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Effect of Elevated Temperature Interaction with Elevated Carbon Dioxide on Physiological Quality of Groundnut (*Arachis hypogaea* L.) Genotypes under Fate Condition

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Four genotypes of groundnut (*Arachis hypogaea* L.) viz., K-6, Naryani, Darani and K-9 were raised under Free Air Temperature Elevated (FATE) condition. The plants were raised in open rings with elevated temperature (+3°C) and carbon dioxide (550ppm) during rabi 2016 to investigate the effect

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of increased temperature and its interaction with CO_2 on various seed quality parameters. The physiological parameters decreased under elevated CO_2 and temperature. The results revealed that germination ranged from 81.5 to 90 per cent between four genotypes *i.e.,* K-6, Naryani, Darani and K-9. Similarly speed of germination 49 to 83.02, shoot length 14.73 to 7.70, root length ranges from 20.80 to 9.50, and seedling vigour index 123.47 to 98.53 and seedling dry weight 0.919 to 0.100 g. Further, biochemical parameters like SOD, MDA and α amylase content increased at eT + eCO_2 as compared to control treatment. Limited evidence suggests that only short periods of high-temperature stress at critical seed development stages are required to reduce seed vigour, but further research is required. The predicted environmental changes will lead to losses of seed quality particularly for seed vigour and possibly germination.

The present study shows that temperature impacts the groundnut crop's physiological and quality parameters.

Keywords: Climate change; elevated CO_2; temperature; groundnut and FATE.

1. INTRODUCTION

Climatic variability, together with an increase in atmospheric carbon dioxide (CO_2) and temperature do have a lot of implications on agriculture sector. In the last one decade, climate change due to global warming has become an issue of serious concern worldwide for existence of life on earth. According to Intergovernmental Panel on Climate Change (IPCC), climate change defined as "Change in climate over time, either due to natural variability or as a result of human activity". In near future, agriculture will inevitably face challenges caused by global climate change, which might lead to both global and local alteration. It has been reported by federal agencies that CO_2 concentration has increased by approximately 30 per cent since the industrial revolution, which is believed to be responsible for an increase of about 0.66^oC in mean annual global surface temperature. Meanwhile, the temperature is anticipated to increase further by 1.4 to 5.8^oC by 2100 with equally increasing atmospheric CO_2 according to IPCC [1]. Elevated carbon dioxide and elevated temperature is known to impact crop growth and quality. With global climate change, atmospheric carbon dioxide concentration is predicted to rise from today's value of 407 ppm to 550 ppm by 2050 and could reach between 730 and 1010 ppm by 2100 [2]. This, combined with other atmospheric changes, is projected to increase global mean temperatures by 1.4 to 5.8°C [3]. Jaggard et al. [4] concluded that CO_2 enhancement was likely to increase yield of crops and it was 13% to 60% in most C3 crops, however yields of C4 crops are not expected to change. The increasing temperatures may negate these beneficial effects of CO_2 in C3 crops, particularly if they occur during reproductive stages [5,6]. Gornall et al. [7] noted that extreme weather events are more likely to occur in the changed climate of the future, and predicted that over much of the world's crop land the maximum daily temperature high may be increased by around 3°C by 2050. A major challenge is ahead for those who involved in the seed industry to provide crop cultivars that can sustain as well as improve the future food grain production in a changing climate [8,9,10]. Climate warming may result in a shift in germination from spring to autumn. Seed dormancy has been naturally modified by changing environment and promotes germination at an undesirable time.

Successful crop production in any environment depends initially on the quality of the seed being sown. The term 'seed quality' is used in practice to describe the overall value of a seed lot for its intended purpose [11] and includes the components of species and cultivar purity, seed mass (size), physical purity, germination, vigour, moisture content and seed health. The present research study examines the effects of increased temperature and its interaction with increased CO_2 on three of these seed quality components, seed mass, germination and vigour of groundnut (*Arachis hypogaea* L.) genotypes.

