IDENTIFICATION AND VALIDATION OF HIGH WATER USE EFFICIENT AND BETTER ROOT TYPE SUNFLOWER (*Helianthus annuus* L.) INBREDS AND PARENTAL LINES FOR IMPROVED DROUGHT TOLERANCE

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BENGALURU
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SHASHIDHARA, K.N.

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Thesis submitted to the
University of Agricultural Sciences, Bengaluru
in partial fulfillment of the requirements
for the award of the degree of

MASTER OF SCIENCE (Agriculture)
IN
CROP PHYSIOLOGY

BENGALURU **July 2009**

Affectionately dedicated to
My Father K. Narasimhaiah
Mother Rama Devi
Brother Bharath
And Grand Mother
Hanumakka

DEPARTMENT OF CROP PHYSIOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
GKVK, BENGALURU

CERTIFICATE

This is to certify that the thesis entitled "**Identification and validation of high water use efficient and better root type sunflower (*Helianthus annuus* L.) Inbreds and parental lines for improved drought tolerance**" submitted in partial fulfillment of **Master of Science (Agriculture)** in **Crop Physiology** to the University of Agricultural Sciences, Bengaluru is a record of research work carried out by **Mr. SHASHIDHARA, K. N.**, ID No **PAK 5166** under my guidance and supervision and that no part of the thesis has been submitted for the award of any other degree, diploma, associateship, fellowship or any other similar titles.

BENGALURU
JULY 2009.

(B. Mohan Raju)
Major Advisor

Approved by:

Chairman: ______________________________
(B. Mohan Raju)

Members:

1. ______________________________
(T. G. Prasad)

2. ______________________________
(M. S. Sheshshayee)

3. ______________________________
(Y. G. Shadakshari)

4. ______________________________
(E. Gangappa)

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Bengaluru

July, 2009 *SHASHIDHARA, K. N.,*

ಮೈಸೂರು ಜರ್ನಲ್ ಆಫ್ ಅಗ್ರಿಕಲ್ಚರಲ್ ಸೈನ್ಸಸ್‌ನಲ್ಲಿ ಪ್ರಕಟಿಸಲು ಎಂ.ಎಸ್ಸಿ. (ಕೃಷಿ) ಬೇಸಾಯ ಶಾಸ್ತ್ರದ ಸಂಶೋಧನ ಪ್ರಬಂಧದ ಸಂಕ್ಷಿಪ್ತ ವರದಿ

ಕೃಷಿ ವಿಶ್ವವಿದ್ಯಾನಿಲಯ. ಜಿ.ಕೆ.ವಿ.ಕೆ., ಬೆಂಗಳೂರು– 560 065

1. ಸಂಶೋಧನ ಪ್ರಬಂಧದ ಹೆಸರು : ಕರ್ನಾಟಕದ ದಕ್ಷಿಣ ಅರೆಮಲೆನಾಡಿನಲ್ಲಿ ಮೆಕ್ಕೆಜೋಳದ ಸುಸ್ಥಿರ ಉತ್ಪಾದನೆಗೆ ಪೋಶಕಾಂಶಗಳ ನಿರ್ವಹಣೆ

2. ವಿದ್ಯಾರ್ಥಿಯ ಹೆಸರು : ಶಶಿಧರ್,ಸಿ.ಯು.

3. ಮುಖ್ಯ ಸಲಹೆಗಾರರ ಹೆಸರು ಮತ್ತು ವಿಳಾಸ : ಡಾ. ಹೆಚ್.ಕೆ. ವೀರಣ್ಣ
ಯೋಜನಾ ಸಂಯೋಜಕರು
ಕೃಷಿ ವಿಜ್ಞಾನ ಕೇಂದ್ರ, ನವಿಲೆ
ಶಿವಮೊಗ್ಗ.

4. ಪದವಿ : ಎಂ.ಎಸ್ಸಿ. (ಕೃಷಿ)

5. ಮುಖ್ಯ ವಿಷಯ : ಬೇಸಾಯಶಾಸ್ತ್ರ ವಿಭಾಗ

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8. ಪ್ರಬಂಧದಲ್ಲಿರುವ ಒಟ್ಟು ಪುಟಗಳ ಸಂಖ್ಯೆ : 130

9. ಪ್ರಬಂಧದಲ್ಲಿರುವ ಒಟ್ಟು ಕೋಷ್ಟಕ ಪಟ್ಟಿಗಳ ಸಂಖ್ಯೆ : 26

10. ಪ್ರಬಂಧದಲ್ಲಿರುವ ಒಟ್ಟು ಚಿತ್ರಗಳ ಸಂಖ್ಯೆ : 10
11. ಪ್ರಬಂಧದಲ್ಲಿರುವ ಒಟ್ಟು ಛಾಯಾ ಚಿತ್ರಗಳ ಸಂಖ್ಯೆ : 6

11. ವಿದ್ಯಾರ್ಥಿಯ ಸಹಿ : (ಶಶಿಧರ್,ಸಿ.ಯು)

12. ರವಾನಿಸುವ ಅಧಿಕಾರಿಯ ಹೆಸರು ಮತ್ತು ಸಹಿ :

ಡಾ॥ ಎನ್. ಕೃಷ್ಣಮೂರ್ತಿ
ಪ್ರಾಧ್ಯಾಪಕರು ಮತ್ತು ಮುಖ್ಯಸ್ಥರು
ಬೇಸಾಯ ಶಾಸ್ತ್ರ ವಿಭಾಗ
ಕೃಷಿ ವಿಶ್ವವಿದ್ಯಾನಿಲಯ, ಜಿ.ಕೆ.ವಿ.ಕೆ.,
ಬೆಂಗಳೂರು – 560 065

ಪ್ರಮಾಣ ಪತ್ರ

ನಾವು ಈ ಮೂಲಕ ಪ್ರಾಮಾಣಿಕರಿಸುವುದೇನೆಂದರೆ, ಈ ಸಂಶೋಧನ ಪ್ರಬಂಧದ ಯಾವುದೇ ಒಂದು ಭಾಗದ ನಕಲನ್ನು ಯಾವುದೇ ವಿಜ್ಞಾನಿಗೆ ಪರಾಮರ್ಶೆನೆಗಾಗಿ ಗ್ರಂಥಾಲಯದ ಮಾಹಿತಿ ಕೇಂದ್ರದಿಂದ ಎರವಲು ಪಡೆಯಲು ನನ್ನಿಂದ ಯಾವುದೇ ಅಭ್ಯಂತರವಿರುವುದಿಲ್ಲ.

ದಿನಾಂಕ : 09–08–2008
ಸ್ಥಳ : ಬೆಂಗಳೂರು

ವಿದ್ಯಾರ್ಥಿಯ ಸಹಿ
(ಶಶಿಧರ್,ಸಿ.ಯು)

ಕರ್ನಾಟಕದ ದಕ್ಷಿಣ ಅರೆಮಲೆನಾಡಿನಲ್ಲಿ ಮೆಕ್ಕೆಜೋಳದ ಸುಸ್ಥಿರ ಉತ್ಪಾದನೆಗೆ ಪೋಶಕಾಂಶಗಳ ನಿರ್ವಹಣೆ

ಶಶಿಧರ್,ಸಿ.ಯು.

ಸಾರಂಶ

ದಕ್ಷಿಣ ಅರೆಮಲೆನಾಡು ಭಾಗದಲ್ಲಿರುವ ಶಿವಮೊಗ್ಗದ ನವಿಲೆ ಆವರಣದಲ್ಲಿ ಪ್ರಾಯೋಗಿಕ ಅದ್ಯಯನವನ್ನು 2007ರ ಮುಂಗಾರಿನಲ್ಲಿ ಮರಳು ಮಿಶ್ರಿತ ಕೆಂಪು ಮಣ್ಣಿನಲ್ಲಿ ವಿವಿಧ ಪೋಶಕಾಂಶಗಳ ನಿರ್ವಹಣೆಯಿಂದ ಮೆಕ್ಕೆಜೋಳದ ಬೆಳವಣಿಗೆ ಮತ್ತು ಇಳುವರಿ ಹಾಗೂ ಮಣ್ಣಿನ ಗುಣಧರ್ಮಗಳ ಮೇಲಾಗುವ ಪರಿಣಾಮವನ್ನು ಅದ್ಯಯನ ಮಾಡಲು ಸಂಶೋಧನೆಯನ್ನು ಕೈಗೊಳ್ಳಲಾಯಿತು.

ಈ ಪ್ರಯೋಗದ ಉಪಚಾರಗಳಲ್ಲಿ ವಿವಿಧ ಬಗೆಯ ಸಾವಯವ ಮೂಲದ ಪೋಶಕಾಂಶಗಳನ್ನು (ಕೋಳಿ ಗೊಬ್ಬರ, ಪ್ರೆಸ್ ಮಡ್) ಕೊಟ್ಟಿಗೆ ಗೊಬ್ಬರದ ಸಾರಜನಕ ಅಂಶದ ಆಧಾರದ ಮೇಲೆ ಶೇಕಡ 100 ಮತ್ತು 125ರ ಶಿಫಾರಸ್ಸು ಮಾಡಿದ ರಸಾಯನಿಕ ಗೊಬ್ಬರಗಳ ಜೊತೆ ಬಳಸಲಾಯಿತು, ಇದರೊಂದಿಗೆ ಲಘು ಪೋಶಕಾಂಶಗಳಾದ ಬೊರಾಕ್ಸ್ (ಪ್ರತಿ ಹೆಕ್ಟೇರಿಗೆ 1 ಕೆ.ಜಿ.), ಸತುವಿನ ಸಲ್ಫೇಟ್ (ಪ್ರತಿ ಹೆಕ್ಟೇರಿಗೆ 10 ಕೆ.ಜಿ.) ಸಹ ಬಳಾಸಲಾಯಿತು ಹಾಗೂ ಸೆಣಬನ್ನು ಮೆಕ್ಕೆಜೋಳದ ಅಂತರ ಸಾಲುಗಳಲ್ಲಿ ಬೆಳೆದು 45 ದಿನಗಳ ನಂತರ ಹಸಿರು ಗೊಬ್ಬರವಾಗಿ ಮಣ್ಣಿನಲ್ಲಿ ಸೇರಿಸಲಾಯಿತು.

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಆರ್.ಸಿ.ಬಿ.ಡಿ ಮಾದರಿಯ ನಕ್ಷೆ ಬಳಸಿ ಅದರಲ್ಲಿ 10 ಉಪಚಾರಗಳನ್ನು 3 ಯತಾರ್ಥ ತಾಕುಗಳಲ್ಲಿ ಅಳವಡಿಸಲಾಯಿತು. ಈ ಎಲ್ಲಾ ಬಗೆಯ ಉಪಚಾರಗಳಲ್ಲಿ ಅತ್ಯಂತ ಹೆಚ್ಚಿನ ಗಿಡದ ಬೆಳವಣಿಗೆ (195.05 ಸೆ.ಮೀ), ಪ್ರತಿ ಗಿಡಕ್ಕೆ ಹಸಿರು ಎಲೆಗಳು (7.4), ಒಟ್ಟು ಒಣ ಪದಾರ್ಥದ ತೂಕ ಪ್ರತಿ ಗಿಡಕ್ಕೆ (365.16 ಗ್ರಾಂ) ಮತ್ತು ಇಳುವರಿಯಲ್ಲಿ ಹೆಚ್ಚಿನ ಧಾನ್ಯ (65.71 ಕ್ವಿಂಟಾಲ್ ಪ್ರತಿ ಹೆಕ್ಟೇರಿಗೆ) ಹಾಗೂ ಹುಲ್ಲಿನ ಇಳುವರಿಯು (9.16 ಟನ್ ಪ್ರತಿ ಹೆಕ್ಟೇರಿಗೆ) ಉಪಚರಿಕೆ ಶೇ. 125 ರಷ್ಟು ಶಿಫಾರಸ್ಸು ಮಾಡಿದ ರಸಾಯನಿಕ ಗೊಬ್ಬರ + ಕೋಳಿ ಗೊಬ್ಬರ (ಕೊಟ್ಟಿಗೆ ಗೊಬ್ಬರದ ಸಾರಜನಕ ಅಂಶದ ಆಧಾರದ ಮೇಲೆ) ದಿಂದ ಪಡೆಯಬಹುದಾಗಿದೆ. ಆದರೆ, ಕೇವಲ ಶೇ. 100ರಷ್ಟು ಶಿಫಾರಸ್ಸು ಮಾಡಿದ ಉಪಚರಿಕೆಯಲ್ಲಿ ಈ ಮೇಲಿನ ಎಲ್ಲಾ ಬೆಳವಣಿಗೆ ಮತ್ತು ಇಳುವರಿಯ ಮಾಪಕಗಳು ಕಡಿಮೆಯಿದ್ದುದು ಕಂಡುಬಂದಿದೆ. ಶೇ. 125ರಷ್ಟು ಶಿಫಾರಸ್ಸು ಮಾಡಿದ ಮಾಡಿದ ರಸಾಯನಿಕ ಗೊಬ್ಬರದೊಂದಿಗೆ ಉಪಚರಿಕೆಯಲ್ಲಿ ಕೋಳಿ ಗೊಬ್ಬರವನ್ನು (ಕೊಟ್ಟಿಗೆ ಗೊಬ್ಬರದ ಸಾರಜನಕ ಅಂಶದ ಆಧಾರದ ಮೇಲೆ) ಬಳಸಿದ ಉಪಚಾರದಲ್ಲಿ ಅತಿ ಹೆಚ್ಚಿನ ಸಾರಜನಕ, ರಂಜಕ ಮತ್ತು ಪೊಟ್ಯಾಷ್ (145.97, 46.83 ಮತ್ತು 183.33 ಕೆ.ಜಿ. ಪ್ರತಿ ಹೆಕ್ಟೇರಿಗೆ) ಹೂ ಬಿಡುವ ಸಂದರ್ಭದಲ್ಲಿ ಹಾಗೂ (185.86, 64.13 ಮತ್ತು 180.24 ಕೆ.ಜಿ. ಪ್ರತಿ ಹೆಕ್ಟೇರಿಗೆ) ಕಟಾವಿನ ಸಂದರ್ಭದಲ್ಲಿ ಮೆಕ್ಕೆಜೋಳದ ಗಿಡಗಳು ಹೀರಿಕೊಂಡಿರುವುದನ್ನು ಗಮನಿಸಲಾಯಿತು, ಹಾಗೂ ಬೆಳೆಯ ನಂತರದ ಮಣ್ಣಿನ ವಿಶ್ಲೇಷಣೆಯಿಂದ ಲಭ್ಯವಿರುವ ಹೆಚ್ಚಿನ ಸಾರಜನಕ, ರಂಜಕ ಮತ್ತು ಪೊಟ್ಯಾಷ್ (212.09, 91.90 ಮತ್ತು 168.11 ಕೆ.ಜಿ ಪ್ರತಿ ಹೆಕ್ಟೇರಿಗೆ) ಶೇ. 125 ರಷ್ಟು ಶಿಫಾರಸ್ಸು ಮಾಡಿದ ರಾಸಾಯನಿಕ ಗೊಬ್ಬರ + ಕೊಟ್ಟಿಗೆ ಗೊಬ್ಬರ ಮತ್ತು ಸೆಣಬಿನ ಹಸಿರು ಗೊಬ್ಬರ ಬಳಸಿದ ಉಪಚಾರದಲ್ಲಿ ಗಮನಿಸಲಾಯಿತು. ಶೇ. 125 ರಷ್ಟು ಶಿಫಾರಸ್ಸು ಮಾಡಿದ ರಾಸಾಯನಿಕ ಗೊಬ್ಬರದ ಜೊತೆಗೆ ಕೋಳಿಗೊಬ್ಬರ (ಕೊಟ್ಟಿಗೆ ಗೊಬ್ಬರದ ಸಾರಜನಕ ಅಂಶದ ಆಧಾರದ ಮೇಲೆ) ಬಳಸಿದ ಉಪಚಾರದಲ್ಲಿ ಅತಿ ಹೆಚ್ಚಿನ ಆದಾಯ : ಖರ್ಚಿನ ಅನುಪಾತ (2.6) ಮತ್ತು ಇದಕ್ಕೆ ಅನುಸಾರವಾಗಿ ಶೇ. 100 ರಷ್ಟು ಶಿಫಾರಸ್ಸು ಮಾಡಿದ ರಾಸಾಯನಿಕ ಗೊಬ್ಬರ + ಕೊಟ್ಟಿಗೆ ಗೊಬ್ಬರ + ಸತುವಿನ ಸಲ್ಫೇಟ್ + ಬೊರಾಕ್ಸ್ ಬಳಸಿದ ಮತ್ತು ಬಳಸದ ಉಪಚಾರದಲ್ಲಿ ಆದಾಯ : ಖರ್ಚಿನ ಅನುಪಾತ (1.9)ನ್ನು ಗಮನಿಸಲಾಯಿತು.

ಡಾ.ಹೆಚ್.ಕೆ.ವೀರಣ್ಣ
(ಪ್ರಧಾನ ಸಲಹೆಗಾರರು)

ಬೇಸಾಯ ಶಾಸ್ತ್ರ ವಿಭಾಗ
ಜಿ.ಕೆ.ವಿ.ಕೆ.
ಕೃ.ವಿ.ವಿ. ಬೆಂಗಳೂರು

THESIS ABSTRACT

1. Title of thesis	Identification and validation of high water use efficient and better root type Sunflower (*Helianthus annuus* L.) inbreds and parental lines for improved drought tolerance
2. Full name of the Student	Mr. K.N.SHASHIDHARA
3. Name and address of the major advisor	Dr. B.Mohan Raju Associate Professor Department of Crop Physiology, UAS, GKVK, Bangalore 560 065
4. Degree awarded	Master's degree in Crop Physiology
5. Year of award of Degree	2009
6. Major Subject	Crop Physiology
7. Total number of pages in thesis	126
8. Number of words in the thesis abstract	1710
9. Number of tables in thesis	22
10. Number of figures in thesis	31
11. Number of flow charts in the thesis	0
12. Number of plates in the thesis	13
13. Signature of Student	
14. Signature and name of the forwarding authority	**V.R. SASHIDHAR** **Professor and Head** **Dept of Crop Physiology,** **UAS, GKVK, Bangalore 560 065**

Certificate

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Date: 24-07-2009 **Signature of Student**
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ABSTRACT

Among the several traits associated with drought tolerance, roots, water use efficiency and intrinsic tolerance at cellular level found to have some practical relevance under water limited conditions. Hence in the present study, one hundred and twenty lines of sunflower comprising parents and inbreds were evaluated in root structures for some of the relevant physiological traits associated with drought tolerance. Accordingly, a wide and significant genetic variability was found in several traits such as total leaf area, specific leaf area, root length, root volume, root dry weight, total dry matter and water use efficiency measured through carbon isotope discrimination approach. Existence of such a wide genetic variability in sunflower lines enabled to identify highly contrasting lines for roots, WUE and TDM and accordingly, a good number of lines with different drought tolerance traits have been identified.

In order to check the consistency of traits and to test the relevance of roots under water limited condition, a subset of sunflower lines from contrasting groups were again evaluated in root structures by imposing moisture stress during active vegetative phase. The results of the study revealed that, even with a small subset of sunflower lines, a significant genetic variability in several of the physiological traits associated with drought tolerance was found both under control and stress condition to indicate that the material is diverse. A strong and significant correlation was also found for various traits such as total leaf area, root dry weight, TDM and $\Delta^{13}C$ between 1st and 2nd experiment to indicate that the traits are consistent and heritable. Further, the high root types performed well over low root types under moisture stress condition to indicate that roots are important for imparting drought tolerance in sunflower. Overall, the existence of wide genetic variability and presence trait donor parents in sunflower will certainly help in sunflower improvement programme.

(B.Mohan Raju)

Signature of the Student **Major Advisor**

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Introduction

I. INTRODUCTION

Sunflower (*Helianthus annuus.*L), an important oilseed crop has occupied a prominent place among oilseed crops and contributes about 12% to the world edible oil production (Anonymous, 1992). Globally, the crop is grown in an area of 20 Mha with a total production of 12-24 M tones. In India, sunflower is cultivated approximately in an area of 2.16 Mha with an annual production 1.32 M tones. In fact, large area under sunflower in the country is being cultivated under rainfed situations where intermittent moisture stress is most prevalent. Although the area under sunflower has increased over the years in the country, the productivity is still the lowest among the sunflower growing countries. As against an average productivity of 1110-1500 kgs/ha recorded in many other countries, its productivity is only about 550 kgs/ha in India (Directorate of Oilseeds Research Report, 2005).

The decreased productivity in oilseeds in general and sunflower in particular is mainly attributed to the intermittent abiotic stresses such as moisture stress, high temperature and salinity stress the crop experiences during growing season. Of these aboitic stresses, moisture stress is the most predominant one as it causes more than 70% reduction in biomass and seed yield in sunflower (Uma Shaanker, 1991). The worldwide reduction in sunflower yield is attributed to drought stress than any other stresses (Dragovic and Maksimovic, 1995). According to USDA Agricultural weather facility report (2005), oilseed production was down by 2% in 2005 from 2004 due to dry weather conditions. In Spain for instance, sunflower crop suffered substantially from drought stress with 41% reduction in production. The available literature therefore unequivocally proved that, the moisture stress is the major factor for reduced productivity in sunflower. Therefore, considering the importance of oilseeds in human diet as well as looking into the demand and to reduce the import of oilseeds, it is necessary to develop drought tolerant sunflower hybrids and varieties for sustained or improved productivity under water limited conditions.

Although sunflower has good potentiality to tolerate drought effects due to its well developed root system, the productivity is still affected by drought. If drought tolerant sunflower hybrids/ varieties are developed, sunflower can be grown successfully under water limited conditions. In this direction, several physiological, morphological and phenological traits have been shown to play a significant role in crop adaptation to drought stress during dry soil conditions (Ludlow and Muchow, 1990; Subbarao *et al*., 1995). The root traits such as root biomass, root length density and root depth have been proposed as the main drought tolerance traits to be considered for sustained seed yield under terminal water limited conditions (Ludlow and Muchow, 1990; Turner *et al*., 2001; Keshiwagi *et al*., 2006). In fact, Baldini *et al.,* (1993) have reported that, wild sunflower species have shown smaller reduction in dry matter production and suggested that, the wild species have drought avoidance mechanisms like higher root/shoot ratio under drought conditions and hence such traits are to be exploited for crop improvement programmes.

As breeding for absolute yields under water limited conditions becoming more difficult, it is now being projected that, several stress relevant traits must be pyramided at different levels of crop breeding programme through trait based breeding approach. Among several traits, the ability of plant to harness water from deeper soil profile associated with roots, water use efficiency (WUE) and relatively high intrinsic tolerance are important traits which need to be exploited (Passiuora, 1996). The practical relevance of root traits and WUE under water limited conditions has been established in many crops. Although these traits are important and relevant under water limited environments, their actual quantification/ assessment poses a serious problem. In this scenario, easily measurable/ quantifiable approaches/ techniques are required to exploit these traits and incorporate in crop improvement programmes. A recent theory linking carbon isotope discrimination for WUE and O^{18} enrichment for transpiration rates provided necessary tools in assessing the variability for WUE and root traits (based on transpiration rates). With these techniques, quantification of some of the relevant drought tolerance traits are now becoming easy and hence trait based breeding can be adopted with greater success. Further, incorporating some of these traits will help the plants to cope up with drought successfully. This approach of trait based breeding is cheapest and most successful to cope up with drought situations (Yordanov *et al*., 2000). Since these traits are heritable, once they are introduced into the material, they will be a permanent source for drought tolerance (Rauf, 2008). Therefore, any trait leading towards drought tolerance shall be considered ideal only when it shows strong positive correlation with seed yield, moderate to high heritability, cheaper and easily measurable and hopefully determined before flowering so that the undesired plants can be rogued out successfully.

Trait based breeding approach has been successful in some of the crop species. In fact, in one such breeding effort to increase WUE in wheat, Richards *et al.,* (2002) and Condon *et al.*, (2004) have succeeded in releasing a superior variety of wheat for water limited conditions. Similar efforts to improve transpiration efficiency resulted in rice cultivar with good growth and productivity under water limited condition (Li *et al.*, 2005). These results clearly demonstrated the relevance of superior drought tolerance traits for improved productivity under water limited conditions. In order to go for trait based breeding approach, a source material with desirable traits is needed. Therefore, a wide genetic variability for the traits of relevance should exist in the crops. Further, techniques should also be available to quantify these traits. Once the trait donor/parents are identified, the superior trait can be used for crop improvement programmes.

In the present study, with an objective of developing sunflower hybrids tolerant to drought conditions, an attempt was made to screen the parental lines and inbreds of sunflower for some of the relevant drought tolerance traits such as roots, WUE and biomass. Being a highly cross pollinated crop, one would expect wide genetic variability for different traits including drought tolerance traits. In fact, a large number of people in the past have reported a significant genetic variability for traits related to water status, osmotic adjustment, root characteristics, gas exchange parameters, seedling traits and drought susceptibility index in sunflower (Lambrides *et al.,* 2004; Kiani *et al.*, 2007a; Rauf and Sadaqat, 2008a).

With this back ground, an attempt was made in the present study to screen and select sunflower parental lines and inbreds for superior drought tolerance traits. The objectives set aside were

1. Physiological assessment of sunflower inbreds / parental lines and selection of contrasts based on root traits, water use efficiency (WUE) and total dry matter (TDM)

2. Testing of the selected contrasting lines for various physiological traits as well as to check the relevance of roots under water limited condition
3. Characterization of sunflower inbreds and parental lines for WUE and root traits

Review of Literature

II. REVIEW OF LITERATURE

Sunflower (*Helianthus annus* L.), an important oilseed crop of the country after groundnut and rapeseed mustard has replaced many of the traditionally grown crops such as sorghum, bengal gram etc., Although it is a crop of temperate countries, it has adapted very well to the Indian conditions. In India, it is grown predominantly as a rainfed crop both in kharif and rabi season.

Over the years, the area under sunflower has increased and in fact in the last one or two years, sunflower has extended its tentacles even to the non-traditional sunflower growing areas. As per the recent information, sunflower is growing in an area of 2.16 M ha with a total production of 12.24 lakh tones (Shankergoud *et al.,* 2006). An estimated 75% of the 2.16 million ha where sunflower is currently being cultivated in India is under sub- optimal conditions of soil water and other resources where stress alone is attributed to over 70% reduction in sunflower yields. The loss in yield due to stress is estimated to be between 10% to as high as 70% under severe moisture stress conditions (Uma Shaanker, 1991).

Considering the importance of sunflower oil in human diet, sustaining or even increasing sunflower productivity under water-limited conditions is most important. Further, looking into the impact of drought stress on productivity and the commercial importance of this crop, it is essential to improve drought tolerance in sunflower.

Drought: An International dilemma in sunflower

Significant area of cultivation of most oilseed crops including sunflower is under rainfed conditions where water availability is the overriding limitation for achieving potential productivity. In fact in most crops, the yield loss due to moisture stress alone is more than all other stresses put together (Kramer, 1980). Hence, moisture stress is considered as the most overriding abiotic factor that limits productivity in most crops including sunflower.

Over the years it has been observed that, the worldwide reduction in sunflower yields has been associated with drought (Dragovic and Maksimovic, 1995). According to USDA Agricultural weather facility report (2005), oilseed production in 2005 was down by 2% from 2004 due to drier than normal weather. In Spain for instance, sunflower crop suffered substantially from the effects of drought with a 41% decrease in production (USDA Agriculture weather facility report, 2005). Similarly in US, drought was a key factor responsible for yield losses of up to 20% (Reddy *et al*., 2004). In Pakistan, nearly 50% yield loss occurred due to drought (Rauf and Sadaquat, 2007a). The available information therefore unequivocally proved that, drought stress is the major cause for yield reduction in many crops including sunflower.

Effects of drought stress on sunflower

Sunflower is categorized as a low to medium drought sensitive crop. It has been found that, both quantity and distribution of water has a significant impact on achene and oil yield in sunflower (Krizmanic *et al*., 2003; Reddy *et al*., 2003; Iqbal *et al*., 2005). Intensity of yield reduction by drought stress depends on the growth stage of crop, the severity of the drought and tolerance of genotypes (Lorens *et al*., 1987). Although drought stress affects every developmental stage of sunflower, maximum reduction in yield was experienced when drought occurred during the reproductive stage (Karaata, 1991; Reddy *et al*., 1998; Reddy *et al*., 2003; Vijay, 2004). It was found that, maximum reduction in yield occurred when drought was imposed during flowering (Karaata, 1991). Similarly, Vijay (2004) studied the response of achene yield to irrigation at four stages viz., 15-20 days after seeding (DAS), capitulum initiation (30-35 DAS), flowering (50-60 DAS) and grain development (70-80 DAS) and showed that, the maximum achene yield was obtained with irrigation at the flowering stage. In contrary to the above, it was also shown that, the maximum effect and yield reduction occurs when stress coincides with active vegetative phase. In fact Ravishankar *et al*., (1991), Ganesh Kumar (1994) and Keerthi (2000) while evaluating sunflower genotypes for stress tolerance observed that, maximum biomass and seed yield reduction occurred when moisture stress coincided with active vegetative phase.

Drought during the vegetative phase of the plants affects both final biological and economic yields. During vegetative development, it reduces the main stem height, stem diameter, number of nodes or leaves and leaf area (Agele, 2003; Turhan and Baser, 2004) while an increase in root length occurs at the expense of above-ground dry matter. This has been observed by a higher root-to-shoot ratio obtained under drought stress condition (Sobrado and Turner, 1986). The reduction in vegetative biomass results in lower plant surface area, which reduces the radiation use efficiency and photosynthetic activities (Stockle and Kiniry, 1990; Badr *et al*., 2004; Germ *et al*., 2005). This finally lowers assimilation of photosynthates during the reproductive phase, which reduces head diameter. Reduction in head diameter further decreases the number of rows per head and number of achenes per head and results in correlation of yield components to severity of drought (Fereres *et al.,* 1986; Gimenz and Fereres, 1986; Alza and Fernandez-Martinez, 1997; Rauf and Sadaqat, 2007a).

On the other hand, stress during the flowering stage causes abortion of ovaries, embryo, sterility of pollen and decreases in leaf area index. This reduces the number of achenes per head, 100-achene weight and fertile achenes per head (Baldini and Vannozzi, 1999; Reddy *et al*., 2003). However, a reduction of 100-achene weight may occur due to lower photosynthates production as a result of excessive loss of leaves at this stage. It has been estimated that, stress during vegetative phase causes 15-25% yield reduction, whereas more than 50% reduction can occur if stress coincides with the flowering stage (Reddy *et al*., 2003). However, minimal damage has been found to occur if drought was imposed during the achene filling stage (Karam *et al*., 2007). At this stage, the plant responds to the stress by early, abrupt leaf senescence (Rauf and Sadaqat, 2007b) and mobilization of stem reserves to the developing achenes (Baldini and Vannozzi, 1999; Rauf and Sadaqat, 2007b).

Breeding for drought tolerance

As most of the cultivated hybrids evolved under optimum conditions, breeding for drought tolerance is essential. 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 when drought occurs. High yield is the ultimate objective of any breeding program. 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 stomata closure allow the plant to limit water losses, but also lead to reduced dry matter production and therefore reduced final seed yield (Fischer and Wood, 1979; Karamanos and Papatheohari, 1999).

As drought events occur at different phases during the growing season, field selection becomes complicated due to high variability associated with multiple interactions contributing for drought tolerance of crops. This contributes to a large genotype × environment (G x E) interaction that may explain the slow progress in developing new cultivars of crops for drought conditions (Fukai *et al*., 1999). In most cases, no clear cause of the G x E interaction has been identified because of lack of information about the environment (such as weather or soil) or the genotypes themselves (Voltas *et al*., 2002). Several indices have been proposed to describe the behavior of a given genotype under stress and non-stress conditions (Fischer and Maurer, 1978; Sojka *et al*., 1981; Lin and Binn, 1988; Yadav and Bhatnagar, 2001).

Sunflower drought tolerance breeding

Breeding for drought tolerance is a major objective for boosting sunflower yield worldwide. However, a very few reports are available regarding the success of breeding sunflower against this stress. Most of the efforts have been intensified to cope up with the drought through agronomic practices. The main focus of the research has been to ameliorate the effect of drought through soil conditioning, seed priming, exogenous foliar spray of certain osmoprotectants and deficit irrigation practices. These treatments have been shown to improve the performance of sunflower genotypes under drought stress conditions (Meo *et al*., 1999; Khaliq, 2004; Iqbal *et al*., 2005).

From a breeding point of view, efforts were restricted to the screening of breeding material and hybrids or formulation of selection criteria under drought stress. Local and introduced genetic materials were evaluated for yield, yield components and physiological traits under drought conditions to discriminate between tolerant and sensitive lines (Tahir *et al*., 2002; Rauf and Sadaqat, 2007a; Rauf and Sadaqat, 2008a; Rauf *et al*., 2008a). Apart from the field or pot screening experiment, in vitro screening and selection for drought tolerant callus has also been carried out by plating the cell suspension on agar-solidified medium containing PEG (Hassan *et al*., 2004). Plants developed through this approach grew better than the non-selected lines under various levels of water deficit induced by polyethylene glycol (PEG). This could probably because of higher amounts of K+, Na+ and N; lower amounts of Ca++ and P in the selected lines than in the non-selected lines.

For drought tolerance breeding, emphasis should be given for traits that confer drought tolerance in the plant and also show positive association with yield. Over the past decades, plant breeders have focused on some traits that were incorporated to plant survival under stress conditions such as lower leaf canopy and reduced transpiration (Fischer and Wood, 1979; Karamanos and Papatheohari, 1999). Often, these traits were not necessarily positively associated with high yield. As a result, breeders continue to develop cultivars that yield poorly under stress conditions. Therefore, analysis of plant traits with significant effects on drought tolerance and high yield potential under stress conditions seems to be necessary (Richards, 2006). In this sense, wide genetic variability for drought tolerance traits is envisaged.

Genetic variability for drought tolerance in sunflower

Most cultivated hybrids or open pollinated varieties evolved under near optimum agronomic conditions often have some common parentage and history of origin. Therefore, breeding for drought tolerance must expand genetic variability. This depends on the incorporation of diverse germplasm so that the potential sources of drought tolerance may be identified and subsequently incorporated to ensure yield when drought occurs.

The first approach for the development of a drought tolerant line is to screen high yielding germplasm accompanied by superior yield contributing traits. It is likely that this germplasm may also contain extensive variation for stress tolerance traits (Vasal *et al*., 1997). Results showed the existence of significant variation between the sunflower genotypes for yield under drought stress. Field experiments at Cordoba, Spain conducted by Fereres *et al.*, (1986) to evaluate the yield responses to drought of 53 sunflower genotypes showed that there was substantial variability among genotypes for stress tolerance. Similar kind of variability for stress tolerance was also reported by Ravishankar (1990), Ravishankar *et al.*, (1991), Ganesh Kumar (1994), Keerthi, (2000) in sunflower**.** Similarly, water stress revealed a high genetic variability among traits related to water status, osmotic adjustment, root characteristics, gas exchange parameters, seedling traits and drought susceptibility index when a large number of sunflower genotypes belonging to diverse origins were evaluated **(**Chimenti *et al*., 2002; Lambrides *et al*., 2004; Turhan and Baser, 2004; Kiani *et al*., 2007a; Rauf and Sadaqat, 2007a; Rauf and Sadaqat, 2008a).

How to manage drought stress?

Drought can be managed by modifying the plant morphology or incorporating some of the traits that help the plants to cope up with drought stress successfully (Yordanov *et al*., 2000). Thus, genetic modification is usually the most successful and cheapest strategy to cope up with the drought. Since modifications within plant morphology and physiology introduced through breeding are heritable, once introduced into a breeding material, it will be a permanent source of drought tolerance. Heritable changes within a crop with the aim of improving drought tolerance can be broadly considered as breeding for improved drought tolerance.

There are numerous strategies utilized in breeding for drought stress in a crop species such as induction of earliness for drought escape, modification of certain plant traits that leads toward drought resistance and introduction of drought tolerance traits associated with high yield. Strategy for breeding drought stress depends upon the intensity, frequency and timing of drought occurrence. A strategy to produce a high yielding genotype combined with traits leading towards drought tolerance is desirable provided that the traits conferring drought tolerance also show strong positive correlation with yield. A trait leading toward drought tolerance should be considered ideal if it shows a strong positive correlation with achene yield, moderate to high heritability, cheaply and easily measured.

The direct selection for yield in dry environments is inefficient due to a large seasonal variation in weather and high genotype × environment interaction resulting in low heritability for yield. In this scenario, it is suggested that 'trait- based' breeding approach be adopted to improve the productivity under drought. Several of the underlying physiological traits such as water acquisition associated with better root characteristics, higher water use efficiency, better water conservation strategies and high intrinsic stress tolerance at cellular level could contribute substantially to yield under stress and therefore they need to be incorporated in drought tolerance breeding programme.

Traits for drought tolerance breeding

Recently, a conceptual model to indicate how the component traits are associated with drought tolerance has been proposed (Fisher and Turner, 1978; Blum, 1988; Richards *et al.,* 2001 and 2002; Araus *et al*, 2002; Chaves *et al*., 2003). According to this model, acquisition of water from deeper layer associated with roots, low canopy temperature, osmotic adjustment, high HI, stem CHO reserves, WUE etc, appears to be the important traits which can be used to improve the drought tolerance of crop plants.

Traits associated with drought tolerance and their relevance

Improving crop productivity in drought prone environment is a daunting challenge because of the involvement of many traits and their interaction with the environment. Conventional breeding and more recently, a trait based breeding approaches and wild crossing have achieved significant impact in crop improvement programs.

A conceptual model for traits associated with main drivers of yield under drought indicated that, it is not a single trait or two are conferring drought tolerance but rather many traits are indeed involved in conferring drought tolerance of crop plants (Fig 1; Reynolds and Tuberosa, 2008). Accordingly, water uptake associated with good root system, transpiration efficiency, resource partitioning more specifically, pre-anthesis partitioning to stem carbohydrates and grain harvest index have relevance under water-limited conditions. In addition, photo protection either through leaf morphology or through pigmentation or antioxidants, better water conservation strategies and high intrinsic stress tolerance at cellular level are often considered as the most relevant physiological traits under drought. In fact, a significant genetic variability for these traits exist (Sheshshayee *et al*., 2003) and several high yielding varieties through improvements in these traits have also been developed (Richards *et al*., 2002; Condon *et al*., 2004; Li *et al*., 2005).

With breeding for absolute yields under water limited conditions becoming more difficult, it is being projected that, several relevant stress adaptive traits must be pyramided to significantly enhance the levels of drought tolerance in crop plants through a "trait-based" breeding approach (Yordanov *et al*., 2000). Significant improvement in productivity through enhanced WUE (Condon *et al*., 2004; Impa *et al*., 2005) and root traits (Li *et al*., 2005) have been demonstrated implying the relevance of these traits in breeding for stress tolerance as well as improved productivity. However, these are complex, multi-gene controlled quantitative traits and hence molecular marker assisted breeding is essential to pyramid them onto the cultivated background.

