PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.1413

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RESEARCH ARTICLE

Influence of exogenous abscisic acid on morpho-physiological and yield of maize (*Zea mays* L.) under drought stress

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OPEN ACCESS

ARTICLE HISTORY

Received: 27 July 2021
Accepted: 07 February 2022

Available online
Version 1.0 (Early Access): 14 March 2022

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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CITE THIS ARTICLE

Ramya S, Arulbalachandran D, Ramachandran M. Influence of exogenous abscisic acid on morpho-physiological and yield of maize (*Zea mays* L.) under drought stress. Plant Science Today (Early Access). https://doi.org/10.14719/pst.1413

Abstract

Abscisic acid (ABA) is a naturally occurring plant hormone, it's also known stress hormone, that act the plant responses to abiotic stresses, especially drought. Maize production losses due to drought prominently affect economics and livelihoods of millions of peoples. The current investigation the role of ABA in drought-stress tolerance of maize. The influence of drought stress and foliar spray of abscisic acid different concentrations (25, 50, 75 and 100 µM) were analysed on morphological, physiological and biochemical parameters. The present results revealed a most effective to increased after drought stress imposed with 75 µM ABA treated plants. Exogenous abscisic acid acts as a scavenger of ROS for mitigating the injury on cell membranes under drought were observed in the opening of stomata. Histochemical detection of more accumulation ROS (H_2O_2 and $O_2^{\bullet -}$) was detected in drought stress shoot compared to ABA treated shoot. Fourier Transform Infrared Spectroscopic (FTIR) study, ABA treated leaves indicated the presence of different functional groups. This study shows that can provide vital insights into maize leaves drought responses and could be beneficial in identifying novel drought tolerance characters. Drought and abscisic acid treatment increased the endogenous foliar abscisic acid level, specifically at 75 µM concentration. The exogenous abscisic acid application effectively ameliorates the adverse effect of drought stress to improve the drought resistance. In conclusion, the level of 75 µM concentration ABA was better growth characteristics, biochemical alterations and yield under drought stress.

Keywords

Zea mays, ABA, Water deficit, FTIR, Biochemical analysis, SDS-PAGE

Introduction

Drought stress is one of the most severe abiotic stresses across the world which is seriously hampering the productivity of agricultural crops (1). Maize (*Zea mays* L.) is the third most important cereal crop in India. Maize (also referred to as corn) is preferred in Southern and Eastern Africa, Central America and Mexico. In addition, maize plays a pivotal role in the agricultural economy, providing food for a more extensive population and raw material for industries and feeding animals. In India, maize is grown in 9.2 million ha, with 28.64 million tonnes, and the average productivity is 3.0 tonnes per ha in Tamil Nadu. Maize is cultivated in 0.31 million hectares with a production of 0.95 million tonnes and productivity of 3 tonnes per ha (2). However, the current crisis in maize production is due to ineffective water manage-

ment and limited resource and expensive due to higher demand by industry and urban consumption and parallels the groundwater depletion at an alarming rate (3). During the drought period, the photosynthetic pigment is damaged and decreases the activities of Calvin cycle (4). The growth, photosynthetic pigments, physiological, biochemical, cellular and molecular components are affected by drought condition (5, 6). The drought-tolerant crops have been evolved by assimilation and adaptation mechanisms, including antioxidant defence systems (7).

Phytohormones are considered the main signals during stress conditions, and almost all processes in a plants life are directly or indirectly influenced (8). Especially, ABA considered one of the important hormone, that trigger various acclimation processes under drought conditions. Exogenous ABA spray stimulated the synthesis of proteins in different species (6). ABA plays a vital role in providing plants under drought conditions to signal stomata closure and reduced shoot expansions and ABA is involved in vigorous root growth and other modifications. Its regulates the expression of numerous stress-responsive genes and synthesises LEA proteins, dehydrins and other protective proteins (9). Chlorophyll fluorescence (CF) imaging is a common non-destructive technique for analysing stress levels in various crops since these characteristics provide information on both mechanical detail and the extent of stress damage in plants. Using CF imaging techniques and photochemical measurements in various plants, including lettuce, researchers discovered that drought stress had various effects on photosynthetic processes (10). Fourier transform infrared (FTIR) spectroscopy is a technique for determining the vibrations of chemical bonds and generating a spectrum that can be used to determine a sample's biochemical profile. FTIR spectroscopy is a powerful and rapidly developing technology that has the potential to help researchers better understands whether maize reacts to drought. Multivariate analysis methods such as principal component discriminate function analysis can be used to extract critical information from a spectrum (11).