2. MATERIALS AND METHODS

Free Air Temperature Elevation (FATE) system consisting of nine rings with 8m diameter was used to assess the impact of elevated temperature (eT) of ambient+ 3°C and its interaction with elevated CO_2 (eT+eCO_2) of 550ppm and compared with ambient control (aT). For each treatment three rings were used. The crop canopy temperature was increased in both eT and eT+eCO_2 treatments by using infra-red

heaters (1000W) at 1.2m above the crop canopy and the set temperature increase was maintained and monitored through IR sensors with SCADA and PLC. In eT+eCO_2 treatment, the enhanced concentration of CO_2 was maintained by injecting the pure CO_2 from perforated PU tubing fixed at periphery of the ring. The release of the CO_2 is regulated and maintained by dedicated control system with NDIR CO_2 analyser linked with sampling point at the centre of the ring as well as wind speed and direction [27].

Genotypes were procured from department of crop science CRIDA Hyderabad and the harvested seeds were evaluated for physiological and biochemical quality parameters.

Special features genotypes were drought tolerant (Dharani, K-9 and K-6), withstands up to 35 days dry spell, Uniform maturity, High SMK%, Attractive pods, Moderate stature, Tolerant to low light conditions, early maturity (Naryani) Popular among farmers for its quality attributes in Rayalaseema (AP), Telangana and Tamilnadu.

2.1 Germination (%)

The germination test was conducted in eight replicates of 50 seeds each by following paper method. The seeds of four groundnut genotypes obtained from aT, eT and eT+eCO_2 were germinated using the rolled towels and placed in the walk-in seed germination room which was maintained at 25 ± 2°C temperature and 90 ± 5% RH. The number of normal seedlings in each replication of individual genotype for each treatment were counted on 10th day and the mean germination was calculated and expressed in percentage.

2.2 Speed of Germination

Seeds were placed for germination on paper medium with eight replication of 50 seeds and the daily germination counts were taken up to the final count (10^{th} day). The speed of germination was calculated by using formula suggested by Maguire [12].

$$\text{Speed of germination} = \frac{X1}{Y1} + \frac{X2-X1}{Y2} + + \frac{Xn-(Xn-1)}{Yn}$$

2.3 Shoot and Root Length (cm)

From the germination test, ten normal seedlings were randomly selected from each treatment on 10th day and the shoot length was measured from the tip of shoot to the hypocotyl point and the mean length was calculated and expressed in centimeters. The root length was measured from tip of root to the hypocotyl point and the mean length was calculated and expressed in centimeters.

2.4 Seedling Dry Weight (mg)

The ten normal seedlings used for measuring root and shoot length were taken in butter paper and dried in a hot-air oven maintained at 70°C temperature for 24 h. Then, the seedlings were removed and allowed to cool in a desiccator for 20 minutes before weighing in an electronic balance. The average weight was calculated and expressed in mg/10 seedlings.

2.5 Seedling Vigour Index (SVI-1)

The seedling vigour index was worked out by multiplying the germination percent and total seedling length [23].

Seedling Vigour Index = Germination % × Total seedling length (cm).

2.6 Superoxide Dismutase (IU/g F.wt)

SOD activity was estimated by recording the decrease in absorbance of the enzyme by soaking 500 mg seeds in water for 24 hours and seeds were homogenized in 0.1 ml of phosphate buffer (pH 7.5) along with chilled acetone. The extract was centrifuged at 10000 rpm for 20 min at 4°C and supernatant was used as enzyme source. 3 ml of reaction mixture containing 0.1 ml of 1.5 M Na_2CO_3, 0.2 ml of 200 mM methionine, 0.1 M of 3 Mm EDTA, 0.1 ml of 2.25 mM NBT, 1.5 ml of 100 mM potassium phosphate buffer (pH 7.5), 1ml of distilled water and 50 µl of enzyme samples. The tube without enzyme was taken as control. Reaction was started by adding 0.1 ml 60 µM riboflavin and placing the tubes below a light source of two 15 W fluorescent lamps for 15 min. The reaction was stopped by switching off the light and covering the tubes with black cloth. Absorbance was recorded at 560 nm.

2.7 Malondialdehyde (mg/g F.wt)

Measurement of MDA content was determined by the thiobarbituric acid (TBA) reaction as described by Ali et al. [25], with slight modifications. Approximately 0.5 g seed samples were crushed in pestle and mortar and

homogenized with 2 ml of 0.1 % trichloro acetic acid (TCA) and centrifuged at 10,000 rpm for 10 min. After centrifugation, 1 ml of the supernatant was mixed with 4 ml 0.5% TBA in 20% TCA and incubated in hot water (95°C) for 30 min. Thereafter, it was cooled immediately on ice to stop the reaction and centrifuged at 10,000 rpm for 5min. Absorbance at 532 and 600 nm was determined, and MDA concentration was estimated by subtracting the non-specific absorption at 600 nm from the absorption at 532 nm, using an absorbance coefficient of extinction (155 mM^{-1} cm^{-1}).