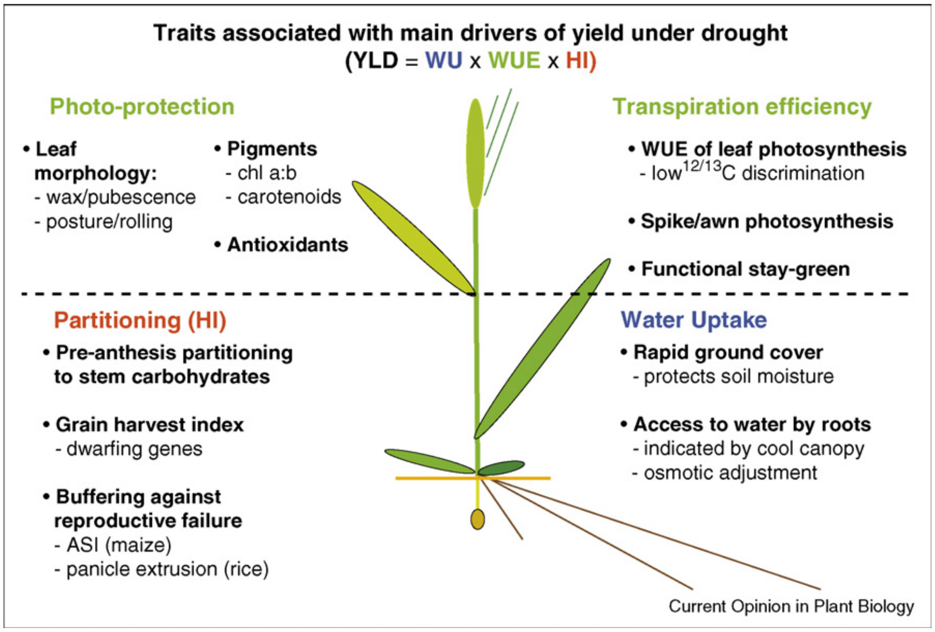


Fig 1: Traits associated with yield under drought

Among the several traits, the ability of plants to harness water from deeper soil profiles associated with deeper root system and the efficiency of water use for biomass production are often the most relevant physiological traits that deserve exploitation.

he first analysis of water requirement of crops and estimate of genotypic variability in water use efficiency (WUE) was reported almost a century back by the pioneering work of Briggs and Shantz (1913). They inferred that, plant growth and production of biomass depend on the amount of water used (WU) for growth as well as WUE. A simple mechanistic relationship of these two parameters (TDM=WU x WUE) describing the variability in these two traits largely determines variations in crop growth rates.

Despite the opinion by Sinclair *et al*., (1984) that, the exploitable variation in WUE is small, it contributes considerably to the productivity. However, more recent studies have shown that, considerable variability in WUE does exist in several plant species (Martin and Thorstenson, 1988; Ebdon *et al*., 1998; Ehab, 2006; Kiani *et al*., 2007b) that can indeed be exploited through breeding.

The main emphasis of the present day crop improvement programme is therefore to breed for drought tolerance traits rather than drought tolerance *per se*. Root traits, high water use efficiency, moisture conservation associated with epicuticular waxes and intrinsic tolerance at cellular level are the most relevant traits for improving drought tolerance. Introgressing these traits onto elite genetic background alone will substantially improve tolerance to water deficit conditions (Kaya and Ibrahim, 2004).

Importance of roots for drought tolerance

The genetic improvement of root traits by conventional method is rather slow due to difficulty in measuring root dynamics and below ground environment. In fact, people have shown the relevance of root biomass especially root length on biomass production in several systems (Sinclair and Muchow, 2001). People have also shown the much-awaited reward when they have incorporated root traits like root length, root biomass and root pulling force in their breeding programme for improved root system to achieve better productivity. Like water use efficiency, the variability for root traits can also be assessed through ^{18}O enrichment studies as it is considered to be a powerful time integrated surrogate for transpiration rate and hence the better root system (Bindu Madhava *et al*., 2005; Sudhakar, 2005)

Root morphology is one of the most well studied putative drought tolerance traits for potential use for yield improvement under drought condition. The root system of a plant is important while considering for drought tolerance breeding. Certain root characteristics such as root length, root biomass and lateral root density would determine the efficiency of water extraction from the soil. A deeper root system would allow water extraction from deeper soil profiles and thus it is expected that, the plant will perform better under moisture stress when its growth is dependent on water stored deeper in the soil. In fact, in one of the studies, it was shown that, sunflower with deep and extensive root system can extract water from up to 270 cm (Gimenz and Fereres, 1986; Rachidi *et al*., 1993). Thus, root system has a practical relevance under water-limited conditions.

Under stress, root length seems to be increased in plants. In fact Pace *et al*., (1999) have reported that, drought-stressed seedlings showed some increase in root length but a reduced diameter. On the other hand, Prior *et al*., (1995) showed that, inadequate soil moisture reduced root elongation. In another study, Plaut *et al*., (1996) have showed that the soil moisture deficit reduced root length and density. Therefore, a number of different seedling traits have been suggested as important to counter drought effects. These include, lateral and tap root weight, lateral root number and root-to-shoot ratios (Cook 1985; Pace *et al*., 1999).

Genetic variability for roots

Several workers have reported genetic variability for root traits in different systems. Recent experiments conducted at our center has reveled significant genetic variability for root traits in groundnut (Shashidhar, 2002), recombinant inbred lines of rice (Ayyappa, 2004), mulberry germplasm accessions (Sudhakar, 2005), sunflower

(Vikram, 2008). Thus, it appears that, the genetic variability for root traits exist in crops and one can exploit these traits for crop improvement programmes.

Genetic improvement of root traits by conventional breeding methods has been rather slow due to difficulty in measuring the root dynamics, their interactions with the below ground environment and lack of understanding of the rhizosphere. It would be rewarding to exploit relatively easily measured root traits like root length, root density, root weight and root pulling force (O'Toole and Soemartono, 1981) in breeding programs. Sinclair and Muchow (2001) in their simulation experiments demonstrated an increase in biomass and yield when root growth was better. These studies emphasize the importance of breeding to improve root system to achieve better productivity. Increasing the water extraction would exhaust the soil water resources resulting in the end season stress (Condon *et al.,* 1990). However, in areas where there is sufficient water in the deeper profiles, deeper root system would significantly increase the total biomass as well as yield (Sinclair and Muchow, 2001). Therefore, from an agricultural point of view, better water mining from deeper layers of the soil associated with superior root mechanisms has relevance. Crop improvement programs have largely ignored to exploit the variability in root traits essentially due to the inefficient methods to accurately determine the root biomass.

Biomass production in plants is strongly associated with total transpiration. Thus, water acquisition is an important determinant of total biomass in any crop (Passioura, 1983; Angus *et al.,* 1990; Matsui and Singh, 2003; Taiz and Zeiger, 2006). In this context, extraction of water from deeper soil profile and using it for transpiration has been recognized as an important trait for biomass production. Extraction of water from deeper soil profile is associated with roots and hence root traits are considered as an important trait for drought tolerance (Li *et al.,* 2005). Drought during seed forming stages has been shown to reduce yield by 56–85% (Nageswara Rao *et al*., 1989). In this scenario, breeding for drought tolerance has been considered as an important strategy in alleviating this problem. Holbrook *et al*., (2000) have demonstrated that, breeding for drought tolerance can also be an effective strategy for alleviating yield loss. However, progress in breeding for drought tolerance has been slow because of the complexity of the trait. A better understanding of the underlying mechanisms of drought tolerance should accelerate the progress in breeding for this trait.

Drought tolerance may be enhanced by improving the ability of the crop to extract water from the soil (Wright and Nageswara Rao, 1994). Deep rooting, root length density (RLD) and root distribution have been identified as drought adaptive traits (Passioura, 1983; Turner, 1986; Ludlow and Muchow, 1990; Matsui and Singh, 2003; Taiz and Zeiger, 2006) that can be used as selection criteria for drought tolerance. Variation among genotypes for shifting root distribution downwards in response to drought has been found in cowpea (Matsui and Singh, 2003), white clover (Annicchiarico and Piano, 2004) and chickpea (Yusuf Ali *et al*., 2005; Benjamin and Nielsen, 2006; Kashiwagi *et al*., 2006). In contrast, Benjamin and Nielsen (2006) found that, water deficit did not affect root distribution in soybean. In this regard, Rucker *et al.,* (1995) found that, some genotypes with large root systems under non-stress conditions gave high yield under drought conditions and they suggested that, these genotypes possessed drought avoidance traits. However, the direct assessment of deep rooting, root length density and root distribution under different water regimes to see how plants respond to drought in terms of these traits has not been clearly demonstrated.

In crop plants, selection for drought tolerance in the past has primarily been based on biomass production and pod yield under drought conditions. The mechanisms by which the resistant genotypes achieve high yield under drought are not well understood. Information on the ability of these drought-resistant crop plants to alter root distribution contributing to high yield under water stress might reveal the avoidance mechanism and could result in the development of improved breeding strategies for drought tolerance.

Water use efficiency and its relevance as a drought tolerance trait

Amongst a number of strategies evolved by plants to adapt to water limited conditions, the efficient use of water for biomass production is considered as another important trait. Water use efficiency (WUE) is often referred to as transpiration efficiency (TE), which is defined as the ratio of the amount of biomass produced over a specific crop growth period to the total amount of water transpired during that period.

The importance of WUE has known for almost a century ever since the documentation of significant genetic variability by Brigs and Shantz in 1913. The relevance of WUE in determining productivity was proposed by Passioura (1986) in his yield model. Accordingly, yield is a product of transpiration, WUE and harvest index.

$$\text{Yield} = T \times \text{WUE} \times \text{HI}$$

Where,

T = Total transpiration or water use
WUE = Water use efficiency
HI = Harvest index

Despite the opinion by Sinclair *et al*., (1984) that, the exploitable variation in WUE is small, more recent studies have shown that considerable variability in WUE does exist in several plant species (Martin and Thorstenson, 1988; Virgona *et al*., 1990; Ehdaie *et al*., 1991; Nageswara Rao *et al*., 1993; Ebdon *et al*., 1998) that can indeed be exploited through breeding.

The physiological processes associated with WUE have been fairly well elucidated. At a single leaf level, WUE is the ratio of the carbon assimilation to the transpiration rate (A/T) and is expressed as

$$A/T = \{gc\ (Ca - Ci)\}/\{gw\ (ei - ea)\}$$

Stomatal conductance regulates the diffusion of both CO_2 and water vapour. At a constant CO_2 concentration in air (Ca), the intercellular CO_2 concentration (Ci) is a function of photosynthetic CO_2 consumption. Similarly, water vapour diffusion is dependent on the gradient of water vapour pressure between leaf (ei) and air (ea). It is apparent that, the stomatal diffusive characteristics and the chloroplast capacity to fix carbon determine the difference in WUE (Caemerrer and Farquhar, 1981). Depending on the dominant factor that regulates the variability in WUE, species or genotypes are often classified under conductance and / or capacity types (Farquhar and Lloyd, 1993; Udaya Kumar *et al*., 1998b). But low success in WUE is attributed to lack of proper quantification techniques to assess genetic variability in WUE. In fact, earlier workers have reported considerable extent of genetic variability in many crop species by several approaches to assess the genetic variability for water use efficiency. These include gas exchange studies, gravimetric approaches and carbon isotope discrimination techniques. Table 1 depicts the genetic variability reported by several workers in different systems to indicate the existence of variability for this important trait.

Determination of WUE

Physiological WUE can be evaluated by measuring CO_2 fixed and transpiration rate. These measurements are usually performed on a single leaf over a limited period of time and the biomass produced is a function of photosynthetic rate. Hence, WUE at a single leaf level is the ratio of carbon assimilation rate to the transpiration rate. This approach is however not reliable because, the measurements are time instantaneous and diurnal variations accounted for variation in WUE cannot be captured with this approach.

In another approach called gravimetric approach, the water transpired and biomass accumulated over a specific period of crop growth is quantified to arrive at WUE. Though this approach is quite accurate and integrates the diurnal as well as seasonal variations, it is notoriously cumbersome and hence cannot be used for large-scale screening. Due to these difficulties with the said approaches, progress in assessing genetic variability in WUE was very slow. Hence, carbon isotope discrimination technique has been developed as a powerful, dependable, time averaged surrogate estimate of WUE (Farquhar *et al.,* 1982; Farquhar and Richards, 1984; Farquhar *et al*., 1989b). The ratio of ^{13}C to ^{12}C in plants varies from that in the air (source). Differences in the physical properties of the isotopes cause different fractionation during the incorporation of CO_2 into the dry matter. Variations in the ratio of ^{13}C to ^{12}C between C3 and C4 species has been employed in ecological studies but more recently, it was established that, the ratio of ^{13}C to ^{12}C is related to the ratio of the internal to external partial pressures of CO_2 (Ci/Ca). This in turn is related to the WUE (Farquhar *et al.,* 1982; Farquhar and Richards, 1984). Deviation of the carbon isotope fractionation in plant organic matter from that of the air is referred to as carbon isotope discrimination

Table 1: Genetic variability for water use efficiency reported in several systems

Species	**Reference**	**Year**
Sunflower	Ravishankar Virgona *et al.,* Farquhar *et al.,* Ouda, A.S	1988 1990 1995 1999
Ground nut	Rao *et al*., Hebbar *et al*., Roy Stephen	1995 1994 1995
Common bean	Ehleringer, and Osmond Ehleringer, *et al*.,	1989 1991
Cow pea	Shashikumar Ismail and Hall Hall *et al*., Ashok Ashok *et al.,*	1983 1992 and 1993 1993 1996 1999
Soybean	Arun	1985
Wheat	Farquhar and Richards Condon *et al.,* Ehadi and Waives Ehadi Condon and Hall Al-Hakimi *et al*.,	1984 1990 1993 1995 1997 1997
Finger millet	Sashidhar Uma	1987 1987
Barely	Hubick and Farquhar	1989
Tomato	Martin *et al.,*	1999
Potato	Jefferies and Mackerron	1997
Chickpea	Gangadhar	1995
Pigeon pea	Devaraj Achar	2000

($\Delta^{13}C$). Discrimination against $^{13}CO_2$ in favor of $^{12}CO_2$ during CO_2 diffusion into sub-stomatal cavities and during photosynthesis in C_3 plants is closely related to TE integrated over crop growth period (Farquhar and Richards, 1984; Richards *et al.*, 2002). This carbon isotope discrimination ($\Delta^{13}C$) approach forms a promising method for improving WUE in plants (Condon and Richards, 1992).

Stable Isotope of carbon and its relevance in assessing WUE

Naturally, carbon is present in two stable isotopic forms i.e. ^{12}C and ^{13}C of which, ^{12}C constitutes to 98.9% of all the atmospheric carbon. Though the isotopes have very similar chemical properties, they differ in physical properties. The heavier isotope, ^{13}C is slow to diffuse through air and during enzyme-catalyzed reactions. Thus, it results in what is known as isotope effect that leads to reduction in the heavier isotopic composition in the product. In fact, plants are known to discriminate against the heavy isotope of carbon during photosynthesis (O'leary, 1988) and hence this discrimination can be used for assessing WUE.

Carbon isotope discrimination $\Delta^{13}C$ in plants at different steps of photosynthesis

The fractionation of carbon isotope during photosynthesis involves several distinct biochemical and biophysical processes. These processes have different tendencies to discriminate between ^{12}C and ^{13}C and the overall discrimination of a particular plant will be a function of the mechanism it uses for CO_2 fixation and the relative balance of the processes that participate in photosynthesis. During photosynthesis, CO_2 must diffuse from the atmosphere to the chloroplast stroma. Since $^{12}CO_2$ diffuses faster than $^{13}CO_2$, several fractionation processes occur along this diffusion path so that the CO_2 available at the sites of carboxylation is always significantly depleted in ^{13}C compared to the atmosphere (O'leary, 1988).

Relationship between $\Delta^{13}C$ and WUE

Plants discriminate against the heavy isotope of carbon ($\Delta\ ^{13}C$) during photosynthesis. However, the extent of discrimination depends on the Pi and hence ^{13}C content in the plant samples has been emerged as a potential tool to quantify Pi. Several reports confirm the close relationship between Pi and $\Delta^{13}C$. Therefore, $\Delta^{13}C$ could be a time-integrated estimate of Pi. Since, Pi /Pa ratio predominantly determine the variations in WUE and Δ, a strong relationship between $\Delta^{13}C$ and WUE can be expected and explained by the following equation proposed by Farquhar *et al*., (1989b).

$$WUE = \{(I - \theta)(b - d - \Delta) / 1.6v(b-a\}$$

Where, θ is the proportion of fixed CO_2 lost in respiration, v is the leaf air vapour pressure gradients, $\Delta^{13}C$ are negatively related to WUE.

The selection for low $\Delta^{13}C$ among and within cultivated North American alfalfa germplasm may provide opportunities for modest improvement (5-15%) in $\Delta^{13}C$. Introgression of low $\Delta^{13}C$ from exotic germplasm would offer additional opportunities to improve WUE in alfalfa. $\Delta^{13}C$ was negatively correlated to shoot WUE in many systems. Accordingly, $\Delta^{13}C$ and WUE were negatively correlated in tomato (Martin and Thorstenson, 1988) cowpea (Ismail and Hall, 1992) and kentuky blue grass (Ebdon *et al*., 1998).

$\Delta^{13}C$ can serve as selection criteria for WUE in breeding programmes. Considerable amount of genetic variations for $\Delta^{13}C$ has been documented in several crop species. Significant variations for $\Delta^{13}C$ within population and among other alfalfa germplasms (Ray and Townsend, 1998 and Ray *et al*., 1999), barley (Craufurd *et al*., 1991), wheat (Ehdaie *et al*., 1991; Condon and Richards, 1992), crested wheat grass (Read *et al*., 1991), cowpea (Ismail and Hall, 1992; Anyia and Herzog, 2003), peanut (Nageswara Rao *et al*., 1993) have been shown. A significant variation for $\Delta^{13}C$ in canola has been observed and ranking of canola genotypes for $\Delta^{13}C$ was constant across years and locations. A significant variation was also found within and between botanical types of peanut in WUE and $\Delta^{13}C$ with Virginia botanical types having higher water use efficiency (Craufurd *et al*., 1991). The available literatures therefore suggest the existence of genetic variability for an important trait WUE.

Water use efficiency is a quantitative trait and a complex trait influenced by both environmental and intrinsic factors. Environmental factors affecting WUE are vapour pressure deficit (VPD), light and temperature, soil volume and moisture stress. Increase in leaf to air vapour pressure differences substantially increases the transpiration and thereby decreases WUE (Fischer and Turner, 1978; Stanhill, 1986). Low VPD increases crop growth rates and lead to an increase in WUE. This could be realized by planting the crops early in the season where VPD is generally lower (Keatinge and Cooper, 1983). Increase in VPD reduces apparent photosynthesis in both stressed and non-stressed plants and thus reduces intrinsic WUE (Turner, 1986).

Heat stress reduces WUE through deleterious effects on carboxylation and respiration and increases water use via effects of increased vapour pressure deficit associated with higher temperature and leaf air temperature difference. On the other hand, moisture stress increases TE/WUE, which is associated with higher A/gs and low Pi (Farquhar and Richards, 1984). Moisture stress induced increase in WUE is often associated with greater reduction in gs than in A (Mayland *et al.*, 1993; Wright *et al.*, 1983).

Traits based breeding approach for the development of new cultivars for water scarce environments- Success stories

A physiological understanding of plants responses to drought has often been sought on the pretext that, this understanding will assist plant breeders develop high yielding varieties for water-scarce environments. However, despite an extensive literature on plants response to drought, there are few documented examples where a physiological understanding of drought has been identified as the traits that limit yield under drought and where these traits have been used in successful crop improvement programs to enhance crop yields.

A few of the success stories where physiological traits were used for breeding programme to develop cultivar for drought situations are presented here.

Extended crop duration in wheat

Breeding a variety with an extended duration of vegetative period capitalizes on higher intrinsic water-use efficiency as more growth occurs during winter when the exchange of CO_2 for water is highest (high transpiration efficiency). Furthermore, less water is lost by direct evaporation from the soil surface and early growth is faster because, soil temperatures are warmer. Further, a longer vegetative duration also extends the duration of root growth allowing utilization of water and nutrients deep in the soil profile. Nutrient-use efficiency is also greater with extended crop duration (Batten and Khan, 1987). A good example of the advantage of extending the duration of crops in dry temperate environments is in chickpea where sowing in autumn rather than the normal spring period doubled the yields (Keatinge and Cooper, 1983).

Breeding for narrow xylem vessel to increase the hydraulic resistance of roots

Most cereals have a dual root system composed of seminal roots that develop from the seed and nodal roots. In wheat, there are typically three seminal axes that grow from each seed. As the seminal roots develop well before the nodal roots, they grow deepest into the subsoil. When it is dry, crops are largely reliant on subsoil water and this water must pass through the single xylem vessel in each axis. If plants use this water too fast during the vegetative period, then little will be available for grain filling. Use of subsoil water is slowed if there is a large hydraulic tolerance in the seminal roots. In this regard, Passioura (1972) proposed that, breeding for a narrow xylem vessel in the seminal roots of wheat should increase the hydraulic tolerance and force the plants to use the subsoil water more slowly. An attractive feature of this proposal was that, if the soil is wet, and then there would be no growth penalty as the nodal root system that is very extensive in the topsoil can adequately supplies the crop with water. A breeding program in wheat was initiated after developing a screening protocol for xylem vessel diameter in the seminal roots after identifying suitable genetic variation. An understanding of the genetic control and of the environmental factors associated with xylem vessel diameter was also investigated to ensure that, a breeding program would be successful (Richards and Passioura, 1981a and 1981b). The breeding program reduced the xylem vessel diameter of two Australian commercial wheat varieties from 65 to less than 55 mm. In the field trials in Eastern Australia, narrow vessel selections averaged over both genetic background and yielded 8% more than the unselected controls in the driest environments.

Anthesis-silking interval (ASI) in maize

In maize, drought that occurs from mid to late vegetative stage onwards does not affect the timing of tassel anthesis, but delays the process of ear silking. In segregating genotypes under drought (for the same anthesis date), a lengthening of the ASI period is an indicator of poor tolerance to drought, is negatively correlated with grain yield and has a higher heritability than yield (Bolanos and Edmeades, 1996; Chapman and Edmeades, 1999). The reason for the silk delay is that the drought-susceptible genotypes allocate less assimilates to ear growth when the ears are quite small. Even if these silks are pollinated separately, many of the grains will abort resulting in a low grain number per ear. Recurrent selection for low ASI, high fertile ear number per plant, small tassels and delayed senescence resulted in substantial increases in partitioning to early ear growth and successful grain set. Substantial genetic variation has been found in tropical maize populations and recurrent selection for the ASI resulted in significant increase in grain yield when the selected populations were grown under drought as well as under low soil nitrogen (Bruce *et al*., 2002). This germplasm has also performed well under drought in southern and eastern Africa (Banziger *et al*., 2005) and across most drought prone locations in both tropical and temperate sites (Bruce *et al*., 2002).

Nitrogen fixation in soybean

Nitrogen fixation in legumes is very sensitive to soil drying. In dry soils, this results in a reduced supply of N to the plant and lower yields (Sinclair *et al*., 1987; Purcell and King, 1996). There is substantial genetic variation in N_2 fixation sensitivity to soil drying in soybean (Sall and Sinclair, 1991) and a method to select for sensitivity has been developed. Soil drying results in the accumulation of ureides in the leaves of soybean (Sinclair and Serraj, 1995) and this is thought to inhibit further nodule activity. These products of nitrogen fixation are associated with sensitivity to water deficit. It has been proposed that, screening for petiole ureide levels would be an effective initial screen to identify soybean lines whose nitrogen fixation is more tolerant of soil drying (Sinclair *et al*., 2000). Little is known about the genetic control of N_2 fixation sensitivity. However, the variety Jackson, which is more tolerant of N_2 fixation in drying soils has been used as a parent in a breeding program and high yielding lines in the absence of irrigation have been identified in multi site trialing (Sinclair *et al*., 2004). These lines are now being used in breeding programs in the USA for improved tolerance to drought.

Transpiration efficiency in wheat

Where water is scarce, any improvement in the efficiency of photosynthesis per unit of transpiration i.e., transpiration efficiency (TE) should improve crop yields provided the efficiency of conversion of biomass to grain (harvest index) does not decrease. Farquhar *et al.,* (1982) proposed that, the isotopic composition of plant carbon should reflect differences in transpiration efficiency in C-3 species. There are several isotopic forms of carbon that occur naturally in the biosphere. ^{12}C is the most common form and accounts for 98.9% and ^{13}C accounts for almost all of the rest. Plants actively discriminate against ^{13}C during photosynthesis. This discrimination can occur firstly during the diffusion of CO_2 from the air into the sub-stomatal cavities and secondly, during the biochemical fixation of CO_2 into simple sugars. The result is that, plants have less ^{13}C than the atmosphere. In this regard, Farquhar and Richards (1984) have demonstrated that, the degree of discrimination is related to TE in wheat and that there was a genetic component in the extent of this discrimination. Carbon isotope discrimination is negatively correlated with TE. The relationship between carbon isotope discrimination and TE was confirmed in many C-3 crops (Condon and Richards, 1993). However, it has also been shown that this relationship does not always translate to improved grain yields and it depends on soil water availability (Condon *et al.,* 1987 and 2002).

Osmotic adjustment in wheat

As soil dries or evapotranspiration increases, leaf water potential declines. To minimize water loss from cells and to maintain cellular function, cells accumulate solutes. This process is called osmotic adjustment or osmoregulation. It is an active process where solutes increase in plant cells so as to maintain leaf hydration and turgidity. There has been some conjecture as to whether osmoregulation is important in crops as its benefit is most evident at yield levels that some consider uneconomical (Serraj *et al*., 2002). However, in the best studied case, (Morgan and Lecain, 1991;

Morgan, 2000) identified an osmoregulation “or” gene in wheat, which was associated with increased grain yield particularly under conditions of high evaporative demand. The recessive ‘or’ gene associated with potassium accumulation (Morgan, 2000) is common in Australian wheat breeding programs and trials conducted using backcross lines with and without the “or” gene and more random fixed lines with and without the ‘or’ gene shows that, higher yields are associated with the gene in the most stressed environments.

Stay-greenness in sorghum

Post-anthesis drought is a common feature of sorghum crops. In Australia, sorghum crops gradually deplete soil water resulting in leaf senescence and low yields. Stay-green lines have been identified which retain more green leaves under terminal drought compared with lines and hybrids without the stay-greenness trait and the stay-green lines have a higher yield of grain and biomass (Borrell *et al*., 2000). Several different sources of genetic variation for stay-greenness have been found in sorghum native to Africa. In these lines and hybrids derived from them, more nitrogen is allocated to leaves from early growth stages resulting in higher specific leaf nitrogen (SLN) (Borrell and Hammer, 2000). After anthesis, it is proposed that the higher SLN delays leaf senescence and allows further uptake of soil water and nitrogen (Borrell and Hammer, 2000). Leaf senescence is delayed and this enhances both radiation use efficiency and transpiration efficiency resulting in higher yields. In a rain-out shelter experiment, a stay-green hybrid retained some photosynthesis for an additional 15 days longer than a senescent hybrid counterpart (Borrell *et al*., 2001). Different types of stay-greenness have been recognized (Thomas and Howarth, 2000). Some are cosmetic and are not photosynthetically active, whereas others are associated with greater biomass accumulation. The genetic control of stay-greenness in sorghum is not well understood. However, up to 11 genomic regions have been reported as being important for stay greenness in sorghum (Tao *et al*., 2000; Xu *et al*., 2000).

Material and Methods

III. MATERIAL AND METHODS

One of the major constraints for productivity in agriculture is drought. Of the 1370 M ha of arable land in the world, only 274 M ha is irrigated which accounts for only 20% of the arable land. The remaining 80% of the cultivable land remained unproductive due to various reasons and the prominent among them is intermittent moisture stress as most of the crops are being cultivated in these areas under rainfed conditions. Due to unpredictable as well as uneven distribution of rainfall throughout the cropping seasons, the crop yields are invariably low. In this scenario, it is imperative to develop drought tolerant crop varieties with reasonably good yields. Selection for high yields under drought stress has already been exploited in most crops and further yield improvement is less likely. In this scenario, it is now argued that, trait based breeding should be adapted to improve productivity under water limited conditions.

For this trait based breeding approach, genetic variability for some of the relevant physiological traits associated with drought tolerance should exist in crop plants in question. Further, in addition to the genetic variability, suitable techniques / approaches to quantify the trait of interest should also exist. In the present study, with an objective of exploiting the genetic variability for some of the relevant physiological traits that confer drought tolerance such as roots and water use efficiency, an attempt was made to screen parents and inbreds of sunflower. The methodology followed in the present study is presented below.

3.1 Selection of seed material and their physiological assessment

The parental lines of sunflower comprising cytoplasmic male sterile lines (CMS lines / A lines) and fertility restorer lines (R lines) were procured from different AICRP (All India Coordinated Research Center) centers of the country. All together, 63 parental lines were procured from six different AICRP centers of the country. In addition to the parental lines, 57 inbred lines developed at AICRP center, Bangalore were also included for screening. Overall, 120 sunflower lines were tested in the present study for genetic variability in some of the relevant drought tolerance traits such as roots, water use efficiency (WUE) and such other traits. The list of parents and inbreds used in the present study is given in table 2.

Location

All the experiments were conducted on temporary root structures built above ground at the Department of Crop Physiology, University of Agricultural Sciences, GKVK, Bangalore. The site is situated at 12 ^{0} 58^{1} North latitude, 77 ^{0} 35^{1} East longitude with an altitude of 930 m above mean sea level (MSL). The first experiment with 120 lines of sunflower inbreds and parents was conducted during summer 2006 (February to May, 2006) and a confirmatory experiment (2nd experiment) with selected lines during summer 2007 (March to June, 2007)

Development of temporary root structures to screen parental lines / inbreds of sunflower for root traits and other relevant drought tolerance traits.

Some of the physiological traits such as roots, WUE and water conservation strategy associated with wax, osmotic adjustment, intrinsic tolerance at cellular level have relevance under water-limited conditions. Roots, an important trait associated with water acquisition is difficult to extract form the field grown plants and hence actual quantification of roots is extremely difficult/ rather impossible in the field grown plants. Therefore, in order to extract the roots completely from the soil, it is now envisaged to raise the plants on temporary root structures built above the ground. Since one of the objectives of the present study was to screen sunflower lines for root traits, temporary root structures were built above ground. In fact, through this approach, one can easily extract the roots completely and quantify the root traits such as root length, root volume, root length density, root weight etc. The details of the development/ construction of temporary root structures are presented below.

Development of temporary root structures

During summer 2006, temporary root structures made of solid cement blocks with a dimension 18.20 mts long x 3.00 mts wide x 1.20 mts tall were built above ground. A central partitioning wall which runs parallel to the entire length of 18.20 mts was also

Table 2: List of parental lines and inbreds of sunflower used for drought tolerance traits studies in root structures.

Sl No	Parents / inbreds	Sl No	Parents / inbreds	Sl No	Parents / inbreds	Sl No	Parents / inbreds	Sl No	Parents / inbreds	Sl No	Parents / inbreds
1	10A	**21**	CMS597A	**41**	NDR4	**61**	RHA859	**81**	IB 31	**101**	IB 82
2	11A	**22**	CMS851A	**42**	NDR7	**62**	DRSF109B	**82**	IB 34	**102**	IB 84
3	16A	**23**	CMS-L(DS) 207A	**43**	NDR-8	**63**	DRSH129B	**83**	IB 35	**103**	IB 85
4	18A	**24**	CMS-L-71-106A	**44**	P 69R	**64**	IB 03	**84**	IB 37	**104**	IB 86
5	234A	**25**	CMS-LSKP-11A	**45**	P62R	**65**	IB 04	**85**	IB 38	**105**	IB 87
6	31A	**26**	COSF-1A	**46**	P72R	**66**	IB 07	**86**	IB 41	**106**	IB 89
7	32A	**27**	FMS400A	**47**	P74R	**67**	IB 08	**87**	IB 42	**107**	IB 90
8	336 A	**28**	6D-1	**48**	P78R	**68**	IB 09	**88**	IB 43	**108**	IB 91
9	3A	**29**	6D-5-3-5	**49**	PKV103R	**69**	IB 10	**89**	IB 44	**109**	IB 92
10	44A	**30**	95-C-1	**50**	PKV104R	**70**	IB 11	**90**	IB 45	**110**	IB 93
11	60A	**31**	95-C-2	**51**	R-16	**71**	IB 13	**91**	IB 47	**111**	IB 97
12	CMS 17A	**32**	586R	**52**	R649	**72**	IB 15	**92**	IB 48	**112**	IB 98
13	CMS 67-1A	**33**	AK1R	**53**	R801	**73**	IB 18	**93**	IB 52	**113**	IB 99
14	CMS L-84-A	**34**	PKV 101R	**54**	RHA 265	**74**	IB 20	**94**	IB 55	**114**	IB 100
15	CMS LDSF-2A	**35**	LRHA 332	**55**	RHA 272	**75**	IB 21	**95**	IB 56	**115**	IB 105
16	CMS103A	**36**	LRHA-P-2	**56**	RHA 278	**76**	IB 22	**96**	IB 66	**116**	IB 108
17	CMS104A	**37**	LRHA-W-5	**57**	RHA 354	**77**	IB 26	**97**	IB 72	**117**	IB 110
18	CMS155A	**38**	LRHA-W8	**58**	RHA23	**78**	IB 27	**98**	IB 75	**118**	IB 115
19	CMS234A	**39**	NDR 6	**59**	RHA288	**79**	IB 28	**99**	IB 78	**119**	IB101
20	CMS45A	**40**	NDR-1	**60**	RHA296	**80**	IB 29	**100**	IB 79	**120**	IB118

made (Plate 1). After complete curing of the walls, the root structures were filled with red soil mixed with farm yard manure (FYM). Later, these structures were watered and allowed the soil to be compacted. This process of watering the root structures and compacting the soil was continued for about 15 days and once the soil was completely compacted, leveling was made. The idea of compacting the soil was to achieve bulk density of soil in root structure close to the field condition. Once the leveling was made, the seeds of all the 120 lines were sown separately in rows (please refer table 2 for the list of inbreds and parental lines of sunflower). Each row in fact represented a separate inbred / parental line. Sowing was done at the recommended spacing of 30 cm X 60 cm and for each genotype, two replications were maintained. Seeds of each genotype were dibbled at 5 different places on the same row at an interval of 30 cm. Before sowing, 50% N and entire dose of P and K fertilizers were applied as a basal dose and the remaining 50% N fertilizer was top dressed at star bud stage (recommended dosage of NPK fertilizer is 60:90:60 kg/ha). Once the seeds were germinated and the seedlings attained an age of around 15 days, thinning was carried out and one seedling per hill was maintained. All together, five plants were maintained in each row representing a particular inbred / parental line. During star bud stage, earthing-up was done with the addition of remaining 50% nitrogen in the form of urea. Regular watering and plant protection measures were taken up as and when required to ensure healthiness of plants throughout the experimental period.

3.1.1 Physiological assessment of sunflower parents and inbreds

When the plants were in active vegetative phase (from 40 DAS to 50 DAS; Plate 2), several growth and physiological measurements were made (Plate 3). These include Specific leaf area (SLA), Total leaf area (TLA), leaf chlorophyll content measured through SPAD and gas exchange parameters. The methodology followed for measuring the said parameters are given below.

3.1.1.1 Determination of specific leaf area (SLA)

Specific leaf area (SLA), an indication of leaf thickness was measured by taking the fully expanded leaf, more specifically, the fifth leaf from the top. Higher SLA

Plate 1: A view of temporary root structures built for phenotyping

Plate 3: Watering and measurements of Physiological parameters in root structures

indicates that the leaves are thinner, while lower SLA indicates more thickness of leaves. Generally, genotypes with low SLA are considered as relatively drought tolerant compared to high SLA genotypes and in fact this approach is being followed to screen genotypes for drought tolerance and narrowing down the number from a very large number to a manageable numbers.

Fifth leaf from the top was collected and immediately, the leaf area was measured using leaf area meter. Later, the leaf was kept for drying at 80^0 C for 3-4 days and once the leaf was dried, leaf weight was taken and SLA was computed as per the equation given below.

$$\text{SLA} = \frac{\text{Leaf area}}{\text{Leaf weight}} \ \text{cm}^2/\text{g}$$

3.1.1.2 Determination of total leaf area (TLA)

Total leaf area, an indication of total photosynthesizing area of the plant was measured following non destructive method proposed for sunflower by Nanja Reddy *et al.,* (1995). According to this method, leaf area was determined by counting the total number of leaves initially and multiplying it with a constant value 0.55 to arrive at the position of the leaf from the top to be considered for length and width measurement. If this value is turned out to be a fraction, then the value was rounded off to the nearest number and that positioned leaf from the top was used for measuring the length and width of leaf. Once the length and width was measured, this value was again multiplied with a constant value 0.69 and the total number of leaves to arrive at the total leaf area, which was expressed in cm^2.

3.1.1.3 Measurement of leaf chlorophyll content using SPAD chlorophyll meter

Leaf nitrogen status is normally manifested with the leaf chlorophyll content. A device developed by, Minolta corp., Ramsey, measures the light attenuation at 430 nm (the peak wavelength for chlorophyll a and b absorption) and that at 750 nm (near infrared) with no transmittance. The unit less value measured by the chlorophyll meter (SPAD-502) is termed as SCMR (SPAD Chlorophyll Meter Reading) and is a good estimate of chlorophyll content and hence N content. The SPAD meter (Soil Plant Analysis Development) is simple hand held equipment that operates with DC power (Volts) and is portable.

In order to measure the leaf chlorophyll content and hence the leaf nitrogen status, portable SPAD meter was clamped onto the leaf at different positions as well as on different leaves (3rd, 5th, 7th leaf from the top) of the plant and the SPAD reading was measured. The mean of SCMR reading was taken out in the end and presented as average SPAD value.

3.1.1.4 Assessing genetic variability in WUE using gas exchange parameters at single leaf level

Genetic variability for photosynthetic and transpiration rate and hence WUE can be measured at single leaf level using photosynthetic gas exchange parameters. The instrument that measures gas exchange parameters is known as photosynthetic system / Infra Red Gas analyzer (IRGA). Using portable photosynthesis system, LI- 6400 (LICOR 6400, Lincoln, Nebraska, USA), gas exchange parameters were measured and recorded. This instrument works on the principle that, any hetero-atomic gas molecules including CO_2, H_2O, NH_3, CO, SO_2, N_2O, NO and gaseous hydrocarbons like CH_3 have characteristic absorption spectra in the infrared region. Therefore, absorption of radiation by a specific hetero-atomic molecule is directly proportional to its concentration in a given air sample.

This instrument apart from measuring the assimilation rate can also measures the following parameters:

a. Volume flow rate of dry air into the cuvette ($cm^3 s^{-1}$)
b. Photon flux density incident on cuvette (μ moles $m^{-2} s^{-1}$)
c. CO_2 concentration and water vapour pressure of air entering and leaving the leaf cuvette.
d. Leaf area (cm^2)

e. Boundary layer resistance to water vapour ($m^2 s^{-1} mol^{-1}$)
f. Leaf temperature

Using several physical constants and measured parameters, the equipment computes the following gas exchange parameters:

1. Stomatal conductance to water vapour (g_s) ($mol\ m^{-2} s^{-1}$) k
2. Photosynthetic rate (A) [μ mol $m^{-2} s^{-1}$]
3. Transpiration rate (T) ($mol\ m^{-2} s^{-1}$)
4. Sub-stomatal cavity CO_2 concentration (C_i) (ppm)

In order to measure the gas exchange parameters, the healthy leaf preferably 5th leaf from the apex was clamped onto the leaf chamber and after stabilization of the readings; several gas exchange parameters were recorded. These include, net photosynthesis ($\mu mol\ m^{-2} s^{-1}$), stomatal conductance ($mol\ m^{-2}s^{-1}$), transpiration rate ($mol\ m^{-2} s^{-1}$) and intercellular CO_2 concentration (Ci, ppm). The measurements were made at an ambient CO_2 concentration of 360 ppm and a PPFD of 1200 $\mu mol.m^{-2}s^{-1}$ (Nataraja and Jacob, 1999). Photosynthetic gas exchange measurements were made only in high and low SLA type inbreds and parents of sunflower as measurement of gas exchange parameters in all the genotypes takes several days. Further, the data generated out of such measurements which spread over for days cannot be compared across the genotypes because of the time/ age difference between the genotypes measured on the first day and the genotypes measured on the last day.

