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INFLUENCE OF HIGH TEMPERATURE ON ROOT SYSTEM ARCHITECTURE TRAITS IN SUNFLOWER (*HELIANTHUS ANNUUS* L.) GENOTYPES

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ABSTRACT

Roots grown in plant growth chambers annexed with image analysis could be potent for screening root traits to understand their morphology and architecture prior to field experimentation. The experiment was carried out to study the effect of increasing temperature (25, 30, 32.5, 35, 37.5 and 40°C) on germination and root parameters of 10 inbred lines and 4 hybrids of sunflower during a seven day period. The objective of the study was to investigate the effect of increasing temperature on root traits so that the breeding programme may be benefited. The root traits studied include root length, projected area, surface area, root volume, root average diameter, root dry weight of sunflower lines. Sampling was done and the data was recorded by using win RHIZO software. Analysis of data revealed that there was a significant difference between genotypes and treatments for the characters under study. The average root diameter was highest at 40°C. Primary root growth, projected area, surface root area and root dry weight averaged over all genotypes was greatest in the 30 – 32.5°C temperature range whereas, root volume was high at 30°C and maximum germination percentage was observed at 25°C. Hybrids DRSH-1 and CSFH-12205 and CMS Lines - 127B, 70B, 42B, AKSF-6-3B showed better germination and root traits at high temperatures (HT). The relationship between sunflower root growth and temperature may be of value in determining planting dates and the root traits studied could be indicators of high temperature tolerance.

Key words : Temperature, Root length, Root diameter, win RHIZO, Germination percentage.

Introduction

Sunflower with optimal temperature of 25–30°C for germination and growth is more sensitive to abiotic stresses than other plant species (Rauf *et al.*, 2012). Temperatures >30°C pose stress on the plant (Qadir *et al.*, 2007) that can cause irreversible damage to plant function and development thus strongly affecting the root growth (Hatfield and Prueger *et al.*, 2015), affects the plant developmental and physiological processes (Liu *et al.*, 2022).

Roots provide anchorage and support for the shoot by taking up water and nutrients from the soil and transporting them to above ground plant parts, storing carbohydrates and other reserves and are a site of biosynthesis of hormones (Zhu *et al.*, 2011). Plants with different root characteristics respond, adapt and survive in different environments. Root responses to HT include several modifications in structure and function (Atkinson and Urwin, 2012) to adapt and cope with stresses (Aidoo, *et al.*, 2016).

The spatial distribution of all root parts in a particular

environment and which is affected by the external environment is referred to as Root System Architecture (RSA) (Lynch, 1995). Global change results in variation of RSA which impacts plant performance by affecting nutrition acquisition (de la Fuente Canto *et al.*, 2020). RSA response to HT is species specific (Gray and Brady, 2016) as the effect of increasing temperature on root growth of plant seedlings can be promotive (Lahti *et al.*, 2005), inhibitive (Takahashi *et al.*, 2019), or first promotive and then inhibitive after an optimum temperature is reached (Seiler, 1998). Even the species growing in same habitat, have species-specific RSA responses to increased temperature (Bardgett *et al.*, 2014). Therefore, it is important to screen and breed crop cultivars with better RSA, which can adapt to stresses and have improved water and nutrient efficiency (He *et al.*, 2013). The magnitude of heat stress effect rapidly increases as temperature increases above a threshold level (Gray and Brady *et al.*, 2016).

An increasing population requires cultivars that can continue to be productive in stress environment and are capable of efficient resource capture from the soil. Understanding the root phenology with higher yield and increased stress tolerance will help breeders to select parents with ideal root phenotypes and develop breeding lines for crop improvement. A clear understanding of the germination and seedling root growth is useful in screening for tolerance of crops and cultivars to HT. It is hypothesized that root growth of sunflower seedlings derived from different inbred lines will have a different response to root temperature due to their diverse genetic backgrounds. This work addresses root growth traits of sunflower seedling adaptation to warming climate i.e., which root traits respond sensitively to temperature change.