2.8 Activity of α -amylase (mm)

The α-amylase activity was analyzed as per the method suggested by Simpson and Naylor [28]. Two gram of agar shreds and one gram of potato starch was mixed together in water to form paste and the volume was made up to 100 ml with distilled water. The homogenous solution of agar-starch mixture after boiling was poured into sterilized petri-dishes and allowed to settle in the form of gel after cooling. The pre-soaked (for 8 hour) and half cut seeds (with their half endosperm and embryo portion intact) were placed in the petri-dishes in such a way that the endospermic part remained in contact with agar-starch gel. The petri-dishes were closed and kept in dark at 30°C. After 48 hour the petri-dishes were uniformly smeared with potassium iodide solution (0.44 g of iodine crystal + 20.008 g potassium iodide in 500 ml distilled water) and excess solution was drained off after few minutes. The diameter of halo (clear) zone formed around the seed was measured in mm and reported as α- amylase activity.

2.9 Statistical Analysis

The data collected were analysed statically by the procedure prescribed by Sundarajan et al. [26]. Critical differences were calculated at 1 % level, wherever 'F' test was significant.

3. RESULTS AND DISCUSSION

The germination % of groundnut genotypes varied significantly for the seed obtained from all treatments. The mean genotypic performance reduced in the seed harvested from eT (86.19%) and eT + eCO_2 (84.88%) from aT (87.81%). Among the genotypes, the germination % of K-6 and Narayani (87.08 and 89.75%) was similar at all treatments while significant decrease was recorded with Dharani and K-9 at eT (83.75% and 83%) and eT + eCO_2 (82.5% and 81.5%) as compared with aT (86.75% and 87.5%). High-temperature stress after physiological maturity can also sometimes reduce germination [24], but more often reduces seed vigour. The relationship between temperature during seed development and subsequent seed germination requires further investigation.

Groundnut genotypes with respect to their speed of germination exhibited significant variation from all treatments. The response was similar to germination % in which the mean performance of genotypes was significantly lower in eT (64.25) and recorded further reduction in eT+eCO_2 (57.60) than that of seeds harvested from aT (72.01). The eT and eT+eCO_2 condition reduced significantly the speed of germination in Dharani and K-9 (64.28 and 71.43) while the impact on K-6 and Naryani was less (59.57 and 63.20).

Mean performance of shoot length of genotypes under eT (12.01cm) was significantly highest followed by eT + eCO_2 (10.93cm) and aT (10.11). However the individual genotype response differed as K-6 and Dharani recorded highest shoot length with seeds harvested from eT + eCO_2 (10.37cm and 13cm) followed by eT (9.80 and 10.67cm) and aT (8.77 and 9.33cm), whereas it was at eT with Narayani and K-9 (14.73 and 12.83cm).

Among the four genotypes, the long roots were produced by Narayani (21.30cm) and K-9 (18.83 cm) of aT. Reduced genotypic mean performance was recorded for root length with the seed harvested from eT (13.88cm) followed by eT + eCO_2 (15.02cm) as compared with aT (17.34). Among the genotypes, lowest root length was registered with K-6 (12.72cm) and it was maintained in all the treatments. Though the seeds from eT (13.88cm) recorded lowest root length with all genotypes, the eT + eCO_2 improved root length in Narayani (16.60cm), Dharani (20.80cm) and K-6 (13.17cm), while it was further reduced in K-9 (9.50).

