Journal of Essential Oil Bearing Plants

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/teop20

Variability in Essential Oil Constituents, Antioxidant Activities and Yield of Elite Small Cardamom Lines (*Elettaria cardamomum* Maton) Under Moisture Deficit Stress

S.J. Ankegowda, M. Alagupalamuthirsolai, R. Sivaranjani, Mohammed Faisal Peeran, K.S. Krishnamurthy & M.S. Shivakumar

To cite this article: S.J. Ankegowda, M. Alagupalamuthirsolai, R. Sivaranjani, Mohammed Faisal Peeran, K.S. Krishnamurthy & M.S. Shivakumar (2022) Variability in Essential Oil Constituents, Antioxidant Activities and Yield of Elite Small Cardamom Lines (*Elettaria cardamomum* Maton) Under Moisture Deficit Stress, Journal of Essential Oil Bearing Plants, 25:5, 1062-1076, DOI: 10.1080/0972060X.2022.2125351

To link to this article: https://doi.org/10.1080/0972060X.2022.2125351

Published online: 11 Nov 2022.

Journal of Essential Oil Bearing Plants

Taylor & Francis
Taylor & Francis Group

https://www.tandfonline.com/journals/teop20 ISSN Print: 0972-060X; ISSN Online: 0976-5026

Article

Variability in Essential Oil Constituents, Antioxidant Activities and Yield of Elite Small Cardamom Lines (*Elettaria cardamomum* Maton) Under Moisture Deficit Stress

S.J. Ankegowda [1]*, M. Alagupalamuthirsolai [2], R. Sivaranjani [2], Mohammed Faisal Peeran [1], K.S. Krishnamurthy [2] and M.S. Shivakumar [1]

[1] ICAR-Indian Institute of Spices Research, Regional Station, Appangala, Madikeri, Karnataka 571201, India

[2] ICAR-Indian Institute of Spices Research, Calicut, Kerala 673012, India

**Corresponding Author:* S.J. Ankegowda *(ankegowdasj@gmail.com)*

Received 28 April 2022; Received in revised form 11 August 2022; Accepted 10 September 2022

Abstract: A field study was conducted at ICAR-Indian Institute of Spices Research, Regional Station, Madikeri, Karnataka during 2018 and 2019 to evaluate elite lines of small cardamom (*Elettaria cardamomum* Maton) for capsule yield, essential oil constituents, photosynthesis and antioxidant enzyme activities under moisture stress condition. Eight small cardamom lines were selected for the study and were grown under irrigated and moisture stress conditions adopting all the package of practices. Photosynthetic gas exchange, relative water content, total soluble proteins and antioxidant enzyme activities were determined at panicle initiation stage during summer. Yield and essential oil components were recorded after harvest. The results showed that moisture stress significantly reduced the soluble protein content and stomatal conductance in the leaves thereby lowering net photosynthetic rate, thus inhibiting growth and yield. Antioxidant enzyme activities viz. catalase, peroxidase, superoxide dismutase and polyphenol oxidase were significantly influenced by moisture stress. Among the genotypes, IC584058 showed bold capsules, higher and less reduction in yield under irrigated and moisture stress condition, respectively. It is suggested that the soluble protein and antioxidant enzymes in leaves play important role in maintaining cell water content and photosynthetic gas exchange during panicle initiation stage leading to less reduction in yield under moisture deficit condition. The essential oil components of three lines namely IC584058, IC584078 and IC584090 showed drastic variation under moisture stress. Though the α-terpinyl acetate content decreased ($P<0.05$), the oxygenated monoterpenes significantly increased in stress tolerant genotype IC584058 which influences antioxidant and antimicrobial activities leading to plant adaptive defense mechanism under moisture.

Keywords: *Elettaria cardamomum* Maton, Moisture stress, Biochemical studies, Drought tolerance, Enzymes.

Introduction

Small cardamom (*Elettaria cardamomum* (L.) Maton, Zingiberaceae), a shade loving herbaceous shrub popularly called "Queen of spices" grown mostly in Western Ghats. It is cultivated extensively in the altitude ranging from 500-1500 m above MSL with an average annual rainfall between 1500 to 5000 mm and annual lowest and highest temperature varying from 10 to 36°C. Cardamom is grown in India with a production of 20,650 MT (Spices Board, 2017-18). Cardamom is a high value (second costliest crop

DOI: 10.1080/0972060X.2022.2125351

in the world after saffron) and high income crop, sensitive to both biotic and abiotic stresses [1,2].

Though Western Ghats region receives good rainfall (1500-5000 mm), the distribution was uneven. Since small cardamom is basically rainfed crops, uneven distribution of rainfall in small cardamom growing tracts leads to reduction in plant growth and development leading to poor yield and in some cases, even leads to the death of plants due to severe water shortage. Reduced soil water content can decrease the yield of cardamom significantly during summer in the months of April and May [2]. The sharp decline in area under cardamom was noticed across the cardamom tracts of Western Ghats in recent decades. The failure of showers during this period results in affects panicle initiation, subsequent growth and crop yield. Summer rain during March, 2008 have benefited the cardamom crop of 2008-2009 up to 20-30%. Therefore, summer rains have positive influence on cardamom. There exists a strong relationship between the water deficit during summer and cardamom production [3].

Reduced plant growth and productivity under drought are caused by altered plant water relations, decreased CO_2 assimilation, cellular oxidative stress, membrane damage of affected tissues, and in some instances, inhibition of enzyme activity [4]. Photosynthesis secured the plant growth; however, moisture deficit stress can cause severe injury to plants [55]. Moisture deficit stress induced stomatal closure leads to decrease in CO_2 availability which directly affects rates of photosynthesis [5,6]. Photosystem II (PSII) is believed to be the most stress sensitive among all photosynthetic functions, evaluation of chlorophyll fluorescence (Fv/Fm) can be used as a quick indicator in any crop [7]. Crop varieties that maintain efficient protection of PSII activity (high Fv/Fm) under water stress conditions, are considered to be stress tolerant [8]. The relative leaf water content of plant decreases to an extent of 60-80% with increase in osmotic potential of the plant cells under water deficit stress [9]. Plants possess an efficient enzymatic antioxidant defense system to cope with ROS-induced oxidative stress [10]. The enzymatic antioxidants (i.e., Superoxide dismutase (SOD), peroxidase (POX), catalase (CAT)) minimize the oxidative damage and ensure the moisture stress tolerance in plants under stressful conditions [11]. These antioxidants have been reported to contribute directly or indirectly in moisture deficit stress tolerance of small cardamom [12]. A relatively higher air temperature during panicle initiation, especially during summer with prolonged dry season (more than two months), may cause abortion of cardamom flowers. High rainfall, air temperature and relative humidity are critical for better capsule setting and quality of cardamom [13,14].

The analysis of plant physiological function, yield and quality of plants exposed to water deficit appears to be a promising approach to identify the deleterious effects of moisture stress in small cardamom. Hence, identification of moisture stress tolerant lines with less reduction in yield and desirable quality under limited water availability is the need of the hour.