Starch is a storage carbohydrate that promotes metabolism in higher plants (12). About half of the photo assimilated carbon is stored as starch in certain plants, to be remobilised later. Amylase, the enzyme most often implicated for the initial attack on starch granules, is responsible for the mobilisation of starch in germinating seeds (13). Proline is also believed to be an antioxidant, helping preserve leaves from lipid peroxidation and osmotic adjustment, free radical scavenger under drought stress condition (14). Proline helps decrease cell osmotic potential drought conditions and stabilises proteins by maintaining the chemical structure (15). There are significant alterations in the stomata opening, photosynthetic reaction centre, electron transport system, or enzyme activity in response to drought stress (16-18).

In the present investigation, we analysed the morphological, physiological, biochemical alterations, FTIR and amylase activity on maize variety under drought and ABA treated. This research will give information on the plants drought-induced exogenous ABA and adaptation, which is will help breeders develop to tolerant variety.

Materials and Methods

The maize variety of Coimatore-6 (CO-6) collected by Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India. The seeds were surface sterilised with 0.1gm mercury chloride ($HgCl_2$) for three minutes with frequent shaking and thoroughly washed with deionised water to remove the mercury chloride (19). The experiments were conducted at Botanical Garden, Department of Botany, Periyar University, Tamil Nadu, India.

The seeds distance was maintained at 15 cm intervals with a spacing plants of 45 cm between the rows. Fifteen seeds were sowed in field conditions (single field), weeds controlled at regular intervals, and agricultural practices such as irrigations, manures, pesticides and insecticides were maintained for proper growth. The seedlings were continuously irrigated up to 55th day for proper growth in field condition. In the treatment phase, the drought stress was induced by withheld water for ten days (56 - 65th) day while control plants continued to normal irrigation of water. After the imposition of drought stress, the visible effects, including folding and wilting of leaves of the maize plants, were observed. The soil moisture content were recorded by soil moisture meter. Plants were treated (morning 7.30 to 8.30 AM) with different concentrations of foliar ABA (25, 50, 75 and 100 µM) for five days (66 - 70th day). After the ABA treatment plants were recovered by water for five days (71-75th day). Then the plants were harvested on the 76th day to analyse the morphology, chlorophyll fluorescence, FTIR and biochemical characters. Yield characters such as tassel counting, tassel length, corn height, corn diameter, and 100 seed weight were measured on the 120th day (20).

Growth characteristics (Cm)

The randomly three plants were collected at 76th day of control, drought stress induced with ABA treated plants. Measured the shoot and root length were expressed in cm.

Fresh weight and dry weight (g)

Plants collected from triplicates of the shoot and root fresh weight recorded. Three plants of each treatment were taken into consideration of recorded in control, drought stress induced and ABA treated plants. The fresh weight was recorded and the plant samples were dried in hot air oven at 70 °C for 48 hrs and weighted as dry weight.

Relative water content (%)

The Relative water content (RWC) was estimated, according to standard methodology (21).

Estimation of photosynthetic pigments (mg g^{-1} FW)

Two hundred mg (200 mg) of shoot materials were crushed with 5 ml of 80 % acetone in pestle and mortar and centrifuged at 4000 g for 15 mins (Model-REMI-C24). The procedure was repeated until the green residue dissipated, and the supernatant was diluted to 20 ml with 80 % acetone. Chlorophyll ‘a’ and ‘b’ contents (22) and the carotenoid

contents (23) were measured at 663, 645 and 480 nm wavelengths and expressed in mg g^{-1} FW. Chlorophyll fluorescence spectra were taken at drought, control and different concentration of foliar ABA leaf samples using the fluorescence spectrometer (Jasco FP 8200- model Japan). The fluorescence was recorded for the major fluorescence bands of treated leaves at 685 nm with the excitation wavelength from 400 to 850 nm.

Quantitative assay for α amylase activity (U/mg protein)

Amylase activity was determined according to the standard method (24). 100 mg of 7^{th} day germinated seed were grained with 2 ml of buffer solution, and the homogenate was spun at 10000 g for 15 min at room temperature. The supernatant was saved. The entire reagent without extract was used as blank. The absorbance and optical density (OD) was read at 540 nm in UV visible spectrophotometer.