3.1.1.5 Determination of WUE based on carbon isotope discrimination ($\Delta^{13}C$) technique

In nature two stable isotopes of carbon namely, ^{12}C and ^{13}C are found. Most of the carbon is ^{12}C (98.9 %) with 1.1% being ^{13}C. The overall abundance of ^{13}C relative to ^{12}C in plant tissue is commonly less than in the carbon of atmospheric CO_2 indicating that the carbon isotope discrimination occurs during the incorporation of CO_2 into plant biomass.

The $\Delta^{13}C$ in plant samples is generally determined using a sophisticated analytical instrument called Isotope Ratio Mass Spectrometer (IRMS). Here, the plant material is converted to CO_2 by combustion to determine the isotopic composition.

Fifth leaf from the top that was used for SLA measurement was taken for carbon isotope discrimination studies. After complete drying of the leaf, the leaf was grounded and made into fine powder. While grinding, care was taken to see that there is no contamination of the sample by washing the ball mill every time with acetone. Approximately, 1mg of fine powdered sample was taken in silver capsules to determine the carbon isotope discrimination using IRMS, a National Facility for stable isotope studies in biological sciences installed at the Department of Crop Physiology, University of Agricultural Sciences, Bangalore. The crimped capsules with the sample were placed sequentially in the carousel of the auto-sampler. The samples are then dropped at precise times along with an injection of pure O_2 into the oxidation reactor. Once the sample is combustioned inside the instrument, it gives the carbon discrimination values ($\Delta^{13}C$ values)

3.2 Evaluation of inbreds and parents of sunflower for root traits and other relevant traits in root structures

At the end of the experiment, i.e. on 58 DAS and just before flowering, the side walls of the root structure was dismantled and soil was washed carefully using a jet of water. Soil adhering to the roots was washed carefully and the plant along with entire root system was taken out from the root structure (Plate 4). The root system was again washed to dislodge the adhered soil particles. Once the plant was removed or harvested, the plant height was measured and the root system was separated from the plant. Later, the number of leaves were counted and five randomly collected leaves from the plant was used for the actual leaf area measurement, while the other leaves were collected and kept inside a separate paper cover for drying. The randomly selected leaves were measured for leaf area using leaf area meter and later, they were also kept for drying. Once the leaves were dried, the total leaf area of the particular plant was determined by cross multiplication.

Once the leaves were collected, the stem portion was cut into pieces and put them for drying to get dry weight of the stem. Similarly in roots, the following observations

Plate 4: Extraction of complete root system of sunflower plants from root structures

were made. This includes number of primary roots, root length of longest root, root volume and once all these parameters were recorded, root was also kept for drying. Root length was measured by taking the root measurements from the collar region up to the tip of the root using measuring scale. Similarly, root volume was measured following the water displacement method proposed by Archimedes. In this method, a known quantity of water was taken in a beaker with graduation. Then, roots were immersed into the beaker containing water and the raise in water level from the initial level was noted down. The difference between the final volume and initial volume is actually the volume of the object (here the roots) expressed in cubic centimeters.

Once leaves, stem and root were dried, they were weighed separately and by adding the weights of all these individual components, total dry matter (TDM) of the plant was arrived at.

3.2.1 Analysis of sunflower lines for drought tolerance traits and grouping of contrasting lines

Several physiological, growth and yield parameters collected from the inbred and parental lines of sunflower were subjected for statistical analysis and the complete data for each inbred/parental line is presented in the form of annexure (Annexure 1). However, as our interest is to look for genetic variability for some of the relevant physiological traits, the range and mean value of the relevant drought tolerance traits were determined. Accordingly, the range and mean values were determined for traits like total leaf area, root length, root volume, root weight, TDM and $\Delta^{13}C$. Further, in order to look for the distribution of sunflower lines for the trait of interest, frequency distribution was also plotted.

Since the main objective of the study was to look for genetic variability for drought tolerance traits and to select inbreds / parental lines with superior drought tolerance traits such as high root and low $\Delta^{13}C$ (which reflects high water use efficiency), an attempt was made to group inbred / parental lines of sunflower into high root dry weight types (hereafter referred as high root types), low root dry weight types (hereafter referred as low root types), high TDM types, low TDM types, low $\Delta^{13}C$ types and high

$\Delta^{13}C$ types. All together, six categories were made and in each category, a good number inbreds / parents with specific traits were identified and selected. In fact in each category, the lines with more than one trait in them were also observed.

In order to validate the results of the 1st experiment with respect to different drought tolerance traits as well as to test the relevance of root traits under water limited conditions, a sub sample of 18 inbred lines/ parental lines were selected from among the six groups made earlier. From each group, 3 to 4 representative lines were selected and while selecting the lines, care was also taken to see that these lines belong to two distinct categories of roots namely, low root types and high root types. This type of selection was made not only to validate the results of the first experiment but also to test the relevance of roots under water-limited condition.

3.3 Confirmatory test and testing the relevance of roots under water limited conditions

A plant with good root system is expected to perform well under water-limited conditions as it has an option of absorbing water from the deeper layer of the soil. In order to study the relevance of the roots in sunflower under water limited conditions, the selected 18 contrasting lines were sown again in root structures during summer season of 2007 (March, 2007) and moisture stress was imposed during the active vegetative phase. As explained in chapter 3.1.3, temporary root structures were built and the seeds of selected lines were sown following the package of practice's recommendations. All agronomic and plant protection measures were taken up to ensure that the plants are normal and healthy. Seeds were sown in two different root structures to impose two treatments namely, control and stress. After seed germination, plant population was maintained at the recommended spacing and regular watering was done for the plants of both the treatments up to 40th day from the date of sowing. From 40 days onwards up to 60 DAS, moisture stress was imposed by withholding watering in one of the root structures. Before the imposition of moisture stress, some of the observations on plant height, leaf area were measured and recorded in both control and stress designated plants. At the end of stress period (after 60 DAS), plants were allowed to recover for 10 days from stress by irrigating the root structures. At the end of recovery period, the recovery growth of plants was recorded. The details of stress imposition like duration of stress period, stage of stress imposition, recovery period and harvesting time are given in table 3.

During the stress period more specifically during the fag end of the stress period when the plants were really experiencing stress, several physiological parameters such as SLA, SCMR (SPAD chlorophyll meter readings) and gas exchange parameters were recorded. After recovery from stress, again plant height and leaf area were recorded in plants of both the treatments. The data generated was then correlated for several traits between control and stress treatments.

3.3.2 Assessment of selected sunflower lines for growth, roots, WUE, TDM and other relevant drought tolerance traits

Just at the beginning of flowering, plants from both the treatments were harvested from the temporary root structures. From the harvested plants, leaves, stem, head and root portions were separated and were kept for drying. Before keeping the leaves for drying, the total leaf area was recorded. Similarly, as root forms one of the important traits for drought tolerance, several measurements were made in the roots. These measurements include number of primary roots, root length, root volume and root dry weight. Once the TDM components were oven dried, their dry weight was measured and the TDM was computed by adding the components of TDM such as total leaf weight, stem weight, head weight and root weight. In addition to these components, the leaf samples collected for different purposes during the experimental period were also included to total leaf weight. Several components contributing for TDM were made to relate with each other and appropriate explanations have been given for the same.

3.4 Examining the consistency of traits across seasons

In order to study whether the drought tolerance traits are consistent across seasons, the data of the selected lines used for confirmatory test were made to correlate with the first season data. Accordingly, the correlation was made for traits such as SLA,

Table 3: Details of moisture stress imposition in selected lines of sunflower

Treatments	Time/ duration	Stage
Moisture stress imposition	40 DAS	Active vegetative phase
Ending of stress period	60 DAS	
Total duration of stress period	20 days	
Alleviation of stress	60 DAS	Reproductive phase
Recovery from stress	70 DAS	
Total duration for recovery	10 days	
Harvest	74 DAS	

Note: DAS- Days after sowing

total leaf area, root weight, TDM and $\Delta^{13}C$ and the results were discussed based on the correlation results.

3.5 Relevance of root traits under water limited conditions

While selecting the sunflower lines for confirmatory test out of the 120 lines evaluated during first season, care was taken to see that the selected lines belong to two distinct groups namely low root types and high root types. Accordingly, out of the 18 lines selected for confirmatory test, the lines coming from low root types, low TDM types and high $\Delta^{13}C$ types incidentally showed low root dry weight where as the lines coming from high root types and low $\Delta^{13}C$ category showed high roots. Although the lines from high TDM types possessed high biomass, their root biomass was moderate and hence these two lines namely, IB 91 and IB 98 were not included under any category and accordingly, their data was not taken into account while testing the relevance of low root types and high root types under water limited conditions. However, for other analysis, the data from these two lines were included. Therefore with the same set of material, apart from validating the results of the first experiment, the relevance of roots under water-limited conditions was tested. During the experimental period, several physiological, growth and yield parameters were recorded in both the categories of plants both under control and stress treatment. These parameters include root length, root volume, root weight and TDM etc. Based on the data/ results, the extent of reduction/enhancement in several of these traits between control and stressed plants under high root category as well as low root category were compared and the results were discussed.

Results

IV. RESULTS

Drought has been the major concerns for Crop Scientists all over the world and attempts are on to develop drought tolerant crop varieties in many species. However, the breeding efforts in this direction to develop drought tolerant crop varieties have not given rich dividends mainly because, most often, the drought tolerance in crop plants is associated with low productivity. Therefore, crop varieties / hybrids should not only be drought tolerant but also be productive under water-limited conditions. Selection for high yields under drought stress has already been exploited and further yield improvement through selection is unlikely. However, it is now argued that, through trait based breeding approach it is possible to improve the productivity under water limited conditions (Condon *et al*., 2004; Richards, 2006).

In the present study, with an objective of identifying the sunflower lines with superior drought tolerance traits, an attempt was made to screen sunflower parental lines and inbreds for relevant drought tolerance traits such as roots, water use efficiency with high biomass. All together, 120 lines comprising 63 parental lines and 57 inbreds were tested and the results of several experiments conducted are presented in this chapter.

4.1 Raising of plant material and assessing them for genetic variability for some of the relevant physiological traits associated with drought tolerance

The seeds of all the 120 lines were sown in root structures as per the package of practice’s recommendations. Of these 120 lines, seeds of 13 lines did not germinate and hence the data was collected from the remaining 107 lines. When the plants of these lines attained 35-40 days old, several growth parameters were measured. These measurements include specific leaf area, total leaf area, leaf chlorophyll content and gas exchange parameters. At harvest, root traits such as root length, root volume, root weight and TDM (which includes leaf, stem and head dry weight) were measured. In addition, $\Delta^{13}C$, a surrogate measurement of WUE was also measured in all the sunflower lines and the data on all the parameters is presented in table 4.

Table 4: Genetic variability among sunflower lines for physiological traits associated with drought tolerance.

Trait	Range	Mean	SD	CD @ 0.05	CV (%)
TLA (cm^2)	1791- 10475	5143.00	1715	257.27	18.57
SLA ($cm^2 g^{-1}$)	80 - 262	156.00	29.00	60.80	19.42
Root length (cm)	20.33 - 81.25	43.78	11.88	18.52	20.52
Root volume (cm^3)	20 - 335	112.73	63.98	67.26	21.68
Root dry weight (g)	5.47 - 137.00	39.76	29.50	24.92	23.45
TDM (g)	85.80 - 356.99	184.44	64.30	51.62	19.79
$\Delta\ ^{13}C$ (‰)	18.23 – 24.63	20.95	1.33	0.37	6.67

The results of the study indicated that, there is significant and wide genetic variability for several of those traits mentioned above. Accordingly, total leaf area (TLA), an important part of the plant for food synthesis showed a significant genetic variability. The total leaf area ranged from as low as 1791 cm^2 to as high as 10475 cm^2 with a mean of 5143 cm^2. The data clearly indicated a wide genetic variability for this important trait (Table 4). Like TLA, specific leaf area (SLA), an indication of leaf thickness also showed a significant genetic variability where its value ranged form 79 cm^2g^{-1} to as high as 262 cm^2g^{-1} with a mean of 155 cm^2g^{-1}. The results clearly indicated the existence of genetic variability for leaf thickness as well.

With respect to root traits, a significant and wide genetic variability for traits like root length, root volume and root weight was found (Plate 5). Accordingly, the root length ranged from 20.33 cm to 81.25 cm with a mean root length of 43.78 cm. Similarly, root volume, another important root trait ranged from 20.00 cm^3 to 335.00 cm^3 with a mean of 112.73 cm^3. Further, root biomass ranged from as low as 5.47 g to as high as 137.00 g with a mean of 39.76 g. The data therefore clearly indicated the existence of a wide genetic variability for an important drought tolerance trait, root trait (Plate 5). Further, as the total dry matter (TDM) contributing characters are significantly varied across the lines, one would expect significant genetic variability for TDM as well. Accordingly, in the present study with many sunflower parental lines and inbreds, the TDM ranged from 85.80 g to as high as 356.99 g with a mean of 184.44 g (Table 4). Since high TDM coupled with good root system is essential under water limited conditions, the existence of wide genetic variability in sunflower lines provided an option to select for high TDM types coupled with good root dry matter types.

$\Delta^{13}C$, a surrogate approach to quantify water use efficiency (WUE) also showed a significant variability with its value ranged from 18.23 ‰ to 24.63 ‰ with a mean of 20.95 ‰. In fact, such a wide variability is not so common in many species. However, sunflower being a highly cross-pollinated crop has given us tremendous scope to select for highly desirable trait such as high WUE with its heterogeneity (Table 4).

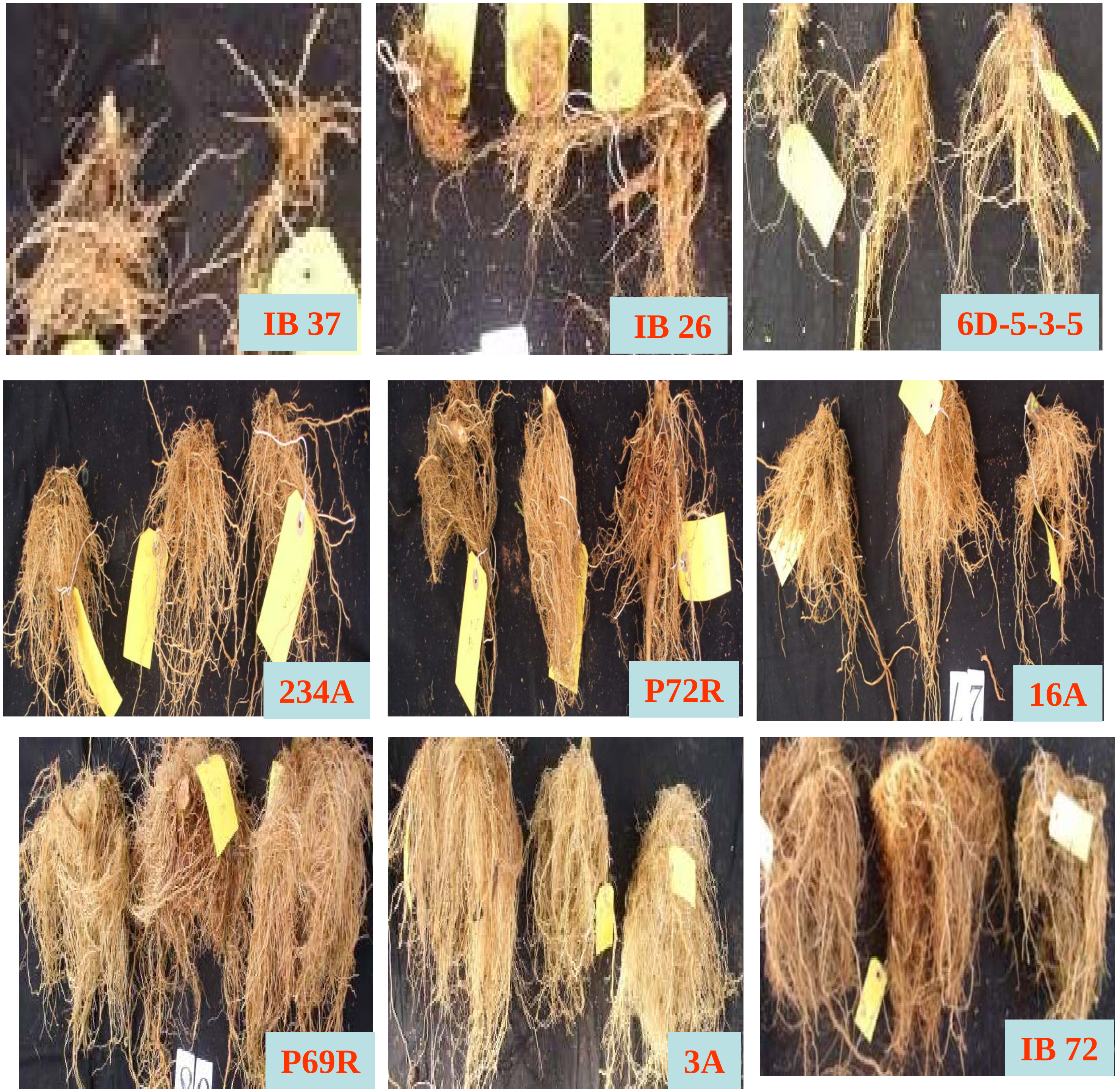


Plate 5: Genetic variability for root traits in inbreds and parental lines of sunflower

The frequency distribution drawn for several of these traits mentioned above showed different types of distribution. Accordingly, the total leaf area showed a normal distribution, while root weight showed somewhat positively skewed distribution with majority of the lines exhibiting low to medium roots. However, with respect to TDM and Δ^{13}C, the frequency distribution curve is neither normal nor positively skewed but lies in between these two categories (Fig 2-5).

The actual growth and physiological parameters of the individual parental lines and inbreds used in this study are presented in Annexure I (107 No's only). Based on the objectives, need and looking into the traits of interest, one can select the parental lines and inbreds for further crop improvement / crop breeding programmes.

In all the parental lines and inbreds of sunflower, how the TDM and its components are related to each other was examined. Accordingly, an attempt was made to correlate many of the growth parameters with TDM. From the results, a positive and significant relationship between TLA and TDM was found to indicate that, the biomass production in sunflower lines (like any other crops) depends on the total leaf area (Fig 6). Similarly, root weight also showed a significant positive relationship with TDM to indicate that TDM depends on the root weight apart from stem weight and total leaf area/ total leaf weight (Fig 7).

In addition to leaf and root traits, an attempt was also made to determine how WUE (determined through carbon isotope discrimination approach) is related to TDM. Accordingly, a significant and negative relationship between TDM and Δ^{13} C was found (Fig 8). The data therefore clearly indicated that, the parental lines / inbreds with high biomass producing ability tend to be high WUE types as well (low Δ^{13}C types). These lines are not only high productive but also high WUE types and hence such lines can be used for crop improvement programme to develop crop varieties / hybrids for water limited conditions. Specific leaf area (SLA), an indication of leaf thickness exhibited no distinct relationship with SPAD readings (Fig 9).

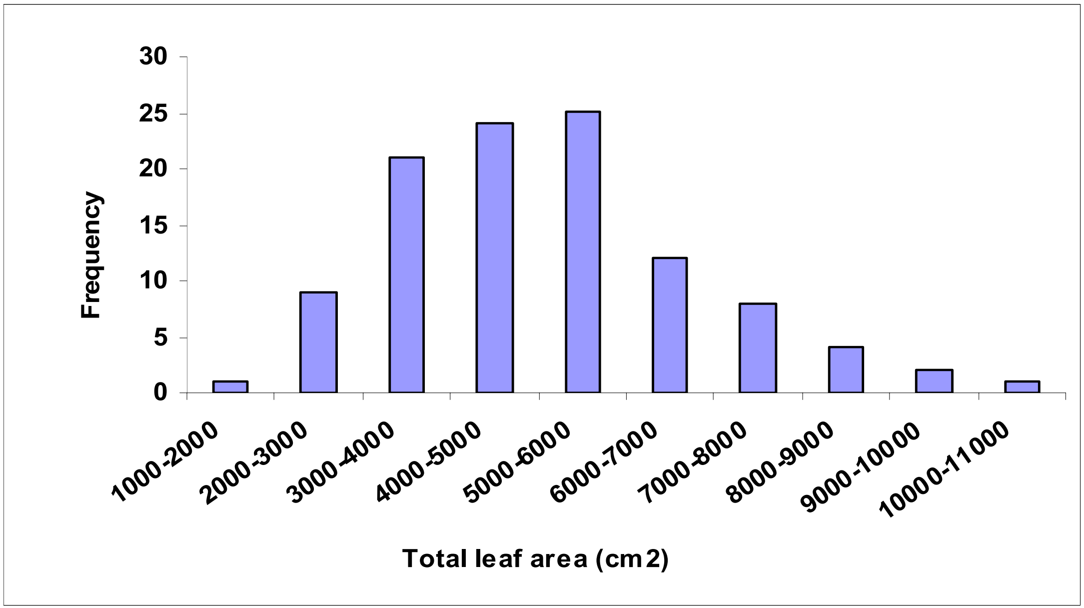


Fig 2: Frequency distribution for total leaf area in parental lines and inbreds of sunflower

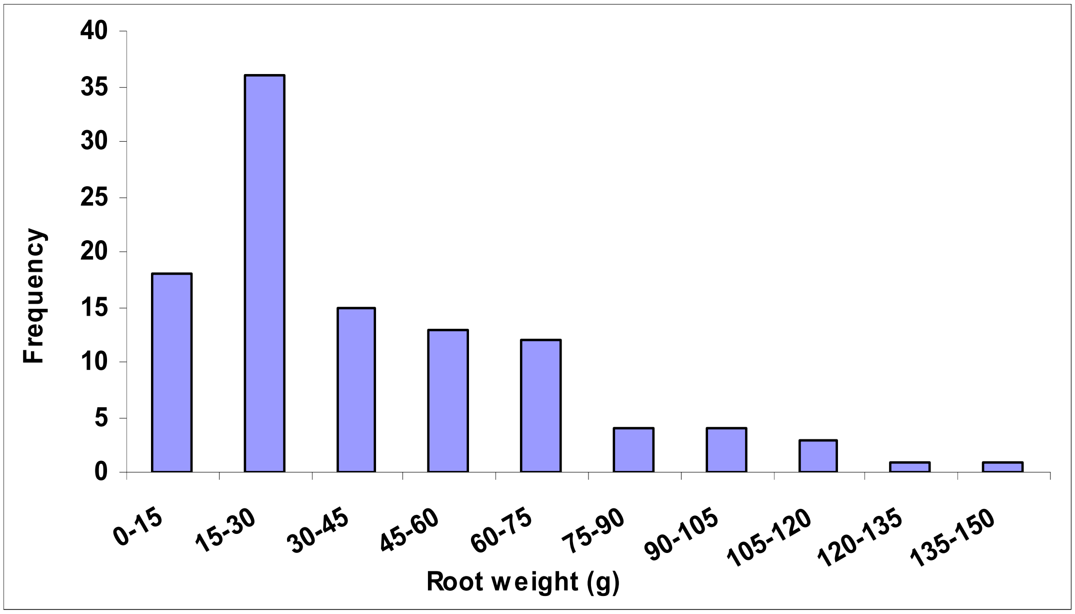


Fig 3: Frequency distribution for root weight (g) in parental lines and inbreds of sunflower

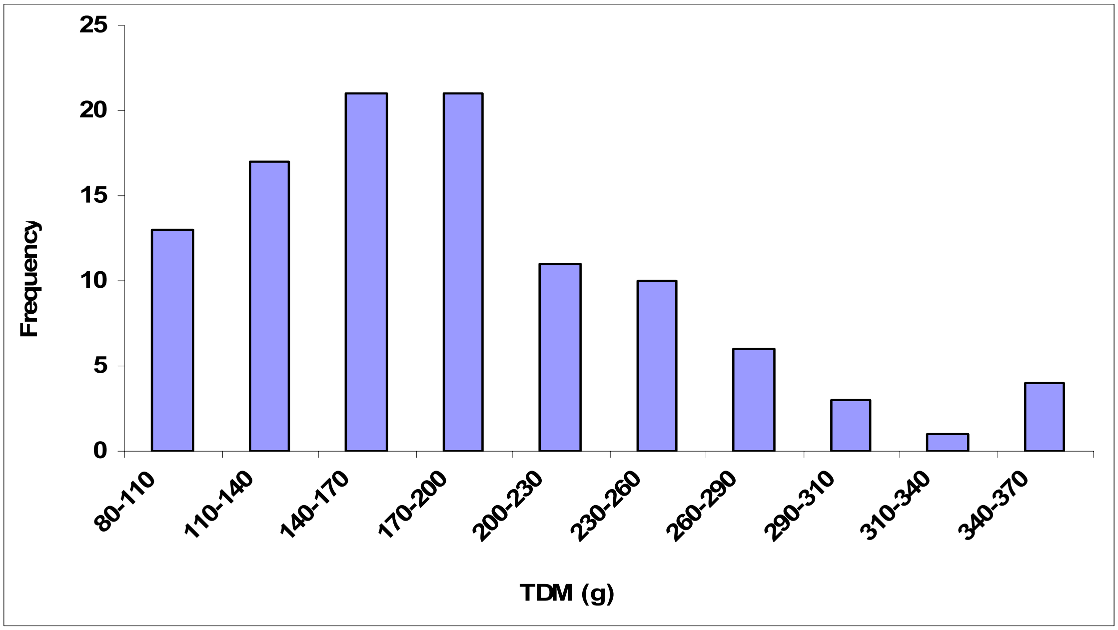


Fig 4: Frequency distribution for total dry matter (TDM) in parental lines and inbreds of sunflower

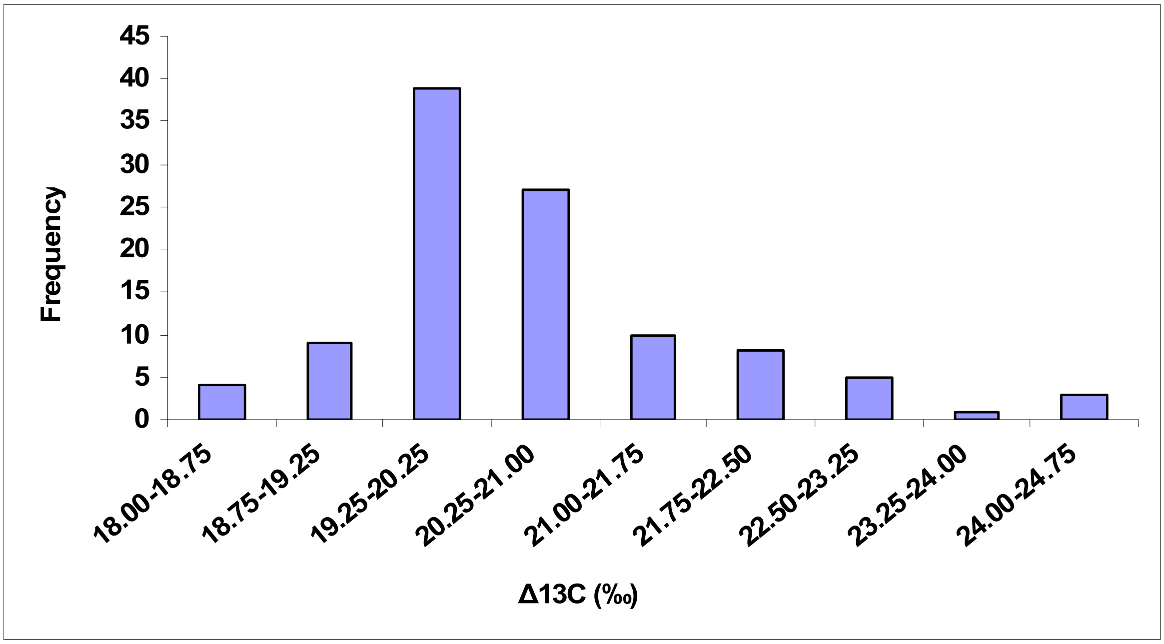


Fig 5: Frequency distribution for $\Delta^{13}C$ (‰) in parental lines and inbreds of sunflower

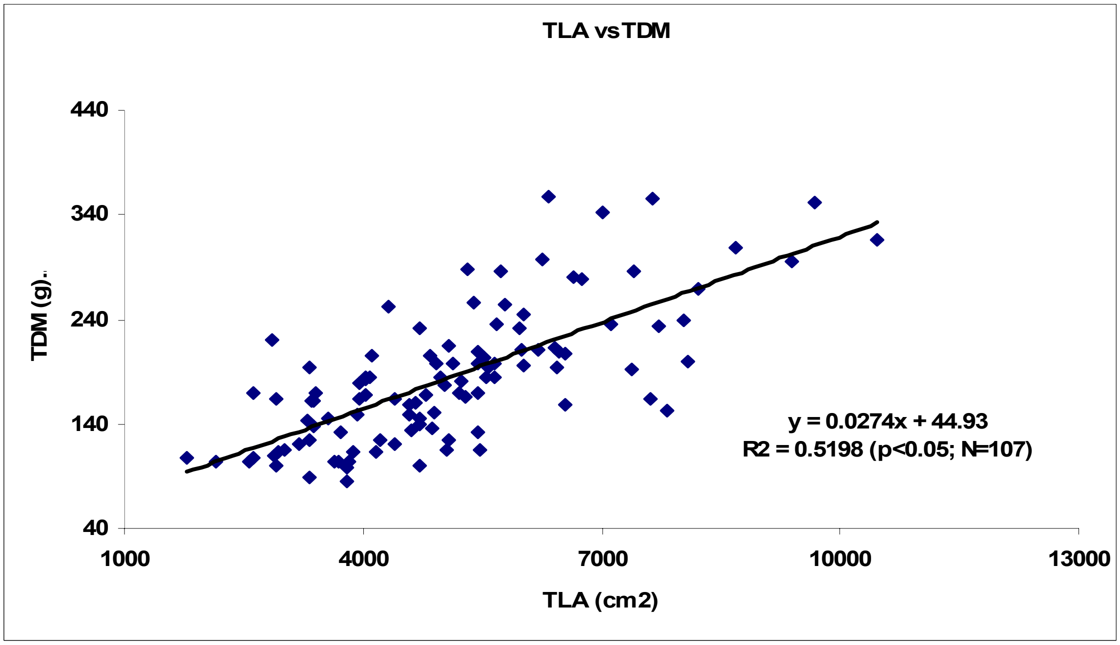


Fig 6 : Relationship between total leaf area (cm^2) and TDM (g) in parental lines and inbreds of sunflower

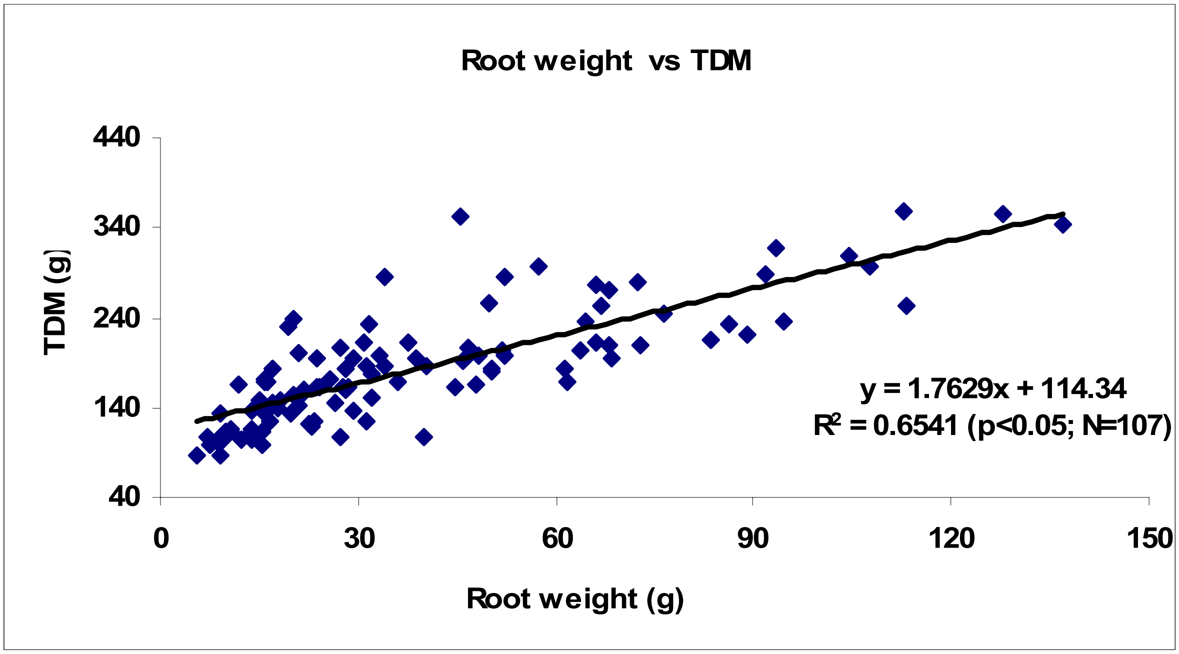


Fig 7: Relationship between root weight (g) and TDM (g) in parental lines and inbreds of sunflower

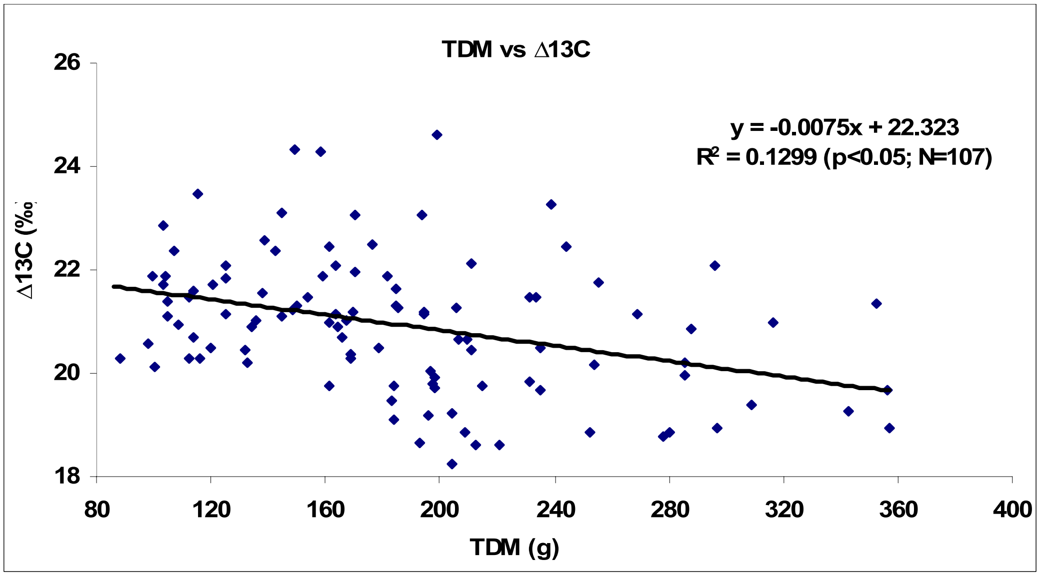


Fig 8: Relationship between TDM (g) and $\Delta^{13}C$ (‰) in parental lines and inbreds of sunflower

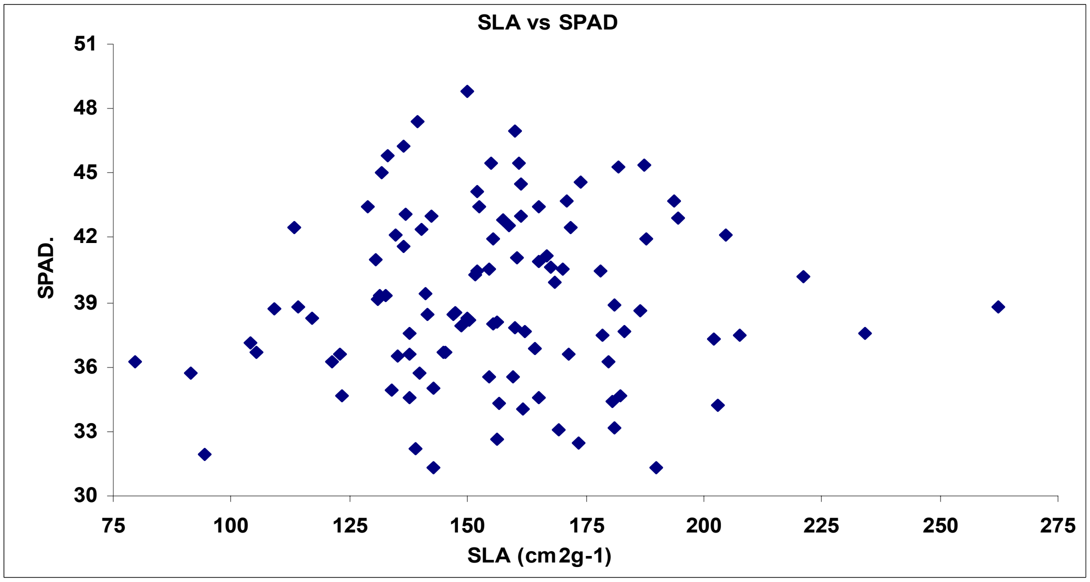


Fig 9: Relationship between SLA (cm^2g^{-1}) and SPAD values in parental lines and inbreds of sunflower

4.2 Genetic variability for WUE assessed at single leaf level using gas exchange parameters

Genetic variability for WUE and other photosynthetic parameters across the parental lines and inbreds were tested following gas exchange approach. For gas exchange measurements, high and low SLA types were selected and with the help of portable photosynthetic system (LICOR-6400 L), gas exchange parameters such as stomatal conductance, transpiration and assimilation rate were measured.

The results of the study indicated a wide and significant variability in gas exchange parameters in the selected parental lines and inbreds (Table 5). Further, a significant and positive relationship between stomatal conductance and assimilation rate (Fig 10) was observed to indicate that, whenever stomata is wide opened, the amount of carbon diffusion will be more and thus, the carbon fixation (assimilation) tend to be more. Similarly, a significant negative relationship was also found between the assimilation rate and WUE measured at single leaf level (A/gs) to indicate that, WUE tend to decrease whenever assimilation rate is more (Fig 11).

4.2.1 Selection and grouping of contrasting lines for different traits

One of the major objectives of the present study was to look for genetic variability for some of the relevant drought tolerance traits and to select the parents and inbreds with superior drought tolerance traits such as high root and high WUE coupled with high biomass. In this direction, an attempt was made to group and select the contrasting lines for traits like roots, $\Delta^{13}C$ (WUE) and TDM. Accordingly for each trait, highly contrasting lines were grouped and selected.