Materials and methods

Experimental site and design

The experiments (under rainfed and irrigated conditions) were conducted in the research farm, ICAR-Indian Institute of Spices Research, Regional Station, Madikeri, Karnataka, India. Two treatments viz rainfed and irrigation treatment were compared in a randomized block design with three replicates with a spacing of 2 m × 2 m in 2018 and 2019. The main plots were irrigation treatment and moisture stress treatment during summer (March to May). Irrigation was provided once in a fortnight for irrigation treatment and for moisture stress treatment, the crop was raised under rainfed condition. The selected eight small cardamom lines were IC349537, IC584058, GG×NKE-12, IC584078, CL668, HS1, Appangala-1 and IC584090. Average temperature was 22.5°C and the mean total rainfall was 317 mm during summer (March to May) of 2018 and 2019. The standard package of practices were followed in all the treatments as per the recommendations of ICAR-Indian Institute of Spices Research, Kozhikode, Kerala, India.

The physiological parameters, including the antioxidant enzyme activities (SOD, POX, CAT and PPO), the content of soluble protein, leaf

water status, photosynthetic gas exchange parameters and chlorophyll fluorescence were determined 10 days after imposition of moisture stress.

Leaf water relation

The leaf relative water content (RWC) was determined using the following equation [15].

RWC(%) = [(FW - DW)/(TW - DW)]×100

Where, FW - fresh weight, DW - dry weight, and SW - saturated weight in water. The dry weight was determined after drying nearly 80°C for 72 hours.

Gas exchange and chlorophyll fluorescence measurement

Photosynthetic gas exchange parameters viz. net photosynthesis rate (A), stomatal conductance (gs) and transpiration (E) were measured in fully expanded 3rd uppermost leaves using a portable photosynthesis system [56] (LCpro-SD Advanced Photosynthesis Measurement System, ADC, England). The photosynthetic photon flux density was maintained at 900 µmol $m^{-2}s^{-1}$. During the treatment period, data were recorded between 9:30 and 11:30 am. The observations were recorded when both A and gs were stable. Same leaf was used for chlorophyll fluorescence measurements immediately after gas exchange measurements. The maximum PS II quantum yield was expressed in terms of chlorophyll fluorescence (Fv/Fm) using the chlorophyll fluorometer (Os-30p) in 10-15 minutes dark adapted leaves between 9:30 and 11:30 am local time according to Strasser [16].

Extraction and determination of antioxidant enzyme activities

Antioxidant enzymes were estimated at panicle initiation stage. Antioxidant enzyme extracts for estimating superoxide dismutase (SOD), Peroxidase (POX) and Catalase (CAT) activities were prepared by homogenizing 100 mg fresh leaves in 10 mL of phosphate buffer. Homogenates were centrifuged at 15000 g for 20 min at 4°C, and the supernatant was collected for enzyme activity assay.

The superoxide dismutase (SOD) activity was measured spectrophotometrically based on inhibition in the photochemical reduction of nitroblue tetrazoilum (NBT)[17]. A volume of 3 mL of SOD reaction mixture (Enzyme extract (100 µL); sodium phosphate buffer (50 mM with pH 7.8); methionine (13 mM); NBT (75 µM); EDTA (0.1 mM) and the riboflavin (2 µM) was added at the end) containing tubes were kept under light. Blank was prepared containing reaction mixture without plant extract and irradiation. The absorbance was recorded at 560 nm. The amount of enzyme extract causing 50% inhibition of photochemical reduction of NBT was defined as one unit of SOD activity and expressed as units/min/g fresh weight (U/min/g fresh weight). Peroxidase (POX) activity was determined by measuring the absorbance of reaction mixture (1 mL phosphate buffer (pH 6.1); 0.5 mL guaiacol (0.25 %); 0.5 mL H_2O_2 (0.1 M and pH 6.0); 0.1 mL enzyme extract) at 470 nm and expressed in µmol of H_2O_2 reduced min-1 mg-1 of plant tissue18. Catalase (CAT) activity was determined by measuring the absorbance of reaction (0.5 mL enzyme extract; 0.5 mL 15 mM H_2O_2) at 240 nm for one minute 19. CAT Activity was calculated using the extinction coefficient (ε240 nm = 40 mM-1 cm-1) for H_2O_2 and mentioned in µmol of H_2O_2 reduced min^{-1} mg^{-1} of plant tissue.

The poloyphenol oxidase (PPO) activity was measured according to Mayer and Harel method [20]. Enzyme extract for PPO activity was prepared by homogenizing 100 mg fresh leaves in 2 mL of sodium phosphate buffer (0.1 M; pH 6.5) at 4°C. The homogenate was centrifuged at 20,000 g for 15 min at 4°C. To start the reaction 200 µL of 0.01 M catechol was added in the reaction mixture (1.5 mL of sodium phosphate buffer (0.1 M; pH 6.5); 200 µL of enzyme extract) and the activity was mentioned as change in absorbance at 470 nm min^{-1} mg^{-1} of plant tissue.

The content of total soluble protein

Soluble protein content was estimated by using Lowry method 21.Frozen leaf samples (0.5 g) were ground in a pestle and mortar using sodium phosphate buffer (0.2 M and pH 7.5). The mixtures were centrifuged at 4,000×g at 4°C for 10 min. The supernatant was collected and used for

protein estimation as per Lowry's method. Absorbance values were recorded spectrometrically at 660 nm and expressed as mg g^{-1} fresh weight. Bovine serum albumin solution was used for the standard curve preparation.

Determination of capsule yield traits

Plants under treatments were allowed to grow up to 4 years, after which observations on dry capsule yield per plant was recorded.

Oleoresin estimation

Cured cardamom capsules were dehusked, and seeds were ground into fine powder. 10 g of powder was used for extraction of oleoresin. Oleoresin estimation was carried out using ASTA22 method. The amount of oleoresin was estimated gravimetrically.

Oleoresin (%) =Weight of residue (g) / Weight of sample (g) × 100

Essential oil estimation

Essential oil was extracted using hydro distillation method on dry whole capsule weight basis [23]. Twenty grams of the whole cured cardamom sample was ground, seeds and husk were transferred to a 1 litre round bottom flask separately, and 500 ml distilled water was added and hydrodistilled for 4 hours. The hydro-distillation in Clevenger apparatus lasted 4 hours until the entire oil was liberated from the sample. The essential oil was carefully collected and dried over anhydrous sodium sulphate, and stored in a sealed glass vial for GC-MS analysis.

The percentage of oil was computed as volume by weight basis:

Essential oil (V/W) (%) = Amount of oil collected (ml) / Weight of sample (g) × 100

GC-MS profiling of essential oil

Qualitative profiling of volatile constituents of cardamom essential oil was done using Shimadzu GC-MS (2010). The separation of compounds was achieved in a Rtx-5 5% Phenyl and 95% dimethylpolysiloxane column with the dimension of 30m x 0.25mm x 0.25µm (L x W x ID). The temperature programming of the column was as follows: 65°C for 2 min, then gradient 3°C min^{-1} to 155°C for 3 minutes. Ion source and interface temperature were set as 200°C and 240°C correspondingly. Other operational parameters were as follows: column oven temperature: 65°C; injection temperature: 240°C; helium flow rate: 1.0 mL min^{-1}; injection volume: 0.2 µL; injection mode: split (1:50 split ratio); acquisition mode: scan; scan range: 40-650 m/z with a scan speed of 1428. The mass spectra of the components were compared with the standard mass spectral library of NIST/WILEY and identified by similarity search [24].

