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Research Article

Impact of elevated temperature on root traits and microbial interaction in cotton (*Gossypium hirsutum* L.) genotypes

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Abstract
Climate change mainly alters the plant phyllosphere and rhizosphere resource allocations. Compared with shoot parameters, there is less information about how roots, especially root system architecture (RSA) and their interactions with others, may respond to elevated temperature changes. These responses could greatly influence different species acquisition of resources and their competition with their neighbours. The main aim of this experiment was to evaluate the effects of ambient temperature (T1) and elevated temperature (+4°C) (T2) in Open-top chamber (OTC) on root traits and microbial interaction changes in cotton (*Gossypium hirsutum* L.). A pot experiment was conducted at the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, during 2020-2021 to investigate the root traits and microbial interactions. Cotton varieties, namely KC3, SVPR6, TSH325, TSH357 and TSH375 were screened at the seedling level for cellular thermo tolerance and further, at the root level, these selected varieties were studied against the elevated temperature condition for 10 days in OTC during the stage of flowering to boll development period along with control temperature condition. Root interactions' intensity and direction may fluctuate as a result of variations in RSA responses between species. Negative root interactions could become more intense under high temperature circumstances and species with bigger roots and greater early root growth had stronger competitive advantages. The present findings showed that elevated temperatures promote various microbial growths in the geothermal regions, enhancing the root angle and root length of cotton species. Among the genotypes, KC3 and SVPR6 performed better under elevated temperatures.

Keywords: Cotton, elevated temperature, microbial interaction, OTC, Root traits

INTRODUCTION

Plants are exposed to different external conditions that affect growth, development and productivity. One of the most crucial factors affecting plant growth is temperature (Gray and Brady, 2016). The fifth assessment of the Intergovernmental Panel on Climate Change (IPCC) predicts that by the end of this century, the average global air temperature will have increased by 0.3 to 4.8°C (Pau *et al.,* 2018).

Cotton is grown under warm climatic conditions. Due to high temperature, shortening of the growth period negatively affects the agronomical properties (Khan *et al*., 2008) especially in early maturing genotypes. Variations in aboveground shoot traits, such as shoot height, leaf morphology and phenology have been the focus of concern and are observed to change with increasing temperature (Meineri *et al.,* 2014). However, the root system architecture (RSA) towards deploying roots in the soil that optimizes the acquisition of water and nutrient has been thought to minimize the negative impacts on elevated temperatures. RSA is mainly affected by various environmental factors such as soil temperature, soil moisture, nutrients and soil pH which adversely affect crop growth and yields. An increase in temperature slows down lateral root growth which in turn effect yields in maize and potatoes were observed (Prince *et al.,* 2019).

Microorganisms are diverse and these are most abundant in geothermal fields on Earth. Some microorganisms can survive at elevated temperatures as 122 °C, challenging our understanding both physical and chemical constraints on life. Recently, researchers have focused on the physico-chemical parameters like temperature, pH and nutrient supply, which shape microbial diversity, activity and community structures in geothermal fields. Microorganisms employ various physiological strategies. Some populations thrive, while others perish (Schimel *et al.,* 2007; Allison and Martiny, 2008), resulting in a shaft in microbial community composition (Acosta-Martinez *et al.,* 2014). Berard *et al.,* 2015 reported that drought and high temperature induced changes in soil microbial physiology, biomass, community structure, diversity and activity and even the extreme climatic events also indirectly changed microbial communities through alters the microbial habitats (Navarro-Garcia *et al.,* 2012).

This paper focuses the belowground growth of plants, similarly the aboveground response could synergistically interact with belowground changes (Belter and Cahill, 2015). Different types of roots have their own special features such as primary root length (Forde 2014), root volume, fresh root weight, dry root weight and root volume helped in determining water absorption and nutrients uptake are the adaptation strategy under deficient conditions (Rewald *et al.,* 2011). The present study aimed for the screening of cotton Genotypes for high temperature to study the plant root traits and microbial interactions

MATERIALS AND METHODS

Seed materials

The seeds of *Gossypium hirsutum* L. were purchased from the Agricultural Research Station (ARS) Srivilliputtur, ARS Kovilpatti and from the Department of Cotton, Tamil Nadu Agricultural University (TNAU), Coimbatore with different size, colour and weight as different genotypes were chosen for the experimental purpose.

Pot culture experiment

A set of pot culture experiments was conducted with cotton varieties of KC3, SVPR6, TSH325, TSH357 and TSH 375 under ambient and controlled (open top chamber condition) during the flowering to boll formation stage.

Crop	Cotton (*Gossypium hirsutum* L.)
Location	Department of Crop Physiology, TNAU, Coimbatore
Statistical design	FCRD (Factorial Completely Randomized Design)
Number of Replications	Three
Number of treatments	Two

Treatment details

T1	Control (Ambient air temperature)
T2	Ambient air temperature + 4^{O}C

Experimental details

Experimental set up

Pot mixture was prepared by mixing thoroughly with red soil, sand and FYM in the ratio of 2:1:1 and filled in plastic pot (35 × 32 cm) with 20 kg of mixture. Four seedswere sown per pot and thinned with two healthy plants per pot. Normal pot culture protection measures were adopted.