With respect to root traits, two groups were made based on root dry weight. These groups include low root dry weight types (hereafter referred as low root types; Plate 6) and high root dry weight types (hereafter referred as high root types; Plate 7 and Table 6 & 7). Totally, 10 lines under low root type category and 15 lines under high root type category were found. In the low root type category, the average root weight was 8.48 g, while in high root types, it was 97.51g. Between low root types and high root types, the

Table 5: Gas exchange parameters in selected lines of sunflower (high and low SLA types)

Parental line / inbred	**gs (mol $m^{-2}s^{-1}$)**	**T (mol $m^{-2}s^{-1}$)**	**A (µmol $m^{-2}s^{-1}$)**	**A/gs**
CMS 104A	1.43	8.74	22.40	15.72
NDR 4	1.91	9.11	36.60	19.13
234A	1.96	8.20	32.90	16.80
IB 13	1.61	7.63	42.80	26.59
R649	2.02	7.42	34.30	16.99
IB118	2.06	8.30	47.80	23.20
CMS-LSKP-11A	1.78	7.87	43.70	24.55
IB82	0.73	5.78	28.20	38.65
Kh04PKV 103R	1.22	6.41	38.80	31.90
IB97	1.15	6.69	34.30	29.83
CMS103A	1.45	6.72	46.60	32.10
IB 85	1.75	6.58	42.90	24.58
NDR-8	1.71	6.43	43.80	25.62
IB34	2.08	7.16	45.69	21.98
IB78	1.76	7.72	40.90	23.30
IB56	1.56	8.14	36.80	23.59
RHA296	1.47	7.63	25.50	17.34
IB28	1.87	7.45	40.20	21.45
6D-1	1.59	9.23	34.40	21.63
R-16	0.72	5.49	31.20	43.11
LRHA-P-2	0.36	3.71	23.60	65.49
RHA859	0.85	5.96	25.00	29.56
18A	0.46	5.23	25.50	55.04
IB31	0.67	5.85	20.20	30.35
CMS597A	0.48	4.77	15.10	31.62
IB29	0.39	4.90	26.10	66.50
10A	1.24	6.94	36.90	29.69
CMS109B	1.43	7.97	35.40	24.73
IB07	1.40	8.74	40.90	29.28
CMS67-1A	1.19	8.20	37.30	31.46
NDR 6	0.39	4.60	20.40	52.26
IB 91	0.41	4.59	25.10	60.65
Kh04 AK1R	1.48	8.24	39.40	26.61
IB77	0.82	6.54	32.80	39.90
IB27	0.73	6.42	30.30	41.40
336A	0.54	5.13	28.00	51.76
IB100	0.58	4.96	27.30	47.41
IB90	0.46	4.46	29.60	64.05
31A	0.49	4.62	19.00	38.89
CMS 101A	0.28	3.60	16.40	58.03
CV (%)	**17.30**	**15.56**	**10.74**	**21.49**
CD @ 0.05	**1.03**	**5.48**	**9.56**	**11.46**

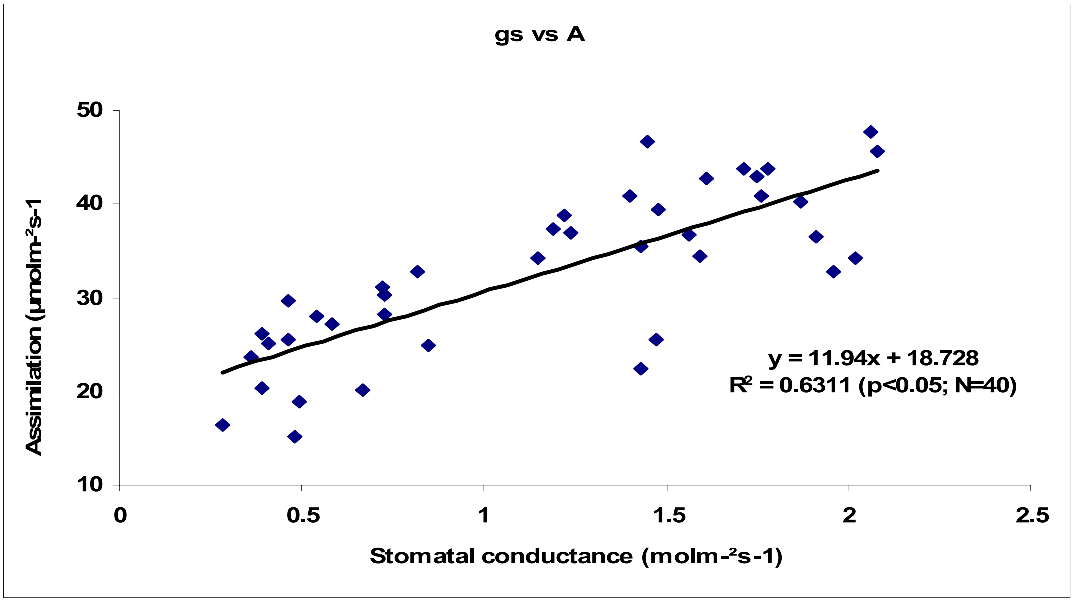


Fig 10: **Relationship between stomatal conductance (mol m^{-2} s^{-1}) and assimilation rate (µmolm-²s^{-1}) in parental lines and inbreds of sunflower**

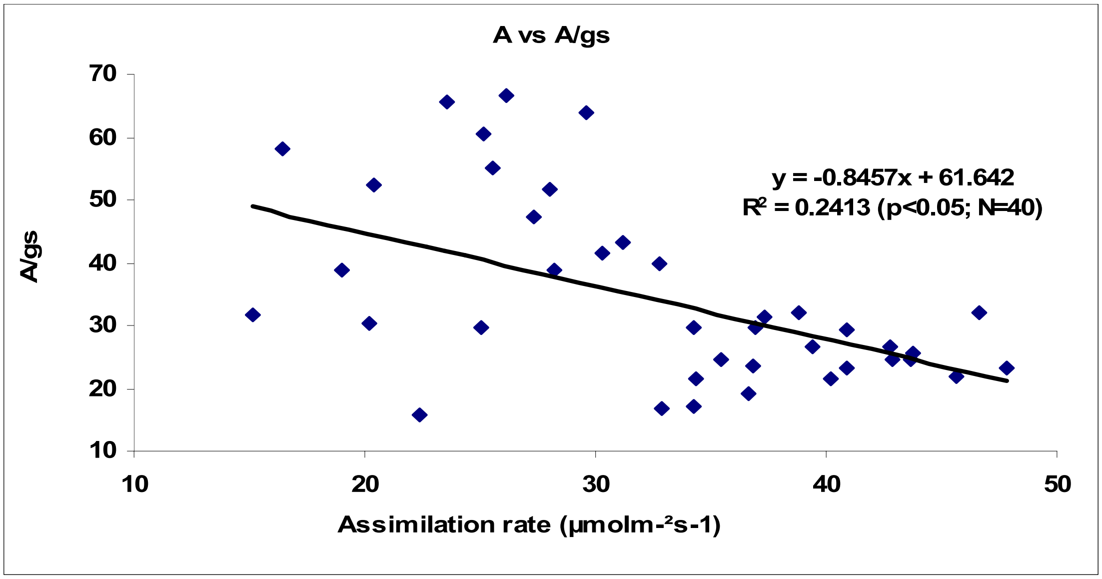


Fig 11: **Relationship between assimilation rate (µmolm-²s^{-1}) and WUE (A/gs) measured at single leaf level in parental lines and inbreds of sunflower**

Table 6: Low root type sunflower lines grouped based on root dry weight

Inbred / parental lines	SLA (cm^2 g^{-1})	TLA (cm^2)	Root dry weight (g)	TDM (g)	Δ ^{13}C (‰)
LRHA-P-2	194	3804	5.47	85.80	22.30
IB 26	180	2619	7.20	108.43	20.93
AK1R	160	2902	7.65	99.74	21.89
R 801	155	2139	8.87	104.74	21.39
RHA 296	178	4703	8.90	100.46	20.13
IB 101	131	3727	8.93	132.33	20.21
RHA 288	155	3637	9.12	103.21	22.86
6D-5-3-5	147	3332	9.30	87.97	20.28
R 649	203	3704	9.40	104.33	21.87
95-C-2	156	4170	9.93	114.08	21.58
MEAN	**166**	**3474**	**8.48**	**104.11**	**21.68**
SD	**22**	**753**	**1.33**	**13.10**	**1.65**

Table 7: High root type sunflower lines grouped based on root dry weight

Inbred / parental lines	SLA ($cm^2 g^{-1}$)	TLA (cm^2)	Root dry weight (g)	TDM (g)	Δ ^{13}C (‰)
PKV103R	80	7021	137.00	342.78	19.26
CMS 597A	165	7635	127.93	355.96	19.69
CMS-L-71-106A	142	4320	113.13	252.20	18.86
CMS-L(DS) 207A	181	6340	112.73	356.99	18.96
336 A	143	6267	107.80	296.75	18.94
IB 10	154	8678	104.33	308.57	19.40
3A	137	5678	94.43	235.13	19.67
IB 66	161	10475	93.28	316.36	20.97
IB 79	139	5322	91.87	287.55	20.85
IB 90	123	8858	89.03	220.88	18.62
11A	182	4725	86.40	231.37	19.82
FMS400A	182	5078	83.55	214.61	19.75
IB 20	145	6023	76.23	244.31	22.44
DRSH 129B	135	5434	72.73	209.52	20.66
IB 86	150	6645	72.27	280.65	18.88
MEAN	**148**	**6567**	**97.51**	**276.91**	**19.79**
SD	**26**	**1712**	**19.39**	**51.50**	**1.04**

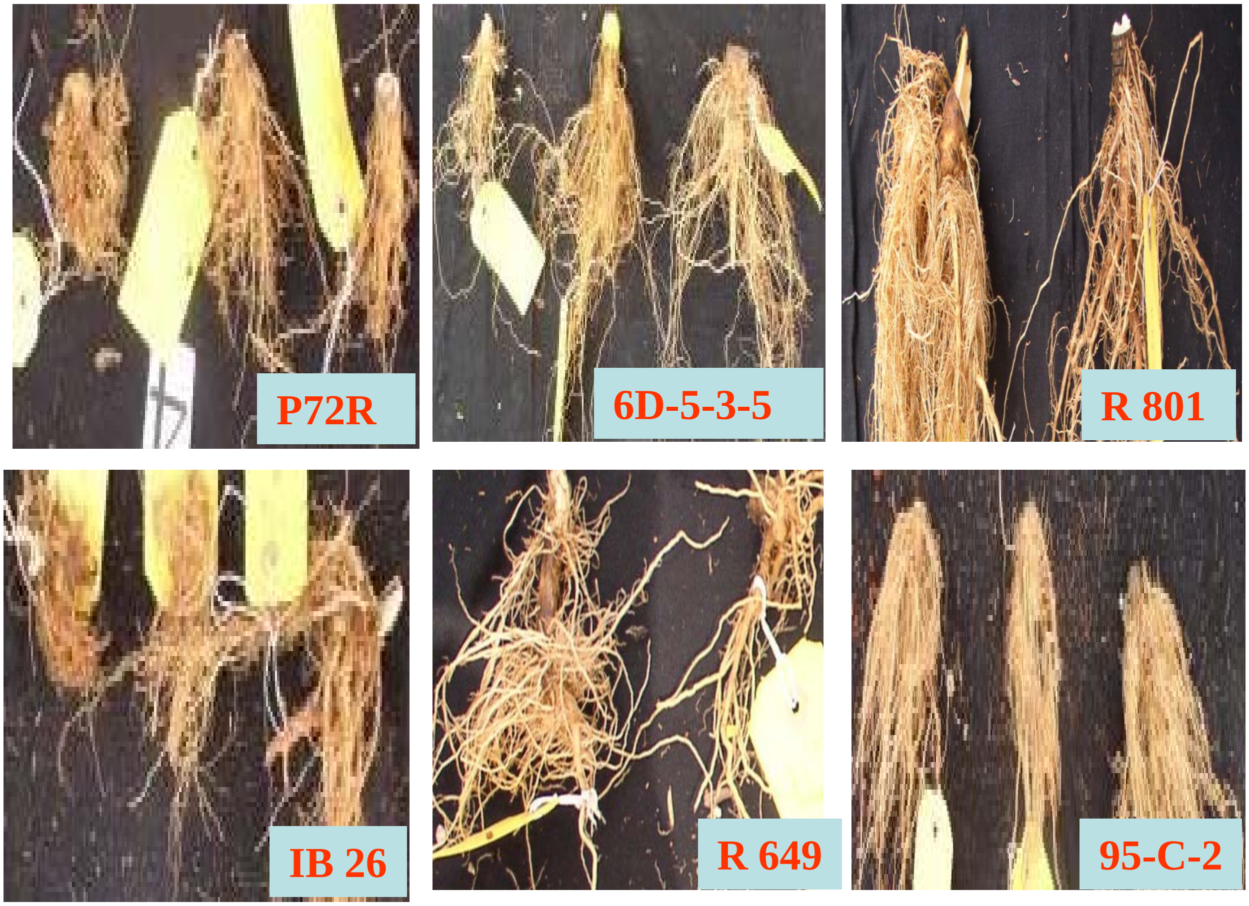


Plate 6: Low root type inbreds and parental lines of sunflower

Plate 7: High root type inbreds and parental lines of sunflower

difference in root weight was almost 12 times to indicate that the genotypes are really contrasting for root traits (Fig 12). Incidentally, the high root types are not only had good root traits but also showed more leaf area, leaf weight and hence increased TDM compared to low root type lines. Interestingly, these high root types also showed relatively high WUE as indicated by low $\Delta^{13}C$ compared to low root types. Further, they also showed lower SLA compared to low root types to indicate that the leaves of high root types are thicker and more productive compared to low root types.

With respect to $\Delta^{13}C$, 16 parents and inbreds of sunflower showed high WUE, which was indicated by low Δ13C values and about 17 lines exhibited low WUE (indicated by high $\Delta^{13}C$ values). The low $\Delta^{13}C$ types (high WUE types) recorded a mean $\Delta^{13}C$ value of 18.94 ‰ as against 23.05‰ recorded in high $\Delta^{13}C$ category (Table 8 & 9 and Fig 13). Like in the previous case with root traits, the high WUE types (low $\Delta^{13}C$) showed more leaf area, high root biomass and high TDM compared to the other contrasting group (high $\Delta^{13}C$ types). Incidentally, these low $\Delta^{13}C$ types also exhibited relatively less SLA compared to high $\Delta^{13}C$ types. The data clearly indicated that, high WUE types are indeed high TDM types and hence one can choose these lines for further crop improvement programme. Although in many cases TDM and WUE are not related, here in sunflower, they showed a good relationship.

With regard to biomass production, low TDM types produced significantly lower TDM compared to high TDM types. Totally, 19 lines under low TDM category and 17 lines under high TDM categories were identified and selected. The average TDM of low TDM types was 105.32 g, while it was 298.49 g in high TDM type category (Table 10 & 11 and Fig 14). The high TDM types by virtue of good root system, leaf area and WUE could able to put on significantly higher biomass compared to lower TDM types. These high TDM types also showed relatively high WUE (indicated by lower Δ^{13} C values) and low SLA compared to low TDM types. The overall results therefore indicated the existence of wide genetic variability for some of the relevant drought tolerance traits such as roots, $\Delta^{13}C$ and TDM and for each trait, a good number of lines with contrasting characters were identified.

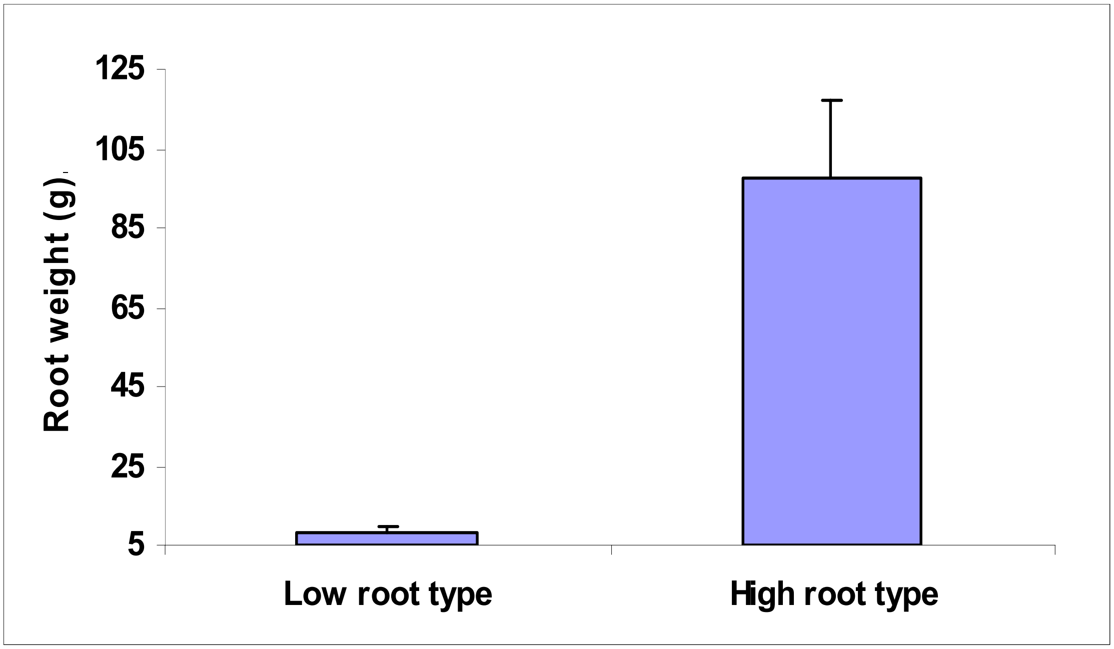


Fig 12: Mean root weight (g) in low and high root type sunflower lines

Table 8: Low $\Delta^{13}C$ (‰) type sunflower lines grouped based on $\Delta\ ^{13}C$ (‰) values

Inbred / parental lines	**SLA ($cm^2\ g^{-1}$)**	**TLA (cm^2)**	**Root dry weight (g)**	**TDM (g)**	**$\Delta\ ^{13}C$ (‰)**
IB13	181	4102	63.83	204.37	18.23
IB92	160	6405	66.20	212.65	18.60
IB 90	123	8858	89.03	220.88	18.62
IB85	94	7391	45.83	192.61	18.66
IB47	156	6743	66.03	277.95	18.77
CMS-L-71-106A	142	4320	113.13	252.20	18.86
IB 11	162	6452	68.00	208.90	18.87
IB86	150	6645	72.27	280.65	18.88
336 A	143	6267	107.80	296.75	18.94
CMS-L(DS) 207A	181	6340	112.73	356.99	18.96
DRSF109B	109	4077	61.43	184.21	19.08
IB97	234	6032	68.55	195.99	19.18
IB07	190	5520	51.93	204.02	19.22
PKV103R	80	7021	137.00	342.78	19.26
IB10	154	8678	104.33	308.57	19.40
IB 38	134	4040	50.25	182.96	19.48
MEAN	**149**	**6181**	**79.90**	**245.16**	**18.94**
SD	**38**	**1495**	**27.12**	**57.84**	**0.33**

Table 9: High Δ ^{13}C (‰) type sunflower lines grouped based on Δ ^{13}C (‰) values

Inbred / parental lines	**SLA ($cm^2 g^{-1}$)**	**TLA (cm^2)**	**Root dry weight(g)**	**TDM (g)**	**Δ ^{13}C (‰)**
R-16	205	8072	21.07	199.17	24.63
32A	150	3935	18.27	148.89	24.33
RHA23	157	6540	21.87	158.10	24.27
95-C-1	159	3024	10.87	115.72	23.46
6D-1	174	8037	20.37	238.90	23.28
IB 09	147	3561	26.40	144.79	23.10
IB 08	195	3337	23.58	193.57	23.08
IB 28	262	5199	25.57	170.27	23.06
RHA 288	155	3637	9.12	103.21	22.86
18A	181	2887	27.20	108.56	22.82
16A	104	4721	17.74	138.82	22.58
CMS155A	139	5028	32.13	176.20	22.50
IB 04	161	3352	23.98	161.42	22.45
IB 20	145	6023	76.23	244.31	22.44
IB 22	164	3299	21.03	142.81	22.39
60A	156	1792	40.03	106.77	22.35
LRHA-P-2	194	3804	5.47	85.80	22.30
MEAN	**167**	**4485**	**24.61**	**158.05**	**23.05**
SD	**34**	**1789**	**15.62**	**45.58**	**0.74**

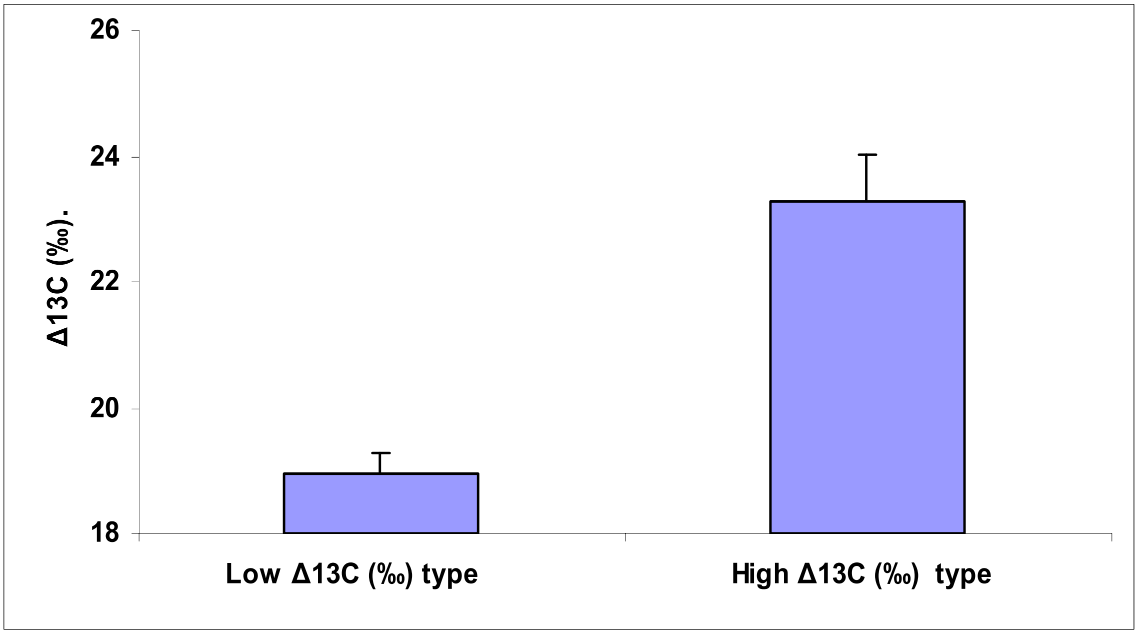


Fig 13: Mean $\Delta^{13}C$ (‰) in low and high $\Delta^{13}C$ type sunflower lines

Table 10: Low TDM type sunflower lines grouped based on TDM

Inbred / parental lines	**SLA ($cm^2 g^{-1}$)**	**TLA (cm^2)**	**Root dry weight (g)**	**TDM (g)**	**Δ ^{13}C (‰)**
LRHA-P-2	194	3804	5.47	85.80	22.30
6D-5-3-5	147	3332	9.30	87.97	20.28
P72R	131	3795	15.60	97.84	20.58
AK1R	160	2902	7.65	99.74	21.89
RHA 296	178	4703	8.90	100.46	20.13
RHA 288	155	3637	9.12	103.21	22.86
CMS L-84-A	172	2560	13.90	103.65	21.71
R649	203	3704	9.40	104.33	21.87
IB 41	178	3834	12.13	104.53	21.08
R 801	155	2139	8.87	104.74	21.39
60A	156	1792	40.03	106.77	22.35
IB 26	180	2619	7.20	108.43	20.93
18A	181	2887	27.20	108.56	22.58
586R	137	3867	15.30	112.40	21.48
P74R	208	2922	13.83	112.56	20.30
95-C-2	156	4170	9.93	114.08	21.58
P78R	104	5065	10.50	114.25	20.71
95-C-1	159	3024	10.87	115.72	23.46
10A	221	5463	13.93	116.02	20.28
MEAN	**167**	**3485**	**13.11**	**105.32**	**21.46**
SD	**28**	**955**	**8.03**	**8.52**	**0.97**

Table 11: High TDM type sunflower lines grouped based on TDM

Inbred / parental lines	**SLA ($cm^2 g^{-1}$)**	**TLA (cm^2)**	**Root dry weight (g)**	**TDM (g)**	**$\Delta\ ^{13}C$ (‰)**
CMS-L (DS) 207A	181	6340	112.73	356.99	18.96
CMS 597A	165	7635	127.93	355.96	19.69
IB91	114	9671	45.45	352.59	21.34
PKV103R	80	7021	137.00	342.78	19.26
IB66	161	10475	93.28	316.36	20.97
IB10	154	8678	104.33	308.57	19.40
336 A	143	6267	107.80	296.75	18.94
IB98	161	9379	57.27	296.09	22.06
IB79	139	5322	91.87	287.55	20.85
CMS103A	91	7401	34.15	285.90	20.20
IB56	105	5732	52.10	285.47	19.97
IB86	150	6645	72.27	280.65	18.88
IB47	156	6743	66.03	277.95	18.77
IB15	167	8212	68.23	269.29	21.13
CMS104A	123	5398	49.88	255.51	21.77
IB118	143	5775	66.70	253.67	20.18
CMS-L-71-106A	142	4320	113.13	252.20	18.86
MEAN	**140**	**7118**	**82.36**	**298.49**	**20.07**
SD	**28**	**1701**	**30.99**	**35.43**	**1.10**

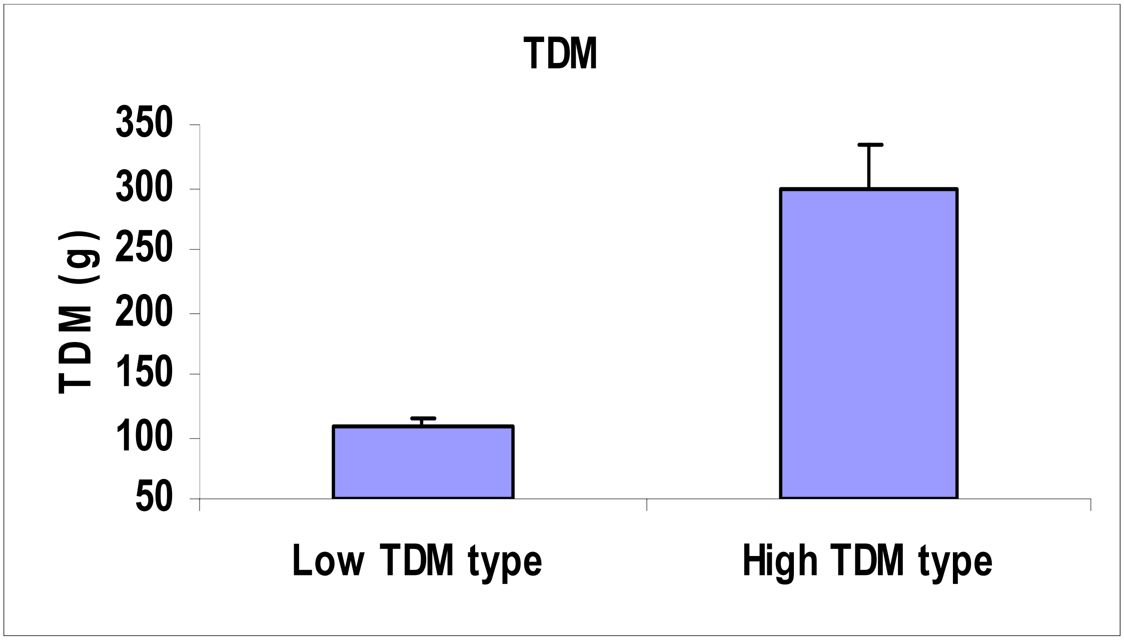


Fig 14: Mean total dry matter (g) in low and high TDM type sunflower lines

4.3 Confirmatory test and testing the relevance of root traits under water limited conditions

In order to validate the results of the first experiment, a sub set of inbreds / parental lines were selected from 6 different groups made earlier. Accordingly, four lines from high root types, two lines from low root types, five lines from low $\Delta^{13}C$ types, 3 lines from high $\Delta^{13}C$ types and two lines each from low and high TDM types were selected (Table 12 A). Although the parents and inbreds were selected from 6 different groups, majority of them fall into two distinct categories of roots namely low root types and high root types. Excepting two lines coming from high TDM types, all other selected lines either fall into low root types or high root types (Table 12 B). This actually paved the way for testing the relevance of roots under water-limited conditions. Altogether 18 parents and inbreds were selected for confirmatory test as well as for assessing their performance under water limited conditions. Basically, the objective here was to test the relevance of roots under water-limited conditions.

4.3.1 Physiological assessment of selected inbreds and parental lines of sunflower under control and stress condition

Out of the 18 sunflower lines used for confirmatory test, only 15 lines were germinated and the rest three lines namely, IB 101 (from low root type category), AK1R (from low TDM category) and 18A (from high $\Delta^{13}C$ category) did not germinate and hence the data is available for 15 out of 18 lines.

For those sunflower lines that have germinated, moisture stress was imposed by withholding irrigation from 40 DAS to 60 DAS (days after sowing). Before the imposition of moisture stress, initial plant height and initial leaf area were measured in plants of control and stress designated blocks. Accordingly, no significant difference in plant height and leaf area between the plants of control and stress treatments was observed to indicate that the plant height and leaf area were similar before the imposition of stress. However, significant difference was observed among inbreds and parental lines within the treatments for plant height and leaf area (Table 13 and Fig 15). Accordingly, some of the sunflower lines and inbreds namely, IB90, CMS106A, CMS207A had shown

Table 12 A: List of sunflower lines selected for confirmatory test/ validate the results of first experiment

Low Root types	**High Root types**	**Low $\Delta^{13}C$ types**	**High $\Delta^{13}C$ types**	**Low TDM types**	**High TDM types**
IB 101* 6D-5-3-5	11 A IB 66 IB79 IB 90	IB 11 IB 86 336A CMS106A CMS 207A	18A* IB 08 6D-1	P72R AKIR*	IB 91 IB 98

Note: Totally, 18 lines representing different groups were selected randomly for confirmatory test

* Seeds did not germinate

Table 12B: List of sunflower lines used for testing the relevance of roots under water limited condition

Inbred / parental line	Root length (cm)	Root Weight (g)	TDM (g)	Δ ^{13}C (‰)
Low root types				
AK1R*	23.75	7.65	99.74	21.89
IB101*	32.25	8.93	132.33	20.21
6D-5-3-5	36.67	9.30	87.97	20.28
P72R	39.50	15.60	97.84	20.58
6D-1	52.00	20.37	238.90	23.28
IB 08	32.50	23.58	193.57	23.08
18A*	44.75	27.20	108.56	22.82
High root types				
IB 11	30.50	68.00	208.9	18.87
IB86	47.67	72.27	280.65	18.88
11A	44.00	86.40	231.37	19.82
IB 90	28.33	89.03	220.88	18.62
IB79	37.00	91.87	287.55	20.85
IB66	56.50	93.28	316.36	20.97
336 A	35.00	107.80	296.75	18.94
CMS207A	34.67	112.73	356.99	18.96
CMS106A	35.67	113.13	252.2	18.86

Note: These lines were picked up from table 12 A based on the root dry weight to test the relevance of roots under water limited conditions

* Seeds did not germinate

Table 13: Initial plant height and initial leaf area recorded in both the treatments before the imposition of stress.

Inbred / parental line	Initial Plant Height (cm)			Initial leaf area (cm^2)		
	Control designated plants	Moisture stress designated plants	Mean	Control designated plants	Moisture stress designated plants	Mean
IB 08	85.00	78.67	81.83	3928	3754	3841
IB 11	68.00	72.50	70.25	3597	3499	3548
IB 66	64.00	63.00	63.50	2606	2683	2645
IB 79	61.33	66.33	63.83	1895	1921	1908
IB 86	87.00	84.00	85.50	4418	4191	4305
IB 90	111.00	105.33	108.17	8122	8014	8068
IB 91	87.33	79.67	83.50	4209	4067	4138
IB 98	75.00	69.33	72.17	5721	5506	5614
11A	69.00	63.33	66.17	2869	2947	2908
CMS 106A	105.67	97.33	101.50	4482	4535	4508
CMS 207A	96.00	94.00	95.00	5509	5133	5321
336 A	85.33	80.67	83.00	5443	5159	5301
6D-1	62.33	56.67	59.50	2936	2880	2908
6D-5-3-5	82.67	76.67	79.67	3372	3260	3316
P72R	92.00	74.67	83.34	2006	2087	2046
Mean	**81.40**	**77.68**		**4074**	**3976**	
CV (%)	**12.57**			**18.76**		
CD @ 0.05for treatments	**NS**			**NS**		
CD@ 0.05 for genotypes	**10.66**			**716**		
CD@ 0.05 for TxG	**10.66**			**1013.12**		

Note: NS- non significant

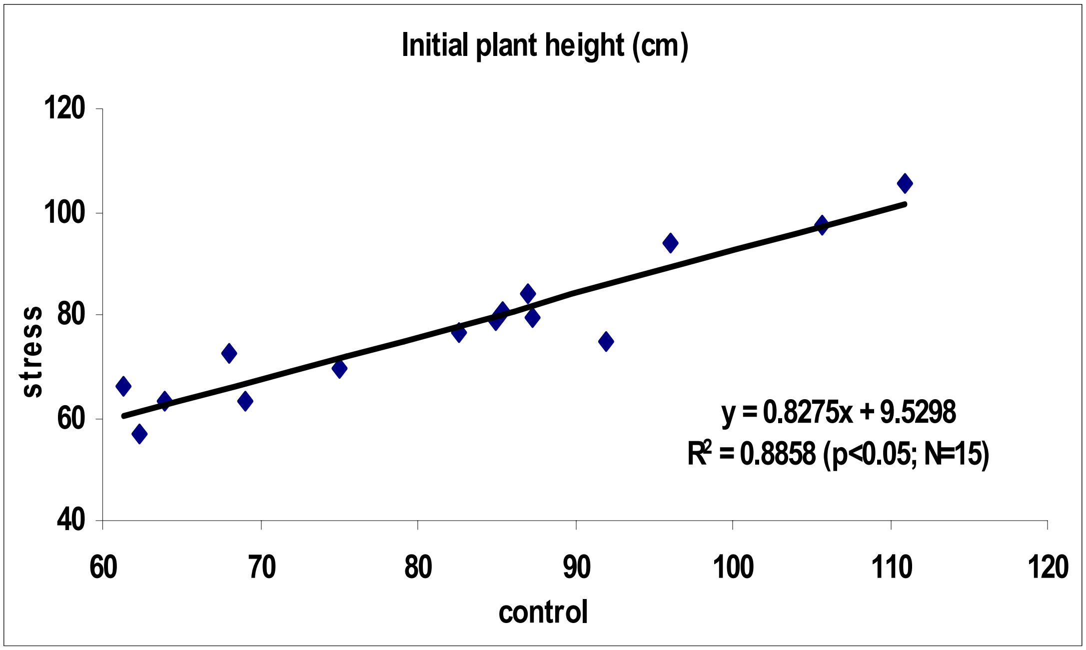


Fig 15: Relationship between initial plant height (cm) of control and moisture stress designated plants before the imposition of stress

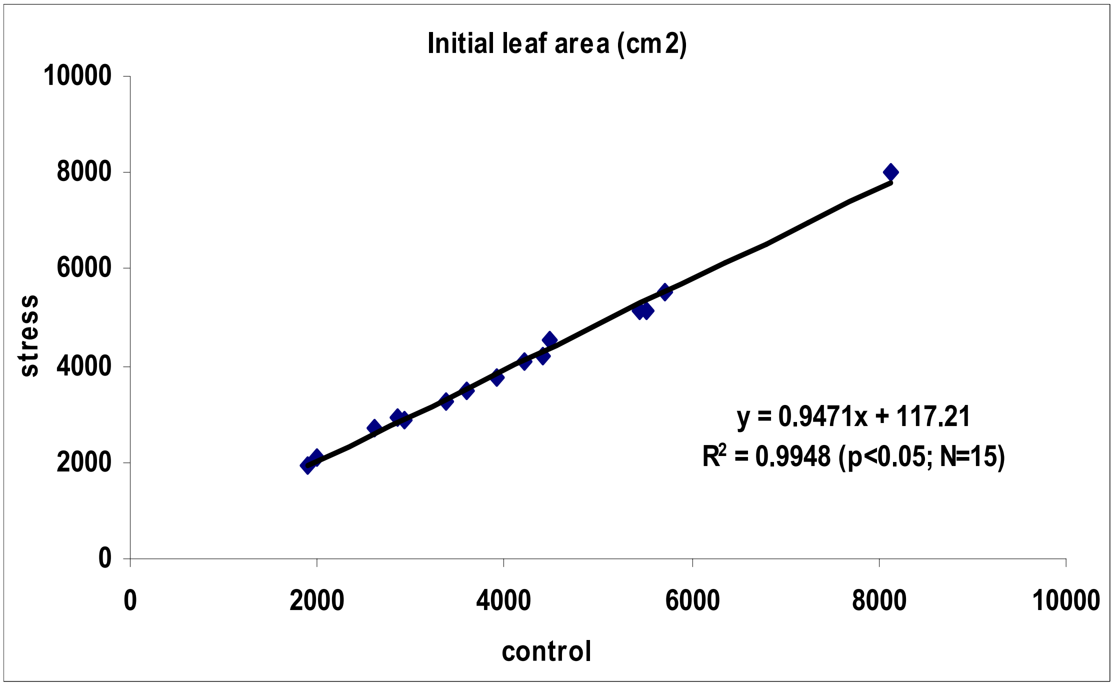


Fig 16: Relationship between initial leaf area (cm^2) of control and moisture stress designated plants before the imposition of stress

significantly more plant height compared to rest of the lines both under control and stress conditions, while lines such as 6D-1, IB66, IB 79 and 11A showed significantly lower plant height both under control and stress conditions. Between inbreds and treatments, IB90, CMS106A and CMS207A found to be superior over rest of the lines.

Although no significant difference in initial leaf area was observed between plants of control and stress designated blocks, a significant genotypic difference for initial leaf area was observed among inbreds and parental lines. Accordingly, sunflower lines namely, IB90, IB 98, CMS 207A and 336A did produce significantly more leaf area compared to other lines even though the time duration was same (Table 13). In fact some of the lines such as IB 79 and P72R showed least leaf area compared to other genotypes both in control and stress designated plants. The overall results imply that, before the imposition of stress, the growth parameters need to be similar in all the treatments. Accordingly, in the present study, no significant difference in any of the parameters observed between plants of control and stress designated blocks before the imposition of stress. Further, the argument was also supported by the fact that, the plant height of control and stress designated plants showed a very strong correlation (Fig 15). Similar kind of strong correlation was also observed between leaf area of control and moisture stress designated plants to indicate that they are similar to begin with (Fig 16).

The specific leaf area, an indication of leaf thickness measured during stress period found to be significantly different in plants of control and stress treatment. Accordingly, the leaves from control plants were thinner, while from stress treatments they are thicker. As against an SLA value of 149 cm^2 g^{-1}observed in control plants, the SLA of stressed plants was 122.71 cm^2 g^{-1} to indicate that they are thicker compared to the leaves of control plants. With respect to SLA across genotypes, CMS 106A, IB 08, IB 11 and 6D-1 have significantly more SLA compared to rest of the lines under control conditions. However under stress condition, CMS 106A and 6D-1 showed significantly more SLA compared to low SLA types such as 336A and IB 66. Interestingly, IB 08 has an SLA of 185 cm^2 g^1 under control condition comes down to 117 cm^2 g^{-1} under stressful condition. This indicates that, at least in few genotypes, the leaves become thicker when exposed to stress conditions. Of the so many lines, CMS 106A was found to possess thinner leaves under both control and stress treatments (Table 14).