Statistical analysis

The experiments were performed in a randomized block design with three replicates. Differences between the treatments as well as among the cardamom lines were tested using the WASP-Web Agri Stat Package 2.0 program. Statistical variance analysis was performed using ANOVA and compared with least significant differences (LSD) at 5% and 1% level. Multiple mean comparison of volatile constituents was done using Duncan's Multiple Range Test (DMRT) [25] with 5% and 1% level of significance.

Results and discussion

Capsule yield

The important physiological stages like panicle initiation and subsequent growth depends on receipt of summer shower from January to May in the cardamom plantation. The drought prevailed during past decades during summer (1982-83) resulted in more than 50% mortality in susceptible cultivars of cardamom3.Earlier studies have also shown that higher air temperature and drought can seriously affect cardamom yields up to 70% in non irrigated areas, therefore, higher summer temperature coupled with drought episodes are considered the strongest climatic limitations across most cardamom growing regions [26]. In our study, a significant reduction in dry capsule yield was observed in all the lines under moisture stress (Table 1). Dry capsule yield of IC584058 and CL668 lines were 74.0 and 73.6 g $plant^{-1}$ respectively, under moisture stress demonstrating them as relatively moisture stress tolerant lines among the eight

elite cardamom lines. Under moisture stress, the maximum yield reduction was observed in IC584090, GG×NKE-12 and HS1 (30.9, 43.9 and 42.7 g plants^{-1}). The dry capsule yield in these lines was highly reduced under moisture stress prevailed during panicle initiation stage, reflecting them as the relatively sensitive lines to moisture stress. Appangala-1 showed medium level of sensitivity to moisture stress (55.8 g plant^{-1}). Earlier study also supported with the present results that the tolerant genotypes of cardamom maintained higher biomass with less reduction in capsule yield under moisture stress condition [48].

Leaf water content (RWC)

Water stress caused a decrease in leaf water content. Plant recovery from desiccation is primarily dependent on the capacity for maintaining higher RWC during desiccation [27]. Relative water content can be used for characterization of cardamom germplasm for drought/moisture stress [49]. In the present study, a significant difference in relative water content (RWC) was observed among lines between moisture stress and irrigation treatments. During stress period, IC 584058 and CL668 lines maintained significantly higher leaf water content (74.8 and 71.4%, respectively) as compared to other lines at panicle initiation stage (Table 1). In controversy, relative water content in cardamom did not show significant variation between water stress and irrigated treatments due to rhizomatous nature of the crop [48]. Studies in rice crop revealed that significant positive correlation between relative water content with grain yield during reproductive stage water stress condition [28].

Photosynthetic gas exchange

Abiotic stress modifies the rate and duration of photosynthesis, transpiration and stomatal conductance [2]. Stomatal closure mechanism is sensitive to water stress [29], it can be used as an indicator of water deficit stress tolerance. Plants with low values of stomatal conductance may occur in water limited environments. In present study, stomatal conductance (gs) varied significantly between irrigation and moisture stress treatments in most of the lines. The line IC584058 maintained lowest gs under moisture stress condition (0.017 mol H_2O $m^{-2}s^{-1}$) followed by IC 349537 (0.019 mol H_2O $m^{-2}s^{-1}$) (Table 1). In contrast, a higher stomatal conductance was observed in GG×NKE-12 (0.027 mol H_2O $m^{-2}s^{-1}$). Among all the lines, while IC584058 maintained the gs of around 0.017 mol $H_2Om^{-2}s^{-1}$, other lines had a slightly increased gs of >0.020 mol H_2O $m^{-2}s^{-1}$ under water deficit stress. Present study revealed that the tolerant line IC5849058 following the water saving mechanism in leaves by maintaining low stomatal conductance under water stress and the similar results were also observed in *Vigna* sp [50].

Photosynthetic rate (A) was also sensitive to water deficit stress condition and it is closely related with biomass production in plants [30]. In agreement with stomatal conductance, the photosynthetic rate was significantly ($P \leq 0.05$) affected in the all the lines under moisture stress (Table 1). A great decrease was observed in HS1. Under water deficit stress, IC584078 recorded 1.89 µmol CO_2 $m^{-2}s^{-1}$ photosynthetic rate while it decreased to <1.65 µmol CO_2 $m^{-2}s^{-1}$ in all the other lines. Maintenance of leaf photosynthetic rate is important to increase the yield potential of cardamombecause the photosynthetic rate of individual leaves which form the canopy, affect dry matter production via photosynthesis within the canopy [2].

Increase in transpiration rate can affect the flowering, capsule setting and the yield of cardamom significantly during summer months (April and May) [2]. Drought tolerant cultivars of bean crop displayed the ability to modulate its stomata and keep a high photosynthetic rate with a low transpiration (High Water Use Efficiency) rate under water limited environment [31]. In the present study, decreased transpiration rate (E) was observed in IC584058 (0.291 mmol H_2O $m^{-2}s^{-1}$) with higher photosynthetic rate compared to other cardamom lines under water deficit condition. It is very clear that the line IC584058 maintained higher leaf water status (as evident from its RWC) through its very sensitive stomata and showing one of the strategies to survive under water deficit condition.

Table 1. Leaf water relation, photosynthetic gas exchange and yield components of elite small cardamom lines under moisture deficit stress

	RWC (%)		g_s (mol m^{-2} s^{-1})		*E* (mmol H_2O m^{-2} s^{-1})		*A* (µmol m^{-2} s^{-1})		Fv/Fm		SP (mg g^{-1} FW)		100 capsules weight (g)		DCY (g $plant^{-1}$)	
Genotypes	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
IC349537	94.5	69.2	0.029	0.019	0.440	0.377	2.21	1.41	0.801	0.779	0.632	0.588	22.2	20.5	65.7	62.0
IC584058	94.2	74.8	0.039	0.017	0.773	0.291	2.64	1.56	0.803	0.789	0.686	0.663	28.8	26.2	119.7	74.0
GG×NKE-12	94.8	70.5	0.029	0.027	0.575	0.439	2.21	0.931	0.815	0.784	0.615	0.634	27.5	26.7	105.4	43.9
IC584078	97.1	58.7	0.036	0.026	0.761	0.459	2.84	1.89	0.811	0.788	0.717	0.666	25.3	23.5	59.4	46.8
CL668	94.6	71.4	0.032	0.022	0.700	0.461	1.68	1.46	0.808	0.777	0.624	0.610	23.6	23.8	74.6	73.6
HS1	93.6	70.1	0.044	0.022	0.917	0.465	3.23	1.52	0.809	0.798	0.621	0.585	21.5	23.2	85.7	42.7
Appangala-1	94.1	70.4	0.031	0.022	0.695	0.381	2.05	1.63	0.820	0.785	0.663	0.644	20.0	19.2	91.8	55.8
IC584090	94.3	66.7	0.031	0.024	0.686	0.377	1.63	1.42	0.822	0.788	0.733	0.672	29.8	27.2	138.4	30.9
Irrigation (I)	2.34**		0.013*		0.326**		NS		0.031**		0.059**		0.582**		NS	
Genotype(G)	1.17**		NS		NS		0.915**		NS		0.03**		1.16**		55.2*	
I*G	3.10**		NS		NS		NS		NS		NS		1.64**		NS	