Materials and Methods

47 sunflower genotypes were screened for HT using temperature induction response (TIR) technique at Indian Institute of Oilseeds Research Hyderabad, India (Aparna *et al.*, 2023). The identified 6 tolerant and 4 susceptible lines along with 4 hybrid checks were evaluated for root structure analysis at six temperature treatments (25°C, 30°C, 32.5°C, 35°C, 37.5°C, 40°C) and replicated for three times in factorial CRD. Seeds were surface sterilized with 1.3% sodium hypochlorite solution, washed with sterile water and placed between two moistened filter paper sheets (50cm wide and 19cm long) covered with plastic cover outside. The papers along with seeds and plastic cover were rolled secured with rubber bands and kept in erect position in a beaker and kept in seed

germinator. Each roll contained 10 seeds and three replicates were used. Each experiment set consisted of growing seeds for seven days at constant temperature ranging from 25 to 40°C. After 7 days the germination percentage was calculated using formula

Germination percentage (GP) % = (Number of seeds germinated / Total number of seeds) × 100

The roots were then separated from the seedling and scanned using a scanner with Win RHIZO software to produce a 2D image. These root images were used to estimate total root volume, root surface area, root length, average root diameter and the root diameter. The root dry weight was taken after drying in oven at 70 °C till they attained a constant weight. All procedures were conducted in accordance with the guidelines.

Statistical analysis

Five plants per replication were collected and the mean data points were used for statistical analysis. The effect of different root zone temperatures on root architecture was analyzed using one-way ANOVA (IBM SPSS). PCA was performed using IBM SPSS software and the values of the first two components were selected and analyzed using SPSS.

Results and Discussion

Germination (G)

G which is determined as the emergence of the root from the seed coat was maximum at 25°C and 30°C (CMS lines -70B, -107B, -125B, CO 2 and DRSH 1) and decreased thereafter (Fig. 1). Inbred line CMS 42B at 25°C, 30°C and 32.5°C, Check CSFH 12205 at 35°C and 40°C and CO-2 at 37.5°C has recorded highest G (Fig. 2). CMS lines-42B, -17B, -107B and CSFH 12205 has showed highest G (Table 1). The results were in accordance with Gay *et al.* (1990) observed the thermal optimum was around 25°C where almost all achenes germinated within 2 days.

Total Root length (TRL)

Inbred line CMS 127B at 25°C, while hybrid checks DRSH 1 at 30, 32.5, 35 and 37.5°C and CSFH 12205 at 40°C recorded higher TRL (Fig. 3). The overall TRL increased up to 32.5°C and decreased thereafter up to 40°C. The average TRL among genotypes was highest in hybrid DRSH 1 and lowest in inbred line CMS 125B. The chronological relationship between temperature and TRL can be seen in Fig. 1. Genotypes CMS 125B, -127B, -135B, -144B, -42B, CO 2, DRSH 1 and KBSH 44 at 30°C, AKSF 6-3 B, CMS 17B, -107B, -70B, ARM 243B and CSFH 12205 at 32.5°C has recorded maximum