With respect to SPAD chlorophyll meter reading (SCMR), which is a reflection of leaf chlorophyll and leaf nitrogen status showed no significant difference between control and stress treatment. However as expected, genotypic differences for SCMR was observed among inbreds and parental lines of sunflower. Accordingly, the inbreds and parental lines such as CMS 207A, CMS 106A, 336A, IB 66 and IB 79 had significantly more chlorophyll compared to most of the other lines under control condition. However under stress condition, CMS 207A, 336A, IB 79 and IB 90 had significantly more chlorophyll content, which was indicated by significantly more SCMR readings. With respect to interaction effects, only CMS 207A had significantly higher SCMR reading compared to IB 08, IB 86, 6D-5-3-5, IB 98 and 11A under control conditions. Incidentally, these parental lines showed higher SCMR values compared to many other parental lines under stress conditions (Table 14). When SLA and SCMR readings of control plants were made to correlate with stressed plants, a significant positive relationship was observed to indicate that, the stress effect on genotypic expression on particular trait is linear (Fig 17 & 18). Further, a significant negative relationship was observed between SLA and SCMR under control condition to indicate that any genotype with low SLA tend to have more chlorophyll content because of the leaf thickness (Fig 19). However under stressful condition, this relationship was not significant (Fig 20).

Water use efficiency assessed at single leaf level

Water use efficiency assessed at single leaf level through gas exchange parameters found to exhibit significant difference not only between treatments but also across parental lines and inbreds. Some of the gas exchange parameters like stomatal conductance, transpiration rate, assimilation rate and water use efficiency (A/gs) were found to be significantly different in plants of control and stress treatment. Under stress condition, stomatal conductance was significantly lower compared to control plants and hence the transpiration rate and assimilation rate of stressed plants were significantly lower. Although, stomatal conductance and assimilation rate were decreased under stress,

Table 14: Specific leaf area (SLA) and SPAD Chlorophyll meter readings (SCMR) of selected lines under control and stress condition during stress period

Inbred / parental lines	SLA (cm^2g^{-1})			SCMR		
	Control	Stress	Mean	Control	Stress	Mean
IB08	185	117	151	34.47	35.29	34.88
IB11	177	134	155	39.58	38.84	39.21
IB66	140	101	121	41.79	37.31	39.55
IB 79	132	120	126	41.76	39.24	40.50
IB86	150	135	142	35.02	38.53	36.78
IB90	134	112	123	39.79	39.40	39.60
IB91	129	115	122	40.61	35.60	38.11
IB98	151	117	134	37.02	37.52	37.27
11A	131	125	128	37.74	37.06	37.40
CMS 106A	207	152	179	36.66	37.60	37.13
CMS207A	115	112	114	46.27	40.86	43.57
336A	111	95	103	42.86	40.54	41.70
6D-1	179	146	163	39.17	37.74	38.46
6D-5-3-5	138	136	137	36.01	34.89	35.45
P72R	156	122	139	38.77	35.64	37.21
Mean	**149**	**123**		**39.17**	**37.74**	
CV (%)	**15.02**			**7.41**		
CD @ 0.05for treatments	**NS**			**NS**		
CD@ 0.05 for genotypes	**23.58**			**1.40**		
CD@ 0.05 for TxG	**33.34**			**8.02**		

Note: NS- non significant

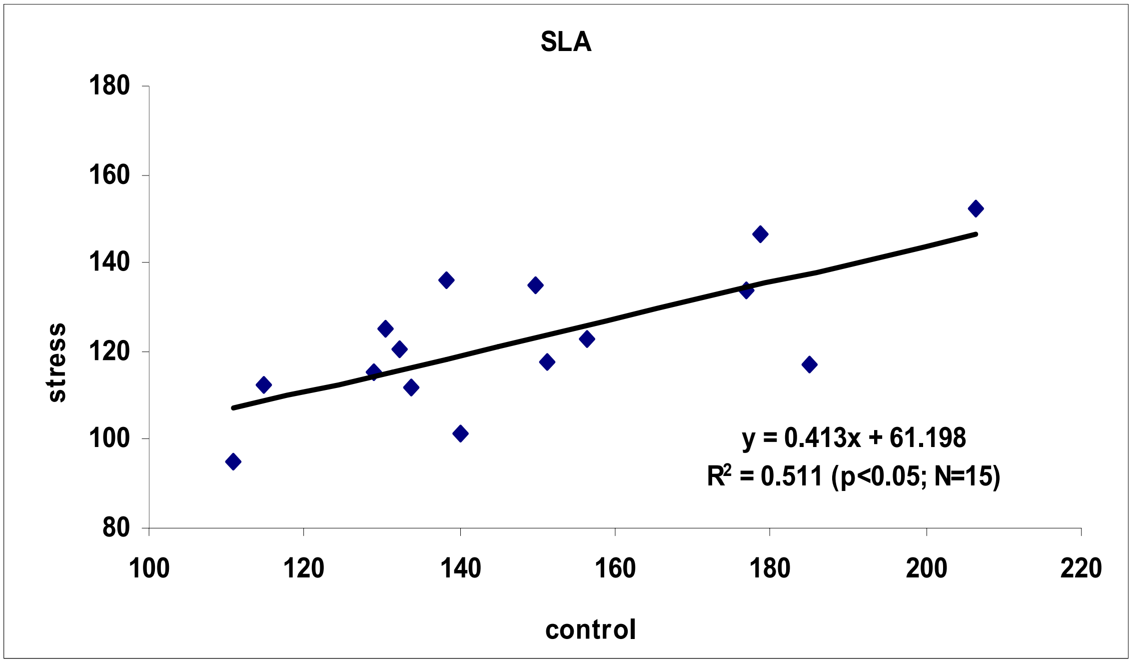


Fig 17: Relationship between SLA ($cm^2 g^{-1}$) of control and stressed plants

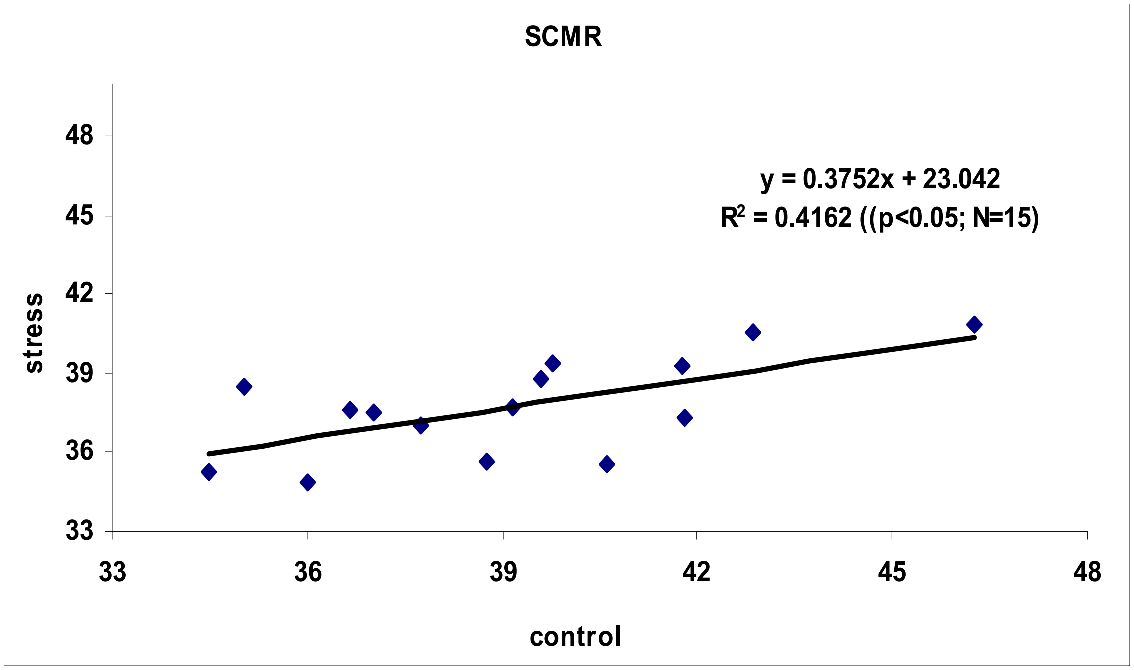


Fig 18: Relationship between SCMR values of control and stressed plants

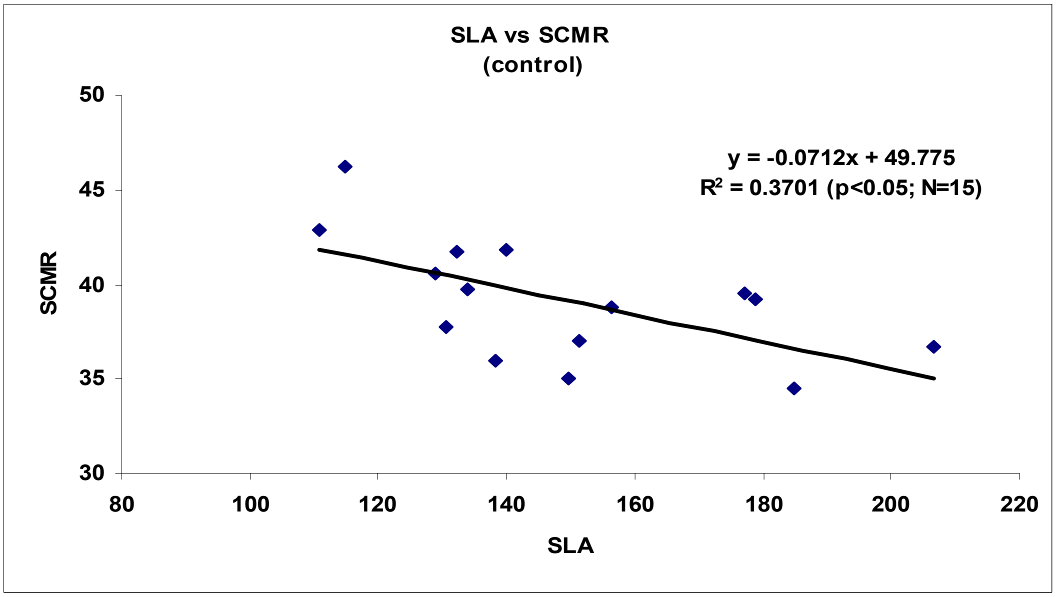


Fig 19: Relationship between SLA and SCMR under control condition

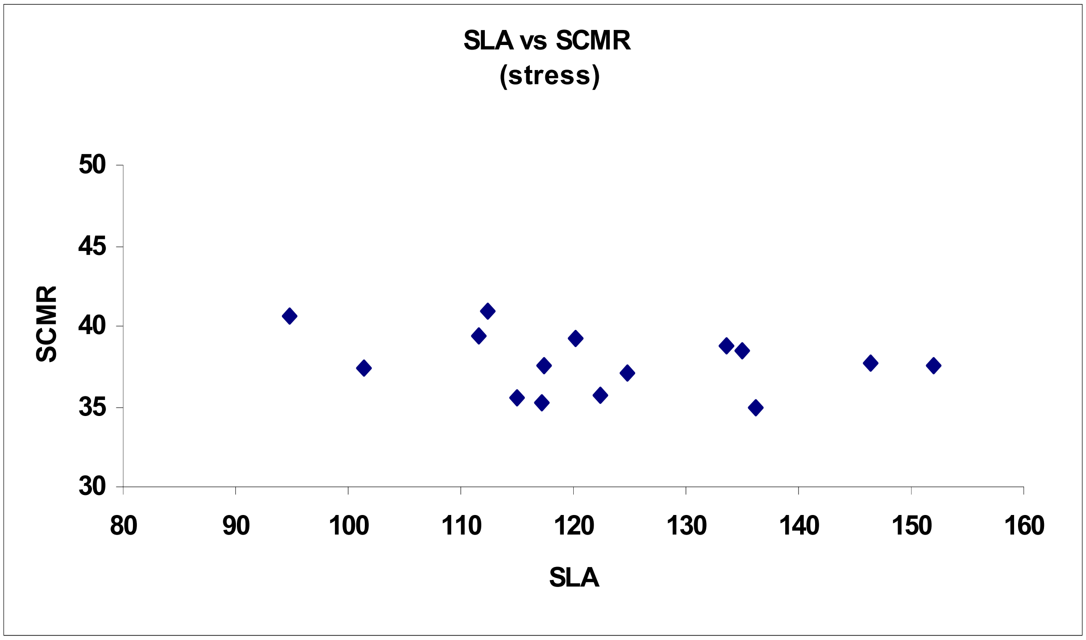


Fig 20: Relationship between SLA and SCMR under stress condition

the extent of reduction was more for stomatal conductance and hence WUE assessed at single leaf level (A/gs) was found to be more in stressed plants. In addition to treatment difference, genotypic difference in many of the gas exchange parameters was found across inbreds and parental lines of sunflower both under control and stress conditions. Accordingly, some of the lines such as IB 08, IB 79, IB 98, 11A, 336 A and P72R had significantly more stomatal conductance under control conditions and hence their transpiration rate and assimilation rates were also significantly higher compared to most of the other lines. However under stress condition, most of these lines showed lower stomatal conductance and hence, less transpiration and less assimilation rates. With respect to WUE (assessed through A/gs), some of the inbreds and parental lines like 6D-1, CMS106A, CMS207A, 6D-5-3-5, IB11 and IB79 found to be superior over other parental lines under control condition. Similarly, lines such as IB11, IB86 and 6D-1 were found to be superior under stressful condition (Table 15).

The increased transpiration and assimilation rates with increased stomatal conductance were shown graphically as well. Accordingly, a strong positive correlation was observed between stomatal conductance and assimilation rate both under control and stress condition (Fig 21A & B). Further, a strong negative relationship between assimilation rate and WUE (A v/s A/gs) was also observed both under control and stress condition (Fig 21 C & D). Interestingly, a very weak and non-significant relationship was observed between plants of control and stress treatment for stomatal conductance (Fig 22A) and assimilation rate (Fig 22B). It appears that, the stress affects stomatal physiology to a greater extent and thus, a significant positive relationship was not found between control and stress conditions for these parameters.

4.3.2 Assessment of sunflower lines for growth, root traits, WUE, yield and yield attributing characters under control and stress conditions

Plant height, total leaf area, leaf biomass, stem and head biomass and root traits were measured in all the sunflower lines at harvest from both control and stress treatments. As moisture stress was created for 20 days, its effect was seen on plant growth and development. Accordingly, the plant height and total leaf area were

Table 15: Gas exchange parameters measured in selected inbreds and parental lines of sunflower under control and stress condition

Inbred / parental lines	gs			T			A			A/gs		
	Control	Stress	Mean	Control	Stress	Mean	Control	Stress	Mean	Control	Stress	Mean
IB08	1.60	0.40	1.00	17.55	10.02	13.79	69.75	29.65	49.70	43.52	75.58	59.55
IB11	0.52	0.20	0.34	9.25	5.85	7.53	35.55	24.90	30.22	68.36	123.27	95.81
IB66	1.23	0.52	0.88	15.85	12.90	14.38	66.63	41.17	53.90	54.76	79.52	67.14
IB 79	1.53	0.86	1.20	18.05	12.87	15.46	65.03	48.93	56.98	42.45	58.31	50.38
IB86	1.21	0.52	0.87	15.33	9.50	12.42	65.22	42.48	53.85	54.10	81.59	67.84
IB90	1.01	0.78	0.90	15.05	15.75	15.40	58.67	47.87	53.27	62.69	63.66	63.17
IB91	0.89	1.25	1.07	13.45	15.10	14.28	57.35	45.60	51.48	68.76	36.38	52.57
IB98	1.27	1.06	1.17	15.63	12.63	14.13	65.28	49.45	57.37	52.57	47.61	50.09
11A	1.25	1.18	1.22	15.63	16.05	15.84	59.73	59.90	59.82	48.39	51.61	50.00
CMS 106A	0.76	0.53	0.65	14.88	12.05	13.47	58.77	41.28	50.03	79.39	77.53	78.46
CMS207A	0.94	0.47	0.71	13.80	11.77	12.79	55.77	41.53	48.65	77.48	88.43	82.95
336A	1.53	0.75	1.14	16.97	14.30	15.64	63.98	38.27	51.13	41.96	51.96	46.96
6D-1	0.84	0.35	0.60	12.21	8.86	10.54	54.08	30.28	42.18	80.50	86.51	83.50
6D-5-3-5	0.83	0.73	0.78	14.67	14.87	14.77	58.88	46.33	52.61	71.28	64.43	67.85
P72R	1.44	0.86	1.15	17.05	12.18	14.62	60.58	46.10	53.34	42.29	60.15	51.22
Mean	**1.12**	**0.70**		**15.44**	**12.31**		**61.41**	**42.25**		**59.23**	**69.77**	
CV (%)	**10.48**			**15.68**			**13.80**			**15.47**		
CD @ 0.05for treatments	**0.15**			**1.16**			**3.80**			**9.42**		
CD@ 0.05 for genotypes	**0.41**			**3.17**			**10.42**			**16.56**		
CD@ 0.05 for TxG	**0.59**			**4.49**			**14.74**			**12.45**		

g_s – Stomatal conductance (mol $m^{-2}s^{-1}$), A –Assimilation rate (µmol $m^{-2}s^{-1}$)
T – Transpiration rate (mol $m^{-2}s^{-1}$), A/gs – Water use efficiency at single leaf level.

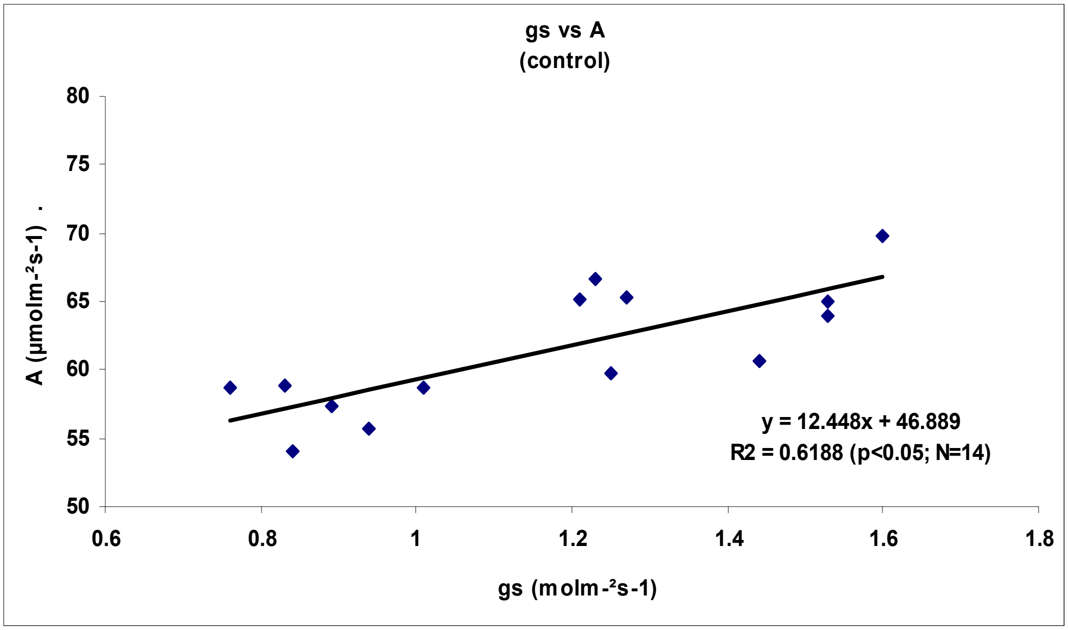


Fig 21A: Relationship between stomatal conductance (mol m^{-2} s^{-1}) and assimilation rate (µmolm-²s^{-1}) in selected lines of sunflower under control condition

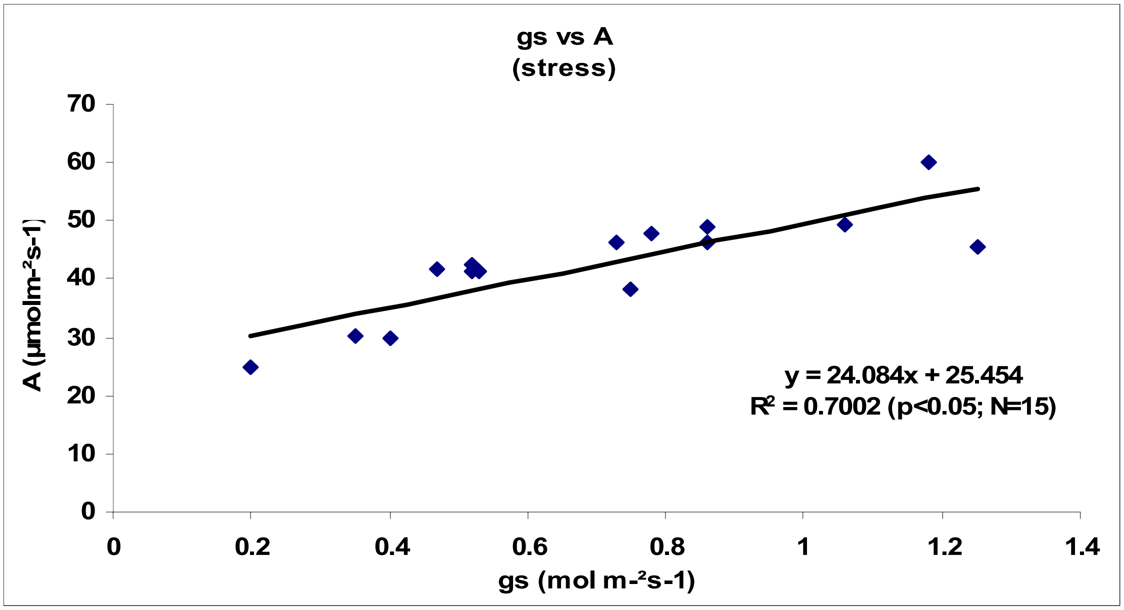


Fig 21B: Relationship between stomatal conductance (mol m^{-2} s^{-1}) and assimilation (µmolm-²s^{-1}) in selected lines of sunflower under stress condition

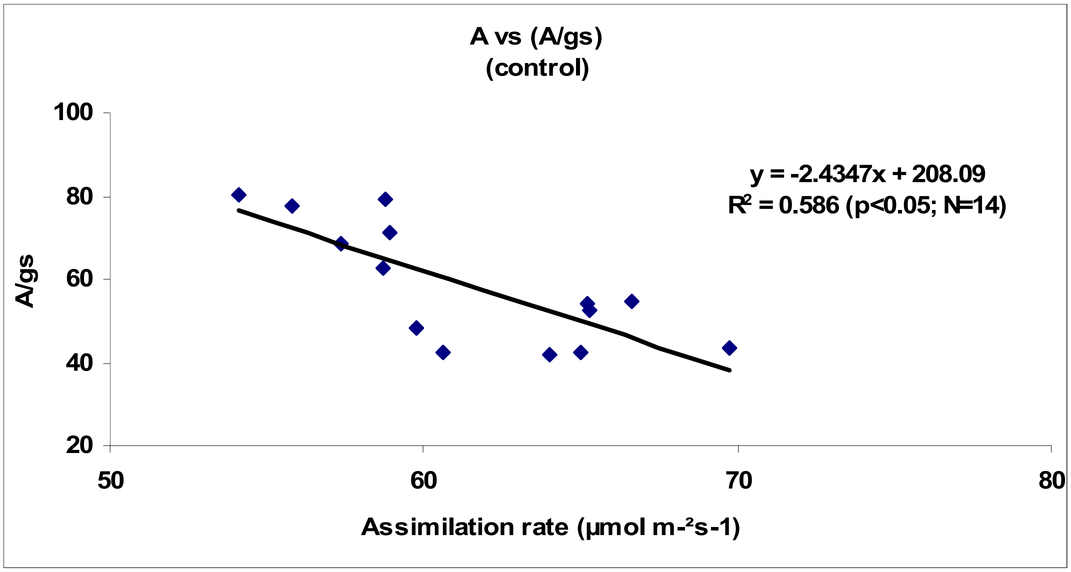


Fig 21C: Relationship between assimilation rate (µmolm-²s^{-1}) and A / gs in selected lines of sunflower under control condition

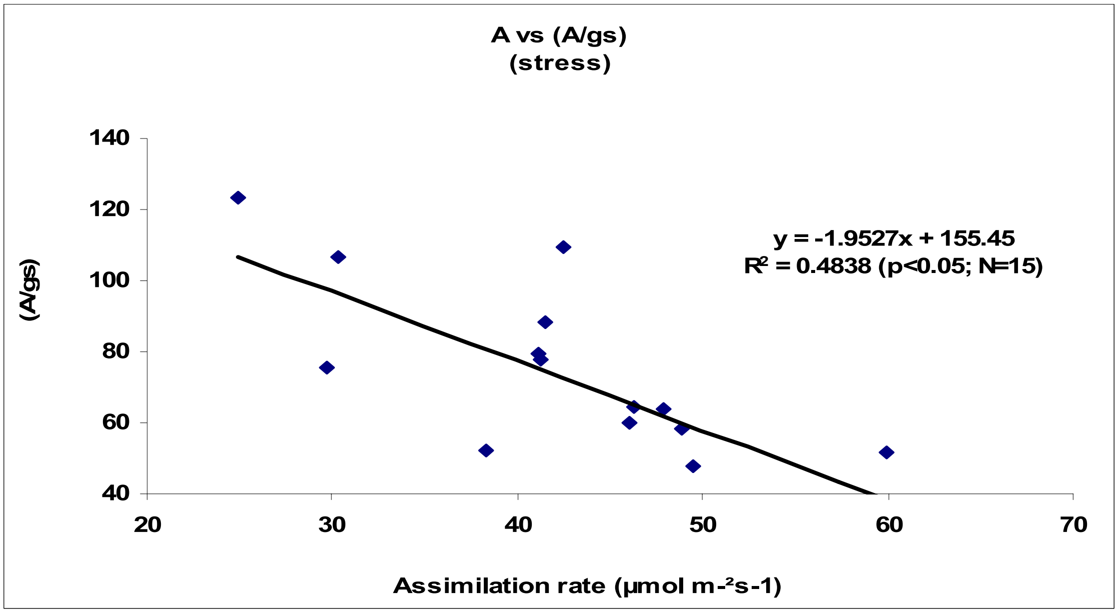


Fig 21D: Relationship between assimilation rate (µmolm-²s^{-1}) and A / gs in selected lines of sunflower under stress condition

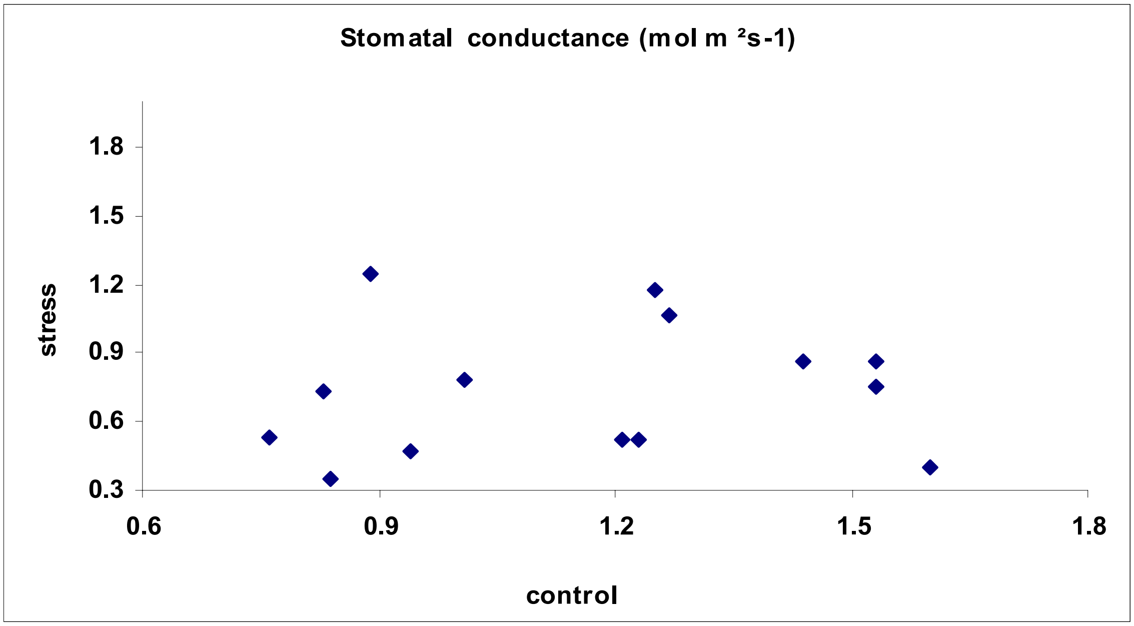


Fig 22A: Relationship between stomatal conductance (mol m-²s^{-1}) of control and stomatal conductance (mol m-²s^{-1}) of stressed plants

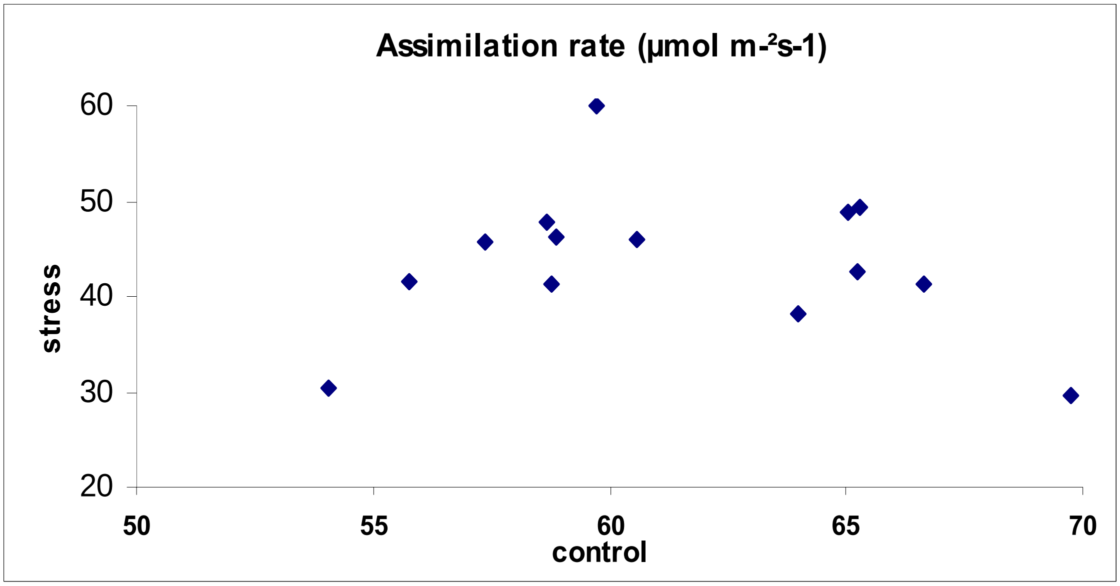


Fig 22B : Relationship between assimilation rate (µmol m-²s^{-1}) of control and assimilation rate (µmol m-²s^{-1}) of stressed plants significantly reduced under stress condition compared to the control condition. As against the average plant height of 141.58 cm recorded in control condition, the stressed plants showed a plant height of 110.54 cm. Similarly, as against the average leaf area of 7095 cm^2 observed in control plants, the stressed plants showed an average leaf area of 3622 cm^2 (Table 16). In fact, almost 50% reduction in leaf area was observed under stressful condition to indicate that, stress causes drastic reduction in leaf area / photosynthesizing area and hence the total biomass production is also going to be reduced drastically under stress condition.

Variability for plant height and total leaf area across inbreds and parental lines both under control and stress conditions was also seen. Accordingly, sunflower lines such as IB90, IB66, IB86, IB91 and CMS106A showed significantly more plant height compared to the lines such as P72R, 6D-5-3-5, IB08 and IB11 both under control and stress treatments (Table 16). With respect to total leaf area, IB90 and IB91 were found to be superior over rest of the inbreds and parental lines of sunflower. Especially inbred IB91 maintained a very good leaf area even under stressful condition to indicate that this is the best inbred as far as leaf area maintenance is concerned under stress condition. Apart from these two inbred lines, IB11 and 336A also showed a significantly higher leaf area compared to most of the other lines under control condition. Interestingly, some of the lines did show least reduction in leaf area under stress condition. These lines are 11A and P72R where the extent of reduction in leaf area was 25.02% and 31.03% respectively over control counterparts. Even the interaction effect of some of the lines such as IB90 and IB91 showed significant superiority over the rest of the lines not only under control condition but also across the treatments (Table 16).

Although there is reduction in plant height and total leaf area under stress, a strong and significant positive relationship was observed between control and stressed plants for plant height as well as total leaf area (Fig 23 and 24). This signifies that, a genotype that performed well under control condition also performs well even under stress condition. In other words, there is linear reduction in plant height and total leaf area under stress condition.

Table 16: Plant height and total leaf area in selected lines of sunflower under control and stress condition after recovery from stress period

Inbred / parental lines	Plant height after recovery period (cm)			Total leaf area after recovery period (cm^2)		
	control	**stress**	**mean**	**control**	**stress**	**mean**
IB08	111.67	95.33	103.50	5746	3116	4431
IB11	110.00	75.50	92.75	7980	2367	5174
IB66	163.67	114.33	139.00	7058	2992	5025
IB 79	143.00	118.33	130.67	4437	2138	3287
IB86	163.33	105.00	134.17	5841	3545	4693
IB90	186.00	133.67	159.83	10521	5260	7890
IB91	162.33	141.67	152.00	13305	7890	10598
IB98	148.33	135.33	141.83	7151	3435	5293
11A	147.00	113.33	130.17	5937	4451	5194
CMS 106A	174.33	142.00	158.17	6670	3120	4895
CMS207A	136.67	111.33	124.00	7598	5231	6414
336A	130.00	88.00	109.00	8615	3168	5892
6D-1	131.33	101.00	116.17	6843	2877	4860
6D-5-3-5	113.33	101.00	107.17	5318	2402	3860
P72R	102.67	82.33	92.50	3402	2346	2874
Mean	**141.58**	**110.54**		**7095**	**3622**	
CV (%)	**12.93**			**13.53**		
CD @ 0.05for treatments	**6.95**			**309.70**		
CD@ 0.05 for genotypes	**19.02**			**848.30**		
CD@ 0.05 for TxG	**26.09**			**1200.30**		

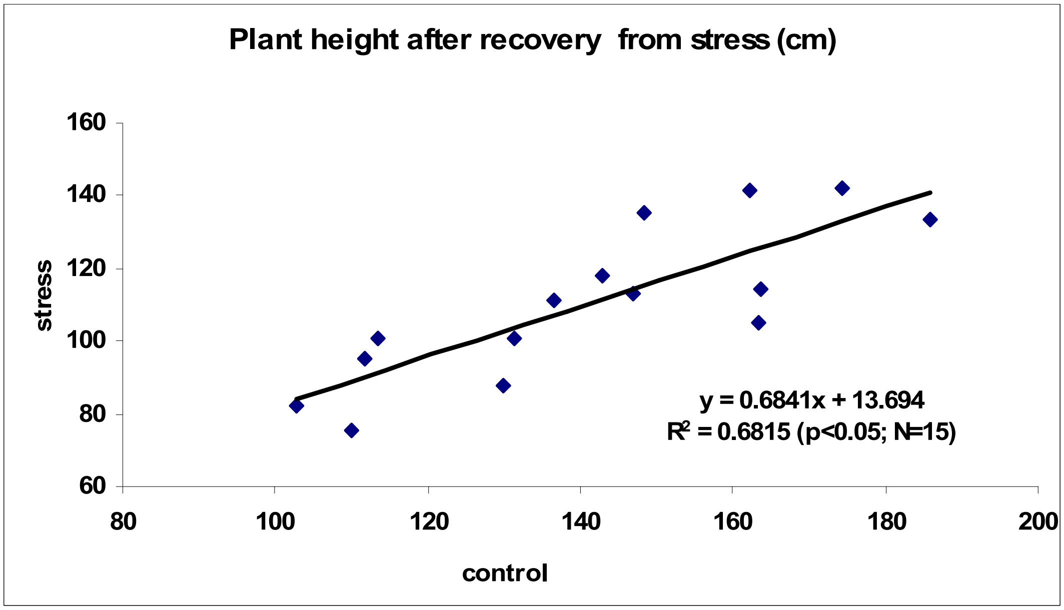


Fig 23: Relationship between plant height of control and plant height of stressed plants after recovery from moisture stress

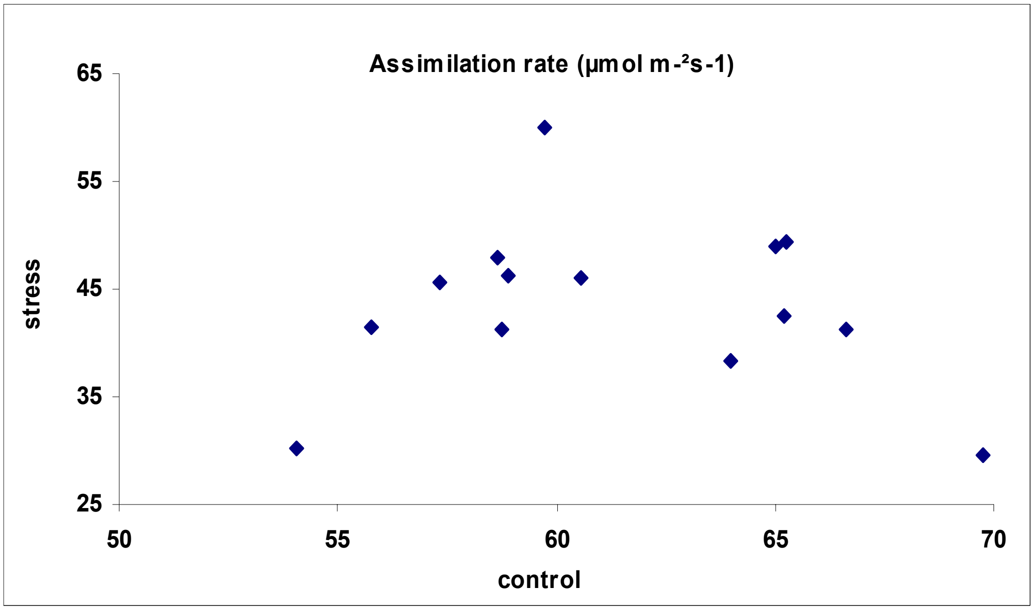


Fig 24: Relationship between total leaf area of control and total leaf area of stressed plants after recovery from stress

Root traits in selected lines of sunflower under control and stress conditions

In the selected lines, several root traits such as number of primary roots, root length, root volume and root weight were measured both under control and stress conditions. As far as the number of primary roots is concerned, there is no significant difference between the treatments. Similarly, although there is a slight increase in root length in plants of stress treatment, the difference was non significant (Table 17). Although no significant difference was found for primary roots and root length between control and stress treatments, within the treatment, a significant difference in number of primary roots and root length was observed among inbreds and parents of sunflower to indicate the presence of genetic variability for these traits. In fact, some of these lines such as IB 08, IB 11, IB 66, IB 98, CMS 106A, 336A, 6D-1 and P72 R had more number of primary roots under stress condition compared to control condition. With respect to root length, majority of the lines showed relatively longer root length in stress condition compared to control condition.