RWC (Relative water content), g_s (Stomatal conductance), *E* (Transpiration rate), *A* (Photosynthetic rate), SP (Soluble protein), DCY (Dry capsule yield). **, * and NS denotes significance level at P< 0.05, P< 0.01 and non-significance respectively

Photosystem II efficiency

Chlorophyll fluorescence analysis may provide a sensitive indicator of stress conditions in plants. In the present study, a significant reduction in chlorophyll fluorescence Fv/Fm value was observed in all lines under moisture stress, while there was minimal reduction in this ratio in HS1 and IC584058 (0.798 and 0.789 under water stress) at panicle initiation stage (Table. 1). The photochemical efficiency of photosystem II is determined by the Fv/Fm ratio which is decreased significantly during water deficit stress [32]. Similarly, winter wheat seedlings indicated that the variety that maintained Fv/Fm was tolerant to water deficit stress, capable of maintain high photosynthetic activity [8].

Soluble protein

Soluble protein is an important component of cell osmotic regulation. Under moisture stress, the concentration of soluble protein significantly decreased in all the lines (Table 1). Under severe drought stress condition the protein synthesis was diminished by giving rise to free amino acids [33]. In present study, the lines IC349537 and HS1 showed a higher magnitude of decrease in soluble protein concentration (0.588 and 0.585 mg g^{-1} FW under water stress) and a lower reduction in soluble protein was observed in IC584058 (0.663 mg g^{-1} FW under water stress). The maintenance of favorable cellular turgor potential under water deficit conditions allows the plant to maintain physiological functions such as stomatal opening, CO_2 assimilation, and cell expansion and development [34].

Antioxidant enzymes

Drought-tolerant plant genotypes are prepared to efficiently detoxify Reactive Oxygen Species by the coordinated action of enzymatic antioxidants; the most important components of which are superoxide dismutase (SOD), catalase (CAT), peroxidase (PRX), ascorbate peroxidase (APX) and polyphenol oxidase (PPO) [52]. Increase of SOD, CAT and POX activities was observed in drought-tolerant genotype, in comparison to the drought sensitive plants of Maize [35]. Further, antioxidant enzymes activities were stimulated by PEG-6000 induced drought stress in cardamom [12]. According to the results represented herein in Fig. 1A, water stress during summer could induce the activity of SOD, CAT, POX and PPO in the various lines of cardamom and the activity of enzymes (SOD, CAT, POX and PPO) involved in reactive oxygen species scavenging varied significantly upon moisture stress treatment. SOD represents the first line of plant defense system against uncontrolled oxidation during unfavorable conditions which converts highly reactive singlet oxygen molecules into more stable hydrogen peroxide [36]. Plant SOD exists in three forms according to the metal ion of their active site; Cu/Zn, Mn and Fe forms [51, 57]. In the present study, SOD activity significantly varied between irrigated (2.03 to 6.3 U/min/g fresh weight) and stressed plants (Ranges from 1.6 to 6.2 $Umin^{-1}g^{-1}$ FW) (Fig. 1A). The increased activity of SOD in some cardamom elite lines under water deficit stress reflects a reasonable degree of tolerance. Conversely, the observed inhibition of SOD in the other lines may be attributed to the adverse impact of water stress protein metabolism or the defect in Cu, Zn, Mn and/or Fe – metals activating the enzyme. Similar results indicating increased SOD in drought tolerant genotypes and suppressed activity in susceptible ones have been formerly highlighted [37,38].

CAT activity also showed similar pattern. CAT activity of stressed plant leaves (Ranges from 1.10 to 1.66 µmol $min^{-1}mg^{-1}$FW) significantly increased in all lines than irrigated plant leaves (Ranges from 1.04 to 1.50 µmol $min^{-1}mg^{-1}$ FW) except IC349537 and IC584090 (Fig. 1B). Perhaps this might be due to the low affinity of CAT towards hydrogen peroxide. The enzyme CAT has low affinity for hydrogen peroxide and effective only in high concentration of hydrogen peroxide [36]. In fact, moisture stress must have induced excessive production of hydrogen peroxide in leaves which was favorable to the enzyme CAT and similar findings have been reported in orchid plants [39].

Moisture deficit stress could induce the activities of POX and PPO in the various elite lines of cardamom but these two enzymes were suppressed in some other lines. Moreover, POX activity was