Biochemical analysis

Estimation of total reducing sugars (mg g^{-1} FW)

Two hundred mg (200 mg) of the shoot and root tissue was crushed with 10 ml of ethanol (80 %), then centrifuged for 15 min at 8000 g. The upper phase contains reducing sugar, was transferred in a fresh tube and reducing sugar content estimated with glucose as a standard (25).

Estimation of total carbohydrate content (mg g^{-1} FW)

One hundred mg (100 mg) of samples were taken in a boiling tube and kept in a water bath for 3 hrs to be hydrolysed in the presence of 5 ml of 2.5 N HCl. Then, the samples were neutralised with solid sodium carbonate until it was made up to 10 ml. Finally, the supernatant was collected after centrifugation for carbohydrate estimated using glucose as standard (26).

Estimation of total soluble protein content (mg g^{-1} FW)

Protein content was determined by following the standard method (27). The absorbance of each sample (replicates from each treatments) was measured at 650 nm with bovine serum albumin (BSA) as a standard.

Protein Separation by SDS-PAGE

The protein was extracted from control, and treated plants of maize by TCA/acetone method were separated by one-dimensional SDS-PAGE using MEDOX-BIO Mini Vertical Slab gel methods (28). The 30 µg of protein sample was loaded in each lane along with pre-stained protein marker (14.4-116 kDa).

Estimation of total amino acids (mg g^{-1} FW)

500 mg of plant tissue were homogenised in five ml of 80 % ethanol. The homogenised sample was centrifuged at 8000 g for 15 min and the supernatant was diluted with 80 percent ethanol to a volume of up to 10 ml for amino acid analysis (29).

Estimation of proline content (mg g^{-1} FW)

The plant material (100 mg) was macerated with 10 ml of 3 % aqueous sulphosalicylic acid. The sample was filtered using Whatman No.1 filter paper. The extract was then pooled and re-extracted using water-soluble sulphosalicylic acid, with an aliquot of up to 20 ml used to estimate proline (30).

Fourier Transforms Infrared Spectroscopy

Treated maize leaves were drought and different concentrations of ABA treated samples were frozen in liquid nitrogen and freeze-dried (lyophilised). The powdered leaf sample was loaded into a Perkin Elmer FTIR spectroscopy (model RX I), was scanned between 400 - 4000 cm^{-1} and had a resolution of 4 cm^{-1}.

Lipid peroxidation (MDA) content (µ mol g^{-1} FW)

A 2 ml aliquot of enzyme solution was added to a tube containing 1 ml trichloroacetic acid (20 % v/v) and 0.5 % thiobarbituric acid (0.5 %). The mixture was boiled at 95 °C for 30 min, cooled to room temperature and then spun for 10 min at 15500 g. Malondialdehyde (MDA) content determines the degree of lipid peroxidation based on the standard method (31).

Stomata conductance

The treated maize leaves were harvested in the field and fixed in formalin-acetic acid-ethanol (FAA; 1:1:9) solution for microscopic examination (32). The stomata opening and closing were measured using a light microscope (image analysis) at power 40x (Olympus make).

Determination of endogenous ABA levels

The control and treated shoot samples were collected from maize, homogenised, then the samples were incubated overnight with 30 ml 80 % cold aqueous methanol in darkness at 4 °C. The extract was centrifuged at 2800 g for 15 mins and the supernatant was collected. Quantification was obtained by comparing the peak areas with known ABA amounts (33).

Histochemical detection of ROS species

The histochemical analysis of DAB (H_2O_2) and NBT ($O_2^{\bullet -}$) was determined (34). The treated and control leaves were collected and immersed in DAB stain (3, 3'- diaminobenzidine 1 mg/l (pH 3.0)) under dark conditions for 12 hrs. The formations of $O_2^{\bullet -}$ in the leaves were spotted by staining of nitro blue tetrazolium (NBT). The isolated leaves were immersed in a 0.5 mg/ml NBT solution in 10 mM potassium phosphate buffer (pH 7.8) at room temperature for eight hrs in the dark to determine superoxide anion accumulation. After the DAB and NBT stain incubation period, leaves were boiled in 95 % (v/v) ethanol for 15 mins and stored in 40 % (v/v) glycerol.