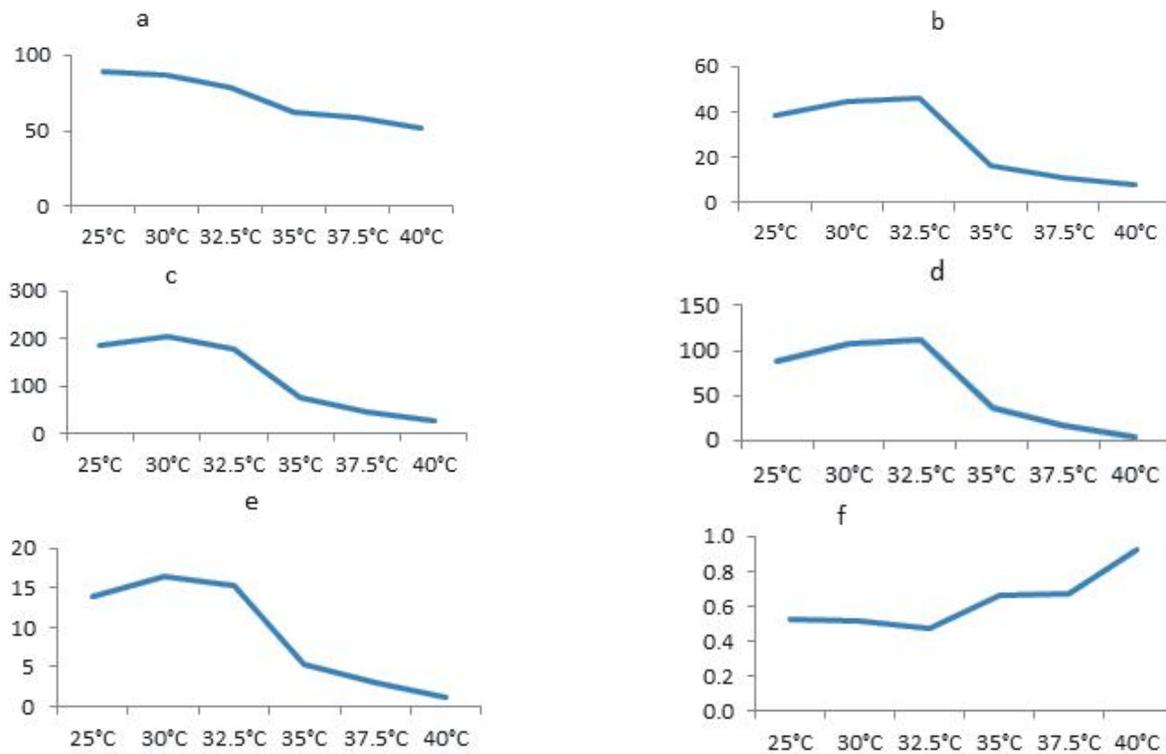


Fig. 1 : Cumulative a) germination percentage b) root dry weight c) root volume d) total root length e) total root area f) root average diameter sunflower genotypes at increasing temperature from 25 to 40°C at 7 days after planting.

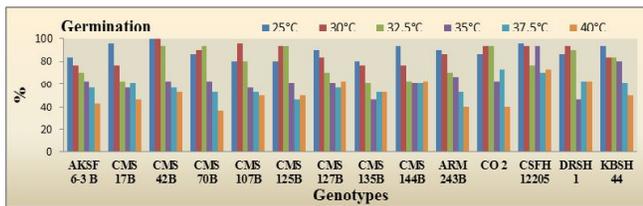


Fig. 2 : Performance of sunflower inbreds and checks for germination (%) at increasing temperature.

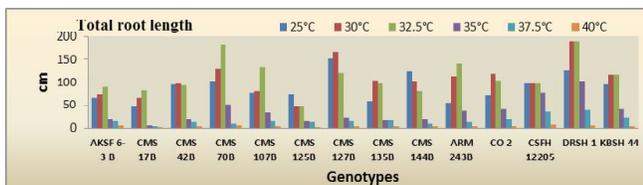


Fig. 3 : Performance of sunflower inbreds and checks for total root length (cm) at increasing temperature.

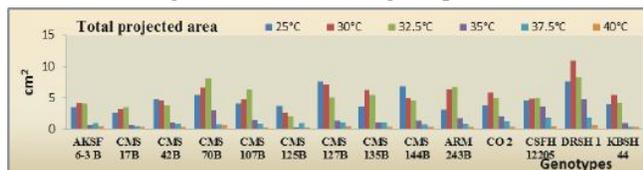


Fig. 4 : Performance of sunflower inbreds and checks for total projected area (cm²) at increasing temperature.

TRL (Table 1).

In general root growth tends to increase with increasing temperature until an optimum is reached above which root growth is reduced. The results were in accordance with Seiler (1998) demonstrated that

sunflower primary and lateral root growth was greatest in the 25–30°C at 10 DAP due to faster meristem cell division rate at increased temperature (Francis and Barlow, 1988). Burholt and Van’T Hof (1971) reported that the individual cell division rate is increased in the sunflower root meristem, cell production rate at the individual cell is increased and at the level of the organ is diminished in the 25-35°C temperature range.

The projected area (TPA) and surface area (TSA)

Inbred line CMS 127B at 25°C has maximum TPA, check DRSH 1 has recorded highest TPA and TSA at 30, 32.5, 35 and 40°C and check CSFH 12205 being at par with all other genotypes showed highest TPA and TSA. The overall TPA and TSA has increased up to 30°C and decreased thereafter up to 40°C (Fig. 1). Among genotypes check DRSH 1 has recorded highest TPA and TSA while inbred CMS 125B and check KBSH 44 has recorded lowest TPA (Fig. 4) and lowest TSA (Fig. 5). A slight decrease in TPA and TSA was observed from 30°C to 32.5°C. Lateral roots increased with increasing temperature up to 30°C and decreased at 32.5°C for seedlings at 7 DAP (Table 2).

The results were in accordance with Seiler (1998) demonstrated that the number of lateral roots increased with increased temperature up to 30°C, then declined at 35°C in sunflower. The branching densities response to a broad range of temperatures is a function of the primary

Table 1 : Principal component analysis of selected traits and the proportion of variation in each principal component.