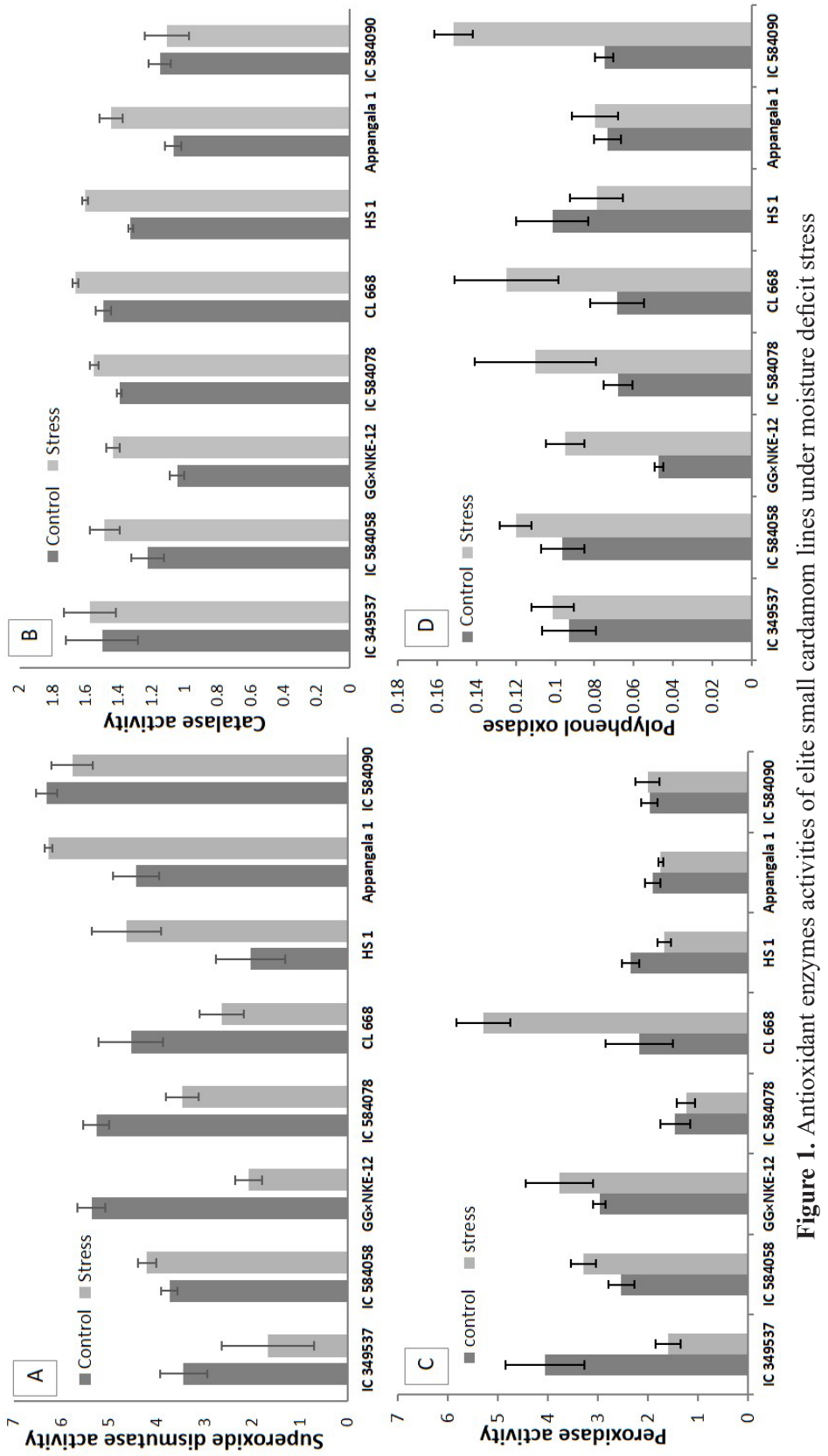

Figure 1. Antioxidant enzymes activities of elite small cardamom lines under moisture deficit stress

induced by stress in IC584058, GG×NKE-12, CL668 (Ranges from 1.45 to 4.06 and from 1.24 to 5.29 µmol $min^{-1}mg^{-1}$ FW under irrigated and stressed plants leaves (Fig. 1C). Enhanced activity of POX was reported in cardamom seedlings as a stress acclimation strategy [12]. Similarly, the cardamom lines IC584058, GG×NKE-12, CL668, and IC584078 showed the enhanced activity of PPO (Ranges from 0.047 to 0.101 and from 0.079 to 0.151 $min^{-1}mg^{-1}$ FW under irrigated and stressed plant leaves, respectively) (Fig. 1D). PPO utilizes O_2 to oxidize phenolics to their corresponding quinones, hence higher PPO activity may indicate more degradation of various toxic substances accumulated because of water stress [40].

Chemical constituents of essential oil

Environmental factors such as drought, high temperature, low temperature and excess rainfall as well as ecological factor such as shade, competition, etc., affects the yield and composition of essential oil in several spices and aromatic crops [41,42]. In cardamom, the oil is dominated by oxygenated compounds and is very little mono- or sesquiterpenic hydrocarbons, all of which are potential aroma compounds. The composition of the ether, 1,8-cineole and the esters, α-terpinyl and linalyl acetates make the cardamom volatiles a unique combination. Alcohol viz. linalool imparts floral woody flavour with citrusy note [53,54]. In our study also, we observed changes in volatile constituents of cardamom essential oil under water deficit stress condition, though the variation was not uniform among the genotypes analyzed (Table 2). In cardamom, 1,8-cineole and α-terpinylacetateare the principle volatile compounds along with α-pinene, sabinene, myrcene, linalool, terpinen-4-ol and α-terpineol. Genotypes IC349537, Appangala-1 and to some extent GCxNKE-12 were found to be non-responsive to drought treatments as no significant changes in major volatile constituents were found between control and stress treatment plants (Table 2). No major changes in volatile profile could be taken as a positive sign that the genotypes are relatively stable in maintaining their flavor profile even in water stress conditions.

The volatile constituents of three genotypes namely IC584058, IC584078 and IC584090 showed drastic variation between irrigated and water stressed plants. Among major volatile compounds, concentration of α-terpinyl acetate reduced significantly ($P<0.05$) in these genotypes as compared to control. As α-terpinyl acetate is the principle flavour compound having mild, sweet-like aroma of cardamom, the decrease in the concentration of this compound in drought stress would lead to decrease in flavour quality of these genotypes under drought condition. In addition to reduction in α-terpinyl acetate and linalool in the genotype IC584078, increase in 1,8-cineole contentwould increase the camphorous flavor of cardamom in this genotype underwater stress.

In the genotype IC584058, decrease in monoterpene hydrocarbon compounds like α-thujene, α-pinene, sabinene, β-pinene, β-myrcene, α-terpinene and ϒ-terpinene was observed with concomitant increase in oxygenated monoterpenes like citral, geraniol and geranial in drought stress plants as compared to control. Similar result was observed in the genotype IC584090. In the genotype GCxNKE-12, significant reduction in monoterpene hydrocarbons like sabinene, myrcene and α-terpinene and ϒ-terpinene was found with the exception of sabinene hydrate where the concentration was found to increase in water stressed plant.

The volatile components were divided into two categories namely monoterpene hydrocarbons and oxygenated monoterpenes to assess the changes in group wise profile upon drought stress. Among them, oxygenated monoterpenes occupied major portion of the volatile components (Table 3). Oxygenated monoterpenes are stable compounds as compared to hydrocarbon monoterpenes which are prone to oxidation [43]. Moreover, the antioxidant and antimicrobial activity of oxygenated monoterpene compounds are higher as compared to hydrocarbon monoterpenes [44]. Based on the comparative analysis, we could say that increase in oxygenated compounds in the genotypes GCxNKE-12, IC58078 and CL668 increased the active compounds in their essential oil. These changes might be con-

Table 2. GC-MS profile of volatile constituents of elite small cardamom lines under moisture deficit stress