Statistical Analysis

A randomised block design (RBD) was used, with three replications, on all parameters. The data from the plants (n=3) was examined using ANOVA, taking into account the repeated measurements of drought, control and ABA-treated plants. The Tukey multiple tests showed that the differences between means were significant at the 5 % ($p < 0.05$) level. SPSS 21.0 software was used for all statistical analyses.

Results and Discussion

In this study, we found that exogenous foliar ABA may partly alleviate the adverse effects of drought-induced oxidative and osmotic stress in maize plants by increasing the levels

of endogenous hormones and antioxidant enzymes. ABA mitigates the detrimental drought on maize plants *via* antioxidant activity, ROS production and endogenous hormone levels, various metabolic pathways of maize plants. Additionally, ROS accumulation in plants impairs organelle integrity, oxidation of cellular components and even cell death. Thus, foliar application of ABA at 75 µM reduced maize's adverse impacts and enhanced growth characteristics, photosynthetic pigment and biochemicals, eventually increasing grain production.

Morphological characteristics

Plant height was increased in 75 µM of ABA (135.8) compared to drought plant (129.5) respective control (Fig. 1A). Plant biomass in terms of fresh weight was decreased in drought-stressed plants (shoot: 50.8; root: 17.4), whereas 75 µM ABA enhanced better recovery (shoot: 54.6; root: 21.8) than control (Fig. 1B). Dry weight was reduced in drought stress plants (shoot: 27.6; root: 13.7). The highest dry weight was observed at 75 µM of ABA (shoot: 33.7; root: 15.9) compared to the respective control (Fig. 1C).

The data presented that drought stress reduced growth characteristics such as plant height, fresh and dry weight than control plants. Exogenous ABA significantly increased in all growth parameters compared to drought plants. Foliar ABA significantly ameliorated the adverse effect of drought stress on growth characters. However, morphological characters of maize plants gradually increased with increasing concentration of ABA. The maximum highest growth was observed in 75 µM of ABA. The plant height showed a significant positive correlation between morphological and biochemical characteristics ($F=5.762$, $p<0.001$).

The increase in all growth parameters observed in maize plants during drought stress after ABA treatment compared to control plants (35). However, exogenous ABA alleviating the adverse effect of low water availability. In