Imposition of stress

A pot culture experiment was maintained under ambient conditions up to the initiation of flowering with a temperature range from 35^{o}C to 41.2^{o}C. After the initiating flowering, one set of pots was retained under ambient condition and another set of pots was kept at open top chamber condition with an elevation of 4^{o}C than the ambient temperature for 10 days, from 60th to 70th day. After 10 days, the pots under OTC were brought out and kept under ambient condition. On 71st day observations were recorded on root parameters and microbial population.

Root fresh weight (g)

Plants were uprooted from the pots and the adhering soil was washed with water. Roots were separated from the shooting part and excess water was removed. Weights of roots were measured using the electronic weighing machine and expressed in grams (g).

Root dry weight (g)

After taking the roots' fresh weights, the roots were kept in oven at 65^{o}C for about 48 hours. Weight of oven dried root samples was measured by using the electronic weighing machine expressed in g.

Root length (cm/pl)

The root length of seedlings was recorded on 70 Days After Sowing (DAS) in randomly selected seedlings from each replication and expressed as cm.

Root volume (ml)

Plants were uprooted from the pots and the adhering soil was washed with the water. Roots were separated from the shoot part and root volume was measured by placing the roots in the measuring cylinder containing a known volume of water. The rise in water level after placing the root is equal to the volume of the root and expressed in ml.

Primary root length (cm)

Primary root length was measured in five plants from each experimental unit with intensive care to save the roots from any external injury. Plants were washed with water and air-dried for some time without any moisture. Primary root length was measured with the help of a measuring scale and expressed in cm.

Root angle

Root angle for each sample was arranged on the plane with a thin cloth so that it should not lose its original identity. Points were chosen for measuring the angle where they emerged and had better resistance against the mechanical shock to maintain their configuration. Root angle was measured with the help of a protractor, as the angle at which a secondary root will emerge from the primary root was indicated as the angle between primary and secondary roots. The angle between the secondary and tertiary roots was used to show the angle at which the tertiary root will emerge from the secondary root. The angle of emergence was determined by pooling and averaging all of the root angles between primary and secondary roots or between secondary and tertiary roots that were observed in the root system.

Soil microbial population (CFU/g)

Rhizospheric soil from pots was collected randomly according to the treatments. One gram of soil (treatment-wise) was mixed in 9 ml sterilized water to give 10^{-1} dilution. By serially transferring 1 ml of each dilution to 9 ml sterilised water blanks, subsequent dilutions up to 10^{-6} were obtained. By plating on the appropriate media, namely Nutrient Agar, Martins Rose Bengal Agar, and Ken Knight Agar media, the populations