Volatile compounds	LRI*	IC349537**		IC584058**		GCxNKE-12**		IC584078**		CL668**		HS1**		Appangala 1**		IC584090**	
		C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S
α-thujene	926	0.13	0.14	0.19^{A}	0.16^{A}	0.19	0.16	0.20^{A}	0.14^{B}	0.27^{A}	0.21^{B}	0.25	0.24	0.15	0.15	0.17^{A}	0.14^{A}
α-pinene	933	0.96	0.99	1.39^{A}	1.12^{B}	1.29	1.06	1.25^{A}	0.98^{B}	1.77^{A}	1.50^{B}	1.67^{B}	1.71^{A}	1.04	0.99	1.08^{A}	0.90^{A}
Sabinene	974	2.53	2.65	3.04^{A}	2.74^{B}	3.33^{A}	3.08^{B}	3.30^{A}	3.04^{B}	3.19	3.28	3.22	3.34	2.58	2.71	3.34	3.16
β-pinene	978	0.28	0.32	0.40^{A}	0.34^{B}	0.38	0.31	0.36^{A}	0.28^{B}	0.51^{A}	0.44^{A}	0.47	0.48	0.34	0.33	0.33^{A}	0.30^{A}
β- Myrcene	991	1.60	1.53	1.71^{A}	1.40^{B}	1.90^{A}	1.59^{A}	1.85^{A}	1.44^{B}	1.98^{A}	1.77^{B}	1.92^{A}	1.81^{A}	1.53	1.57	1.72^{A}	1.43^{A}
Octanal	1005	0.14	0.14	0.19	0.22	0.18	0.17	0.16	0.16	0.11	0.10	0.11	0.10	0.11	0.13	0.20^{B}	0.23^{A}
α-terpinene	1018	0.17	0.22	0.15^{A}	0.11^{A}	0.27^{A}	0.19^{B}	0.21	0.15	0.23^{A}	0.20^{B}	0.21	0.21	0.18	0.24	0.19	0.12
1,8-cineol	1037	42.08	40.92	48.05	47.52	42.03	42.65	38.07^{B}	43.20^{A}	44.19	45.34	45.61	47.37	39.38	39.52	35.92	38.49
β-ocimene	1048	0.11	0.12	0.08	0.08	0.09	0.07	0.08^{A}	0.05^{A}	0.13	0.11	0.11	0.12	0.12	0.13	0.07	0.06
ϒ-Terpinene	1060	0.35	0.43	0.35^{A}	0.30^{A}	0.53^{A}	0.39^{B}	0.48^{A}	0.33^{A}	0.48^{A}	0.41^{B}	0.43	0.41	0.40	0.47	0.40	0.29
Sabinene hydrate	1071	0.54	0.81	0.78^{B}	1.21^{A}	0.65^{B}	1.03^{A}	0.70^{A}	1.05^{A}	0.87	1.03	0.89	1.11	0.57^{B}	0.83^{A}	1.11^{B}	1.33^{A}
α-terpeinolene	1091	0.29	0.32	0.25	0.24	0.49	0.31	0.32^{A}	0.25^{A}	0.27^{A}	0.24^{B}	0.25	0.22	0.25	0.29	0.25	0.26
Linalool	1105	5.77	5.97	1.45^{B}	1.88^{A}	1.46	1.73	1.58	1.69	2.34	2.63	2.26	2.36	5.73	5.97	1.71	1.87
Terpinen-4-ol	1183	2.70	2.50	2.34	2.49	2.89	2.67	2.78^{A}	2.44^{A}	2.32^{A}	2.06^{B}	2.09^{A}	1.93^{A}	2.58	2.41	2.37	2.38
α-terpineol	1198	4.74	4.79	5.28^{A}	6.01^{A}	4.58	3.88	4.78	4.67	4.20	4.23	3.88	4.06	4.43	4.48	4.94^{B}	5.55^{A}
Nerol	1233	0.10	0.10	0.02	0.02	0.03	0.03	0.02	0.02	0.04	0.04	0.03	0.04	0.10	0.09	0.02	0.03
Neral	1246	0.34	0.33	0.36^{B}	0.55^{A}	0.34	0.33	0.39	0.38	0.20	0.22	0.20	0.19	0.29	0.31	0.31^{A}	0.43^{A}
Geraniol	1260	2.33	2.40	0.75^{B}	1.59^{A}	2.20	3.07	2.86	2.82	2.35	2.19	2.26	2.17	3.19	2.42	2.71^{A}	3.04^{A}
Geranial	1276	0.66	0.58	0.71^{B}	1.06^{A}	0.54	0.50	0.65	0.62	0.35	0.35	0.33^{A}	0.27^{A}	0.55	0.56	0.61^{A}	0.76^{A}
Borneol acetate	1291	0.04	0.04	0.04	0.05	0.05	0.04	0.04	0.03	0.04	0.04	0.03	0.04	0.04^{A}	0.03^{B}	0.06	0.05
Ocimenyl acetate	1322	0.21	0.22	0.23	0.23	0.27	0.26	0.25^{A}	0.20^{B}	0.35^{A}	0.30^{B}	0.30	0.31	0.25	0.23	0.22	0.20
Terpinyl acetate	1359	32.23^{A}	32.81^{A}	31.14^{A}	29.86^{A}	35.09	35.48	38.35^{A}	35.16^{A}	31.76	31.68	31.74	29.90	34.08	34.40	40.21^{A}	37.59^{B}
Neryl acetate	1388	1.24	1.18	0.62	0.46	0.79	0.70	0.87^{A}	0.60^{A}	1.31^{A}	1.16^{A}	1.06^{A}	1.13^{A}	1.49	1.24	1.36^{A}	0.86^{B}
Nerolidol	1567	0.51	0.53	0.56	0.44	0.49	0.37	0.51^{A}	0.34^{B}	0.80^{A}	0.56^{A}	0.74^{A}	0.54^{A}	0.67	0.55	0.78^{A}	0.58^{B}

*Linear Retention Indices as calculated by comparing retention time of series of n-alkanes. **Means with common superscript are statistically significant at P<0.05 and means with different superscript are statistically significant at P<0.01 using DUNCAN's Multiple Range Test

Table 3. Composition of different classes of volatile constituents upon moisture deficit stress in small cardamom lines

Genotypes	Treatments	% Volatile compounds		
		Monoterpene Hydrocarbons**	Oxygenated monoterpenes**	Others**
IC349537	Control	8.07	91.43	0.65
	Stress	8.57	90.91	0.67
IC584058	Control	9.56	89.89	0.74
	Stress	9.49	90.08	0.66
GCxNKE-12	Control	10.16^{A}	89.36^{B}	0.67
	Stress	9.16^{B}	90.48^{A}	0.53
IC584078	Control	9.92^{A}	89.57^{B}	0.67^{A}
	Stress	8.85^{B}	90.82^{A}	0.49^{B}
CL668	Control	10.32^{A}	88.88^{B}	0.91^{A}
	Stress	9.82^{A}	89.63^{A}	0.65^{A}
HS1	Control	10.03^{A}	89.24	0.85^{A}
	Stress	10.18^{A}	89.28	0.64^{A}
Appangala-1	Control	8.09	91.25	0.78
	Stress	8.67	90.78	0.68
IC 584090	Control	9.73	89.50	0.97^{A}
	Stress	9.39	90.04	0.81^{A}

**Means with common superscript are statistically significant at P<0.05 and means with different superscript are statistically significant at P<0.01 using DUNCAN's Multiple Range Test

sidered as plants adaptive defense mechanism against water shortage or drought like conditions. Some studies reported that the content of oxygenated monoterpenes reduced under moderate and severe drought stress condition in Saliva dolomitica with increase in some of the oxygenated sesquiterpenes [45] as opposed to our study where we found increase in oxygenated monoterpenes in some of the genotypes. Moreover, they observed the reduction in sesquiterpenes hydrocarbon and an increase in oxygenated sesquiterpenes. In Basil (Ocimum basilicum) crop, the plants subjected to 50% field capacity increased major phenolic and monoterpene hydrocarbon compounds as compared to control and 75% field capacity samples [46]. They also proved that, drought stress influenced the expression of some of the genes involved in terpenoid and phenolic biosynthetic pathway. The variation observed in our study could also be due to modulating the expression profile of some of the genes involved in terpenoid biosynthetic pathway [47]. Study like this would throw light on the effect of moisture stress on flavour quality of cardamom. Furthermore, it also helps in the identification of suitable genotypes to be cultivated under drought prone area without affecting the flavor profile of cardamom capsules.