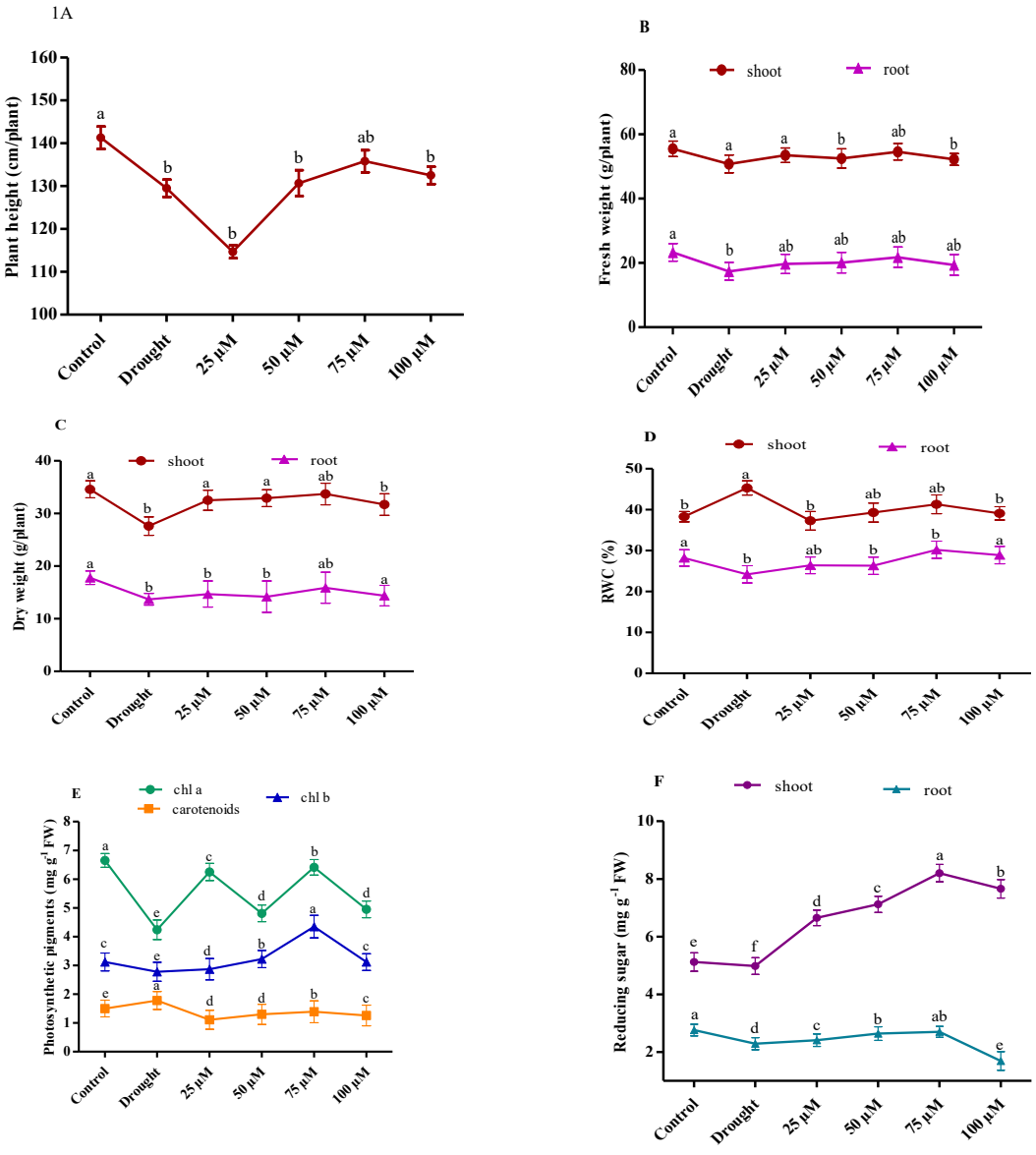

Fig. 1. The Effect of different concentration of ABA under drought stress (A) Plant height (B) Fresh weight (C) Dry weight (D) Relative water content (E) Photosynthetic pigments (F) Reducing sugar at 76th day plant. The same subset letters indicate no significant difference ($p< 0.05$) according to Tukey multiple range tests.

contrast relative water content was increased in drought-stressed plants (shoot: 45.3; root: 24.2) compared to ABA treated plants (shoot: 41.3; root: 20.2) at 75µM (Fig. 1D). The relative water content of drought-induced maize shoot showed a significant positive correlation with a carotenoid ($p<0.05$) and MDA content. Similar, results were observed for RWC, which increased in drought and foliar ABA in aromatic rice plants, in RWC helping plants better survive stress conditions and improve plant growth and development (36).

α amylase activity

Interestingly, our results revealed that the amylase activity involved in growth regulation and stress resistance. During the present study, the treatment were significantly different from control and each other treatments, which may be due to the difference in starch content. The α amylase activities were higher in the seeds from the maize plants treated with a maximum 75 µM of ABA concentration compared to the drought stress induced seeds (Fig. 2). Amylase activity an active role in starch hydrolysis during seed germination. It may also be responsible for the maintaining the required water potential by providing soluble sugars during the seed germination phase. Strong starch degrading activity is present in the cell wall of plants. Our results confirm that the amylase activity in lentil seeds decreased under PEG induced drought stress, and suggested that the variation in stress sensitivity of contrasting to osmoregulate drought stress (37).

Photosynthetic pigments

The photosynthetic pigments (chl-a 4.24; chl-b 2.78) under drought conditions considerably decreased while the carotenoid content increased under drought (drought: 1.78) compared with the control. The results observed that plants with foliar ABA lead to increased photosynthetic pigments all concentrations. However, the higher Chl contents were measured in 75 µM ABA treated maize shoot of (chl-a: 6.41 chl-b: 4.35; carotenoid: 1.39) compared to the drought and control, respectively (Fig. 1E). The results confirmed that drought stress affects the photosynthesis rate of maize plants. The reduced contents of chlorophyll a, b and carotenoid were observed during the vegetative stage in *Zea mays*. However, under drought stress conditions, foliar ABA caused an increase in the contents of photosynthetic pigments.

The results showed that plants treated with foliar application of ABA to increased photosynthetic pigments all concentrations. In the present investigation, drought stress significantly altered the concentration of chlorophyll (F=3.107) and carotenoid (F=0.591) content in drought plants. However, the enhanced recovery was observed at 75 µM of ABA compared to the drought and control.