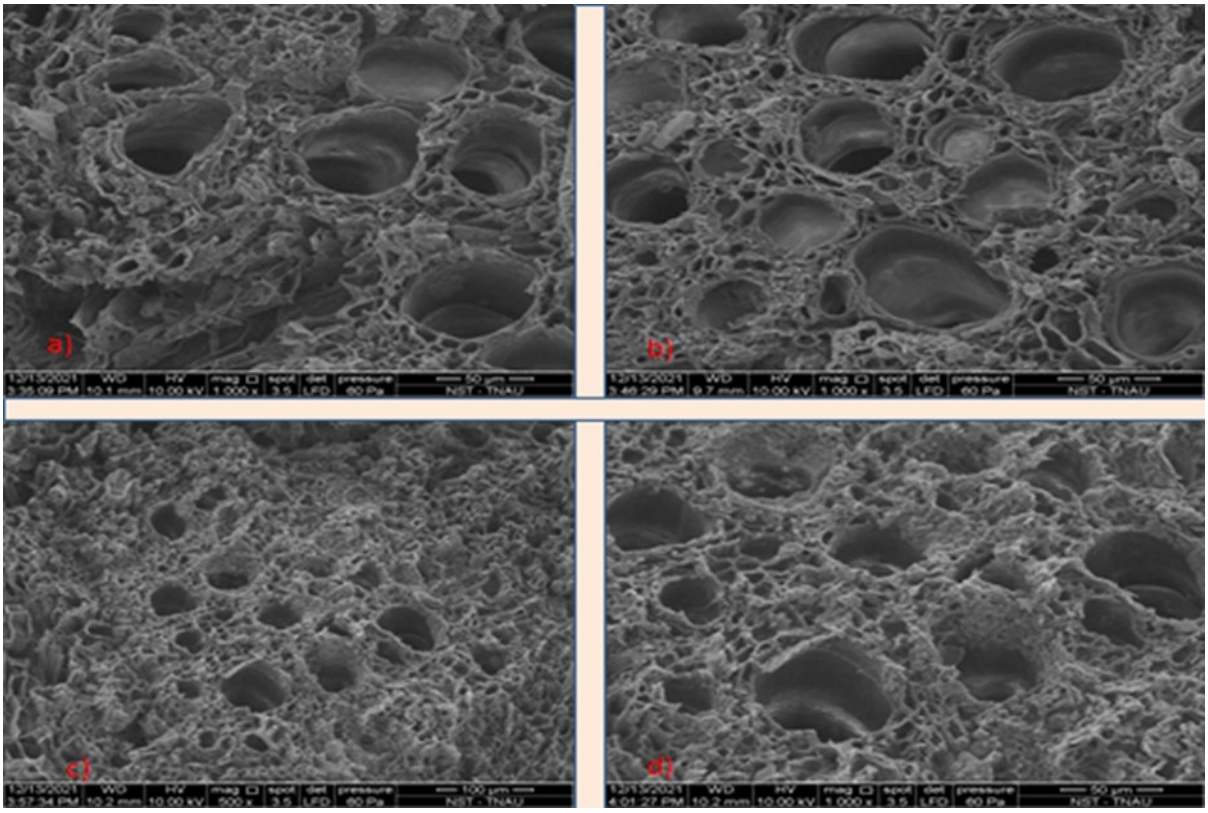

Fig. 1. *SEM images of root cells showing that plants under high temperature stress (T2) had shown increased root diameter area in both the genotypes, KC3 and SVPR6 a) T1- Control (KC3), b) T2- +4^{O}C (KC3), c) T1- Control (SVPR6), d) T2- +4^{O}C (SVPR6)*

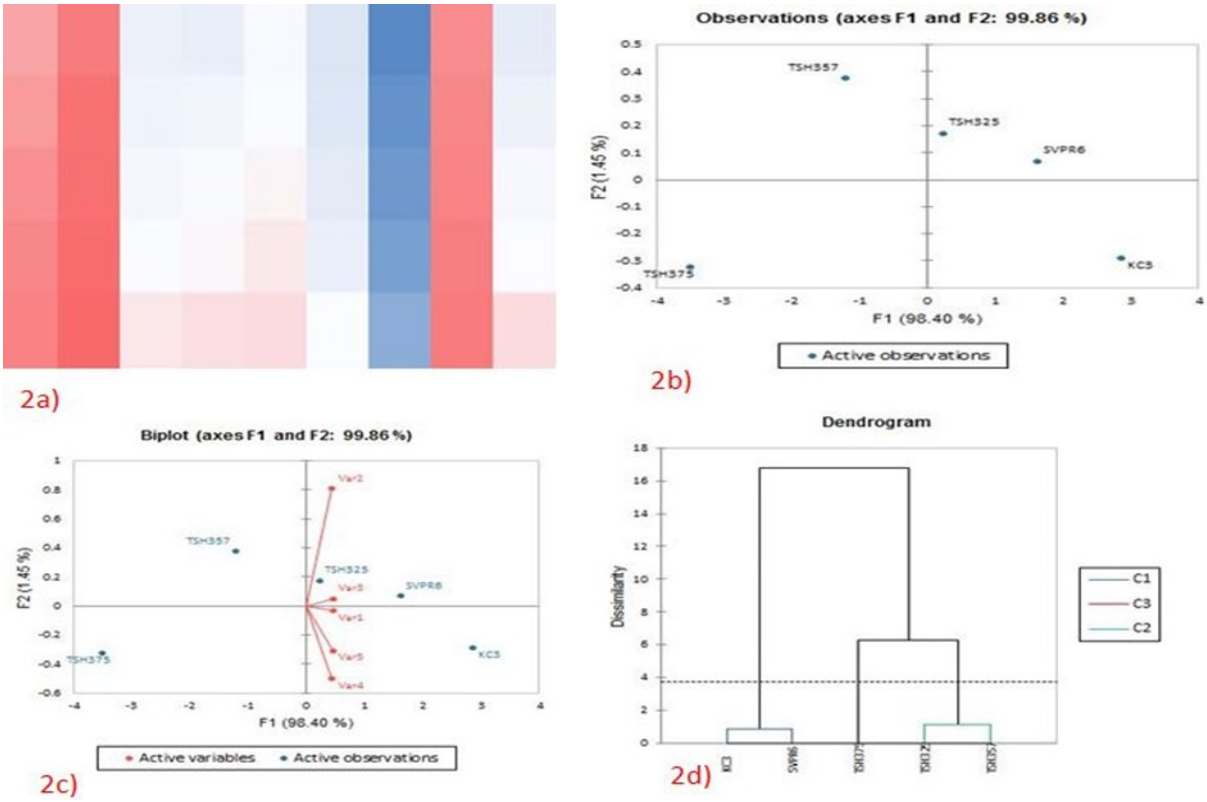

Fig. 2. *2a) Diagram represents the heat map for all the root parameters and microbial interactions. 2b, 2c) PCA for all the 5 genotypes which shown as observations as root parameters such as root length, root angle, bacterial population, fungi population, actinomycetes population. 2d) Dendrogram picture which shows the tolerant genotypes and susceptible genotypes*