	PC 1	PC 2
TPA35	.915	
TSA35	.915	
RV35	.889	
TSA40	.878	
TPA40	.878	
TSA30	.855	-.405
RLV25	.854	
TRL35	.836	.222
RDW35	.828	.291
RLV35	.811	.277
TPA30	.806	-.366
RDW37.5	.793	.455
TRL40	.793	.238
TRL30	.785	-.338
TPA37.5	.760	.437
TSA37.5	.760	.437
RDW40	.745	.222
TPA25	.744	-.201
RLV30	.738	-.137
RV30	.717	-.508
RLV37.5	.706	.645
TRL37.5	.699	.504
TSA25	.698	-.218
RV37.5	.696	.356
TPA32.5	.694	-.496
TSA32.5	.682	-.579
RDW25	.679	
RLV32.5	.675	-.497
AD37.5	-.674	-.182
TRL32.5	.666	-.497
TRL25	.659	
RV40	.651	-.278
RDW30	.638	-.391
RV25	.617	-.286
RLV40	.530	.188
AD25	-.501	-.345
G35		.723
RV32.5	.579	-.609
G40	.472	.479
AD35	-.481	
AD40	-.377	-.118
AD30	-.445	-.142
RDW32.5	.396	-.289
G30	.395	.229

Table 1 continued...

Table 1 continued...

G32.5	.301	.122
AD32.5	-.178	.165
G25		.360
G37.5	.389	.514
Eigen Value	21.9	5.8
Variance (%)	45.7	12.1
Cumulative (%)	45.7	57.9

Table 2 : Coefficient of variation values ($P < 0.05$) of different root traits.

	G	TRL	TPA	TSA	AD	RV	RDW
LSD (G)	6.2	6.2	0.43	1.7	0.005	25	3.5
LSD (T)	4	4.1	0.28	1.1	0.003	17	2.3
LSD(G*T)	15.1	15.4	1.05	4.1	0.11	62	8.6

G-Genotype, T-Temperature, G*T-Genotype* Temperature interaction.

root length and number of lateral roots (Zhu *et al.*, 2011).

Average diameter (AD)

The AD of roots was decreased up to 32.5°C and increased thereafter up to 40°C (Fig. 1) for all the lines except for CMS -107B and -144B. CMS 135B at 25, 30 and 32.5°C, CMS 125B at 35 and 40°C and CMS 17B at 37.5°C (Fig. 6) has recorded highest AD. Among the genotypes CMS 125B has recorded highest and CSFH 12205 has recorded lowest AD (Table 3).

Root Length/volume (RL/V)

The RL/V of roots was increased up to 30°C and decreased thereafter up to 40°C (Fig. 1) for all the lines except for CMS 70B, -107B, -135B, ARM 243B, and check CO 2 RL/V increased up to 32.5°C and decreased thereafter (Table 3). At 25°C check DRSH 1 (164.45 cm³), followed by inbreds CMS 144B (124.99 cm³), CMS 127B (117.28 cm³), at 30°C inbred CMS 127B (151.9 cm³) followed by check DRSH 1 (125.09 cm³), inbred CMS 144B (123.04 cm³), at 32.5°C inbreds CMS 127B (118.97 cm³) followed by CMS 107B (117.12 cm³), check DRSH 1 (116.98 cm³), at 35°C checks DRSH 1 (72.83 cm³) followed by CSFH 12205 (64.04 cm³), inbred ARM 243B (35.91 cm³), at 37.5°C checks CSFH 12205 (47.19 cm³) followed by DRSH 1 (30.13 cm³), CO 2 (21.75 cm³), at 40°C inbred AKSF 6-3B (5.41 cm³) followed by check CSFH 12205 (5.36 cm³), inbred CMS 70B (5.32 cm³) has recorded maximum RL/V. Among the genotypes, check DRSH 1 (85.71 cm³) has recorded highest and inbred CMS 17B (20.91 cm³) has recorded lowest RL/V (Fig. 7).

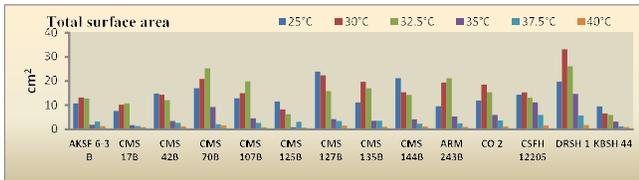


Fig. 5 : Performance of sunflower inbreds and checks for total surface area (cm²) at increasing temperature.