There was significant difference in response trend of seedling biomass of groundnut genotypes for eT and eT + eCO_2. Genotypic mean performance of seedling dry weight was found be non-significant for the groundnut seeds harvested from all three treatments. The seeds of eT (0.615mg) showed reduced seedling weight as compared with aT and eT + eCO_2 (0.754mg)

Table 1. Germination % and speed of germination of groundnut genotypes at aT, eT and eT+eCO_2 condition

	Germination %						Speed of germination				
	K-6	**Narayani**	**Dharani**	**K-9**	**Mean**		**K-6**	**Narayani**	**Dharani**	**K-9**	**Mean**
aT	87.25	89.75	86.75	87.50	87.81	aT	62.22	65.00	77.78	83.06	72.01
eT	88.00	90.00	83.75	83.00	86.19	eT	59.22	62.83	66.06	68.89	64.25
eT+eCO_2	86.00	89.50	82.50	81.50	84.88	eT+eCO_2	57.28	61.78	49.00	62.33	57.60
Mean	87.08	89.75	84.33	84.00		Mean	59.57	63.20	64.28	71.43	
	T	G	T x G				T	G	T x G		
SEM	0.63	0.73	1.26			SEM	1.18	1.37	2.36		
CD @ 1%	2.44	2.82	NS			CD @ 1%	4.17	0.47	10.18		

Table 2. Shoot length and root length of groundnut genotypes at aT, eT and eT+eCO_2 condition

	Shoot length (cm)						Root length (cm)				
	K-6	**Narayani**	**Dharani**	**K-9**	**Mean**		**K-6**	**Narayani**	**Dharani**	**K-9**	**Mean**
aT	8.77	11.83	9.33	10.50	10.11	aT	12.83	21.30	16.40	18.83	17.34
eT	9.80	14.73	10.67	12.83	12.01	eT	12.17	13.27	13.17	16.90	13.88
eT+eCO_2	10.37	12.67	13.00	7.70	10.93	eT+eCO_2	13.17	16.60	20.80	9.50	15.02
Mean	9.64	13.08	11.00	10.34		Mean	12.72	17.06	16.79	15.08	
	T	G	T x G				T	G	T x G		
SEM	*0.48*	*0.55*	*0.96*			SEM	*0.96*	*1.11*	*1.92*		
CD @ 1%	*NS*	*2.20*	*NS*			CD @ 1%	*NS*	*NS*	*7.65*		

Table 3. Seedling dry weight of groundnut and Seedling vigour index genotypes at aT, eT and eT+eCO_2 condition

	Seedling dry weight (mg/10 seedlings)						Seedling vigour index				
	K-6	**Narayani**	**Dharani**	**K-9**	**Mean**		**K-6**	**Narayani**	**Dharani**	**K-9**	**Mean**
aT	0.100	0.854	0.842	1.223	0.755	aT	108.27	123.47	111.07	117.00	114.95
eT	0.217	0.737	0.681	0.826	0.615	eT	109.97	118.33	106.50	111.73	111.63
eT+eCO_2	0.511	0.695	0.891	0.919	0.754	eT + eCO_2	108.20	117.60	114.80	98.53	109.78
Mean	0.276	0.762	0.805	0.989		Mean	108.81	119.80	110.79	109.09	
	T	G	T x G				T	G	T x G		
SEM	0.101	0.117	0.202			SEM	1.28	1.47	2.55		
CD @ 1%	NS	0.465	NS			CD @ 1%	NS	0.47	10.18		

Table 4. MDA and SOD content of groundnut genotypes at aT, eT and eT+eCO_2 condition

	MDA Content						SOD Content				
	K-6	**Narayani**	**Dharani**	**K-9**	**Mean**		**K-6**	**Narayani**	**Dharani**	**K-9**	**Mean**
aT	0.241	0.258	0.255	0.293	0.262	aT	3.113	3.434	3.139	2.991	3.169
eT	0.322	0.292	0.239	0.253	0.277	eT	3.502	2.644	2.579	2.562	2.822
eT+eCO_2	0.234	0.200	0.285	0.234	0.238	eT+eCO_2	1.056	1.456	1.807	1.906	1.557
Mean	0.266	0.250	0.260	0.260		Mean	2.557	2.512	2.508	2.487	
	T	G	T x G				T	G	T x G		
SEM	0.008	0.010	0.017			SEM	0.070	0.081	0.140		
CD @ 1%	NS	NS	0.067			CD @ 1%	0.279	NS	0.558		

Table 5. α- amylase of groundnut genotypes at aT, eT and eT+eCO_2 condition

	α- amylase (mm)				
	K-6	**Narayani**	**Dharani**	**K-9**	**Mean**
aT	2.10	2.36	2.38	2.28	2.28
eT	2.02	2.35	2.40	2.26	2.26
eT + eCO_2	2.14	2.22	2.41	2.23	2.25
Mean	2.09	2.31	2.40	2.25	
	T	G	T x G		
SEM	*0.038*	*0.043*	*0.076*		
CD @ 1%	*NS*	*0.175*	*NS*		