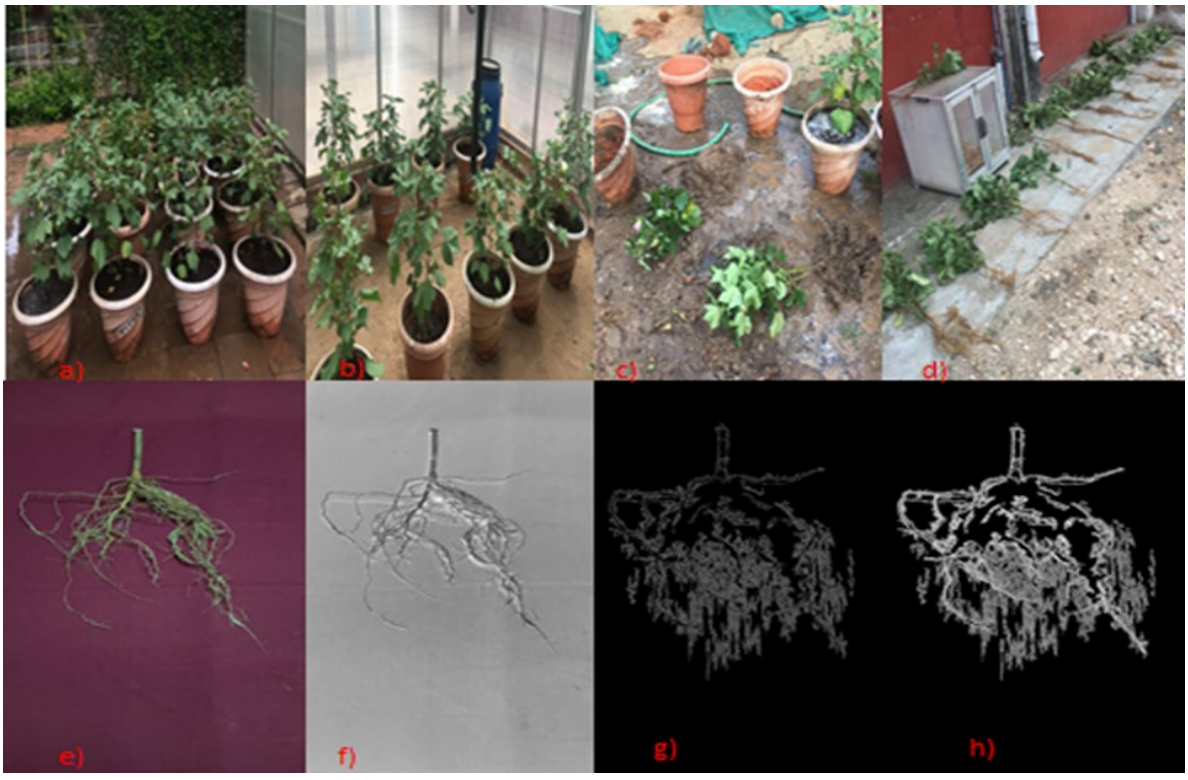

Fig. 3. *Root analysis software Giaroots used to capture the images of root architecture pics (f, g and h), a) 70DAS of control plants, b) Plants under elevated temperature, c) Separation of roots from pot, d) Discarded plants from pots, e) Cleaned image, f) Gray image, g) Tinned image h) Threshold image*

of bacteria, fungi, and actinomycetes were determined. The inoculated plates were kept for incubation at 30°C ± 1°C and emerging colonies were counted. The microbial population was expressed in colony- forming units (CFU/g) of the soil.

Root anatomical studies

Root anatomical characters were observed by using Scanning electron microscope (SEM) with the model of Quanta 250. Root sample spread on double sided conductive carbon tap mounted on the stub and coated with sputter and placed the sample in chamber of SEM.

Giaroots software

For both the control plants (T1) and the plants that were subjected to high temperatures (T2), pictures of the root systems were taken using the Gia roots software. Images are displayed in Fig. 3.

Statistical analysis

SPSS Statistics version 23.0 software (http:// www.spss. com) was mainly used for doing the following statistical analysis. The mean values of each parameter were identified and examined using analysis of variance to determine the significance for all the genotypes and treatments.