On the contrary to the said results, a significant difference in root volume and root weight was observed between plants of control and stress treatment (Table 17). In fact, over 40% reduction in root volume and over 35% reduction in root weight were observed in plants imposed with stress (Table 17; Plate 8 and 9). The results therefore clearly indicated that, stress affects root growth in sunflower. Apart from difference in root volume and root weight observed between the treatments, genotypic differences within the treatment were also observed. Accordingly, lines such as IB 90 and CMS 106A showed significantly higher root volume compared to most of the other lines. Even the root weight also found to be significantly higher compared to the other lines. In addition to these two lines, CMS 336A also showed significantly higher root biomass compared to other lines. Interestingly, the inbreds and parental lines with more root volume exhibited more root weight to indicate that they are positively related. Although root volume and root weight were reduced under stress, some of the inbreds and parental lines did maintained the root biomass and hence per cent reduction in root weight under stress condition was relatively lower in some of these lines. Accordingly, IB 66, IB 79, IB 86, 11A and CMS 207A showed less than 30% reduction in root weight under stress, while

Table 17: Root traits in selected lines of sunflower under control and stress condition

Inbred / parental lines	**No. of primary roots**			**Root length (cm)**			**Root volume (cm^3)**			**Root dry weight (g)**		
	control	**stress**	**mean**	**control**	**stress**	**mean**	**control**	**stress**	**mean**	**control**	**stress**	**mean**
IB08	9.00	12.33	10.67	44.00	50.33	47.17	60	36	48	20.50	9.83	15.17
IB11	10.50	16.00	13.25	38.00	36.00	37.00	135	53	94	52.27	33.55	42.91
IB66	10.00	13.00	11.50	39.67	47.33	43.50	141	48	95	54.20	40.60	47.40
IB 79	13.33	12.00	12.67	52.67	41.67	47.17	107	60	83	35.00	24.73	29.87
IB86	19.33	11.00	15.17	39.67	40.67	40.17	107	82	94	45.67	36.05	40.86
IB90	14.33	13.33	13.83	71.00	72.33	71.67	130	103	117	78.47	44.83	61.65
IB91	14.67	8.00	11.33	59.33	65.00	62.17	150	88	119	63.17	40.97	52.07
IB98	9.67	13.00	11.33	40.33	53.33	46.83	117	63	90	48.40	32.30	40.35
11A	9.67	9.33	9.50	35.00	52.33	43.67	107	67	87	34.27	24.53	29.40
CMS 106A	14.00	14.67	14.33	56.33	80.33	68.33	180	110	145	61.13	40.20	50.67
CMS207A	17.00	16.33	16.67	60.00	41.00	50.50	133	85	109	43.70	30.06	36.88
336A	10.33	17.00	13.67	46.33	56.67	51.50	130	87	108	66.00	38.97	52.48
6D-1	10.67	13.00	11.83	53.67	56.33	55.00	67	37	52	18.23	8.70	13.47
6D-5-3-5	13.67	7.67	10.67	53.00	35.33	44.17	90	26	58	13.33	5.73	9.53
P72R	10.33	14.00	12.17	41.33	28.00	34.67	63	35	49	13.00	7.23	10.12
Mean	**12.43**	**12.71**		**48.69**	**50.44**		**114**	**65**		**43.16**	**27.89**	
CV (%)	**23.07**			**19.65**			**20.33**			**15.74**		
CD @ 0.05for treatments	**NS**			**NS**			**7.79**			**2.37**		
CD@ 0.05 for genotypes	**3.34**			**11.27**			**21.34**			**6.48**		
CD@ 0.05 for TxG	**4.72**			**15.94**			**30.18**			**9.18**		

Note: **NS-** non significant

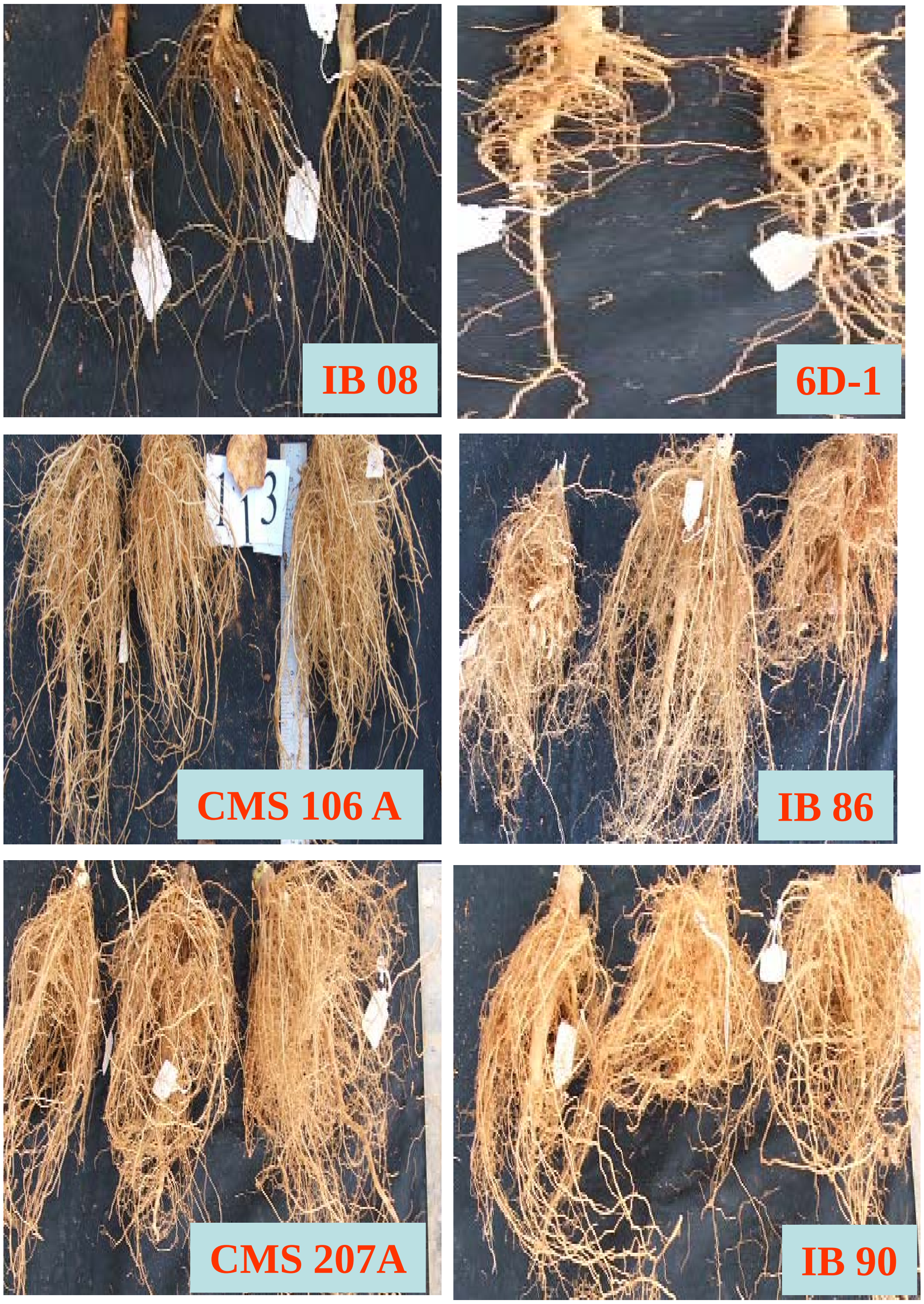


Plate 8: Genetic variability for roots in the second season under control condition

Plate 9: Genetic variability for roots in second season under stress condition

lines such as IB 08, 6D-1, P72 R and 6-D-5-3-5 showed more than 50% reduction in root weight to indicate that some of the lines have intrinsic ability to tolerate the stress effect by maintaining relatively good root system under stress condition (Table 21). The extent of reduction in root weight under stress condition was positively related to control condition to indicate that there is linear relationship between control and stress treatments for this particular trait (Fig 25).

Variability in TDM and its components

The TDM and its components such as leaf weight, stem weight, head weight and root weight were found to be significantly more under control condition compared to stress condition. The results therefore indicated that, whenever stress occurs, the biomass contributing traits are affected and hence there is reduction in each of these components leading to reduced biomass and yield under stress condition. Under stress not only the leaf area is reduced but even the photosynthetic rate also comes down. This results in reduced resource synthesis and allocation into some of the vegetative and reproductive organs leading to less biomass and yield production under stress. In the present study, due to reduced allocation of photosynthates under stress to different organs, the stem weight, leaf weight, root weight and head weight were reduced leading to reduced TDM under stress condition (Table 18). However, genotypic differences were observed for several of these traits both under control and stress conditions. Accordingly, some of the genotypes did produce significantly more leaf biomass, stem biomass, head and root biomass. For instance, IB 91 has produced a leaf biomass, stem biomass, head and root biomass of 122.28 g, 109.90 g, 69.43 g, 63.17 g respectively, while P 72R produced leaf biomass, stem biomass, head and root biomass of 30.19 g, 37.07 g, 20.87 g, 13.00 g respectively under control condition. The TDM of IB 91 was 364.78 g, while for P72R, it was only 101.12 g. Same is true for stress condition where IB 91 showed higher TDM of 192.63 g, while P72R did accumulate a biomass of only 49.38 g. Apart from IB 91, there are other lines namely IB 11, IB 90, IB 66, CMS 207A, CMS 336A and CMS 106A which maintained relatively good biomass both under control and stress conditions, where as the other lines even at the same growth conditions failed to produce relatively good biomass to indicate the existence of genetic variability for TDM and its contributing traits (Table

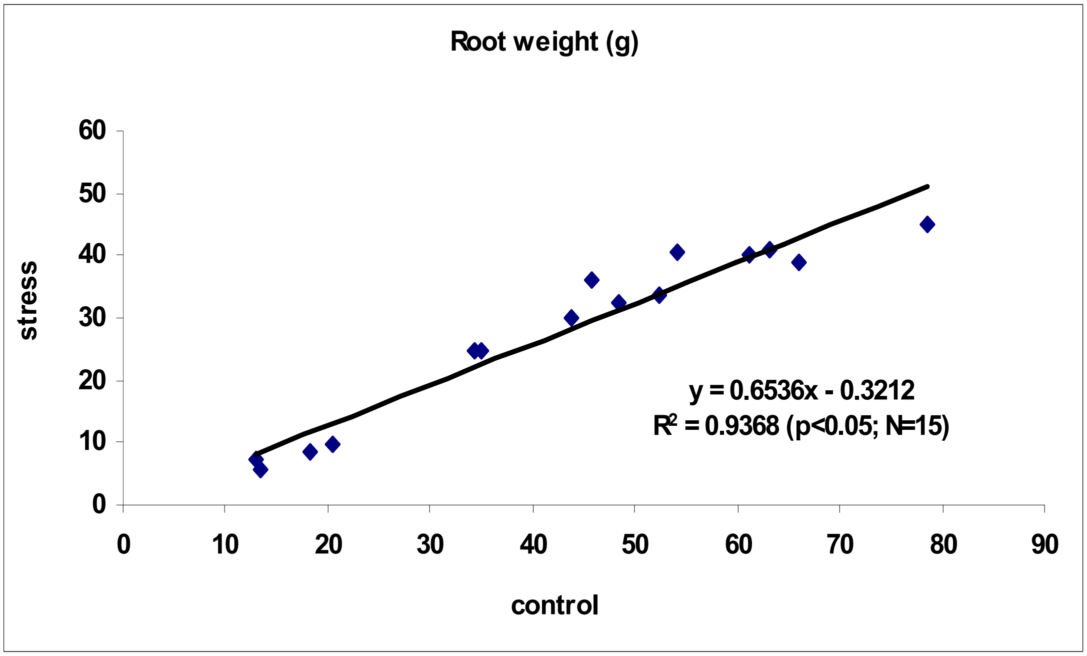


Fig 25: Relationship between root weight (g) of control and root weight of stressed plants

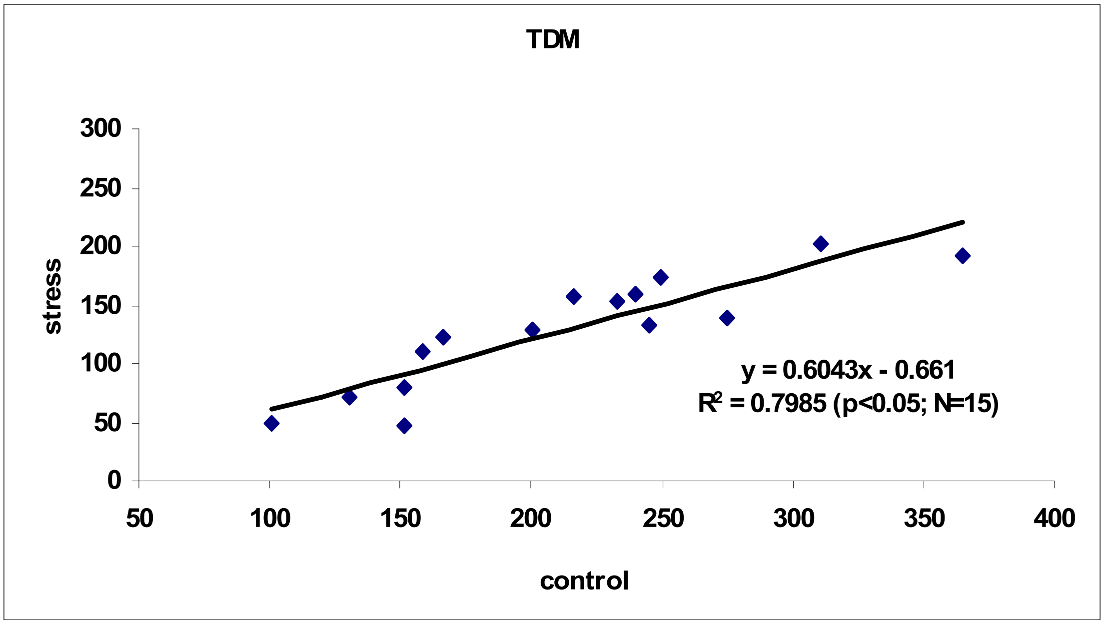


Fig 26: Relationship between TDM (g) of control and TDM of stressed plants

Table 18: Total dry matter and its components in selected lines of sunflower under control and stress condition

Inbred / parental lines	Leaf weight (g)			Stem weight (g)			Head weight (g)			Root dry weight (g)			TDM (g)		
	control	stress	mean	control	stress	mean	control	stress	mean	control	stress	mean	control	stress	mean
IB08	32.50	20.48	26.49	48.50	29.80	39.15	29.00	11.70	20.35	20.50	9.83	15.17	130.50	71.81	101.16
IB11	78.95	39.53	59.24	102.25	43.00	72.63	41.70	23.00	32.35	52.27	33.55	42.91	275.17	139.08	207.13
IB66	77.48	30.36	53.92	69.50	53.70	61.60	31.47	28.80	30.13	54.20	40.60	47.40	232.65	153.46	193.05
IB 79	41.97	28.83	35.40	50.00	32.97	41.49	31.70	23.67	27.69	35.00	24.73	29.87	158.67	110.20	134.44
IB86	45.16	26.77	35.97	73.77	40.63	57.20	35.80	25.23	30.52	45.67	36.05	40.86	200.39	128.68	164.54
IB90	89.37	41.73	65.55	86.80	67.37	77.08	56.33	48.63	52.48	78.47	44.83	61.65	310.97	202.56	256.76
IB91	122.28	66.37	94.33	109.90	57.10	83.50	69.43	28.20	48.82	63.17	40.97	52.07	364.78	192.63	278.71
IB98	65.30	40.10	52.70	70.17	60.40	65.28	32.33	24.97	28.65	48.40	32.30	40.35	216.20	157.77	186.99
11A	47.50	35.07	41.29	49.97	38.93	44.45	34.30	24.43	29.37	34.27	24.53	29.40	166.04	122.96	144.50
CMS 106A	69.20	40.55	54.88	76.60	52.33	64.47	32.60	26.10	29.35	61.13	40.20	50.67	239.53	159.18	199.36
CMS207A	64.77	40.90	52.84	90.23	57.43	73.83	50.37	44.23	47.30	43.70	30.06	36.88	249.07	172.63	210.85
336A	56.50	33.56	45.03	89.53	35.87	62.70	32.80	24.17	28.49	66.00	38.97	52.48	244.83	132.56	188.70
6D-1	38.47	15.15	26.81	66.87	44.37	55.62	27.50	11.23	19.37	18.23	8.70	13.47	151.07	79.45	115.26
6D-5-3-5	45.04	14.86	29.95	59.17	13.50	36.33	33.70	12.66	23.18	13.33	5.73	9.53	151.24	46.75	98.99
P72R	30.19	10.54	20.37	37.07	16.90	26.98	20.87	14.70	17.78	13.00	7.23	10.12	101.12	49.38	75.25
Mean	**60.31**	**32.32**		**72.02**	**42.95**		**37.33**	**24.78**		**43.16**	**27.89**		**212.81**	**127.94**	
CV (%)	**13.92**			**14.56**			**12.61**			**15.74**			**7.48**		
CD @ 0.05for treatments	**2.71**			**3.09**			**1.88**			**2.37**			**5.48**		
CD@ 0.05 for genotypes	**7.42**			**8.46**			**5.16**			**6.48**			**15.01**		
CD@ 0.05 for TxG	**10.49**			**11.98**			**7.30**			**9.18**			**21.23**		

18). The data therefore clearly indicated that, wide genetic variability for TDM contributing traits exists in sunflower parents and inbreds and one can exploit these traits for crop improvement programme.

The TDM of control and stressed plants were correlated to study how this trait is related to each other under control and stress condition. Accordingly, a significant positive relationship was observed between the plants of control and stressed plants for total biomass to indicate the linear reduction in TDM under stress (Fig 26). Further, as TDM is a dependent factor, many parameters regulating / controlling the TDM production was made to correlate with each other to know how these TDM contributing parameters are controlling the TDM production. Accordingly, a significant and positive relationship between TLA and TDM was found both under control and stress conditions. This relationship was stronger in control condition (Fig 27A), while under stress condition, although the relationship was significant, it was not stronger (Fig 27B). It appears that under stress, leaf area reduced to a greater extent and hence, the contribution of leaf area/ leaf biomass to TDM is low compared to the control condition. Like leaf area, root weight also contributes for TDM and a positive and significant relationship was found between root weight and TDM to indicate that, whenever root biomass is more, the TDM production is also higher (Fig 28A & B). Interestingly, this relationship was stronger under stress condition because, whenever there is stress, the plants try to produce more root biomass and contributes more to TDM (Fig 28A & B). Further, the TDM production of control plants significantly correlated with stressed plants to indicate the degree of response of the genotypes to stress condition was linear (Fig 26).

Genetic variability in WUE

Water use efficiency (WUE) assessed through carbon isotope discrimination technique showed no significant difference between control and stress treatment (Table 19). Although increased WUE was expected under stress condition, it did not happen in the present study. The plausible reason for this observed result could be the problem with the sample as the same SLA sample was used for analyzing $\Delta^{13}C$. This SLA sample was collected a few days after the imposition of moisture stress where probably the plants have not really experienced the stress and thus the results for control and stress treatment

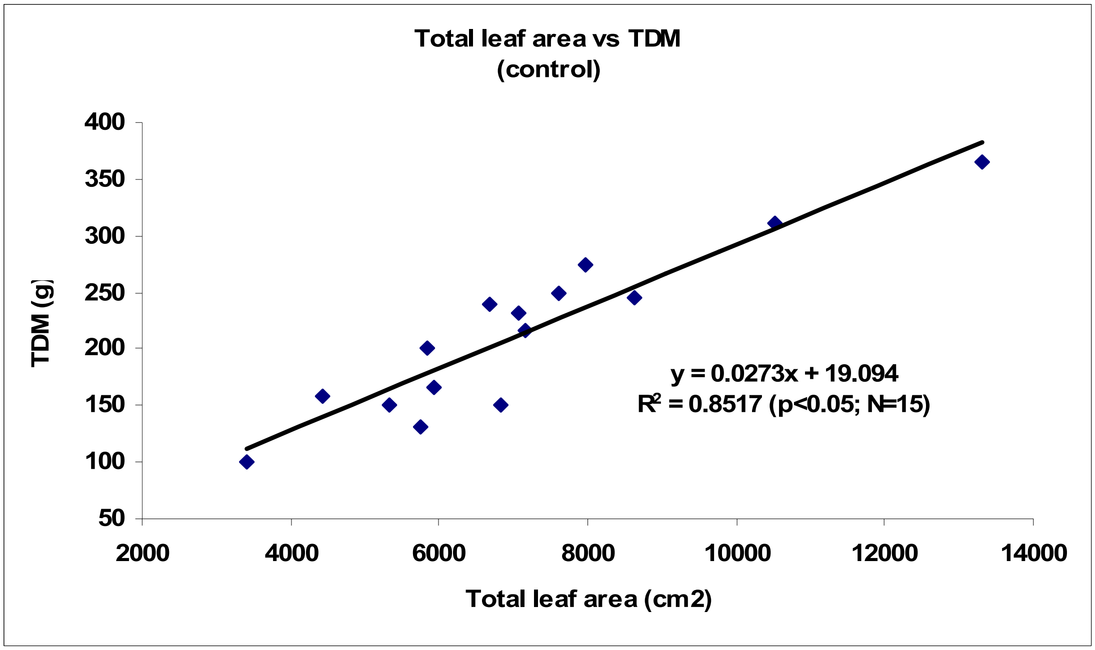


Fig 27A: Relationship between total leaf area (cm^2) and TDM (g) under control condition

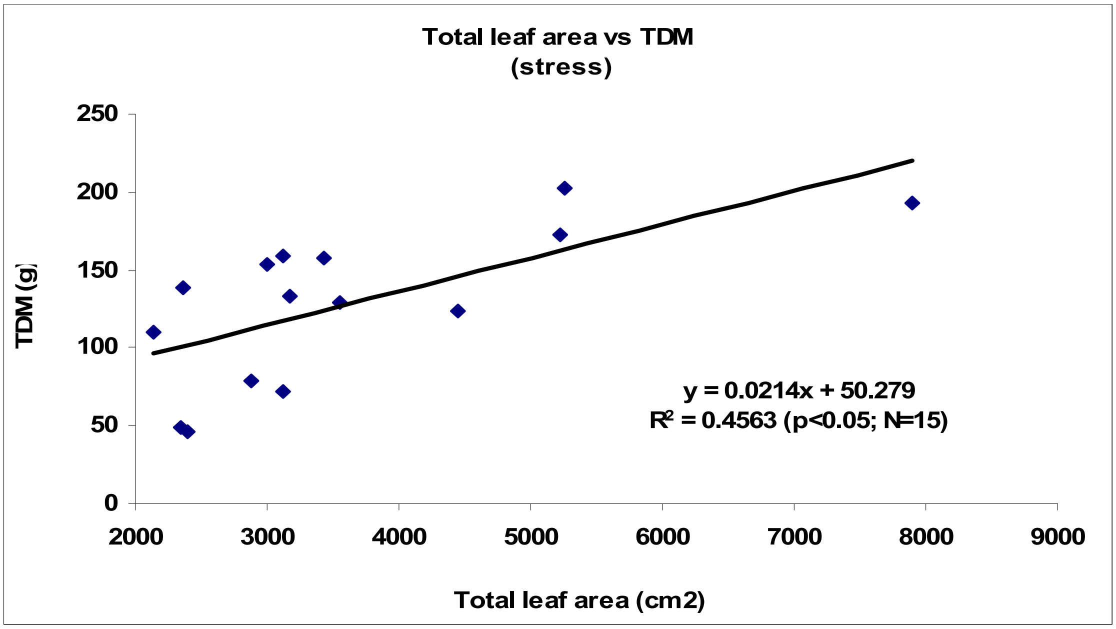


Fig 27B: Relationship between total leaf area (cm^2) and TDM (g) under stress condition

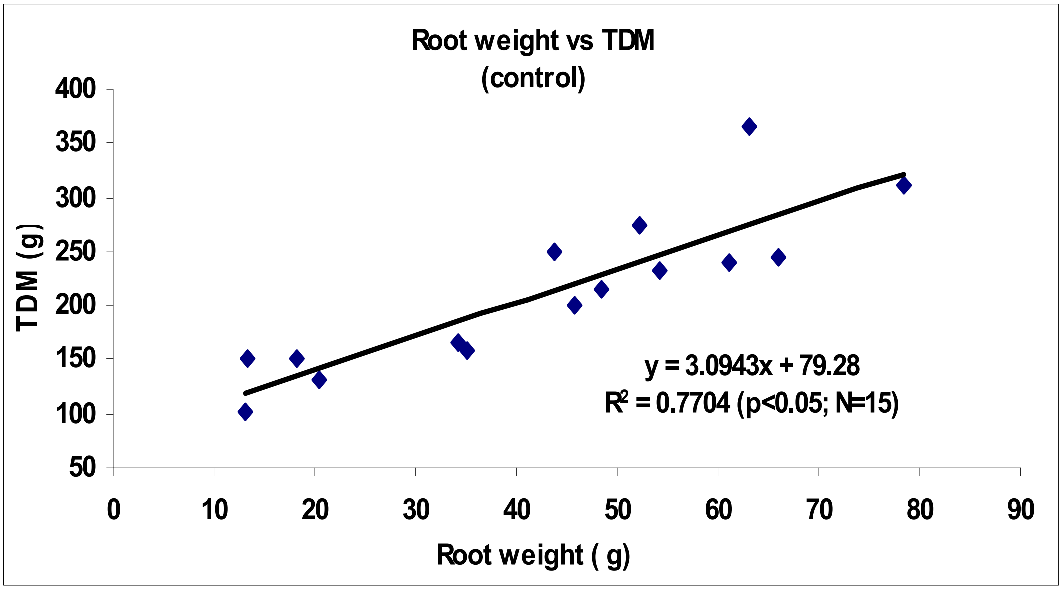


Fig 28A : Relationship between root weight (g) and TDM (g) under control condition

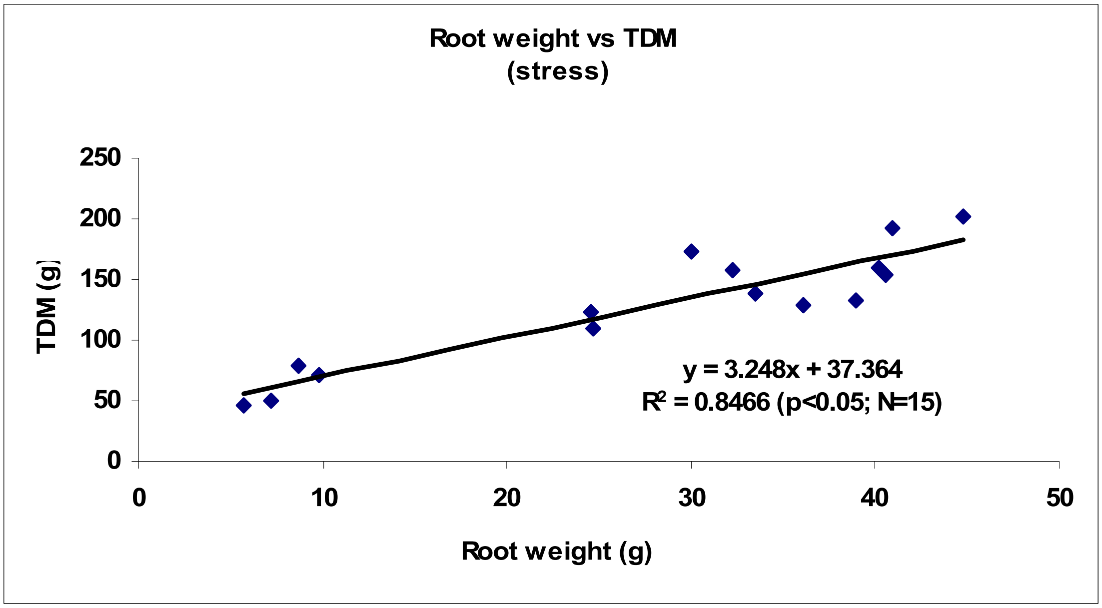


Fig 28B : Relationship between root weight (g) and TDM (g) under stress condition

Table 19: $\Delta^{13}C$ values in selected sunflower lines under control and stress condition

	$\Delta^{13}C$ (‰)		
Inbred/ Parental line	**Control**	**Stress**	**mean**
IB08	21.35	22.04	21.69
IB11	22.77	22.53	22.65
IB66	22.30	22.41	22.35
IB 79	21.25	21.74	21.49
IB86	20.93	21.70	21.32
IB90	22.32	22.15	22.23
IB91	22.77	-	-
IB98	21.78	22.18	21.98
11A	22.99	23.25	23.12
CMS 106A	20.79	21.70	21.25
CMS207A	23.30	22.59	22.95
336A	22.02	21.81	21.91
6D-1	21.80	22.27	22.04
6D-5-3-5	21.31	21.82	21.56
P72R	21.72	22.05	21.89
Mean	**21.96**	**22.16**	
CV (%)	**3.26**		
CD @ 0.05for treatments	**NS**		
CD@ 0.05 for genotypes	**1.21**		
CD@ 0.05 for TxG	**0.86**		

Note: **NS-** non significant

for $\Delta^{13}C$ were significantly not different. However, significant difference was observed for $\Delta^{13}C$ (‰) values across sunflower lines both under control and stress conditions. Accordingly, a few of the lines such as CMS 106A, IB86, IB79 and 6D-5-3-5 showed lower $\Delta^{13}C$ (‰) values (high WUE) compared to IB 11, IB 91, 11A and CMS 207A under control condition. However under stress condition, excepting 11A, no other parent/ inbred lines showed significant difference for $\Delta^{13}C$ (‰) values.

With respect to correlation studies, the correlation was stronger for $\Delta^{13}C$ (‰) between plants of control and stress treatment to indicate that the change in $\Delta^{13}C$ under stress is linear to that of control (Fig 29A). The stronger relationship also suggests that, the sampling can be made either from control or stress treatment to assess the WUE in sunflower. The relationship drawn between TDM and $\Delta^{13}C$ (‰), showed no relationship both under control and stress treatment to indicate that biomass production and WUE are not related (Fig 29B & C). Like in many other cases, the WUE and TDM are not related among sunflower lines tested.

Genetic variability in the selected lines of sunflower under control and stress condition

Although the number of lines used for confirmatory test was low, a significant genetic variability was observed for several traits such as TLA, SLA, root length, root volume, root weight, TDM and $\Delta^{13}C$ both under control and stress conditions. Accordingly, the TLA ranged from 3402 to 13305 cm^2 with a mean of 7094.94 cm^2 in control condition, while in stress condition, it ranged from 2137 to 7890 cm^2 with a mean of 3622.42 cm^2. Similarly, root weight another important trait ranged from as low as 13 g to as high as 78.47 g with a mean of 43.16 g under control condition (Plate 8), while in stress condition, it ranged from 5.73 g to 44.83 g with a mean root weight of 27.89 g (Plate 9). The TDM ranged from 101.12 g to as high as 364.78 g with a mean TDM of 212.94 g under control condition, while in stress condition, it ranged from 46.75 g to 202.56 g with a mean of 127.94 g (Table 20). As expected, under stress condition, the mean values of these traits are low compared to their control counterparts. For instance, as against a mean TLA of 7094 cm^2 in control condition, it was only around 3622 cm^2 in stress condition. Almost 50% reduction in TLA was observed under stressful condition.

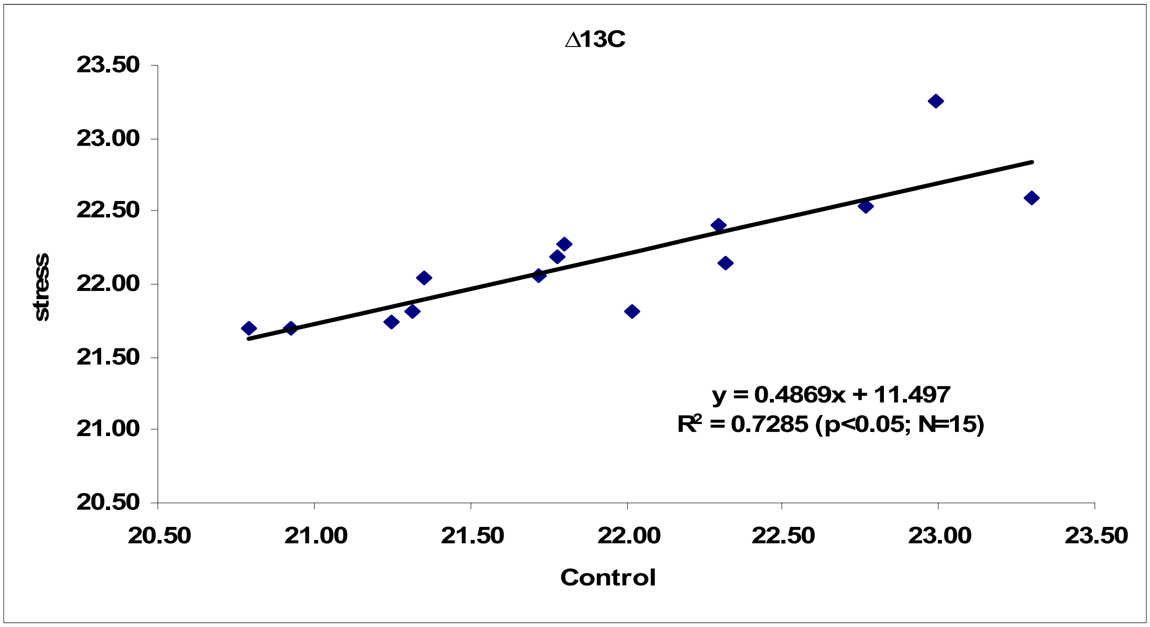


Fig 29A: Relationship between $\Delta^{13}C$ (‰) of control and $\Delta^{13}C$ (‰) of stressed plants

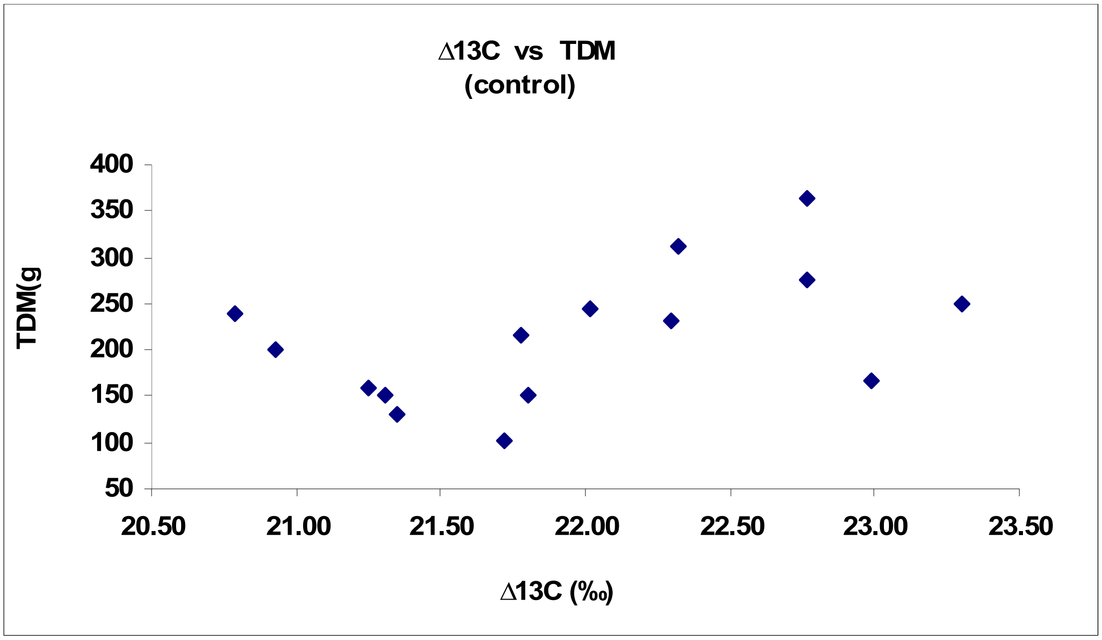


Fig 29B: Relationship between Δ1^{3}C (‰) and TDM (g) under control condition

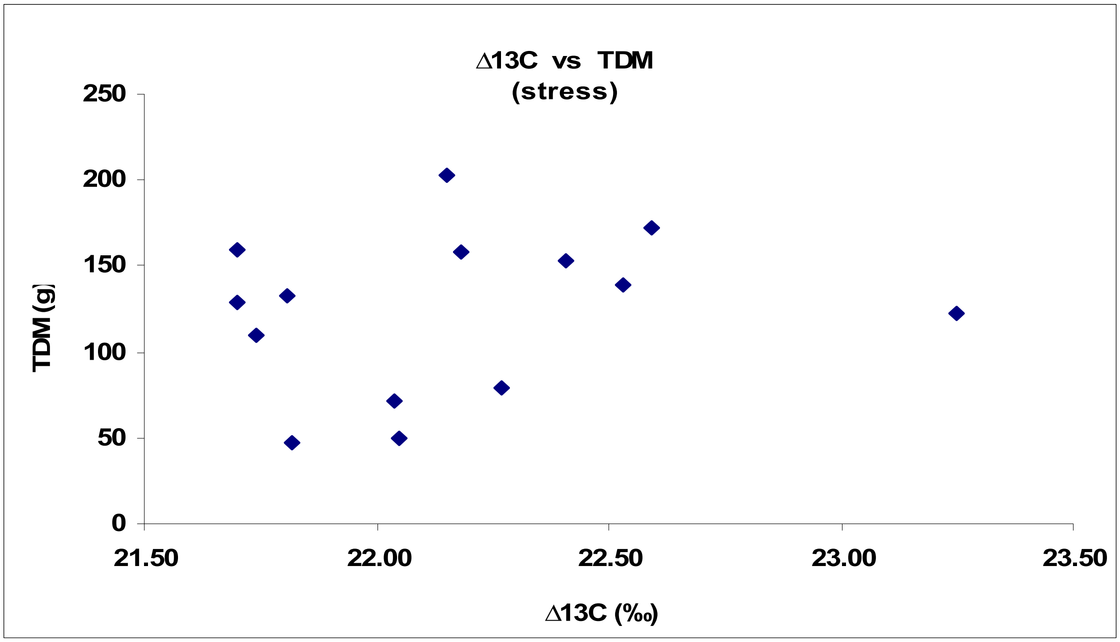


Fig 29C: Relationship between Δ^{13}C (‰) and TDM (g) under stress condition

Table 20: Genetic variability for physiological traits associated with drought tolerance in selected lines of sunflower tested under control and stress conditions

Trait	Treatment	Range	Mean	SD	CD @ 0.05	CV (%)
TLA (cm^2)	**Control**	3402 - 13305	7094.90	2427.39	688.76	13.11
	Stress	2137 - 7890	3622.42	1530.19	363.91	12.98
SLA ($cm^2\ g^{-1}$)	**Control**	110 - 206	148.95	27.27	19.37	17.43
	Stress	94 - 152	122.71	15.76	9.45	10.3
Root length (cm)	**Control**	35.00 - 71.00	48.69	10.26	8.54	23.37
	Stress	28.00 - 80.33	50.44	14.35	5.77	15.34
Root volume (cm^3)	**Control**	60 - 180	114.38	33.97	17.79	22.11
	Stress	25 - 110	65.21	26.44	8.16	18.33
Root dry weight (g)	**Control**	13.00 - 78.47	43.16	20.37	5.49	16.64
	Stress	5.73 - 44.83	27.89	13.76	2.01	13.06
TDM (g)	**Control**	101.12 - 364.78	212.94	71.82	12.38	7.82
	Stress	46.75 - 202.56	127.94	48.56	7.71	8.56
$\Delta\ ^{13}C$ (‰)	**Control**	21.70 - 23.25	22.16	0.43	0.62	3.44
	Stress	21.20 - 23.25	22.16	0.43	0.37	2.07

With respect to roots, although there is a slight increase in mean root length, the other root traits such as root volume, root weight were found to be decreased under stress condition. Overall, around 30 to 40% reduction in root weight and root volume was observed due to moisture stress.