Conclusions

Eight elite small cardamom lines were evaluated for moisture deficit stress tolerance under field condition during the fifth successive crop season. Significant variation was recorded for growth, yield and quality parameters between the treatments and the lines. The line IC584058 having compact flowering, bold capsule, higher yield under irrigated condition also showed less re-

duction in yield under moisture deficit condition, indicating that it is relatively tolerant to moisture stress. The study also revealed that it has sensitive stomata, maintained higher leaf water content and better gas exchange and higher antioxidant activities under moisture stress conditions. Among the lines, it also showed increased in active oxygenated monoterpenes like citral, geraniol and geranial under moisture stress which might be confer tolerance to moisture deficit stress. The lines IC349537, Appangala-1 and to some extent GCxNKE-12 showed no significant changes in major volatile constituents between treatments but with significant yield reduction under moisture deficit stress. Hence, the line IC584058 could be used in breeding programme to develop high yielding and climate resilient cardamom genotype which is urgently required under climate change scenario.

Acknowledgments

This work was supported and funded by the Government of India, ICAR-Indian Institute of Spices Research, Kozhikode, Kerala.

References

1. **Ankegowda, S.J. (2011).** Impact of irrigation on cardamom production, Indian Journal of Horticulture. 68: 581-582.
2. **Murugan, M., Shetty, P.K., Ravi, R. and Subbiah, A. (2009).** The Physiological Ecology of Cardamom (*Elettaria cardamomum* M) in Cardamom Agro Forestry System. International Journal of Environmental Research. Winter. 3(1): 35-44.
3. **Prasada Rao and G.S.L.H.V. (2016).** Weather extremes and plantation crops in the humid tropics. Mausam. 67(1): 251-258.
4. **Abid, M., Ali, S., Qi, L.K., Zahoor, R., Tian, Z., Jiang, D., Snidor, J.L. and Dai T. (2018).** Physiological and biochemical changes during drought and recovery periods at tillering and jointing stages in wheat (*Triticum aestivum* L.). Sci Rep. 8: 4615.
5. **Chaves, M.M., Flexas, J. and Pinheiro, C. (2009).** Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Annals of Botany. 103: 551-560.
6. **Lawlor, D.W. and Cornic, G. (2011).** Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell and Environment. 25: 275-294.
7. **Jansen, M., Pinto, F., Nagel, K., Dusschoten, D., Fiorani, F., Schneider, H., Walter, A. and Schurr, U. (2014).** Non-invasive phenotyping methodologies enable the accurate characterization of growth and performance of shoots and roots. In: Genomics of Plant Genetic Resources, (eds) Tuberosa, R., Graner, A. and Frison, E. (Dordrecht: Springer), 467-487.
8. **Zlatev, Z. (2009).** Drought-Induced Changes in Chlorophyll Fluorescence of Young Wheat Plants, Biotechnology & Biotechnological Equipment, 23: sup1, 438-441.
9. **Singh, R., Pandey, N., Naskar, J. and Shirke P.A. (2015).** Physiological performance and differential expression profiling of genes associated with drought tolerance in contrasting varieties of two Gossypium species. Protoplasma. 252: 423-438.
10. **Ashraf, U., Kanu, A.S., Mo, Z., Hussain, S., Anjum, S.A., Khan, I., Abbas, R.N. and Tang, X. (2015).** Lead toxicity in rice: effects, mechanisms, and mitigation strategies-a mini review. Environ. Sci. Pollut. Res. 22: 18318-18332.
11. **Sharma, P., Jha, A.B., Dubey, R.S., and Pessarakli, M. (2012).** Reactive oxygen species, oxidative damage and antioxidative defense mechanism in plants under stressful conditions. J. Bot. 217037.
12. **Alagupalamuthirsolai, M., Ankegowda, S.J., Mohammad, F.P. and Sharon, A. (2020).** Establishment of a rapid screening method for drought tolerance of small cardamom by using polyethylene glycol-6000. Int. J. Chem. Stud. 8(2): 1750-1755.
13. **Manoj, O., Xavier, R.B.A., Vadiraj, P.M., Suresh, K. and Remashree, A.B. (2018).** Quality appraisal of small cardamom (*Elettaria cardamomum* Maton) sourced

from A, B and C Zones of CHR in Idukki district of Kerala, India. Journal of Essential Oil Bearing Plants. 21(5): 1315-1326.

14. **Alagupalamuthirsolai, M., Ankegowda, S.J., Murugan, M., Sivaranjani, R., BalajiRajkumar and Akshitha, H.J. (2019).** Influence of Light Intensity on Photosynthesis, Capsule Yield, Essential Oil and Insect Pest Incidence of Small Cardamom (*Elettaria cardamomum* (L.) Maton), Journal of Essential Oil Bearing Plants. 22: 1172-1181.
15. **Jones, M.M. and Turner, T.C. (1978).** Osmotic adjustment in leaves of sorghum on response to water deficits. Plant Physiol. 25: 591-597.
16. **Strasser, R.J., Srivastava, A. and Govindjee. (1995).**Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria. Photochem. Photobiol. 61: 32-42.
17. **Dhindsa, R.A., Plumb, D.P. and Thorpe, T.A. (1981).** Leaf senescence: correlated with increased permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J. Exp. Bot. 126: 93-101.
18. **Castillo, F.I., Penel, I., and Greppin, H. (1984).** Peroxidase release induced by ozone in Sedum album leaves: involvement of Ca^{2+}. Plant Physiol. 74: 846-851.
19. **Aebi, H. (1984).** Catalase in vitro. Meth. Enzymol. 105: 121-126.
20. **Mayer, A.M. and Harel, E. (1979).** Polyphenol oxidases in plants. Phytochemistry. 18: 193-215.
21. **Lowry, O.H., Rosebrough, N.J. and Farr, A.L. (1951).** Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-276.
22. **ASTA (1978).** Official Analytical Methods of the American Spice Trade Association. 2ndEdn. American Spice Trade Association Inc. N.J, pp. 38-41.
23. **ASTA (1997).** Official Analytical Methods of American Spice Trade Association, 4th edition, Washington DC, USA.
24. **Adams, R.P. (2007).** Identification of essential oil components by gas chromatography-massspectrometry. Allured Publishing corpotion: Carol Stream, Illinois.
25. **Duncan, D.B. (1955).** Multiple range and multiple F tests. Biometrics. 11: 1-42.
26. **Murugan, M., Shetty, P.K., Anandhi, A. and Ravi, R. (2012).** Present and future climate changein Indian cardamom hills: Implications for cardamom production and sustainability. British Journal of Environment & Climate Change. 2: 368-390.
27. **Blum, A., Mayer, J., Golan, G. and Sinmera, B. (1999).** Drought tolerance of a doubled- haploid line population of rice in field. In: Genetic improvement of rice for water limited environment. Ito, P., Toole, J.C. and Hardy, B. (eds). IRRI, pp. 319-330.
28. **Kumar, S., Dwivedi, S., Singh, S., Jha, S., Sathee, L., Rajamanickam, E., Singh, O. and Bhatt, B. (2014).** Identification of drought tolerant Rice genotypes by analysing drought tolerance indices and morpho-physiological traits. SABRAO J. Breed Genet. 46: 217-230.
29. **Medrano, H. (2002).** Regulation of photosynthesis of C_3 plants in response to progressive drought: stomatal conductance as a reference parameter. Ann. Bot. 89: 895-905.
30. **Zhao, W., Liu, L., Shen, Q., Yang, J., Han, X., Tian, F. and Wu, J. (2020).** Effects of Water Stress on Photosynthesis, Yield, and Water Use Efficiency in Winter Wheat. Water. 12(8): 2127.
31. **Tavera, V.M., Aguirre Mancilla, C.L., Acosta Gallegos, J.A., Rámirez Pimentel, J.G., Mireles Arriaga, A.I., and Ruiz Nieto, J.E. (2018).** Mechanisms for water-use efficiency between bean cultivars tolerant to drought are different. Acta Scientiarum. Agronomy. 40(1): e39378.
32. **Mamnouie, E., Fotouhi Ghazvini, R., Esfahany, M. and Nakhoda, B. (2006).** The Effects of Water Deficit on Crop Yield and the Physiological Characteristics of Barley (*Hordeum vulgare* L.) Varieties. Journal of Agricultural Science & Technology. 8: 211-219.