RESULTS AND DISCUSSION

Root fresh weight

Data on fresh root weight are furnished in Table 1, showing the reduction in root fresh weight among the genotypes. Higher root fresh weight was produced in KC3 (13.0gms) and lower root fresh weight in TSH375 (6.6gms) when compared to the remaining genotypes. Among the treatments, elevated temperature (+4°C) treatment (T2) produced lower root fresh weight (7.4gms) compared to control (T1) (11.6gms). Coming to genotype and treatment interaction relationship, genotypes KC3 (15.3gms) and SVPR6 (13.7gms) grown under control conditions treatment (T1) showed significantly ($P<0.01$) higher root fresh weight compared to all other genotype and treatment interactions. It was observed that genotype TSH375 (5.1gms) showed significantly lower fresh root weight grown under elevated temperature (+4°C) treatment (T2) compared to all other combinations. The decrease in root fresh weight was due to a thin deeper root system even though the root length was more. Roots cannot bear the more volume of water to sustain their life because high temperature induces and it makes the roots to no availability of water though it effects as moisture stress. Besides as there is an increase in temperature, there will be a decrease in availability of water (Nord and Lynch, 2009)

Root dry weight

Data recorded on dry root weight are furnished in Table 2, which were significantly reduced. Higher root dry weight was produced in KC3 (5.40gms) and lower root dry weight in TSH375 (1.85gms) when compared to other genotypes. Among the treatments, elevated temperature (+4°C) treatment (T2) produced lower root dry weight (2.84gms) when compared to control (T1) (3.78gms). Coming to genotype and treatment interaction relationship, the genotypes KC3 (6.30gms, 4.5gms) grown under control (T1) and elevated temperature treatment (T2) showed significantly higher dry weight compared to all other genotype and treatment interactions. It was observed that genotype TSH375 (1.60gms) showed significantly ($P<0.01$) lower root dry weight grown under elevated temperature (+4°C) treatment compared to all other combinations. Root dry matter was generally reduced under elevated temperature, which might be due to the senescence of roots and retardation in the development of root meristem due to the up-regulation of ABA (Bita and Gerats, 2013). Present study results indicated that the reduction in total dry matter production severely impacted the high temperature. Disturbance in the enhancement of the root vascular system under heat stress decreased source movement for the lateral root production and reduced root biomass (Singh *et al.,* 2012). However,

Table 1. Effect of high temperature on root fresh weight (g) in cotton genotypes

Genotypes	Control (T1)	High temperature (+4°C) (T2)	MEAN
KC3	15.3	10.7	13.0
SVPR6	13.7	8.5	11.1
TSH325	11.5	6.8	9.15
TSH357	9.4	5.9	7.65
TSH375	8.1	5.1	6.60
MEAN	11.61	7.4	
	TREATMENT	**GENOTYPE**	**INTERACTION**
CD	0.41	0.66	0.93
SE(m)	0.14	0.22	0.31
SE(d)	0.19	0.31	0.44

the root biomass of maize genotypes were increased under increase in temperatures upto certain level but under higher temperatures, it was reduced sigtnificantly (Xia *et al.,* 2021)

Root length

Data on root length represented in Table 3, showed that among the genotypes, higher root length was produced in KC3 (40.30cms), which significantly (P<0.01) differed with SVPR6 (37.95cms) and lower root length in TSH375 (25.75cms) when compared to remaining genotypes. Among the treatments, elevated temperature (+4^{o}C) (T2) produced a higher root length (36.72cms) when compared to control (T1) (31.7cms). Coming to genotype and treatment interaction relationship, genotypes KC3 (42.4cms) and SVPR6 (39.8cms) grown under elevated temperature (+4^{o}C) treatment (T2) showed significantly higher root length compared to all other genotype and treatment interactions. It was observed that genotype TSH375 (23.4cms) showed significantly (P<0.01) lower root length grown under control (T1) compared to all other combinations. Deeper root systems can reduce water deficit by increasing plant water uptake mechanisms in warmer weather (Mueller et al., 2013), which may be better adapted to water deficit which comes after higher temperature with deeper roots (Nord and Lynch, 2009). Increases in temperature were typically accompanied by decreases in the availability of water for the plant (Nord and Lynch, 2009). Lateral roots (lateral root numbers) typically comprise the major portion of root systems, accounting for approximately 90% of the total root length (Zobel *et al.,* 2007). The formation of lateral roots will improve the root system, and ability to sink, which encourages the growth of longer roots and, consequently, a higher ability to access soil resources (Varney and Canny, 1993; Postma *et al.,* 2014). In the current study, the increase in root length under elevated temperature conditions was around 15.8% compared to control, whereas Nagel *et al.* (2009) observed that there was 18% increased root length in maize.

Root volume

Data on root volume represented in Table 4 were significantly decreased. Among the genotypes, higher root volume was produced in KC3 (42.0cc) and lower root volume in TSH375 (24cc) when compared to other genotypes. Among the treatments control (T1) produced a higher root volume (35.4cc) when compared to elevated temperature treatment (+4^{o}C) (T2) (29.6cc). Coming to the genotype and treatment interaction relationship, genotypes KC3 (45cc, 39cc) under control (T1) and

Table 2. Effect of high temperature on root dry weight (g) in cotton genotypes

Genotypes	Control (T1)	High temperature (+4^{O}C) (T2)	MEAN
KC3	6.30	4.50	5.40
SVPR6	4.10	3.20	3.65
TSH325	3.60	2.80	3.20
TSH357	2.80	2.10	2.45
TSH375	2.10	1.60	1.85
MEAN	3.78	2.84	
	TREATMENT	**GENOTYPE**	**INTERACTION**
CD	0.15	0.24	0.34
SE(m)	0.05	0.08	0.11
SE(d)	0.07	0.11	0.16