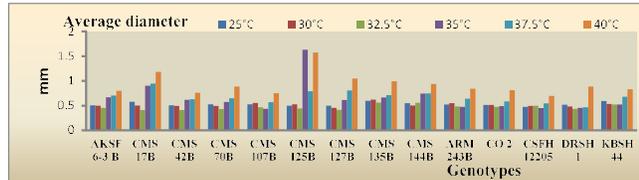


Fig. 6 : Performance of sunflower inbreds and checks for average diameter (mm) at increasing temperature.

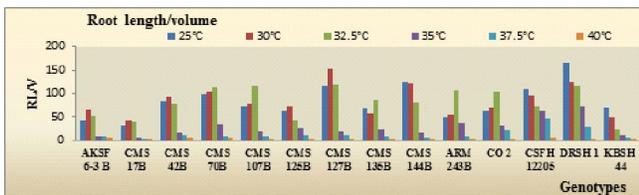


Fig. 7 : Performance of sunflower inbreds and checks for root length/volume (cm/m³) at increasing temperature.

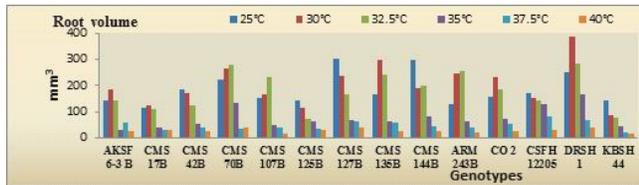


Fig. 8 : Performance of sunflower inbreds and checks for root volume (mm³) at increasing temperature.

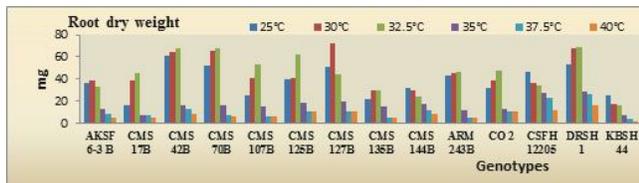


Fig. 9 : Performance of sunflower genotypes for root dry weight (mg) under increasing temperature.

Root volume (RV)

Inbred line CMS 125B at 25°C, checks DRSH 1 at 30, 32.5, 35 and 40°C and CSFH 12205 at 37.5°C showed highest RV (Fig. 8). Average RV was increased up to 30°C and decreased thereafter (Fig. 1). Among genotypes DRSH 1 has recorded maximum and KBSH 44 has recorded minimum RV. The decrease in RV was more prominent from 35°C.

Root dry weight (RDW)

Inbred lines CMS 42B at 25°C, CMS 127B at 30°C and check DRSH 1 from 32.5 to 40°C recorded maximum RDW. RDW was maximum for lines at 32.5°C, except for CMS 144B, CSFH 12205, KBSH 44 (maximum RDW

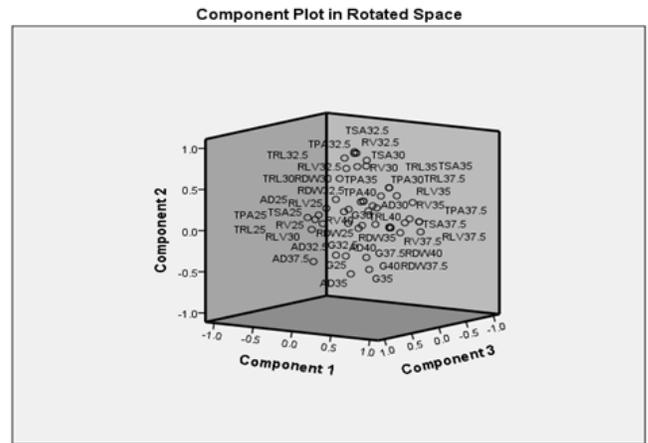


Fig. 10 : Principal component analysis of selected root traits across 14 sunflower genotypes and six temperatures.

at 25 °C), AKSF 6-3 B and CMS 127B (maximum RDW at 30 °C) suggesting that roots has prospect to grow up to 32.5°C (Fig. 1). The decrease in RDW was observed from 35°C to 40°C (Fig. 9). Among genotypes DRSH has recorded maximum and KBSH 44 has recorded minimum average RDW. RDW reflects the allocation of assimilate to the roots (Iqbal *et al.*, 2020). Several genera of plants respond differently to the influence of root temperature on root growth for RDW (Cooper, 1973).