33. **Monreal, J.A., Jimenez, E.T., Remesal, E., Morillo-Velarde, R., García-Maurino, S. and Echevarría, C. (2007).** Proline content of sugar beet storage roots: Response to water deficit and nitrogen fertilization at field conditions. Environ. Exp. Bot. 60: 257-267.
34. **Serraj, R. and Sinclair, T.R. (2002).** Osmolyte accumulation: can it really help increase crop yield under drought conditions? Plant Cell Environ. 25: 333-341.
35. **Chugh,V., Kaur, N., Grewal, M. and Gupta, A. (2013).** Differential antioxidative response of tolerant and sensitive maize (*Zea mays* L.) genotypes to drought stress at reproductive stage. Indian journal of biochemistry & biophysics. 50: 150-8.
36. **Cruz de Carvalho, M.H. (2008).** Drought stress and reactive oxygen species: Production, scavenging and signaling. Plant Signaling & Behavior. 3(3): 156-165.
37. **Ren, J., Sun, L.N., Zhang, Q.Y. and Song, X.S. (2016).** Drought tolerance is correlated with the activity of antioxidant enzymes in *Cerasus humilis* seedlings. Biomed. Res. Int. pp. 1-9.
38. **Siddiqui, M.H., Al-Khaishany, M.Y., Al-Qutami, M.A., Al-Whaibi, M.H., Grover, A., Ali, H.M., Wahibi, M.S. and Najat, A.B. (2015).** Response of different genotypes of faba bean plant to drought stress. Int. J. Mol. Sci. 16: 10214-27.
39. **Mohd. Aminur F.S., Khor S.P., Chin, C.K. and Sreeramanan, S. (2015).** Biochemical and physiological responses to polyethylene glycol (PEG) treatment in protocorm -like bodies (PLBs) of Aranda Broga Blue Bell. Emirates Journal of Food and Agriculture. 27(10): 776-783.
40. **Bardees, M.M. and Aldesuquy, H.S. (2017).** Impact of osmotic stress on seedling growth observations, membrane characteristics and antioxidant defense system of different wheat genotypes, Egyptian J. Basic and Applied Sciences. 4: 47-54.
41. **Petropoulos, S.A., Daferera, D., Polissiou, M.G. and Passam, H.C. (2008).** The effect of water deficit stress on the growth, yield and composition of essential oils of parsley. Sci. Hort. 115: 393-397.
42. **Selmar, D. and Kleinwachter, M. (2013).** Influencing the product quality by deliberately applying drought stress during the cultivation of medicinal plants. Industrial Crops and Products. 42: 558-566.
43. **Dhifi, W., Bellili, S., Jazi, S., Bahloul, N. and Minf, W. (2016).** Essential Oils' chemical characterization and Investigation of some Biological Activities: Medicines (Basel). 3(4): 25.
44. **Mezza, G.N., Borgarello, A.V., Grosso, N.R., Fernandez, H., Pramparo, M.C. and Gayol, M.F. (2018).** Antioxidant activity of rosemary essential oil fractions obtained by molecular distillation and their effect on oxidative stability of sunflower oil. Food Chemistry. 242: 9-15.
45. **Casera, C.M., Chitarrab, W., D'Angiolillod, F., Perroneb, R., Demasia, S., Lovisoloa, C., Pistellie, L., Pistellid, L. and Scariota, V. (2019).** Drought stress adaptation modulates plant secondary metabolite production in *Salvia dolomitica*. Industrial Crops and Products. 129: 85-96.
46. **Mandoulakani, B.A., Eyvazpour, E. and Ghadimzadeh, M. (2017).** The effect of drought stress on the expression of key genes involved in the biosynthesis of phenylpropanoids and essential oil components in basil (*Ocimum basilicum* L.). Food Chemistry. 139: 1-7.
47. **Anjali, N., Nadiya, F., Thomas, J. and Sabu, K.K. (2019).** Identification and characterization of drought responsive microRNAs and their target genes in cardamom (*Elettaria cardamomum* Maton). Plant Growth Regul, 87: 201-216.
48. **Ankegowda, S.J. and Krishnamurthy, K.S. (2008).** Evaluation of small cardamom accessions for moisture stress. Journal of Spices and Aromatic Crops Volume 17(2): 172-176.
49. **Ankegowda,S.J. and Krishnamurthy, K.S.(1998).** Effect of moisture stress on growth of cardamom (*Elettaria cardamomum* Maton). In water and Nutrient management

for sustainable production and quality of spices (Eds.) A K Sadanandan, K S Krishnamurthy, K Kandamman and V S Karikanthianath. Indian Society for Spices, Calicut. pp. 158-161.

50. **Iseki, K., Takahashi, Y., Muto, C., Naito, K. and Tomooka, N. (2018).** Diversity of Drought Tolerance in the Genus Vigna. Front. Plant Sci. 9: 729.
51. **Huseynova, I.M., Aliyeva, D.R. and Aliyev, J.A. (2014).** Subcellular localization and responses of superoxide dismutase isoforms in local wheat varieties subjected to continuous soil drought. Plant Physiol Biochem. 81: 54-60.
52. **Heidari, M. and Golpayegani A. (2012).** Effects of water stress and inoculation with plant growth promoting rhizobacteria (PGPR) on antioxidant status and photo-synthetic pigments in basil (*Ocimum basilicum* L.). J. Saudi Soc. Agric. Sci. 11: 57-61.
53. **Korikanthimath, V.S., Mulge, R. and Zachariah, T.J. (1997).** Variation in yield and quality characters of cardamom clones. J. Med. and Aromat. Plant Sci. 19(4): 1024-1027.
54. **Lewis, Y.S., Nambudiri, E.S. and Phillip, T. (1966).** Composition of cardamom oils perfume. J. Essent. Oil Res. 57: 623-626.
55. **Osakabe, Y., Osakabe, K., Shinozaki, K. and Tran L-SP. (2014).** Response of plants to water stress. Front. Plant Sci. 5: 86.
56. **Khan, F., Upreti, P., Singh, R. (2017).** Physiological performance of two contrasting rice varieties under water stress. Physiol Mol. Biol. Plants. 23: 85-97.
57. **Khaulood, A., Hemida, Abdullah, Z.A., Eloufey, Mohamed, A., Seif El-Yazal and Mostafa, M. Rady. (2017).** Integrated effect of potassium humate and α-tocopherol applications on soil characteristics and performance of *Phaseolus vulgaris* plants grown on a saline soil, Archives of Agronomy and Soil Science. 63(11): 1556-1571.