Table 3. Effect of high temperature on root length (cm) in cotton genotypes

Genotypes	Control (T1)	High temperature (+4^{O}C) (T2)	MEAN
KC3	38.2	42.4	40.30
SVPR6	36.1	39.8	37.95
TSH325	32.5	35.6	34.05
TSH357	28.6	33.1	30.85
TSH375	23.4	28.1	25.75
MEAN	31.7	36.72	
	TREATMENT	**GENOTYPE**	**INTERACTION**
CD	1.42	2.45	3.12
SE(m)	0.47	0.75	1.07
SE(d)	0.67	1.05	1.51

Table 4. Effect of high temperature on root volume (cc) in cotton genotypes

Genotypes	Control	High temperature (+4^{O}C)	MEAN
KC3	31.7	34.5	33.10
SVPR6	29.33	32.7	31.02
TSH325	26.57	29.8	28.19
TSH357	24.38	27.3	25.84
TSH375	22.21	24.6	23.41
MEAN	26.84	29.78	
	TREATMENT	**GENOTYPE**	**INTERACTION**
CD	1.15	1.82	2.87
SE(m)	0.39	0.61	0.86
SE(d)	0.54	0.87	1.23

Table 5. Effect of high temperature on primary root length (cm) in cotton genotypes

Genotypes	Control	High temperature (+4^{O}C)	MEAN
KC3	31.7	34.5	33.10
SVPR6	29.33	32.7	31.02
TSH325	26.57	29.8	28.19
TSH357	24.38	27.3	25.84
TSH375	22.21	24.6	23.41
MEAN	26.84	29.78	
	TREATMENT	**GENOTYPE**	**INTERACTION**
CD	1.15	1.82	2.87
SE(m)	0.39	0.61	0.86
SE(d)	0.54	0.87	1.23

Table 6. Effect of high temperature on root angle (deg) in cotton genotypes

Genotypes	Control (T1)	High temperature (+4^{O}C) (T2)	MEAN
KC3	47.37	53.54	50.46
SVPR6	45.26	51.89	48.58
TSH325	41.56	48.71	45.14
TSH357	38.41	44.36	41.39
TSH375	26.82	33.72	30.27
MEAN	39.88	46.44	
	TREATMENT	**GENOTYPE**	**INTERACTION**
CD	1.77	2.81	3.94
SE(m)	0.59	0.94	1.34
SE(d)	0.84	1.33	1.89

Table 7. Effect of high temperature on Microbial population of bacteria in cotton genotypes

Genotypes	Control (T1)	High temperature (+4^{O}C) (T2)	MEAN
KC3	126.5	136.2	131.35
SVPR6	120.3	129.5	124.9
TSH325	115.8	121.6	118.7
TSH357	109.3	115.7	112.5
TSH375	98.5	103.3	100.9
MEAN	114.08	121.26	
	TREATMENT	**GENOTYPE**	**INTERACTION**
CD	4.72	7.46	9.21
SE(m)	1.58	2.51	3.55
SE(d)	2.24	3.55	5.02

elevated temperature treatment (+4°C) (T2) showed significantly (P<0.01) higher root volume compared to all other genotype and treatment interactions. It was observed that genotype TSH375 (20cc) showed significantly (P<0.01) lower root volume grown under elevated temperature (+4°C) treatment (T2) compared to all other combinations. Root volume is mainly based on the amount of water which level the plant can absorb and water use efficiency. Root fresh weight can be directly correlated with the root volume as they may be directly proportional to each other. A decrease in root fresh weight can reduce the amount of root volume uptake as they have a deeper thin root system which is indirectly affected by drought. The present study is in line with the results of Kuroyanagi and Paulsen (1988) that the high temperature restricts the root metabolism and inhibits the root volume in *Phaseolus acutifolius.* In the current study, the decrease in root volume under elevated temperature conditions was around 16.4% compared to control, whereas Xia *et al.,* (2021) observed there was 53.95% reduction in root volume of maize genotypes.