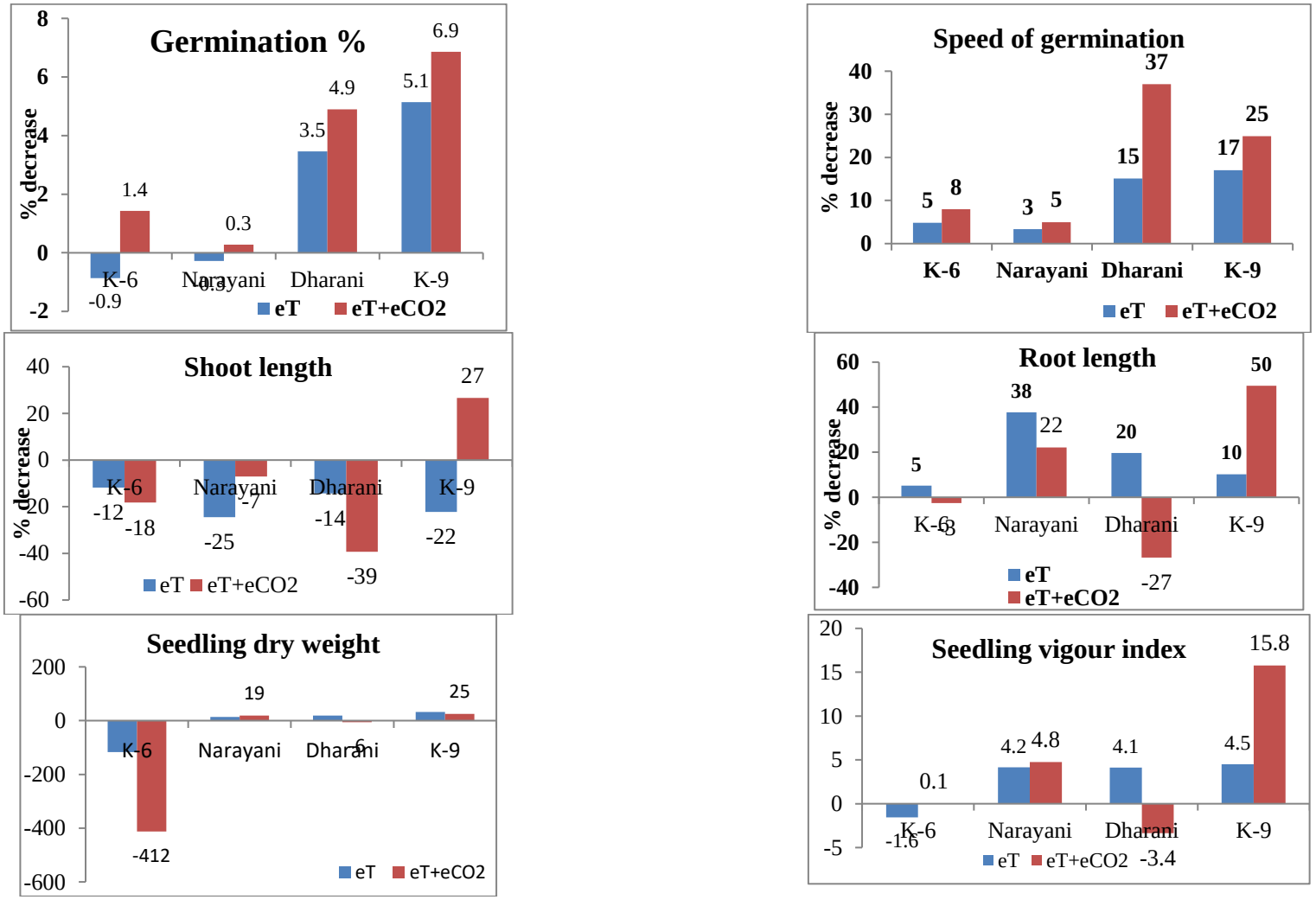

Fig. 1. Graphical distribution of physiological quality of groundnut with % decrease

recorded improved seedling weight over eT revealing that the damage due to high temperature was ameliorated by the eCO_2. Seed mass responses to elevated CO_2 are highly variable, Increasing temperature may decrease seed mass because of an accelerated seed growth rate and reduced seed filling duration.