Root Trait Variability

Two principal components (PCs) were identified with eigen values >1, capturing 57.8% of the total variation in root system architectural traits across the 8 root parameters at six temperature treatments (Table 1). The first component (PC1) represented 45.8% of the variability and influenced by most root traits (TPA, TSA, RV, RL/V, TRL, RDW, AD at 25, 30, 32.5, 35, 37.5, 40°C and germination at 30, 32.5, 37.5, 40°C Table 1). PC2 represented 12.1% of the total variation and was influenced by the TSA and TPA at 25, 30, 32.5, 37.5, TRL, RDW and RL/V at 30, 32.5, 35, 37.5, 40°C, RV and AD at 25, 30, 32.5, 37.5, 40°C and germination at 25, 30, 32.5, 35, 37.5, 40°C (Table 1).

Genotypes distribution of the 8 selected traits under 6 temperature treatments based on PCA regression scores is shown in a biplot (Fig. 10). An analysis of variance (ANOVA) indicated that genotypes, temperatures and interaction were significant sources of variation for germination, TRL, TSA, AD, RV and RDW (p<0.05) (Table 2). The differential response of the sunflower genotypes to root growth at different temperatures (Fig 11A & 11B) is an example of genotypes by environment interaction. The temperature for optimum emergence and growth differ significantly among species (Zhang *et al.*, 2015). The diverse genetic backgrounds of plant species

Table 3: Promising genotypes for different traits at high temperatures.

Trait	Best genotypes at higher temp of 37.5°C or 40°C	
	Checks	CMS lines
Germination (%)	CSFH-12205, DRSH-1	CMS 144B, CMS 127B
Root length (cm)	CSFH-12205, DRSH-1	CMS 70B, AKSF-6-3B, CMS 42B
Root dry weight (mg)	CSFH-12205, DRSH-1	CMS 125B, CMS 127B, CMS 144B, CMS 42B
Root volume (mm ³)	CSFH-12205, DRSH-1	CMS 127B, CMS 70B
Root area (cm ²)	CSFH-12205, DRSH-1	CMS 70B, CMS 127 B, AKSF-6-3B
Root average Diameter (mm)	CSFH-12205, KBSH-44, CO-2	CMS 42B, CMS 107B, AKSF-6-3B

are important in determining root system growth with changes in temperatures both between and within species. Genetic diversity in root growth is important for survival of species in highly variable climates (McMichael and Quisenberry, 1993).

The present study was undertaken to test the hypothesis that root growth of young sunflower seedlings of different lines will have a different response to temperature. In the present paper, we investigated the effect of different temperature on seed germination and root growth. This study confirms that in the absence of other limiting factors the seed germination and seedling root growth were temperature dependent. This observation was inconsistent with past works (Luo *et al.*, 2020). Cooper (1973) reviewed the relationship between root temperature and plant growth for several plant genera and concluded that genera respond differently to the influence of root temperature on root growth for root dry weight, root extension and root branching.

The exposure of sunflower roots to higher temperature than optimum temperatures can have an adverse effect on the growth and development of the root system. Changes in root morphology as a function of changes in temperature are generally characterized by differences in root length, dry weight and branching. In general root growth tends to increase with increasing temperature until an optimum is reached above which root growth is reduced. Optimum temperature for root growth in sunflower was 25°C. If the temperature deviates from optimum, root growth may be affected. Root growth in sunflower genotypes studied was maximum in the 30–32.5°C temperature range.

The genotypes vary in their RSA responses to temperature changes, especially root volume and root area. The overall projected area and surface area has increased up to 30°C and decreased thereafter up to 40°C. A larger root system helps to acquire more resources and increase nutrient uptake under field condition (de Kroon *et al.*, 2003). The differences in root

size of sunflower genotypes to temperature change suggest that Hybrids CSFH-12205 and DRSH-1 and inbreds CMS 127B, CMS 70B, CMS 42B, AKSF-6-3B showed better germination and root traits at high temperatures (Table 3). Root branching intensity exhibited sensitivity to temperature change. Overall total root area increased upto 30°C and decreased thereafter which is consistent with previous reports (Nagel *et al.*, 2009). This might be caused by high temperature (30°C) significantly accelerating the root meristem cell division, thus development of lateral root primordium (Francis and Barlow, 1988). Enhanced root surface area at 30°C that in turn will water uptake (Pregitzer *et al.*, 2000). This increase provides their roots with a greater surface area for absorption per unit root weight or length (Macduff *et al.*, 1986). Several studies made with tomato have shown that the more heat tolerant varieties are those that have a higher root activity or a larger RSA.