In biomass production, almost 40% reduction was observed in stress condition compared to control condition. Interestingly, WUE measured through carbon isotope discrimination technique did not show any difference between control and stress treatments, although higher WUE was expected under stress condition (Table19).

Overall, a wide and significant genetic variability was observed even in the selected lines of sunflower although the number of lines selected for confirmatory test was low. Further, the traits are consistent across seasons and therefore, the identified material can be used for crop improvement programme more specifically for trait based breeding.

4.4 Consistency of traits across seasons

Since one of the main objectives of the present study was to look for consistency of traits across seasons, an attempt was made to correlate many of the traits across seasons. Here, the data of first season experiment was made to correlate with the data of second season experiment. Accordingly, many parameters such as SLA, TLA, root weight and TDM showed significant correlation with the data of second season experiment to indicate the consistency of the traits across seasons (Fig 30A to D). However, no relationship was observed for $\Delta^{13}C$ (‰) between first season and second season experiment (Fig 30E). Although no reasons can be attributed for this, the selection of material for analysis could probably the reason for this. As explained earlier, the time difference in sampling between 1st experiment and the 2nd experiment could probably the main reason for the observed results.

4.5 Relevance of root traits under stress condition

In order to test the relevance of root traits under stress, the selected lines excluding IB 91 and IB 98 (where they fall into neither of the groups because of

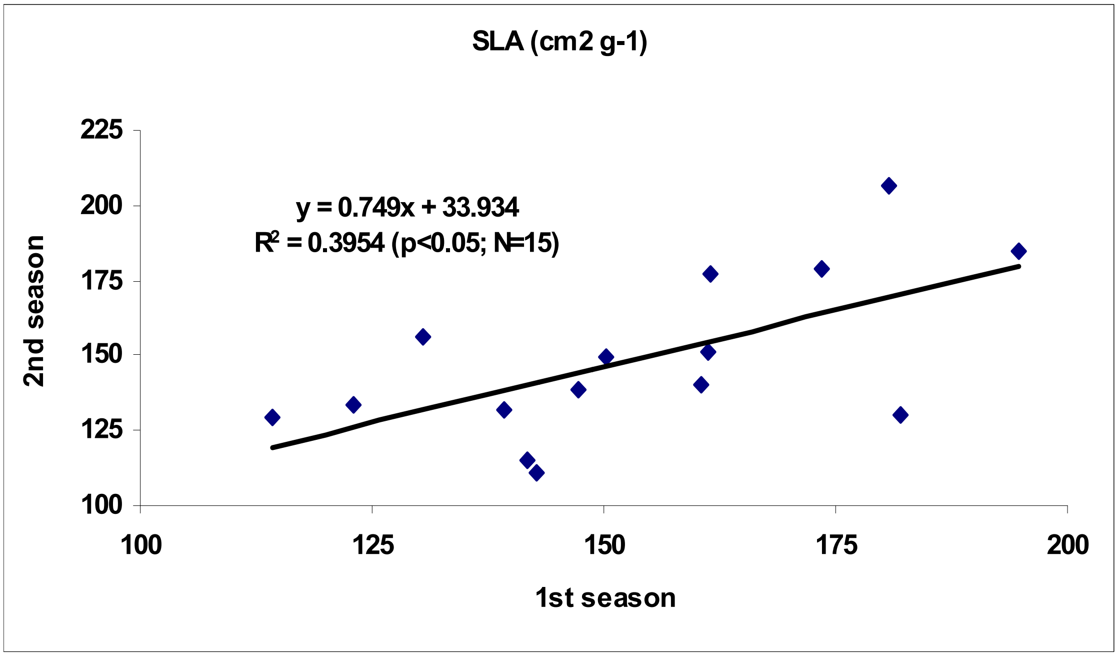


Fig 30A: Relationship between SLA (cm^2 g^{-1}) of 1st season experiment and SLA (cm^2 g^{-1}) of 2nd season experiment

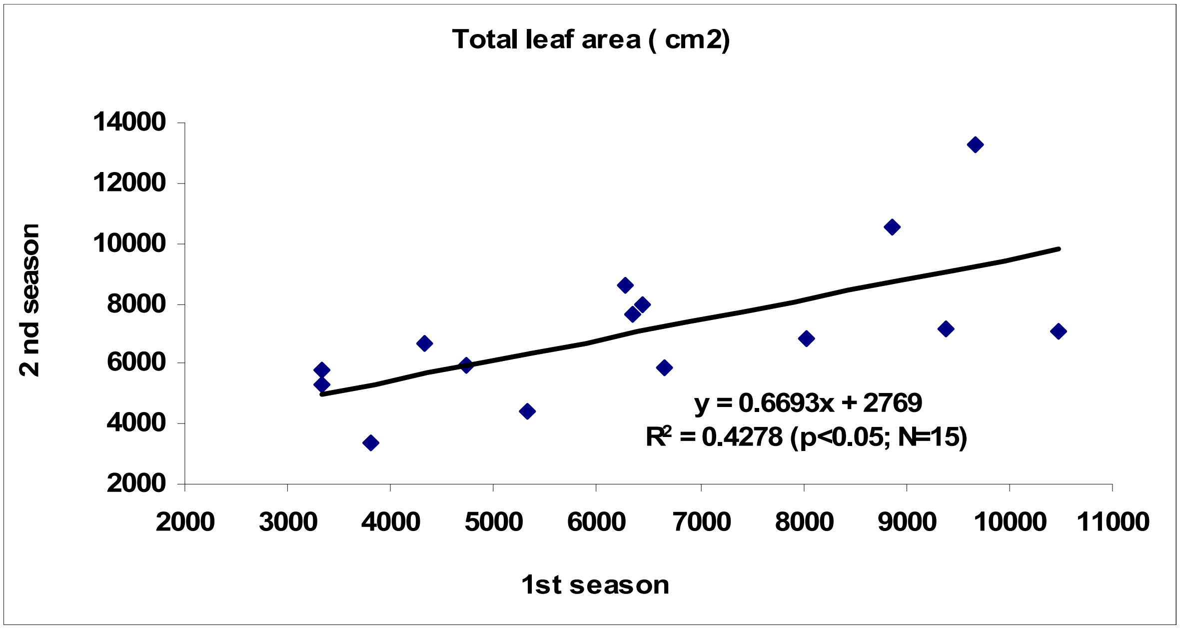


Fig 30B: Relationship between total leaf area (cm^2) of 1st season experiment and total leaf area of 2nd season experiment

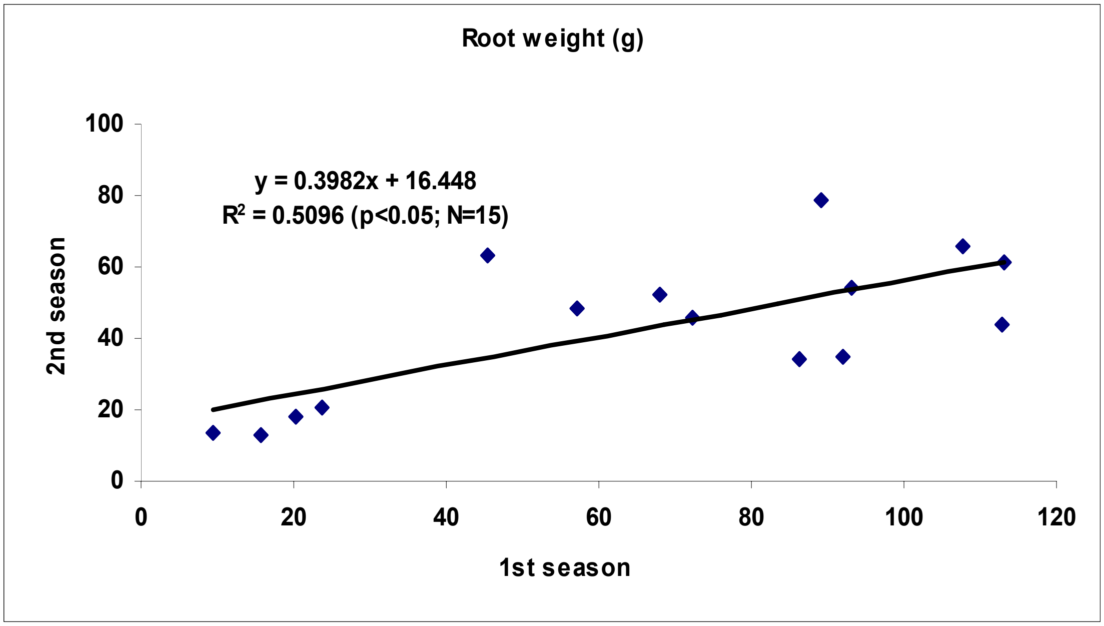


Fig 30C: Relationship between root weight (g) of 1st season experiment and root weight (g) of 2nd season experiment

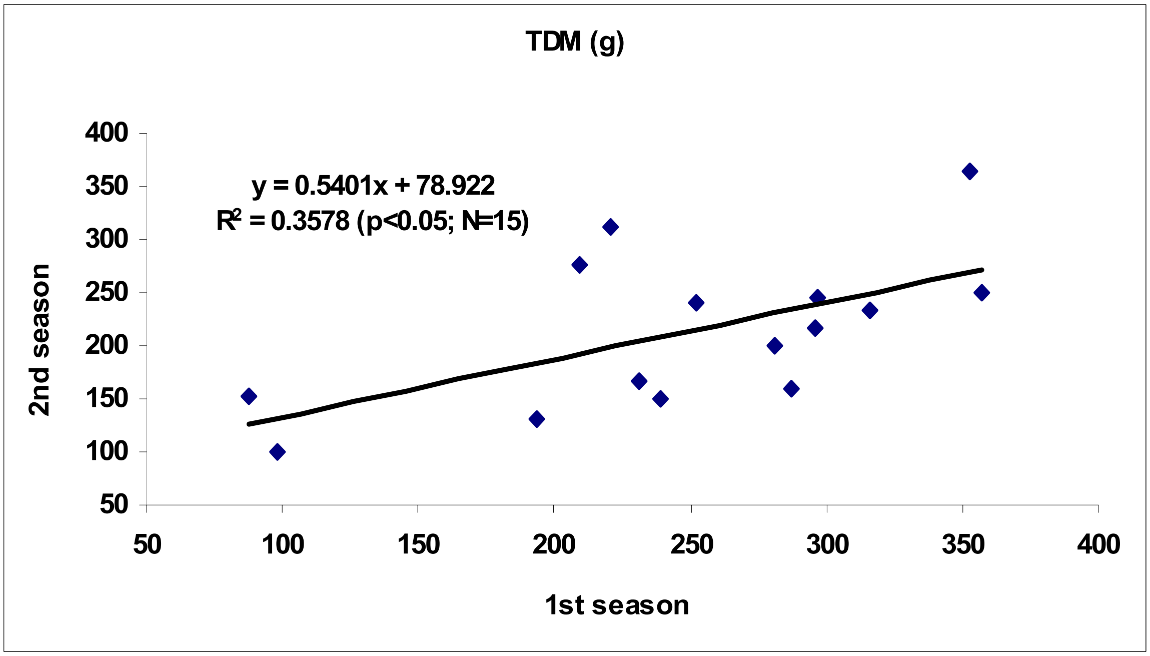


Fig 30D: Relationship between TDM (g) of 1st season experiment and TDM (g) of 2nd season experiment

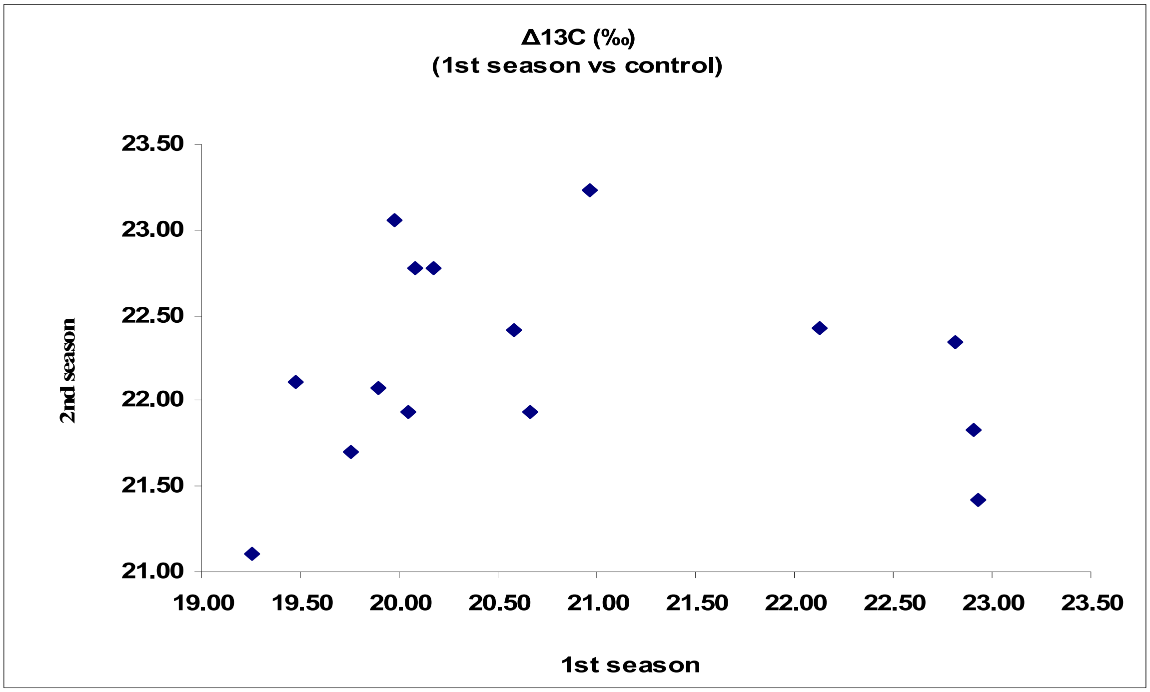


Fig 30E : Relationship between Δ^{13}C of 1st season experiment and Δ^{13}C of 2nd season experiment

moderate roots) were grouped into two categories namely, low root types and high root types. To test the relevance of high root traits under water limited conditions, the data of high root types were compared with low root types. The percent reduction/ enhancement of many of the TDM contributing traits under stress was examined with the control both in low and high root type category. The results of the study indicated that, there is slight enhancement in number of primary roots and root length under stress condition compared to control condition (Table 21). Interestingly, number of primary roots and root length was relatively more for low root type category lines compared to high root category lines. However, with respect to root volume and root weight, the percent reduction under stress was to the extent of 40% and 32% respectively for high root type category (Plate 10), while for low root type category, the extent of reduction in root volume and root weight was 50% and 51% respectively (Plate 11). The data clearly indicated that, high root type inbreds and parental lines maintained relatively good root system under stress condition and hence the extent of reduction in root weight was low compared to low root type category lines (Plate 10 & 11). Interestingly, high root type and low root type inbreds and parental lines continued to maintain similar trend in the second season indicating that these traits are consistent and heritable (Plate 12 & 13).

With respect to reduction in TDM and its components, the extent of reduction in high root type category was relatively low compared to low root types. Accordingly, the reduction in total leaf weight in high root type category was 42%, while in low root category; it was 57% (Table 22). Similarly, for stem weight, head weight and root weight, the percent reduction was 37%, 23% and 32% respectively under high root category, while in low root category, it was 51%, 53% and 51% respectively. With respect to TDM, more than 50% reduction in dry matter was observed for low root type category (more precisely 53%), while for high root type category, the reduction in dry matter was only 36% which is significantly lower compared to low root type category (Table 22 & Fig 31). The data clearly indicated that, the high root types continued to produce more biomass compared to low root types by virtue of relatively good root system under stress condition. While maintaining good root system, they could able to extract water and supplement for overall growth and developmental processes of plants (Fig 31).

Table 21: Percent change in root traits over control in low and high root type sunflower lines

Inbred	**No. of primary roots**			**Root length (cm)**			**Root volume (cm^3)**			**Root dry weight (g)**		
	control	**stress**	**Percent change**	**control**	**stress**	**Percent change**	**control**	**stress**	**Percent change**	**control**	**stress**	**Percent change**
High root types												
IB11	10.50	16.00	+52.38	38.00	36.00	-5.26	135.00	52.50	-61.11	52.27	33.55	-35.81
IB66	10.00	13.00	+30.00	39.67	47.33	+19.33	140.67	48.33	-65.64	54.20	40.60	-25.09
IB79	13.33	12.00	-10.00	52.67	41.67	-20.89	106.67	60.00	-43.75	35.00	24.73	-29.33
IB86	19.33	11.00	-43.10	39.67	40.67	+2.52	106.67	81.67	-23.44	45.67	36.05	-21.06
IB90	14.33	13.33	-6.98	71.00	72.33	+1.88	130.00	103.33	-20.51	78.47	44.83	-42.86
11A	9.67	9.33	-3.45	35.00	52.33	+49.52	106.67	66.67	-37.50	34.27	24.53	-28.40
CMS 106 A	14.00	14.67	+4.76	56.33	80.33	+42.60	180.00	110.00	-38.89	61.13	40.20	-34.24
CMS207A	17.00	16.33	-3.92	60.00	41.00	-31.67	133.33	85.00	-36.25	43.70	30.06	-31.21
336 A	10.33	17.00	+64.52	46.33	56.67	+22.30	130.00	86.50	-33.46	66.00	38.97	-40.96
Mean	**13.17**	**13.63**	**+9.36**	**48.74**	**52.04**	**+8.93**	**129.89**	**77.11**	**-40.06**	**52.30**	**34.84**	**-32.11**
Low root types												
IB08	9.00	12.33	-37.04	44.00	50.33	-14.39	60.00	35.50	-40.83	20.50	9.83	-52.03
6D-1	10.67	13.00	-21.88	53.67	56.33	-4.97	66.67	36.50	-45.25	18.23	8.70	-52.29
6D-5-3-5	13.67	7.67	-43.90	53.00	35.33	-33.33	90.00	25.50	-71.67	13.33	5.73	-57.00
P72R	10.33	14.00	-35.48	41.33	28.00	-32.26	63.33	35.00	-44.74	13.00	7.23	-44.36
Mean	**10.92**	**11.75**	**-12.62**	**48.00**	**42.50**	**-11.56**	**70.00**	**33.13**	**-50.62**	**16.27**	**7.88**	**-51.42**
CV (%)	**23.07**			**19.65**			**20.33**			**15.74**		
CD@ 0.05 for treatments	**1.22**			**4.11**			**7.79**			**2.37**		
CD@ 0.05 for genotypes	**3.34**			**11.27**			**21.34**			**6.48**		
CD@ 0.05 for TxG	**4.72**			**15.94**			**30.18**			**9.18**		

Note: + indicates increase over control

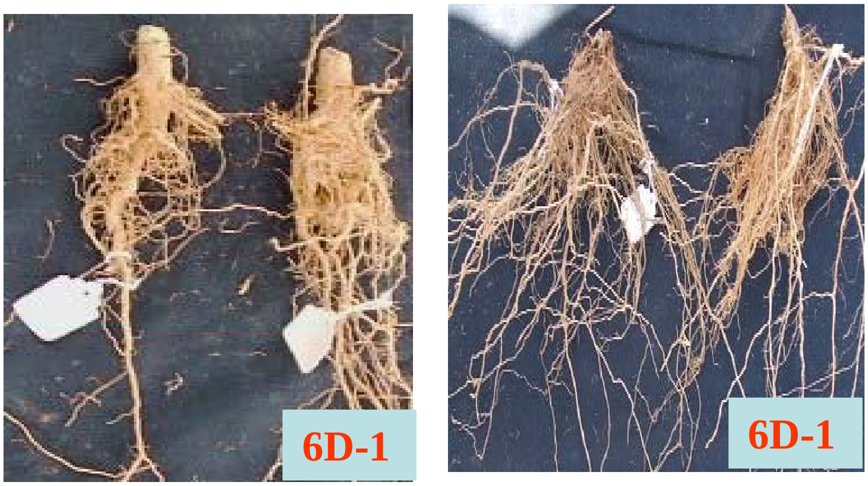
- indicates decrease over control

control Stress

control Stress

Plate 10: Root traits in high root types under control and stress condition

control stress



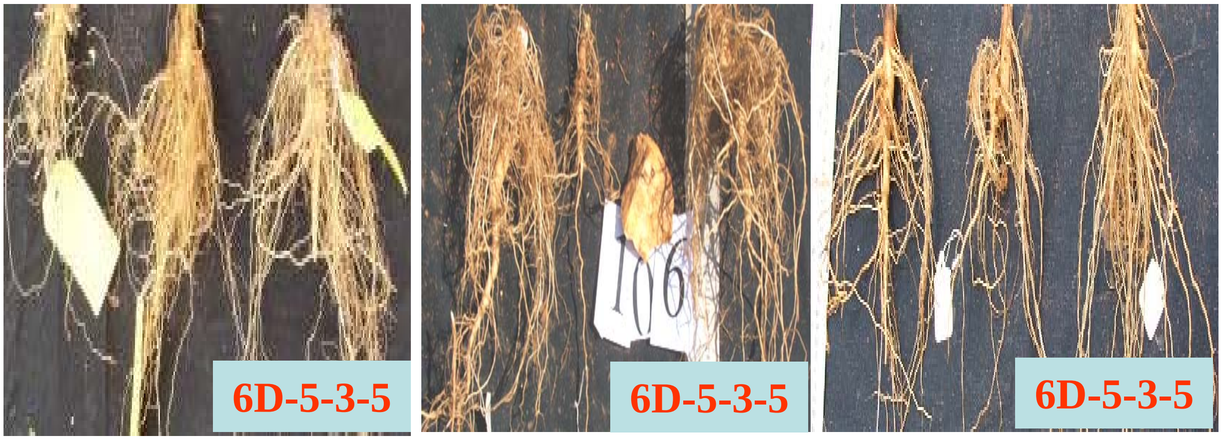
control stress

Plate 11: Root traits in low root types under control and stress condition

1st season 2nd season control 2nd season Stress

1st season 2nd season control 2nd season Stress

Plate 12: Comparison of high root types between 1st and 2nd season



1st season   2nd season   control   2nd season   Stress

1st season   2nd season   control   2nd season   stress

Plate 13: Comparison of low root types between 1st and 2nd season

Table 22: Percent change in TDM and its components over control in low and high root type sunflower lines

Inbred	Total leaf weight (g)		Percent change	Stem weight (g)		Percent change	Head weight (g)		Percent change	Root dry weight (g)		Percent change	TDM (g)		Percent change
	Control	Stress		Control	Stress		Control	Stress		Control	Stress		Control	Stress	
High root types															
IB 11	78.95	39.53	-49.93	102.25	43.00	-57.95	41.70	23.00	-44.84	52.27	33.55	-35.81	275.17	139.08	-49.46
IB 66	77.48	30.36	-60.82	69.50	53.70	-22.73	31.47	28.80	-8.47	54.20	40.60	-25.09	232.65	153.46	-34.04
IB 79	41.97	28.83	-31.30	50.00	32.97	-34.06	31.70	23.67	-25.33	35.00	24.73	-29.33	158.67	110.20	-30.54
IB 86	45.16	26.77	-40.72	73.77	40.63	-44.92	35.80	25.23	-29.53	45.67	36.05	-21.06	200.39	128.68	-35.78
IB 90	89.37	41.73	-53.31	86.80	67.37	-22.39	56.33	48.63	-13.67	78.47	44.83	-42.86	310.97	202.56	-34.86
11A	47.50	35.07	-26.18	49.97	38.93	-22.08	34.30	24.43	-28.78	34.27	24.53	-28.40	166.04	122.96	-25.94
CMS 106A	69.20	40.55	-41.40	76.60	52.33	-31.68	32.60	26.10	-19.94	61.13	40.20	-34.24	239.53	159.18	-33.54
CMS 207A	64.77	40.90	-36.85	90.23	57.43	-36.35	50.37	44.23	-12.18	43.70	30.06	-31.21	249.07	172.63	-30.69
336 A	56.50	33.56	-40.61	89.53	35.87	-59.94	32.80	24.17	-26.31	66.00	38.97	-40.96	244.83	132.56	-45.86
Mean	**63.43**	**35.25**	**-42.35**	**76.52**	**46.92**	**-36.90**	**38.56**	**29.81**	**-23.23**	**52.3**	**34.84**	**-32.11**	**230.81**	**146.81**	**-35.64**
Low root types															
IB 08	32.50	20.48	-36.98	48.50	29.80	-38.56	29.00	11.70	-59.66	20.50	9.83	-52.03	130.50	71.81	-44.97
6D-1	38.47	15.15	-60.62	66.87	44.37	-33.65	27.50	11.23	-59.16	18.23	8.70	-52.29	151.07	79.45	-47.41
6D-5-3-5	45.04	14.86	-67.01	59.17	13.50	-77.18	33.70	12.66	-62.43	13.33	5.73	-57.00	151.24	46.75	-69.09
P72R	30.19	10.54	-65.07	37.07	16.90	-54.41	20.87	14.70	-29.55	13.00	7.23	-44.36	101.12	49.38	-51.17
Mean	**36.55**	**15.26**	**-57.42**	**52.90**	**26.14**	**-50.95**	**27.77**	**12.57**	**-52.70**	**16.27**	**7.88**	**-51.42**	**133.48**	**61.85**	**-53.16**
CV (%)	**13.92**			**14.56**			**12.61**			**15.74**			**7.48**		
CD @ 0.05for treatments	**2.71**			**3.09**			**1.88**			**2.37**			**5.48**		
CD@ 0.05 for genotypes	**7.42**			**8.46**			**5.16**			**6.48**			**15.01**		
CD@ 0.05 for TxG	**10.49**			**11.98**			**7.30**			**9.18**			**21.23**		

Note: + indicates increase over control

- indicates decrease over control

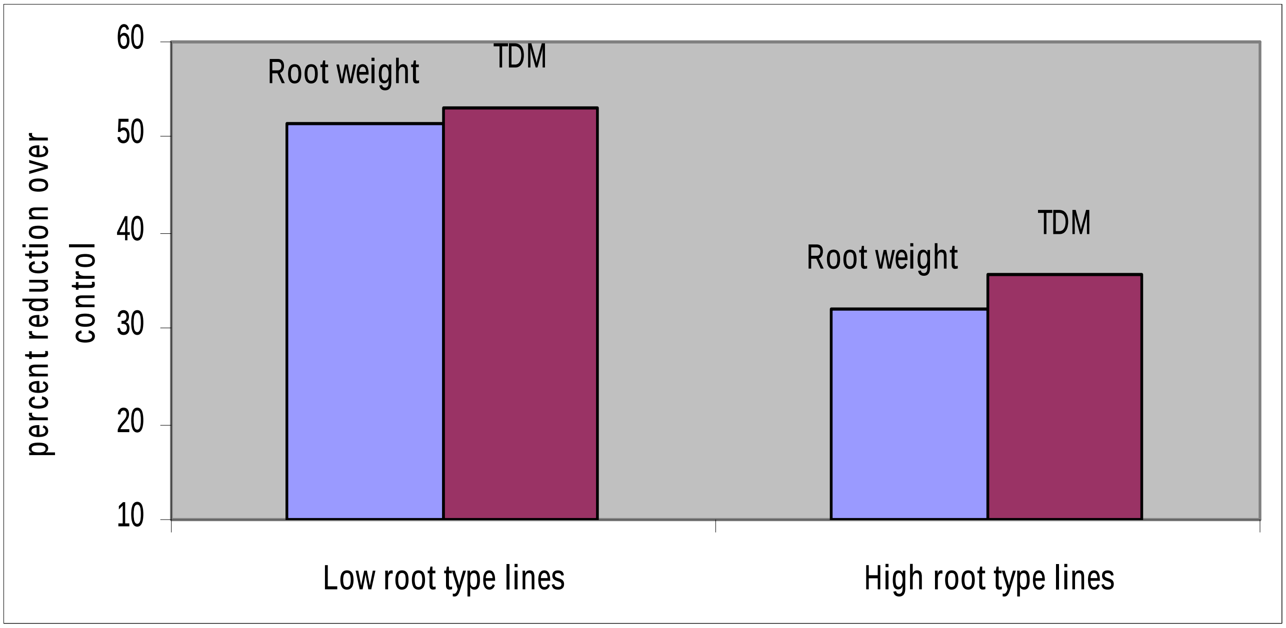


Fig 31: Percent reduction in root weight and TDM under moisture stress condition in low and high root type sunflower lines

Discussion

V. DISCUSSION

Under rainfed agriculture, water availability is the most predominant limitation to achieve potential productivity in most crops. With ever increase in population in tropical countries like India where rainfed agriculture is in vogue, increasing/ sustaining food productivity is a challenging task. In fact in this direction, one of the global research agendas of Crop Scientists all over the world is to increase the productivity of crop plants under water-limited conditions (Richards, 1996 and Richards *et al*., 2002).

Although breeding strategies through selection has improved the productivity under water-limited conditions, further progress is not forthcoming. A narrow genetic variability in yield *per se* among the cultivated varieties, the complex multi-gene regulation of yield and high G x E interactions are often responsible for this slow progress in breeding for yield under water-limited conditions. Further, it is now predicted that, the global food production must be increased by 1.6% to 2.6% annually to meet the forecasting demand of world population by 2020 (Brown and Byrd, 1996). As breeding for absolute yield is now becoming more difficult, it is now being argued that, breeding for constituent physiological traits will provide the necessary breakthrough in yield improvement in crop plants (Araus *et al*., 2002 and Sheshshayee *et al*., 2003).

In order to achieve sustainable productivity under water limited conditions, the trait based breeding approach is being envisaged. In this direction, any trait will be relevant for crop improvement only when it is associated with increased growth rate. From this context, the ability of plants to harness water from deeper soil profile associated with root system and efficient use of water referred to as water use efficiency are often considered as most relevant traits under water limited condition for improved productivity (Richards *et al*., 2002, Sheshshayee *et al*., 2003 and Condon *et al*., 2004). In the present study, with an objective of identifying and selecting sunflower lines with drought tolerance traits, an attempt was made to screen 120 sunflower lines comprising parents and inbreds. Here the objective was to look for genetic variability for drought tolerance traits and to select the lines with drought tolerance traits more specifically root traits and water use efficiency. The relevance of drought tolerance traits to improve the productivity under water-limited condition has been shown by a number of workers in the past. Accordingly, Richards *et al*., (2002) have successfully released wheat variety with high WUE and superior productivity over other varieties. Similarly, a rice cultivar having improved root traits with good growth and productivity under water limited condition was developed by Li *et al*., (2005). This clearly suggests that, the trait-based breeding approach is the likely answer for increased productivity under water-limited condition.

In recent years, rainfed agriculture is becoming unproductive due to so many constraints. Among the so many inputs that the crop requires, water is the most important one and at the same time most limiting. In this scenario, it is envisaged to develop drought tolerant or high water productive crop varieties/ hybrids to improve/ sustain productivity under water-limited conditions (Rauf *et al*., 2008). Since breeding for absolute yield in water limited condition is more difficult, it is being suggested that, several related stress adaptive traits must be pyramided to significantly enhance the level of drought tolerance through the trait based breeding approach. In fact, a significant improvement in productivity has been demonstrated by Condon *et al*., (2004) and Li *et al*., (2005) through enhanced root traits. Realizing the importance of improved productivity under drought in sunflower, an attempt was made to screen and identify lines with desirable traits in the present study.

Genetic variability: An essence for drought tolerance breeding

Most cultivated hybrids or open pollinated varieties evolved under near optimum agronomic conditions and often have same common parentage and history of origin. Therefore, breeding for drought tolerance must expand genetic variability. This depends on the incorporation of diverse germplasm so that potential sources of drought tolerance may be identified and subsequently incorporated to ensure yield when drought occurs. The first approach for the development of a drought tolerant line is to screen high yielding germplasm accompanied by superior yield contributing traits. In the present study with 120 parental lines and inbreds of sunflower, a wide genetic variability was observed for several of the drought tolerance traits including many other physiological traits (Table 4). Thus, breeding for drought tolerance would be possible in sunflower as the genes for the trait of interest is available. In fact, research conducted elsewhere also showed lot of genetic variability for the traits of relevance under stress. With 53 sunflower genotypes, Fereres *et al*., (1986) found substantial variability among genotypes both in dry land and under frequent irrigation condition. Similarly, a high genetic variability among traits related to water status, osmotic adjustment, root characteristics, gas exchange parameters, seedling traits and drought susceptibility index was found in a number of sunflower genotypes belonging to diverse origin (Seiler, 1994; Chimenti *et al*., 2002; Lambrides *et al*., 2004; Turhan and Baser, 2004; Kiani *et al*., 2007a; Rauf and Sadaqat, 2007a; Rauf and Sadaqat, 2008a). Thus, trait based breeding is possible in sunflower

Wild species are potentially important source of abiotic stress tolerance. Therefore, it may be desirable to introgress drought tolerant genes from wild relatives. According to Korrell *et al*., (1996), wild sunflower contains considerable variability for disease, insect pest resistance and tolerance to abiotic stresses like drought and salinity. For drought tolerance breeding, emphasis should be on the traits that confer drought tolerance in the plant and also show positive association with the yield. Over the past decades, plant breeders have focused on some traits that were incorporated to plant survival under stress conditions such as lower leaf canopy and reduced transpiration (Fischer and Wood, 1979; Karamanos and Papatheohari, 1999). Often, these traits were not necessarily positively associated with high yield. As a result, breeders continue to develop cultivars that yield poorly under stress conditions. Therefore, analysis of plant traits with significant effects on drought tolerance and high yield potential under stress conditions seems to be necessary (Richards, 2006).

Does variability exist for drought tolerance traits?

Variability for WUE was first documented by Briggs and Shantz in (1913) and a large body of literature to date has clearly shown the existence of considerable genotypic variation for this exploitable trait. Despite the realization of the importance of WUE, breeding to improve this trait has not always been successful (Udaya Kumar *et al*., 1998b). Two reasons are often attributed for this lack of success. Lack of high throughput screening technique has been one of the major constraints. Though accurate determination of WUE can be made through gravimetrically, this technique owing to its cumbersome nature prohibits large scale screening of germplasm and breeding lines. Measurement of gas exchange has made the determination of WUE at single leaf level quite rapid. However, since gas exchange is a snapshot measurement, it does not account for diurnal and seasonal variations in gas exchange parameters. Therefore, the discovery that plants discriminate against heavy isotope of carbon ($\Delta^{13}C$) during photosynthesis (O'leary, 1981) and association with WUE (Farquhar and Richards, 1984; Farquhar *et al*., 1989a & b) provided a tremendous impetuous to the efforts in assessing genotypic variability in WUE.

To date, numerous studies have experimentally validated the association between $\Delta^{13}C$ and WUE (Ebdon *et al*., 1998; Farquhar *et al*., 1989b; Martin *et al*., 1999; Condon *et al*., 2002; Richards *et al*., 2002). Despite the rapidity of measurement of $\Delta^{13}C$ using IRMS, success in breeding for improving WUE was not normally encountered. A concomitant decrease in total biomass while selecting for high WUE is perhaps the most significant reason for lack of success in breeding for high WUE. This arises because of a strong interdependence between transpiration and TDM. Maximizing WUE through a reduction in transpiration exhibits a significant yield penalty (Richards, 1996; Udaya Kumar *et al*., 1998b and Sheshshayee *et al*., 2003). Therefore, in order to exploit the advantages of WUE, it is essential to increase WUE without substantially decreasing transpiration. This necessitates the determination of genetic variability in both WUE and transpiration. While $\Delta^{13}C$ has been well established as a surrogate measure of WUE, the enrichment of heavy isotope of oxygen is shown to be a good time average reflection of transpiration rate (Sheshshayee *et al*., 2005). A significant negative relationship between WUE and $\Delta^{13}C$ and a positive relationship between transpiration rate and O^{18} enrichment was demonstrated in a number of crop species including sunflower to reiterate the relevance of these approaches for quantifying WUE and root traits respectively (Sheshshayee *et al*., 2003; Knight *et al*., 2006; Farquhar *et al*., 2007; Gessler *et al*., 2008). In fact in the present study, a wide genetic variability in WUE was observed among inbreds and parental lines of sunflower. Accordingly, the $\Delta^{13}C$ value ranged from 18.233‰ to 24.630 ‰ in the sunflower lines tested with a mean $\Delta^{13}C$ value of 20.952 ‰. Similarly, total dry matter (TDM) also showed significant and wide genetic variability among sunflower lines. Wide variability in these traits paved the way for selection and incorporation of these traits in breeding programmes. In fact, wide genetic variability for $\Delta^{13}C$ was also reported by several other workers in different crop plants such as barley (Craufurd *et al*., 1991), wheat (Ehdaie *et al*., 1991; Condon and Richards, 1992), cowpea (Ismail and Hall, 1992; Aniya and Herzog, 2003), peanut (Nageswara Rao *et al*., 1993), Sunflower (Lambrides *et al*., 2004) and tree crops such as teak (Mahadeva Murthy, 2009), cashew (Raju, 2001). Therefore, wide variability seen in sunflower lines could be attributed to the wide diverse lines available in our germplasm pool and depending on the interest of the researchers; the diverse lines with specific traits can be exploited and used them for crop improvement programmes. Presence of wide genetic variability in both WUE and TDM gave us an option to select the lines with desirable traits. In one of the studies, Rauf and Sadaquat (2007a) have identified the promising sunflower inbreds with these traits and suggested to use them for breeding programme. Since most often, high WUE is associated with low TDM, the lines with low $\Delta^{13}C$ (high WUE) and high TDM are worthwhile to identify and incorporate in breeding programme and if these traits are intorgressed and brought together on a single genetic background (more specifically on agronomically superior genotype), the productivity is further improved. In the present study also a number of sunflower lines with desirable drought tolerance traits have been identified and grouped based on the traits and these lines can now serve as source material for further breeding programme (Table 6-11).

Roots as an important drought tolerance trait

In addition to WUE, another important trait that imparts drought tolerance is root traits. Ability of the crop plants to extract water from deeper soil profiles associated with roots is considered as an important drought tolerance trait (Wright and Nageswara Rao, 1994). The root system of a plant is important while considering drought tolerance breeding because, certain root characteristics such as root length, root biomass and lateral root density would determine the efficiency of water extraction from the soil. A deeper root system would allow water extraction from lower soil profiles and thus it is expected that, the plant will perform better under moisture stress when its growth is dependent on water stored deeper in the soil. Sunflower with its deep and extensive root system can extract water up to 270 cm (Gimenz and Fereres, 1986; Connor and Sadras, 1992; Rachidi *et al*., 1993). Its root growth depends upon several factors such as plant genotype, water availability, plant population and soils. Limited research literature is available on the intraspecific variations for the root system in sunflower (O'Toole and Bland, 1987) since determination of root characteristics in the field is very laborious and time consuming. In fact, a few reports have characterized heritable variation in sunflower for root length (Fereres *et al*., 1986; Schneiter, 1992; Rauf and Sadaqat, 2008b). Angadi and Entz (2002) compared root system characteristics and water extraction patterns of dwarf hybrids with hybrids of standard height. Most of the dwarf sunflower hybrids showed lower root length, root length density and root distribution thus showing a positive correlation between plant height and root characteristics studied. An increase in root length and root-to-shoot ratio in response to moisture stress along with the decrease in lateral root length as an adoptive measure for drought stress can be generalized from the experiments conducted on sunflower (Petcu and Stanciu, 2001; Gomes *et al*., 2005; Rauf and Sadaqat, 2008b). Soil characteristics such as compaction and depth also have significant impacts on root growth. A decrease in root area and biomass had been observed with the increase in soil depth and compaction under moisture stress conditions (Petcu and Petcu, 2006).