Among the four groundnut genotypes, the highest seedling vigour index was recorded with Narayani (119.80) for all the treatments. The genotype K-6 with lowest seedling vigour index of aT (108.81) maintained similar values for eT and eT + eCO_2 (109.97 and 108.20) (Table 3). The seeds from eT (118.33, 106.50 and 111.73) recorded reduced SVI as compared with aT (123.47, 111.07 and 117) in Narayani, Dharani and K-9 while eT + eCO_2 improved seedling vigour index only with Dharani (114.80) while reduced with K-9 (98.53).

Reduced seed vigour in soybean and pea was reported by high-temperature stress before physiological maturity [13,21,20] and after physiological maturity [14,22,18,19].

The MDA content of groundnut genotypes was recorded highest with seeds harvested from eT (0.277) except Dharani (0.239). It was also observed that in these genotypes eT+ eCO_2 (0.238) reduced the MDA content lower than aT (0.262) values. The genotype Dharani showed a different response trend as highest values with eT+eCO_2 (0.285) and lowest with eT (0.239).

The seed SOD activity of groundnut genotypes varied significantly obtained from all the treatments. The lowest activity of all the genotypes was recorded in the seeds harvested from eT+eCO_2 (1.557). The eT also reduced the SOD activity in all the genotypes except K-6 (2.557).

Genotypic mean performance of Alpha amylase content were found be decreasing from aT to eT and eT + eCO_2 in case of Naryani (2.36, 2.35 and 2.22mm) and K-9 (2.28, 2.26 and 2.23mm) genotypes similarly showing opposite in case of K-6 (2.10, 2.02 and 2.14mm) and Dharani (2.38, 2.40 and 2.41mm) genotypes seeds harvested from all three treatments.

Physiological attributes revealed that significantly highest germination percentage, speed of germination, seedling vigour index and seedling dry weight (87.81%, 72.01, 114.95 and 0.755g respectively) of seeds harvested from control treatments. Among four genotypes Naryani showed highest percentage of germination (89.75%) and lowest with K-9 (84%). Further, speed of germination and seedling dry weight was highest in genotype K-9 (71.43 and 0.989g), while seedling vigour index was highest in genotype Dharani (110.79).

Among four genotypes, Naryani recorded highest shoot and root length (13.08cm and 17.06cm respectively). Further, seeds harvested from ambient control (aT) showed maximum root length (17.34cm) while seeds harvested from elevated temperature (eT) showed maximum shoot length (12.01cm).

Seed biochemical parameters like MDA, SOD and alpha amylase content as influenced by variation in CO_2 and temperature. Among the treatments under elevated Temperature showed highest MDA content (0.277). Further under control treatment SOD and Alpha amylase recorded highest compare to elevated temperature and CO_2 (3.169 and 2.284 respectively). Between the genotypes K-6 recorded highest MDA and SOD content (0.266 and 2.557 respectively) [15,16,17].

4. CONCLUSION

The environment during seed development and maturation can significantly reduce seed quality (Dornbos 1995; Gusta et al., 2004; Egli et al., 2005; Shinohara et al., 2008), particularly seed vigour. Between the genotypes Naryani showed highest germination %, shoot and root length. Further, K-9 recorded significantly highest speed of germination and seedling dry weight. And under enzymatic activity K-6 is reactive under stress conduction because K-6 recorded highest MDA and SOD content. Enzymatic activity increased under elevated temperature. How likely is it that elevated CO_2 levels and temperature increases of up to 3^0C by 2050 will further increase this loss of seed quality? To answer this question accurately will require substantially more research in order to determine the critical periods during seed development when seeds are sensitive to environmental stresses, and for temperature, how this interacts with the duration of exposure to elevated temperatures which are deleterious to seed quality.

From the information that is available, it can be concluded that predicted environmental changes

will lead to the increased occurrence of loss of seed quality, particularly seed vigour and possibly germination. While seed mass will also change, this does not necessarily imply any negative effect on germination or vigour. To minimize the risk of reductions in seed quality the seed industry will therefore have to consider.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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