The AD of roots was decreased up to 32.5°C and increased thereafter up to 40°C. High root branching intensity usually results in thin root diameter (Kaspar and Bland, 1992).

Genetic variation exists among the lines for root development in response to changes in temperature. Both the optimum temperature for root length development and the temperature response for lateral root initiation differed among the lines. The differential response of the sunflower genotypes to root growth at different temperatures is an example of genotype by environment interaction (McMichael and Quisenberry, 1993). The high significant interaction of genotypes with temperature is confirmed by statistical analysis.

In vitro assays showed that high temperature (> 32.5°C) inhibited primary root growth of sunflower and increased root diameter (Fig. 1). Roots with smaller diameter have shorter lifespan than roots with larger-diameter roots (Baddeley and Watson, 2005) and are more metabolically active with higher C cost to the plant (McDowell *et al.*, 2001). However, roots of small diameter contain greater nutrient concentrations and are

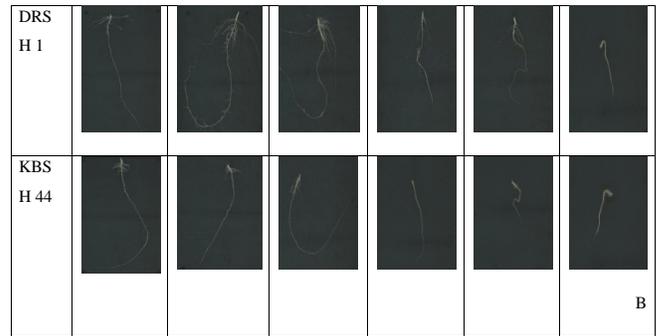
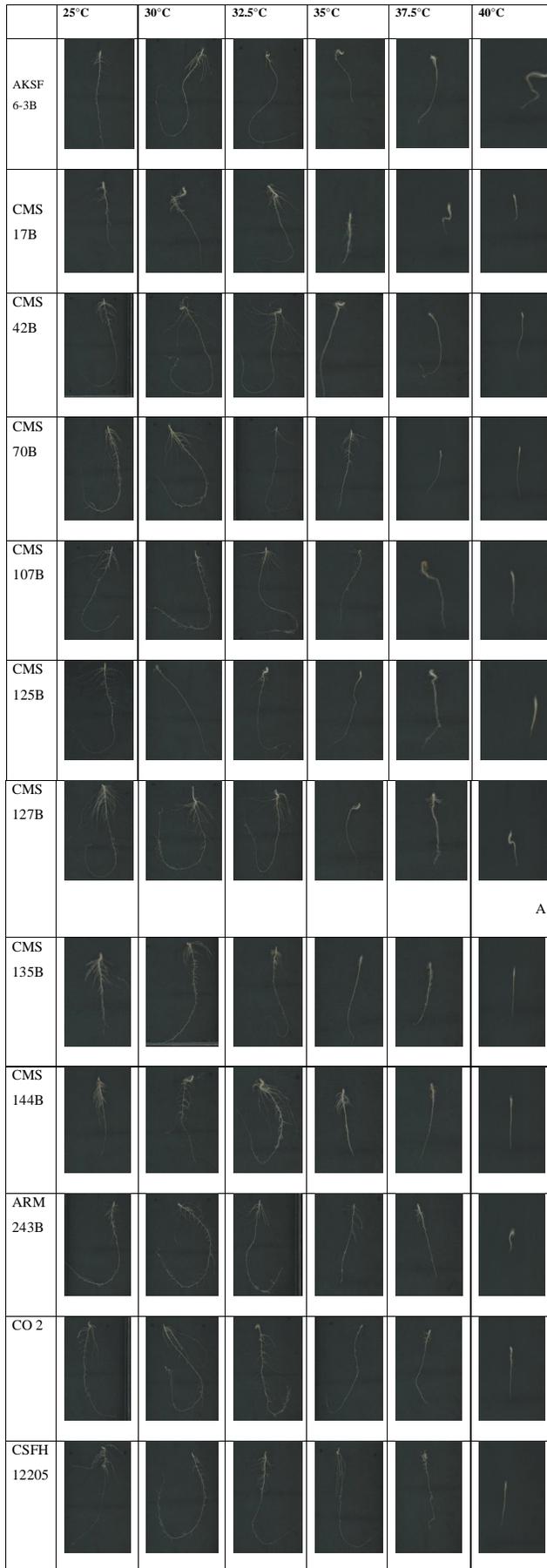