In the present study, a significant genetic variation for roots was noticed among sunflower inbreds and parental lines. In fact, the root biomass ranged from as low as 5.47 g to as high as 137.00 g to indicate wide variability for this trait. Similar kind of wide genetic variability in roots was observed and reported in cowpea (Matsui and Singh, 2003), white clover (Annicchiarico and Piano, 2004) and chickpea (Yusuf Ali *et al*., 2005, Benjamin and Nielsen, 2006; Kashiwagi *et al*., 2006). The well developed root system of these crops would enable them to perform better under water-limited conditions than those with poor root system. This is true because, plants with good root system have an option to absorb more water from the deeper profile of the soil and are expected to survive better under drought or moisture stress conditions. Therefore, any efforts to incorporate this important trait onto an agronomically superior genotype will improve the productivity of crop under drought conditions (Richards, 2006; Rauf, 2008).

Relevance of drought tolerance traits

The relevance of these traits has been adequately explained in a simple mechanistic model proposed by Passioura (1976 and 1986). According to this model, total dry matter (TDM) is a function of total water used for transpiration and WUE. The latter term also referred to as Transpiration efficiency – is the ratio of amount of biomass produced to total water transpired during the specific crop growth period. Recent studies have shown considerable genetic variability in WUE in several crop species (Martin and Thorstenson, 1988; Virgona *et al*., 1990). In India, genetic variability in WUE has been demonstrated in a large number of crops as well as perennial tree species (Ashok *et al*., 1999; Udaya Kumar *et al*., 1998a; Sheshshayee *et al*., 2003; Raju, 2001; Vikram, 2008; Mahadeva Murthy, 2009). These studies clearly suggest that, there is considerable genetic variability for root traits in most of the species, which in fact can be exploited through breeding. However, the major lacuna has been the drudgery involved in assessing WUE in a breeding population. This lacuna has been significantly overcome by the discovery that plants discriminate against heavy isotope of carbon during photosynthesis resulting in the depletion of ^{13}C content in biomass (O'leary, 1981). The deviation of the carbon isotope ratio ($^{13}C/^{12}C$) of plant biomass from that of the air is called carbon isotope discrimination ($\Delta^{13}C$) is related to the partial pressure of CO_2 to that in the ambient air (Pi/Pa) (O'leary, 1981; Farquhar *et al.*, 1982). Since WUE is also related to ratio of CO_2 partial pressure, a strong interrelationship between the $\Delta^{13}C$ and WUE is expected (O'leary, 1981). The link of $\Delta^{13}C$ to Pi formed a framework for proposition that $\Delta^{13}C$ is related to WUE (Farquhar *et al*., 1989b). Till date several experiments have been conducted at our center as well as elsewhere both under field and green house condition to verify the relationship between $\Delta^{13}C$ and WUE (Sheshshayee *et al*., 2003).

In fact, $\Delta^{13}C$ is extensively being used in several laboratories across globe to improve WUE through breeding programmes. It has been noticed that, $\Delta^{13}C$ has broad sense heritability (Richards et *al*., 2002) and has a low G x E interaction (Ismail and Hall, 1992 and 1993; Ashok *et al*., 1999). These aspects render $\Delta^{13}C$ as a potential selectable parameter for crop improvement programme.

Genetic variability for other growth and physiological traits

Variability was not only seen for root traits and WUE among sunflower lines and inbreds, but even for other growth and physiological traits as well. Accordingly, variability was seen in total leaf area per plant, which ranged from 1791-10417 cm^2 with a mean of 5143 cm^2. Similarly, variability was also seen in leaf thickness measured by SLA where it ranged from 80 cm^2 g-^{1} to as high as 262 cm^2 g-^{1}. The total dry matter also found to be varied among the sunflower lines tested. They ranged from 85.80 g to 356.99 g. With respect to root traits, significant and wide genetic variability seen for root length, root volume and root weight. Accordingly, the root length ranged from 20.33 cm to 81.25 cm, while root volume and root weight ranged from 20 cm^3 to 335 cm^3 and 5.47 g to 137.00 g respectively. Water use efficiency assessed through carbon isotope discrimination approach also showed significant variability where $\Delta^{13}C$ values ranged from 18.23‰ to 24.63‰. In addition to this, the genetic variability was also seen for some of the gas exchange parameters like stomatal conductance and assimilation rate. Because of this variability, the WUE assessed at single leaf (A/gs) level also showed significant variation to indicate that the lines differ with each other in carbon fixation efficiency as well. Some of the lines fixed more carbon while others relatively less even though the growth conditions remained same to indicate their intrinsic ability to fix more carbon (mesophyll efficiency). With increased photosynthetic efficiency in some of the lines coupled with more leaf area, the total biomass would be expected to be rather high. Further, majority of sunflower lines in the present study with high root exhibited high total biomass. This is true with many crops because, plant with good root system tends to produce more biomass not only because of its root weight contributing for TDM but also by absorbing more water and nutrients from the soil and supplying it for transpiration and metabolic demands of the plants for increased carbon fixation. Increased assimilate production in such a scenario will facilitate the growth of above ground portion including stem biomass, leaf biomass and hence total biomass. Therefore, it is imperative to have good root system in plants for improved productivity under water-limited conditions.

Wide genetic variability for drought tolerance traits found in sunflower parents and inbred lines is really a boon for scientists to exploit desirable traits and incorporate them for breeding programmes to improve varieties and hybrids for water limited conditions. Presence of inbreds / parental lines with superior drought tolerance traits help the breeders to introgress these traits and come out with highly drought tolerant crop varieties / hybrids. These parents and inbreds with specific traits can also serve as a recipient parent to receive a gene of interest. In a nutshell, a parent / inbred with superior drought tolerance trait/s has many usages in breeding programmes. In fact, this kind of wide genetic variability for root traits, WUE and specific leaf area, total leaf area and total dry matter and many of the gas exchange parameters was also shown in a number of other systems like pigeon pea (Devaraj Achar, 2000), ground nut (Shashidhar, 2002), rice recombinant lines (Rekha, 2003 and Ayyappa, 2004), cotton (Ehab, 2006), coffee (Devaraj Achar, 2007) and sunflower (Vikram, 2008).

Contrasting lines for different traits

Existence of wide genetic variability for different growth and physiological traits including drought tolerance traits paved the way for selection of contrasting lines for different traits. These contrasting lines were so distinct that they can be used for developing mapping population. Similarly, a good number of lines with superior drought tolerance traits such as root traits, WUE coupled with high biomass have been identified in the present study and these lines can be directly used for crop improvement programme either for trait introgression or for receiving foreign gene/s of interest. In fact, some of these lines had a profuse root growth that weighs more than 100 g to indicate the presence of high root type sunflower lines. Similarly, some of the lines also exhibited high WUE (indicated by low $\Delta^{13}C$ values) and even very high TDM of more than 300 g to indicate the presence of good material that can be used for crop improvement programme under water limited conditions. In fact, it has been shown that the introgression of these superior traits onto elite genetic background would substantially improved the tolerance of crops under water deficit condition (Kaya and Ibrahim, 2004). Therefore, the material identified in the present study with superior drought tolerance traits can be used to improve drought tolerance of crop plants by incorporating these traits onto elite genetic background. It is also likely that, the lines with superior traits can serve as recipient parent to receive the foreign genes to further enhance or improve the tolerance and productivity under water limited condition. Thus, the existence of wide genetic variability in sunflower parents and inbreds is really paving the way for development of drought tolerant sunflower hybrids with relatively a good productivity. Since the variability was seen in parents and inbreds, these lines can straight away serve as one of the parents in hybridization programme. Especially the inbreds with superior drought tolerance traits can serve as pollen parent with the existing CMS lines. In case if these lines fail to restore fertility with any of the existing CMS lines, they can as well serve as maintainer lines and therefore ultimately used in conversion programme to develop new CMS lines.

Consistency of traits and relevance of root traits under water limited condition

Introgression of any trait including drought tolerance traits onto elite genetic background has relevance only when the traits are consistent across seasons and locations. In other words, high heritability is rather important to carry forward the identified material under crop improvement programme. In this scenario, an attempt was made to check the consistency of drought tolerance traits by selecting a subset of samples out of 120 lines used earlier. Here in this case, 18 parents and inbreds of sunflower were selected from six different groups made earlier (Table 6-11). In a nutshell, the parental lines and inbreds of sunflower representing the entire strata were selected for confirmatory test. Incidentally, the lines selected from six groups fall into either low or high root type category and hence with the same set of material, the relevance of roots under moisture stress was tested. Of the 18 contrasting lines sown on temporary root structures, the seeds of only 15 lines have germinated and the results of these lines are discussed here under.

In order to study how these lines perform under water limited condition, moisture stress was imposed. Before the imposition of moisture stress, initial plant height and initial leaf area were measured and found no significant difference between plants of control and stress designated blocks. A very strong correlation for plant height and leaf area between control and stress designated plants indeed suggests that, these parameters are similar to begin with before the imposition of stress. It is in fact true that, before the imposition of stress, the plant growth remained to be similar in all the treatments as the plants were grown under similar environmental conditions. Although significant difference was not found between the treatments, the genotypic differences were noticed within the treatment to indicate the existence of genetic variability among the lines tested for plant height and leaf area. Accordingly, some of the lines such as IB90, CMS106A, CMS207A and 336A did put on more plant height and more leaf area compared to other lines to indicate the existence of variability. Such kind of variability was also seen in many other systems to indicate the existence of variability, which can form as breeding material for crop improvement.

Physiological and growth characteristics in sunflower parents and inbreds both under control and stress conditions.

Specific leaf area (SLA), an indication of leaf thickness found to be significantly different in plants of control and stress treatment. Under control condition, SLA was 149 $cm^2 g^{-1}$, while in stress condition, it was 123 $cm^2 g^{-1}$ to indicate that under stress, SLA is reduced. Reduction of SLA under stress is due to reduction in leaf area without concomitant reduction in leaf thickness. In fact, reduction in leaf area under stress is serving as one of the strategies to survive under stress. Reduced SLA under stress has in fact been shown in a number of other systems (Ehab, 2006; Vikram, 2008). Specific leaf area not only differed across the treatments but even within the treatments as well. In fact, more leaf thickness and hence low SLA is found to be a good trait as genotypes possessing low SLA types are expected to be productive with high WUE. In many species, SLA is taken into account while selecting lines for WUE from a very large population (Nageshwar Rao *et al*., 1989).

SPAD chlorophyll meter reading (SCMR), a reflection of leaf chlorophyll / leaf nitrogen content showed no significant difference between control and stress treatment. However, genotypic differences for SCMR were observed among inbreds and parental lines of sunflower. Although SCMR readings were expected to be lower under stress due to degradation of chlorophyll, due to some reasons, SCMR readings in stress did not differ with control. It appears that, the plants did not experience the stress effect at the time of measuring SCMR readings and hence no significant difference was observed between control and stress treatment. However, a positive correlation observed between control and stressed plants for SLA and SCMR readings indicated that, the rate of decrease or increase in these parameters would remain to be similar. Further, a significant negative relationship between SLA and SCMR readings suggested that, any genotype with low SLA tend to contain more chlorophyll and is likely to be productive. In fact, such a strong negative relationship was also observed and reported in many other systems by earlier workers (Shashidhar, 2002; Rekha, 2003 and Ehab, 2006).

Gas exchange parameters particularly stomatal conductance is often implied as initial screening for WUE (Condon and Richards, 1993; Richards *et al*., 2002). Ratio of A/gs or A/T is often considered as instantaneous measurements of WUE. Although gas exchange parameters do not take into account the diurnal variation in assimilation rate and stomatal conductance, still these parameters are valid in determining the variation and hence one can use these parameters for initial screening. In the present study, water use efficiency (WUE) measured at single leaf level revealed that, there is a significant difference between the plants of control and stress treatment. WUE was significantly higher under stress condition compared to control condition. Under stress because of partial closer of stomata, plants lose less water and hence WUE tend to be higher. Although assimilation rate decreased under stress, the rate of decrease was more for stomatal conductance rather than assimilation rate and hence A/gs or A/T tends to be more. Increased WUE under stress has been a well proven concept and the results of the present study are supporting the already proven concept in plants.

Genetic variability for growth parameters under control and stress conditions

Although the number of lines used for confirmatory test was small, a significant genetic variability was found for various growth and yield parameters such as plant height, total leaf area, root length, root volume, root biomass, stem weight, leaf weight and TDM both under control and stress conditions. Under stress treatment, both plant height and leaf area were reduced significantly. However, the extent of reduction in leaf area was very drastic under stress condition to indicate that, whenever the plants experience stress, they reduce their leaf area/ Photosynthetic area as one of the strategies. This reduced leaf area is ultimately reflected in reduced biomass and seed yield in many species including sunflower. In fact in sunflower, seed yields are closely related to maximum leaf area (Muriel and Downes, 1974; Rawson *et al*., 1980; Rawson and Turner, 1983). Under moisture stress where virtually low leaf growth occurs, the seed yields are drastically reduced (Rawson and Turner, 1982). In fact, in the present study also, almost 50% reduction in leaf area was observed under stress. Although the leaf area was reduced by 50% under stress, a significant genotypic difference was observed where some of the inbreds and parents did maintain relatively good leaf area after alleviation of stress and such lines can definitely be considered as best lines for water limited conditions.

In the present study, a strong positive relationship was also observed between control and stressed plants for plant height and leaf area to suggest that, the performance of genotypes under moisture stress can be predicted from that under control condition.

Root traits in selected lines as influenced by moisture stress

Although the number of primary roots and root length did not differ significantly between control and stress treatment, root volume and root weight did differ. Nearly 30 to 40% reduction in root weight and root volume was observed under stress condition to indicate that, whenever the plants are under stress, the root biomass is decreased. However, genotypic differences for the number of primary roots, root length, root volume and root weight were found within the treatment. Incidentally, some of the lines chosen from high root type category maintained high root weight and high root volume both under control and stress conditions. This implies the heritability of such an important trait. Significant difference in root traits was also reported in many other systems by earlier workers both under control and stress condition to suggest the existence of variability for such an important trait (Ehab, 2006; Vikram, 2008). Therefore, these traits can be exploited and used in crop improvement programme.

Apart from the root traits, genotypic differences were also observed in TDM contributing parameters such as total leaf weight, stem weight, head weight and TDM across the parents and inbreds of sunflower. Further between treatments, significant difference in total leaf weight, stem weight, head weight, root weight and TDM were also observed. In fact under stress, the growth and yield contributing parameters reduced considerably and hence, the TDM was drastically reduced under stress condition. However, as pointed out earlier, some of the lines especially those that have come from high root type and high TDM type categories did perform well under water-limited conditions. They did maintain the yield attributing characters at relatively higher rate compared to the other lines. The results again support that, the traits are heritable and one can use these traits for crop improvement programme. Similar effect of moisture stress on sunflower growth and yield has been reported by many workers earlier (Ravishankar, 1990; Ganesh Kumar, 1994) to reiterate the findings of the present study.

Do drought tolerance traits are consistent across seasons?

Use of any trait in breeding programme is relevant only when the traits are consistent and heritable. The consistency and heritability of many physiological traits were examined in the present study and found that, the traits such as SLA, TLA, root weight and TDM were strongly correlated with the first season data to indicate the consistency and heritability of the traits across seasons. However, although high heritability was observed by previous workers for WUE (measured through carbon isotope approach), no correlation was found for $\Delta^{13}C$ values between the first experiment and second experiment. It is likely that, the sampling could be the main cause for not getting the correlation for $\Delta^{13}C$ between the experiments, as the stage of collection of leaf samples for $\Delta^{13}C$ was different.

Do high root types perform better under water-limited condition?

The relevance of root traits was tested under water-limited condition. Accordingly, many physiological, growth and drought tolerance traits were measured in both low root type and high root type inbreds and parental lines both under control and stress conditions. Based on the extent of reduction in each of these traits under stress condition, the results were interpreted. In the present study, the extent of reduction in root volume and root weight was significantly more in low root type compared to high root type inbreds and parental lines of sunflower. Similarly, in TDM contributing parameters such as total leaf weight, stem weight, head weight including TDM also, a significant reduction in the said parameters was observed in low root types compared to high root types. As against 53% reduction in TDM observed in low root types, the reduction was only about 36% in high root types under moisture stress condition over control. The maintenance of relatively high TDM under stress condition by high root types is possibly through the better root system where it helped in absorption of water from deeper soil profiles and supported the growth and maintenance of above ground portion. The results therefore signify the importance of roots under water-limited condition.

Therefore, in order to improve the productivity under water limited condition, the breeding approach should be focused on the component physiological traits that impart drought tolerance. In fact, research efforts of Richards and Condon (1993) and Li *et al.,* (2005) resulted in development of wheat and rice varieties with improved productivity under water limited conditions. Based on WUE and root traits, they have succeeded in developing and releasing suitable varieties. Therefore, it is now realized that, the trait based breeding approach could probably the best approach to develop drought tolerant crop varieties and hence it emphasizes the involvement of crop scientists from different disciplines to work together to address such an important task of developing drought

Summary

VI. SUMMARY

From the time of its introduction to the country, the area under sunflower has increased and presently it has occupied an area of about 2.16 Mha with a total production of 12.24 lakh tonnes. Not only the area, even the productivity of sunflower has increased over the years mainly due to introduction of high yielding hybrids. These sunflower hybrids incidentally are productive only under optimum conditions of irrigation and nutrients. However, majority of them failed to produce reasonably a good yield under moisture and other kinds of stresses. Therefore, in order to improve/sustain productivity under water-limited conditions, it is necessary to develop drought tolerant sunflower hybrids/varieties. As selection for yield *per se* has already been exploited, it is now envisioned that the trait based breeding approach could form as a viable option to improve productivity under water-limited condition. Among several drought tolerance traits, the ability of plants to harness water from deeper soil profile associated with roots, WUE and intrinsic tolerance are important which need to be exploited. In fact, the relevance of roots and WUE under water-limited conditions has been established in many crops and hence it is desirable to target on these traits to improve productivity in sunflower as well. However, in order to go for trait based breeding approach, suitable trait donor parents need to be identified from among the diverse lines available in the germplasm pool.

In the present study with an objective of identifying and selecting sunflower lines with superior drought tolerance traits such as root traits and water use efficiency (WUE), 120 lines of sunflower comprising parents and inbreds procured from different AICRP centers of the country were used. As measurement of root traits become extremely difficult/ impossible in the land grown plants, root structure approach was followed to quantify the root traits. With this approach, roots can be quantified fairly accurately. All the 120 sunflower inbreds and parental lines were grown on temporary root structures built above ground and were evaluated for several physiological traits associated with drought tolerance. These traits include roots, WUE and such other physiological traits associated with drought tolerance directly or indirectly.

In the present study with sunflower parents and inbreds, a wide and significant genetic variability for several physiological traits associated with drought tolerance traits has been found. Accordingly, the genetic variability was seen for total leaf area (TLA), specific leaf area (SLA), root length, root volume, root weight, TDM and $\Delta^{13}C$. Total leaf area, an important part of the plant for photosynthesis and hence for biomass production ranged from 1791 cm^2 to 10475 cm^2. Similarly, root biomass, TDM and $\Delta^{13}C$ values ranged from 5.47g to 137.00g, 85.80g to 356.99g and 18.23 ‰ to 24.63 ‰ respectively. The data therefore clearly indicated the existence of a wide and significant genetic variability for some of the important physiological traits associated with drought tolerance. The genetic variability was also seen for gas exchange parameters among the parents and inbreds of sunflower to indicate that, even in physiological processes variability was seen among the lines tested. Therefore, one can make use of this variability for crop improvement programme. Especially the inbreds/ parents with superior drought tolerance traits would be more useful as they can be used as trait donor parents to introgress the trait onto an agronomically superior background and also can serve as recipient parents to receive some of the relevant stress tolerant gene/s to improve the drought tolerance and productivity further.

As there is wide genetic variability for some of the traits associated with drought tolerance in sunflower parents and inbreds, an attempt was made to identify and select highly contrasting lines for roots, TDM and $\Delta^{13}C$. Accordingly, 10 lines with low roots; 15 lines with high roots; 16 lines with low $\Delta^{13}C$ (high WUE); 17 lines with high $\Delta^{13}C$ (low WUE); 19 lines with low TDM and 17 lines with high TDM were selected. These lines are highly contrasting and can be used for various purposes depending on the objectives. However, use of these materials is possible if the traits are consistent across seasons and locations. In other words, the said traits have relevance only if they are heritable. In this direction, in order to check the consistency of the traits, a few lines from each category that served as representative genotype were selected for confirmatory test. Accordingly, all together 18 lines were selected from six different groups (made earlier) and sown on temporary root structures to assess how these genotypes (inbreds and parents of sunflower) behave in the next season. In addition, the relevance of roots under water-limited condition was tested using the same set of material as the majority of the selected inbreds and parents fall into either low root or high root type category.

Of the18 lines sown in the root structures, the seeds of only 15 lines germinated, while the other three lines did not. For these lines, moisture stress was imposed during active vegetative phase and the relevance of roots under water limited condition was examined. The results of the study indeed revealed that there is significant genetic variability for the drought tolerance traits tested even with 15 lines. This is true not only under control condition but even under stress situation as well. Accordingly, significant variability was noticed for total leaf area, root weight and TDM across inbreds and parents of sunflower and showed variability both under control and stress condition. However, with respect to $\Delta^{13}C$, variability was narrow and in most cases non significant. As the material selected for confirmatory test was diverse, the wide variability was thrown out in the second experiment. Interestingly, even under stressful condition also, the variability was thrown out. The results therefore indicated that the traits are heritable and can be used for further breeding programme.

The consistency of the traits between the experiments was again confirmed through correlation studies. Here, the data of 1st experiment was made to correlate with the 2nd experiment and accordingly, for many traits such as SLA, TLA, root weight and TDM a significant correlation was found to indicate the consistency and heritability of these physiological traits. Although $\Delta^{13}C$ (WUE) is highly heritable, in the present study, probably due to wrong selection of leaf material, correlation was not found. Nevertheless, many other traits are related to each other and hence it can be concluded that the said traits are heritable and one can use them in breeding programme to develop drought tolerant crop varieties.

Apart from examining the consistency and heritability of the traits, the relevance of root traits under water-limited condition was also tested in the present study. Accordingly, the same set of sunflower lines used for confirmatory test was used for checking the relevance of root traits under water-limited condition as majority of the lines (excepting IB 91 and IB 98 which possess moderate roots) fallen into either low or high root type category. From the results, it can be concluded that the high root type inbreds/ parents by virtue of good root system performed better under moisture stress condition compared to the low root type inbreds/ parents. The extent of reduction in leaf area, leaf, stem, head and root biomass as well as in total dry matter (TDM) was significantly lower in high root type inbreds and parents compared to the low root type parents and inbreds. In fact in high root types, the extent of reduction in TDM was only around 35%, while in low root types; it was more than 50% indicating the importance of roots under water limited conditions. Some of the parents and inbreds such as IB 66, IB 79, IB 86, IB 90, 11A, CMS 106A and CMS 207A with good root system really performed well under stress condition compared to the low root type inbreds and parents such as IB 08, 6D-1, 6D-5-3-5 and P72 R. The results therefore signify the importance of roots under water-limited condition.

Overall, the existence of wide genetic variability for physiological traits associated with drought tolerance traits in sunflower parents / inbreds enable the Breeders in exercising option of either introgressing the superior drought tolerance traits or all together develop a new hybrid / variety with improved drought tolerance by straight away using the identified lines with superior drought tolerance traits as a pollen parent. Further, the contrasting lines identified in the present study can be used for developing mapping population.

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ANNEXURE

ANNEXURE- 1

Biometric data of parental lines and inbreds of sunflower evaluated in root structures

Sl No	Inbred / parental lines	SLA (cm^2/ g)	TLA (cm^2)	Total leaf weight (g)	Stem weight + Head weight (g)	Root length (cm)	Root volume (cm^3)	Root Weight (g)	TDM (g)	$\Delta^{13}C$ (‰)	SCMR
1	**CMS104A**	123.48	5397.99	38.63	166.85	47.75	132.50	49.88	255.51	21.77	34.65
2	**DRSH129B**	135.02	5433.85	31.07	105.37	62.00	103.33	72.73	209.52	20.66	36.50
3	**NDR7**	139.99	4663.23	30.66	107.70	53.80	118.00	21.72	161.00	19.74	35.73
4	**IB101**	131.24	3727.25	21.73	101.50	32.25	33.75	8.93	132.33	20.21	39.33
5	**NDR4**	170.22	6541.13	42.00	116.93	65.67	118.33	46.53	206.24	20.67	40.53
6	**6D-5-3-5**	147.34	3331.73	19.07	59.43	36.67	41.67	9.30	87.97	20.28	38.53
7	**CMS851A**	113.35	4858.67	23.47	82.80	36.33	73.33	29.17	135.53	21.03	42.43
8	**R801**	155.33	2139.29	17.53	78.30	35.00	35.00	8.87	104.74	21.39	41.97
9	**234A**	152.55	5445.68	34.23	129.30	46.00	203.33	33.20	196.98	20.04	43.47
10	**IB21**	136.60	3392.05	18.95	104.35	34.25	55.00	14.03	137.66	21.56	46.27
11	**CMS L-84-A**	171.92	2560.37	18.00	71.23	50.00	83.33	13.90	103.65	21.71	42.43
12	**IB13**	181.24	4102.17	30.33	109.90	45.00	153.33	63.83	204.37	18.23	33.15
13	**R649**	202.91	3703.86	22.80	71.47	34.33	46.67	9.40	104.33	21.87	34.20
14	**IB118**	142.94	5774.73	51.05	135.35	60.00	275.00	66.70	253.67	20.18	35.05
15	**P74R**	207.75	2921.70	19.07	79.57	44.00	103.33	13.83	112.56	20.30	37.45
16	**IB86**	150.29	6645.32	53.17	155.10	47.67	233.33	72.27	280.65	18.88	38.20

Sl No	Inbred / parental lines	SLA (cm^2/g)	TLA (cm^2)	Total leaf weight (g)	Stem weight + Head weight (g)	Root length (cm)	Root volume (cm^3)	Root Weight (g)	TDM (g)	$\Delta^{13}C$ (‰)	SCMR
17	**11A**	131.19	4386.10	33.42	74.98	40.50	87.50	23.05	119.91	20.48	39.10
18	**IB82**	137.74	4919.98	33.85	111.88	45.50	172.50	52.10	197.86	19.73	37.60
19	**PKV103R**	79.71	7021.24	57.35	148.40	62.50	300.00	137.00	342.78	19.26	38.20
20	**IB97**	234.02	6032.29	41.48	85.80	44.75	222.50	68.55	195.99	19.18	39.10
21	**P72R**	130.50	3795.42	23.35	58.73	39.50	82.50	15.60	97.84	20.58	37.60
22	**IB52**	140.34	4216.31	33.88	59.83	47.80	128.00	31.10	124.95	21.16	36.23
23	**CMS103A**	91.36	7401.26	33.42	181.80	58.00	90.00	34.15	285.90	20.20	37.57
24	**IB85**	94.36	7391.45	36.83	109.90	58.67	126.67	45.83	192.61	18.66	40.97
25	**NDR-8**	116.92	5448.42	37.10	69.43	81.25	197.50	61.83	168.79	20.38	42.40
26	**IB34**	152.09	3964.83	26.10	102.13	49.75	157.50	50.35	178.74	20.47	35.73
27	**16A**	104.17	4720.53	34.26	86.50	59.00	114.00	17.74	138.82	22.58	31.97
28	**IB78**	151.76	7126.45	49.23	121.08	65.50	166.25	64.50	235.45	20.50	38.30
29	**P78R**	104.26	5064.90	26.35	77.40	29.00	65.00	10.50	114.25	20.71	40.47
30	**IB56**	105.30	5731.90	48.33	184.83	51.00	193.33	52.10	285.47	19.97	37.10
31	**RHA296**	178.46	4702.63	23.83	67.20	35.75	65.00	8.90	100.46	20.13	40.30
32	**IB41**	178.11	3834.46	23.90	68.38	39.00	62.50	12.13	104.53	21.08	37.57
33	**RHA288**	154.76	3637.39	16.40	77.55	33.17	45.00	9.12	103.21	22.86	36.67
34	**IB44**	134.92	4603.81	27.43	86.73	41.25	102.50	19.80	134.13	20.91	37.43
35	**60A**	156.33	1791.70	14.17	52.33	44.67	56.67	40.03	106.77	22.35	40.47

Sl No	**Inbred / parental lines**	**SLA (cm^2/g)**	**TLA (cm^2)**	**Total leaf weight (g)**	**Stem weight + Head weight (g)**	**Root length (cm)**	**Root volume (cm^3)**	**Root Weight (g)**	**TDM (g)**	**$\Delta^{13}C$ (‰)**	**SCMR**
36	**IB 42**	161.95	3942.20	26.23	90.03	31.00	116.67	47.90	164.69	20.92	35.50
37	**95-C-2**	156.33	4169.79	25.88	77.85	53.75	55.00	9.93	114.08	21.58	42.17
38	**IB28**	262.38	5199.02	41.60	103.10	44.67	83.33	25.57	170.27	23.06	32.67
39	**6D-1**	173.57	8036.63	27.47	190.70	52.00	93.33	20.37	238.90	23.28	37.65
40	**IB20**	145.32	6023.34	28.27	139.73	54.67	223.33	76.23	244.31	22.44	38.10
41	**CMS155A**	138.81	5027.61	39.00	104.93	52.00	113.33	32.13	176.20	22.50	38.80
42	**RHA23**	156.64	6540.36	43.70	92.07	61.00	73.33	21.87	158.10	24.27	32.43
43	**95-C-1**	158.98	3023.52	36.14	65.77	45.33	41.67	10.87	115.72	23.46	36.67
44	**IB15**	166.91	8211.83	46.33	154.47	36.00	186.67	68.23	269.29	21.13	32.23
45	**R-16**	204.55	8071.98	42.40	134.93	59.33	96.67	21.07	199.17	24.63	34.30
46	**IB09**	147.10	3560.75	38.05	79.68	38.25	57.50	26.40	144.79	23.10	42.53
47	**LRHA-P-1**	No Germination									
48	**CMS234A**	150.15	5643.29	44.63	105.80	43.67	123.33	34.07	184.91	21.31	42.13
49	**LRHA-P-2**	193.55	3804.28	21.47	58.80	20.33	22.67	5.47	85.80	22.30	38.47
50	**IB66**	160.55	10475.02	83.33	139.50	56.50	232.50	93.28	316.36	20.97	34.70
51	**PKV104R**	155.65	5226.96	36.05	128.80	49.00	90.00	16.90	181.75	21.87	48.80
52	**IB79**	139.30	5321.83	39.07	156.17	37.00	130.00	91.87	287.55	20.85	43.73
53	**P62R**	174.12	7610.43	41.95	77.27	54.67	130.00	44.53	163.75	21.14	41.03
54	**RHA859**	131.91	4961.86	33.70	110.90	47.00	123.33	40.27	185.15	21.27	34.30

Sl No	Inbred / parental lines	SLA (cm^2/g)	TLA (cm^2)	Total leaf weight (g)	Stem weight + Head weight (g)	Root length (cm)	Root volume (cm^3)	Root Weight (g)	TDM (g)	$\Delta^{13}C$ (‰)	SCMR
55	**32A**	150.06	3935.35	32.20	98.13	44.33	78.33	18.27	148.89	24.33	47.40
56	**IB98**	161.33	9379.43	58.13	180.57	43.00	193.33	57.27	296.09	22.06	44.60
57	**18A**	181.00	2886.65	22.53	58.48	44.75	67.50	27.20	108.56	22.82	45.00
58	**IB03**	137.71	6196.86	50.10	129.53	55.00	147.50	30.78	210.95	20.46	38.23
59	**CMS LDSF-2A**	170.79	5141.03	36.40	113.80	56.00	106.67	46.53	197.74	19.81	44.50
60	**IB84**	187.93	5658.53	37.85	111.43	48.25	153.75	48.45	198.02	19.91	38.87
61	**11A**	182.10	4724.90	30.83	113.83	44.00	145.00	86.40	231.37	19.82	36.63
62	**IB31**	187.30	4583.35	31.20	101.47	49.33	78.33	15.20	148.67	21.21	43.67
63	**NDR-1**	167.55	4699.44	36.28	90.94	41.40	96.00	17.14	144.47	21.10	41.93
64	**CMS597A**	165.01	7634.78	57.73	170.13	36.67	226.67	127.93	355.96	19.69	45.27
65	**VRX-3**	No Germination									
66	**IB29**	128.71	5559.06	55.85	99.85	54.75	117.50	28.23	184.15	19.74	40.17
67	**FMS400A**	182.18	5077.94	35.90	94.30	48.50	155.00	83.55	214.61	19.75	38.00
68	**LRHA-16**	No Germination									
69	**852A**	No Germination									
70	**IB48**	168.37	4389.80	31.83	107.90	48.75	107.50	23.65	163.81	21.13	43.40
71	**CMS45A**	160.94	5438.01	28.78	87.50	30.75	38.75	15.75	132.24	20.45	34.67
72	**IB10**	154.37	8677.63	59.28	144.60	48.25	175.00	104.33	308.57	19.40	42.13
73	**10A**	221.07	5462.73	31.08	70.50	55.50	52.50	13.93	116.02	20.28	40.94

Sl No	Inbred / parental lines	SLA (cm^2/g)	TLA (cm^2)	Total leaf weight (g)	Stem weight + Head weight (g)	Root length (cm)	Root volume (cm^3)	Root Weight (g)	TDM (g)	$\Delta^{13}C$ (‰)	SCMR
74	**IB47**	155.58	6742.99	53.30	158.18	73.00	202.50	66.03	277.95	18.77	39.90
75	**3A**	136.67	5677.53	41.63	98.70	78.75	335.00	94.43	235.13	19.67	45.50
76	**DRSF109B**	108.93	4076.92	29.08	93.65	49.75	155.00	61.43	184.21	19.08	40.55
77	**44A**	165.07	7819.00	34.40	98.58	39.50	50.00	20.10	153.42	21.47	40.17
78	**IB07**	190.08	5520.40	31.38	120.58	62.00	100.00	51.93	204.02	19.22	38.00
79	**CMS 67-1A**	152.08	5080.17	24.48	83.22	44.60	60.00	16.46	125.09	21.86	41.63
80	**IB92**	159.97	6405.39	36.43	109.43	51.00	132.50	66.20	212.65	18.60	38.67
81	**NDR 6**	121.36	4836.21	46.23	131.77	31.67	83.33	27.27	205.82	21.25	40.90
82	**IB91**	114.32	9670.83	95.15	211.35	45.50	160.00	45.45	352.59	21.34	31.30
83	**RHA 272**	141.20	3416.09	31.30	123.20	21.67	46.67	15.77	170.27	21.97	44.15
84	**IB 55**	132.81	5277.95	50.27	103.40	41.33	70.00	11.83	165.97	20.70	46.95
85	**LRHA-W-5**	137.82	5968.71	53.67	157.67	29.67	46.67	19.43	231.00	21.48	36.27
86	**IB 26**	179.95	2618.73	23.28	77.63	32.75	20.00	7.20	108.43	20.93	38.80
87	**AK1R**	160.06	2902.17	17.20	74.73	23.75	25.00	7.65	99.74	21.89	39.37
88	**IB 11**	161.54	6452.25	40.20	100.25	30.50	185.00	68.00	208.90	18.87	42.97
89	**P 69R**	159.80	4889.16	33.23	84.63	28.00	73.33	32.20	150.11	21.29	45.50
90	**IB72**	No Germination									
91	**COSF-1A**	163.23	5999.94	65.95	107.65	35.00	95.00	37.50	211.10	22.12	39.30
92	**IB 35**	183.25	2618.84	26.93	106.30	29.00	123.33	36.13	169.41	21.19	34.53

Sl No	Inbred / parental lines	SLA (cm^2/ g)	TLA (cm^2)	Total leaf weight (g)	Stem weight + Head weight (g)	Root length (cm)	Root volume (cm^3)	Root Weight (g)	TDM (g)	Δ^{13}C (‰)	SCMR
93	**586R**	136.73	3866.87	20.80	76.00	31.50	45.00	15.30	112.40	21.48	36.23
94	**IB77**	No Germination									
95	**LRHA 332**	133.13	4785.89	43.80	108.90	32.67	53.33	15.70	168.54	20.29	37.63
96	**IB 04**	161.26	3351.61	29.60	107.65	52.25	102.50	23.98	161.42	22.45	43.07
97	**PKV 101R**	148.67	3208.58	29.50	68.95	33.25	40.00	22.38	120.97	21.72	40.77
98	**IB 22**	164.24	3299.33	29.30	91.90	41.00	46.67	21.03	142.81	22.39	45.83
99	**NDCMS85**	No Germination									
100	**IB27**	No Germination									
101	**336 A**	142.64	6266.62	44.90	137.30	35.00	193.33	107.80	296.75	18.94	43.03
102	**IB 18**	169.25	7717.33	47.55	154.10	34.75	92.50	31.70	233.37	21.46	37.93
103	**207A**	No Germination									
104	**IB 100**	202.23	6438.65	38.55	126.23	32.50	127.50	29.38	194.44	21.17	42.40
105	**CMS-L-71-106A**	141.70	4320.26	32.10	106.80	35.67	186.67	113.13	252.20	18.86	31.30
106	**IB 90**	122.93	8857.70	23.23	108.50	28.33	110.00	89.03	220.88	18.62	33.07
107	**31A**	186.45	4042.53	32.60	120.98	35.50	85.00	31.35	184.97	21.64	37.10
108	**IB 08**	194.65	3337.45	35.55	134.18	32.50	82.50	23.58	193.57	23.08	37.33
109	**RHA265**	No Germination									
110	**CMS-L(DS) 207A**	180.80	6339.76	52.63	190.33	34.67	253.33	112.73	356.99	18.96	38.52
111	**IB 38**	133.75	4040.20	30.45	101.70	40.00	95.00	50.25	182.96	19.48	34.90

Sl No	**Inbred / parental lines**	**SLA (cm^2/g)**	**TLA (cm^2)**	**Total leaf weight (g)**	**Stem weight + Head weight (g)**	**Root length (cm)**	**Root volume (cm^3)**	**Root Weight (g)**	**TDM (g)**	**$\Delta^{13}C$ (‰)**	**SCMR**
112	**CMS 17A**	145.01	2904.03	35.65	100.13	33.00	75.00	27.60	163.51	22.07	36.70
113	**IB 37**	142.32	3385.02	33.08	99.85	36.75	70.00	28.33	161.29	20.99	42.97
114	**RHA 354**	155.00	4580.11	24.93	105.30	34.25	62.50	28.08	158.88	21.88	45.50
115	**IB 45**	171.24	5578.88	32.63	122.83	32.75	97.50	38.75	194.80	21.16	36.57
116	**RHA116**	No Germination									
117	**IB40**										
118	**RHA 278**	165.07	4024.10	29.73	121.55	35.25	60.00	16.15	167.58	21.00	40.13
119	**IB 43**	157.46	3333.10	24.93	76.23	23.00	43.75	23.40	124.86	22.09	34.60
120	**CMS101**	No Germination									