Primary root length

Data on primary root length represented in Table 5, significantly differed among the genotypes. Higher primary root length was produced in KC3 (33.10cms) and lower primary root length in TSH375 (23.41cms) compared to other genotypes. Among the treatments, elevated temperature (+4°C) treatment (T2) produced higher primary root length (29.78cms) when compared to control (T1) (26.84cms). Coming to genotype and treatment interaction relationship, the genotypes KC3 (34.5cms) grown under elevated temperature (+4°C) treatment (T2) were showed significantly higher primary root length compared to all other genotype and treatment interactions. It was observed that genotype TSH375 (22.21cms) showed significantly (P<0.01) lower primary root length grown under control (T1) compared to all other combinations. As the temperature range increases, the amount of water availability will be decreased to the roots as they will be subjected to moisture stress accompanied by decreases in water availability of the plant (Nord and Lynch, 2009), and deeper root systems, which may be better adapted to the water deficit that accompanies greater temperature, can alleviate water deficit situations by boosting plant water uptake capabilities under warm environmental conditions (Mueller et al., 2013). Lateral roots may typically comprise the major portion of root system and account for approximately 90% of the total root length (Zobel *et al.,* 2007). Lateral roots formation will increase the sink strength of the root system, which helps in promoting the development of greater root length and thereby greater soil resource acquisition (Varney and Canny, 1993; Postma *et al.,* 2014). The present study is in line with above mentioned reporters.

Root angle

Data pertaining to root angle, represented in Table 6 significantly differed among the genotypes. Higher root angle was produced in KC3 (50.46) which is on par with SVPR6 (48.58) and a lower root angle in TSH375 (30.27) when compared to other genotypes. Among the treatments, elevated temperature (+4°C) treatment (T2) produced higher root angle (46.44) when compared to control (T1) (39.88). Coming to the genotype and treatment interaction relationship, the genotypes KC3 (53.54) grown under elevated temperature (+4°C) treatment (T2) showed a significantly (P<0.01) higher root angle compared to all other genotype and treatment interactions. It was observed that genotype TSH375 (26.82) showed significantly (P<0.01) lower root angle grown under control (T1) compared to all other combinations. The present study showed that the root angle differed significantly under high temperature. The root angle distribution was higher in high temperatures, which direct the lateral roots towards the upside and those roots are trying to get enough volume of soil to absorb the nutrients and water they need to grow. Though there is limited information about root angle due to high temperature, and it indirectly gets affected by moisture stress. Hammer *et al*. (2009) suggested that the changes in root architecture in terms of root angle directly impacted the growth and yield response of crops. There is a genotypic difference in root angle (Nakamoto and Oyanagi, 1994) that was also found under drought stress however, there is no relevant information reported on the root angle under high temperature.

Soil microbial population (Bacteria, fungi, actinomycetes)

Data pertaining to soil microbial populations are represented in Table 7, 8 and 9. Population of bacteria, fungi and actinomycetes were observed in all the treatments of soil samples during the stage of flowering to boll development period along with ambient condition at 71st day. The Microbial population were found to be higher in plants were grown under elevated temperature (+4°C) treatment (T2) when compared to control (T1). Among the genotypes, KC3, had a higher bacteria, fungi and actinomycetes population in (T2) treatment (131.35 cfu×106, 8.45 cfu×103, 43.85 cfu×104) and which is significantly (P<0.01) on par with SVPR6 and TSH375 had lower (100.9 cfu×106, 5.30 cfu×103, 23.45 cfu×104) when compared to control plants. Among the treatments, higher bacteria, fungi and actinomycetes populations were found in (T2) treatment (121.26 cfu×106, 7.6 cfu×103, 36.7 cfu×104). Genotype and treatment interaction were significantly varied.

Table 8. Effect of high temperature on Microbial population of fungi in cotton genotypes

Genotypes	Control (T1)	High temperature (+4^{O}C) (T2)	MEAN
KC3	42	45.7	43.85
SVPR6	37	41.2	39.10
TSH325	31	36.7	33.85
TSH357	26	33.1	29.55
TSH375	20	26.9	23.45
MEAN	31.2	36.7	
	TREATMENT	**GENOTYPE**	**INTERACTION**
CD	1.4	2.2	3.1
SE(m)	0.48	0.76	1.08
SE(d)	0.68	1.07	1.53

Table 9. Effect of high temperature on Microbial population of actinomycetes in cotton genotypes

Genotypes	Control (T1)	High temperature (+4^{O}C) (T2)	MEAN
KC3	7.3	9.6	8.45
SVPR6	6.8	8.4	7.60
TSH325	6.1	7.9	7.00
TSH357	5.6	6.4	6.00
TSH375	4.9	5.7	5.30
MEAN	6.12	7.6	
	TREATMENT	**GENOTYPE**	**INTERACTION**
CD	0.28	0.45	0.64
SE(m)	0.09	0.15	0.21
SE(d)	0.14	0.22	0.30

Table 10. Correlation analysis table of cotton genotypes under T1 and T2.