Fig. 11 : A & B Root System Architecture of 14 sunflower genotypes at increasing temperature from 25 to 40°C.

more involved in nutrient absorption (Burke and Raynal, 1994). Elevated temperatures may not significantly increase root production, as reduced soil N availability, significantly restricts potential increase in plant growth (King *et al.*, 2005). Majority of sunflower area in the country is now shifted to *rabi* (0.14 m ha in *rabi* and 0.12 m ha in *kharif*) owing mainly to pests and disease problem and low yields resulting due to rain at flowering in *kharif*. Delayed sowing and changing climate both subject the crop to increasing temperatures during crop growth period. The higher soil water content were made available to the crop at normal sowing while in the late season crop, the low status of soil water supply and the percent extractable water have limited root water extraction. Early in the late cropping season, the soil was at field capacity, subsequently after, the status of soil water declined. The status of water available to root system is important in meeting the water requirements of the crop at the different phases of development (Meinke *et al.*, 1993). The efficiency of the root system to extract water is influenced by root growth and distribution (Passioura, 1983). Decreased level of soil water in the root zone is inhibitory to the crop’s physiological activity in late season crop of sunflower, restricting the biomass production and canopy development.

Temperature influence the cell elongation, root growth, root length and extension, initiation of new lateral roots and root hairs, and root branching (Pregitzer *et al.*, 2000). High temperature results differential uptake and partitioning of nitrogen, phosphorous and potassium in different crops (Huang and Xu, 2000; Hussain *et al.*, 2019). The overall root length increased up to 32.5°C and decreased thereafter up to 40°C. Similarly, in sorghum, high root zone temperature reduces the elongation and cell production rate in seminal roots (Pardales *et al.*, 1992). In wheat the increase in temperature reduced the length and number of central late metaxylem in the root tip as an adaptation to limit damage by the changes in water viscosity and root hydraulic conductance produced by heat (Morales *et al.*, 2003).

High temperature alters the membrane stability, proteins and nucleic acids (Vu *et al.*, 2019) alter membrane fluidity and composition causing the activation of calcium (Ca₂C) channels (Mittler *et al.*, 2012). The negative effect of high temperatures usually reduces the surface between root and soil thus reducing the water uptake (Nagel *et al.*, 2009). Alteration of root growth is caused by a decrease in the cell division rate (Joshi *et al.*, 2016).

Conclusion

Though, root growth is affected by higher temperatures, genotypes responded differentially to temperature. Some genotypes *viz.*, CMS lines -42B, -125B, -127B, -144B showed total root area tolerance up to 25°C while other genotypes *viz.*, AKSF 6-3B, CMS 135B, CO 2, DRSH 1, KBSH 44 tolerated temperature up to 30°C and genotypes CMS lines- 17B, -70B, -107B, ARM 243B and CSFH 12205 tolerated temperature up to 32.5°C. The temperature rise from 25-32.5 °C increased all the parameters except root diameter indicating the optimum temperature range could be 25-32.5 °C for the initial growth. Delayed sowing of sunflower due to pest and disease incidence is subjected crop to high temperature stress. The exposure of sunflower roots to higher than optimum temperatures can have an adverse effect on the growth and development of root system. Changes in the root morphology as a function of changes in temperature are generally characterized by differences in root length, dry mass and branching. Knowledge of the root growth at different temperatures has utility in modeling plant growth. The relationship between sunflower root growth and temperature may be of value in determining planting dates. The information presented may be useful in selecting sunflower genotypes that fit a particular environment. Thus, the lines can be grown in different locations based on their tolerance to particular temperature. Further studies are needed to correlate laboratory observations with responses under field conditions.

Ethics approval and consent to participate

The authors declare no conflict of interest.

Consent for publication

Informed consent was obtained from all individual participants included in the study.

Availability of data and material

Data is available in supplementary tables presented Roots supple. Folder

Competing interests : Not applicable

Funding : There is no funding

Authors' contributions

¹Corresponding author has conducted the research work as a part of Ph.D. study, ²provided the material for experiment also supported with designing the experiment & corrected the paper, ³provided facility of WINRHIZO software & corrected the paper, ⁴data analysis .

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List of abbreviations: Germination percentage (GP), Principal Component Analysis (PCA), Germination (G), Total Root length (TRL), The projected area (TPA) and surface area (TSA), Average diameter (AD), Root Length/volume (RL/V), Root volume (RV), Root dry weight (RDW).