-195.50 and 39.64-195.23, respectively in *cms* lines and inbreds.

With respect to root traits, mean root length (cm/pl) did not vary between R-lines (31.17), *cms* lines (21.08-46.00) and inbreds (35.84), though the maximum root length is bigger in R-lines (51 cm/pl). Similarly root volume (cc/pl), another important root trait, ranged from 33.33-77.08 cc with a mean value of 54.06 in R-lines, 18.75-83.33 cc in *cms*-lines and 45.83-75.00 cc in inbreds. The root volume was higher in inbreds. The mean total dry matter (TDM) was less in R-lines (71.76) compared to *cms*-lines (109.70) and inbreds (110.11). *Cms* lines showed maximum TDM (280.88 g/pl).

Since, high TDM coupled with good root system is essential under water limited conditions, the existence of wide genetic variability in sunflower lines provides an option to select for high TDM types with good root dry matter types.

$\Delta^{13}C$ (‰), a surrogate approach to quantify WUE, also showed a significant variability of its value ranging from 20.23 to 23.90 with a mean value of 22.87 for R-lines, 21.55-25.34 for *cms* lines with a mean value of 23.23 and 22.12-24.12 with a mean value of 23.05 for inbreds. Some genotypes from R-lines seem to be more efficient with the respect to WUE. Based on all these traits, genotypes are grouped into high and low- root types, TDM and $\Delta^{13}C$ (‰) (Table 2).

Promising sunflower entries were selected according to the high TDM with a good root system. Accordingly, the best lines are, RES-834-1, R-45, R-64-NB (R-lines), *Cms*-10B, *Cms*-89A, IMS-400A, ARM-246A (*cms* lines), PSN-569 (inbred). Some of the entries showed low $\Delta^{13}C$. They are P-93R (20.23), 11-B (21.89), RCR-114 (21.88), LTRR-341 (21.60), *Cms*-104A (21.71) and *Cms*-336A (21.55). Only one entry, PSN-569 an inbred, exhibited good root system with high TDM and low $\Delta^{13}C$.

Table 2: Grouping of selected sunflower genotypes based on root type and total dry matter

Entries	High root types with high TDM			Low root types with low TDM		
	Genotypes	Root dry weight (g/pl)	TDM (g/pl)	Genotypes	Root dry weight (g/pl)	TDM (g/pl)
R-lines	RES-834-1	25.64	98.74	R-348	5.29	44.15
	R-45	33.03	150.40	P-107-RP2	6.15	38.46
	R-64-NB	23.56	104.54	RHA-6D-1	7.94	46.77
Cms lines	*Cms*-10B	35.18	143.87	*Cms*-248A	6.19	49.78
	Cms-89A	33.43	155.53	ARM-246B	10.61	52.59
	IMS-400A	33.29	165.04	*Cms*-275A	11.65	65.97
	ARM-246A	46.54	280.88	*Cms*-852-B	12.68	81.10
Inbreds	PSN-569	30.25	146.45	IB-29	14.62	51.04

Genetic variability between hybrids and varieties was also observed (Table 3). More leaf area was observed in hybrids and the range for total leaf area (cm^2/pl) varied from 1902.79 to 11064.96 with a mean value of 6074.89 in sunflower hybrids whereas, it is 3763.26 to 7426.16 in varieties with a mean value of 5527.94. The specific leaf area in hybrids ranged from 48.02 to 97.93 with a mean value of 73.65. The range is 76.49 to 128.60 for varieties. The SLA is less in hybrids, indicating higher photosynthetic rate per leaf area.

Table 3: Genetic variability for physiological traits in hybrids and varieties of sunflower

Traits	Hybrids		Varieties	
	Range	Mean	Range	Mean
No. of leaves	21.67-29.83	24.61	18.00-26.50	22.22
Plant height (cm/pl)	120.83-177.42	157.71	43.33-159.17	118.97
Leaf area (cm^2/pl)	1902.79-11064.96	6074.89	3763.26-7426.16	5527.94
SLA (cm^2/g)	48.02-97.93	73.65	76.49-128.60	89.85
Root volume (cc)	54.17-141.67	81.39	50.00-104.17	77.92
Root length (cm/pl)	29.33-45.67	38.13	33.00-41.33	36.30
Root dry weight (g/pl)	19.27-54.81	34.87	25.88-65.20	38.94
Total dry matter (g/pl)	75.36-232.19	166.46	107.57-220.60	150.55
$\Delta^{13}C$ (‰)	22.58-23.88	23.16	22.88-23.95	23.57

Maximum root volume was also observed in hybrids (141.67 cc) and it was established that it was 104.17 cc in varieties. KBSH-44 & RSFH-130 exhibited more root volume. Root length of all hybrids and varieties was similar to the check KBSH-44 & KBSH-41 except DRSH-108.

The TDM ranged from 75.36 to 232.19 g/pl with a mean value of 166.46. Hybrids like TCSH-1, PKVSF-9 and variety TNAU-SUF-7 showed good root system with high TDM. DRSF-108 (hybrid) and CoSFV-5 (variety) showed a low root system and low TDM. As expected, two checks KBSH-44 & KBSH-41 exhibited a very good root system with high TDM. As all the hybrids and varieties showed more $\Delta^{13}C$ (‰) values (range: 22.55-23.95), it is not possible to select any entry with good root system, high TDM and low $\Delta^{13}C$ values.