Correlation matrix					
	Root length	**Root angle**	**Bacteria population**	**Fungi population**	**Actinomycetes population**
Root length	1				
Root angle	0.972**	1			
Bacteria population	0.997**	0.978**	1		
Fungi population	0.988**	0.937**	0.987**	1	
Actinomycetes population	0.996**	0.953**	0.995**	0.995**	1

r value is at 99% significant level and is represented by **

The application or presence of beneficial microorganisms in the rhizospheric soil of a crop represents an environment-friendly tool to secure improved crop production (Hartman *and* Tringe, 2019). The thermal sensitivity of roots in rhizospheric nature was ensured by the soil microbial load, particularly some beneficial bacteria, fungi and actinomycetes (Barreiro *et al.,* 2020). However, the plants associated with several soil microbial communities have benefit their host during plant growth and its development; however, under harsh environments, the beneficial effects of microbes are still underestimated (Weyenes *et al.,* 2009). Present results expressed that the soil microbial load, such as bacteria fungi and actinomycetes, were higher when exposed to high temperature. Though there is a limited temperature, they can grow as normal, but when we increase the temperature, the multiplication rate will be higher in the microbial population. Beyond the optimum level of temperature, it will get reduced. The present findings also endorsed that the microbial load under the temperature stress showed increased bacteria, fungi and actinomycetes.

Root anatomical studies

The root anatomical sections (Fig.1) were observed at the flowering to boll development stage. It was indicat-

Table 11. Elevated temperature data at Open Top Chamber (OTC)

Date (10 Days)	Temperature (°C)	
	Ambient Temperature (T1)	Elevated temperature (+4°C) (T2)
22.6.2021	30.1	34.1
23.6.2021	31.7	35.7
24.6.2021	29.6	33.6
25.6.2021	30.6	34.6
26.6.2021	31.2	35.2
28.6.2021	28.9	32.9
29.6.2021	31.1	35.1
30.6.2021	32.2	36.2
1.7.2021	28.8	32.8
2.7.2021	29.4	33.4

ed that plants under high-temperature stress (T2) had shown increased root diameter area in both the genotypes, KC3 and SVPR6 when compared to ambient control treatment (T1). The present study was similarly endorsed with previous results (Luo *et al.,* 2020).

Correlation analysis

Correlation data is furnished in Table 10. The correlation studies were conducted between root length and root angle with various microbial populations. It was observed that root length was significantly correlated with root angle at 99% significant level. The significant correlation was also observed between root traits with bacterial, fungal and actinomycetes populations. Among the microbial populations, bacterial population was positively correlated with fungal and actinomycetes populations at 99% significant level and vice-versa. It indicates that there was an existence of a symbiotic relationship between various microbial populations in the geothermal field. Wider root angles and deeper root lengths probably promote microbial growth vigorously in the root zones under both control and elevated temperature conditions.

Heat map analysis

The EXCEL STAT programme was used to conduct heat map analysis (Fig. 2a) on root parameters and microbial populations. It is tricky to investigate the high temperature tolerance for all of the root characteristics and microbial populations for all five genotypes due to problems in determining the statistical significance of variances. By using a different colour to symbolise each parameter since each colour indicates a different parameter, all the parameters revealed varied disparities, both favourably and negatively. It is abundantly obvious that the genotypic variations between each of the genotypes included in the current study are to responsible for these variations.

Principal component analysis

The principal component analysis (PCA) performed for five genotypes to select better cotton genotypes under high temperature stress indicated as to how the root characteristics of cotton genotypes were influenced by the high temperature stress (Fig. 2b, 2c). The total variability shown by different principal components, principal component 1 (PC1) and principal component 2 (PC2) contribute 98.40 % and the biplot was plotted between PC1 and PC 2. The better genotypes were chosen based on the PC1. Thus, based on PC1, the following genotypes have exhibited better root characters such as root length, root angle and microbial population growth under high temperature stress condition that include KC3 and SVPR6 genotypes.

Dendrogram analysis

Dendrogram (Fig. 2d) was analysed for five genotypes to observe the significant similarities between five cotton genotypes for root architecture traits and microbial population growth near the root zone. It was observed that KC3 and SVPR6 genotypes were on the same height and indicatde that these genotypes performed significantly better root architecture traits and maintained significant microbiome compared to the remaining genotypes. The heights of the genotypes TSH325 and TSH357 in the dendrogram maintained significantly similar heights next to KC3 and SVPR6. Among five genotypes, TSH375 showed significantly least root architecture traits and also reduced microbial population growth near the root zone.

Conclusion

In the initial stage or establishment stage, plants prioritize root growth only for nutrient uptake mechanisms and physical support, especially in tropical regions for intense competition. The present study concluded that root traits and microbial interactions showed the effect of warming temperature on root systems of plants viz., cotton germplasm and particularly the spatial distribution of root systems was necessary to predict the plant performance and community regenerations in future higher temperatures or warming climatic nature. The findings mainly suggested that root system architecture for different genotypes showed significant differences in all the root traits which are subjected to elevated temperature. Variations of rhizosphere soil responses to temperature change can be greater than that of responses of the phyllosphere among different species. Furthermore, the variations can also explain the direction in root angle, orientation and intensity in plant interactions under climate change, which is mainly important for the success of seedling establishment as well as for growing a better root system. It is suggested to conduct field studies to compare the differences arising in RSA between natural and artificial conditions and to study the allocation of nutrients and N/C ratios between the roots to better understand higher climatic conditions.

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Conflict of interest

The authors declare that they have no conflict of interest.

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