In all the entries screened it was examined how the TDM and its components are related to each other by correlating many growth parameters with TDM. The results indicate that there is a significant positive relationship between the total leaf area and TDM, indicating that the biomass production in sunflower lines depends on the total leaf area. Similarly, root dry weight also showed a significant positive relationship with TDM, indicating that TDM depends on the root weight apart from stem weight and the total leaf area (Figure 1) and a similar graph was obtained between root length and root volume as well. However, $\Delta^{13}C$ values neither related to TDM nor to root dry weight. Furthermore, there is a need for an in depth study in this aspect.

CONCLUSION

High production of biomass in plants is strongly associated with water use and water use efficiency (Paussioura, 1986). Though several approaches are available to assess and quantify the water use efficiency, one of the most spread is carbon isotope discrimination technique. Earlier research workers have shown that the plants are known to discriminate heavy isotope of carbon during photosynthesis (O'Leary, 1981). Thus, one can resort to this approach to assess the genetic variability for water use efficiency (Farquahar *et al.*, 1989; Hubick and Farquahar, 1989).

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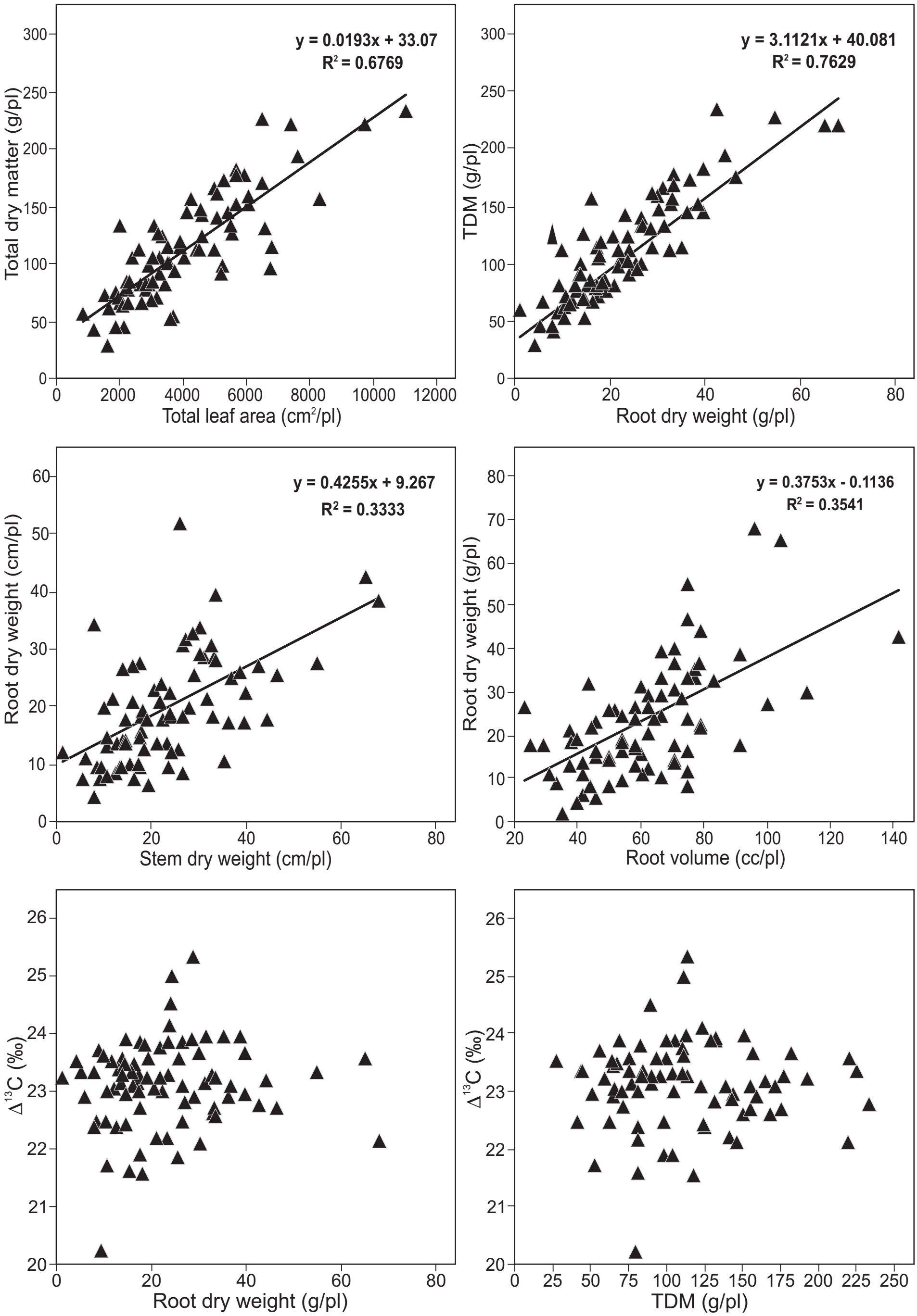

Figure 1: Relationship between TDM, total leaf area, $\Delta^{13}C$ and root traits