Previously reported induction of water stress also resulted in a gradual increase in chl-a and chl-b concentra-

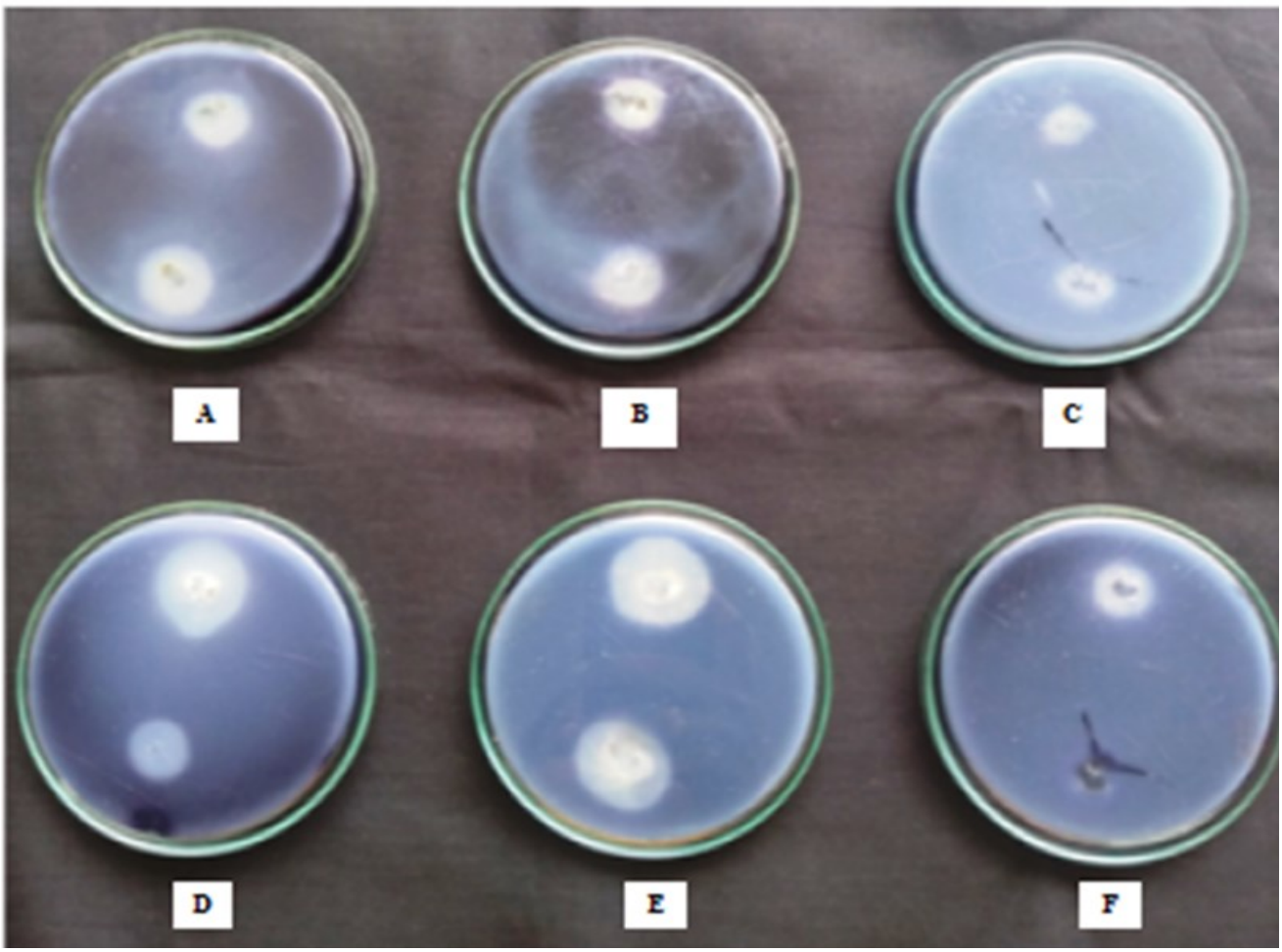

Fig. 2. The α amylase activity effect of different concentrations of ABA under drought stress (A) Control (B) Drought (C) 25 µM ABA (D) 50 µM (E) 75 µM (F) 100 µM ABA at 7th day seedling.

tions in abscisic acid-treated plants (38). Application of ABA which regulates a higher level of photosynthetic pigment content in pearl millet plants. Whereas, carotenoid content was increased in withheld water-induced drought-stressed rice and foliar application of ABA treated plants, carotenoids contents were enhanced (39). In the present study, gradually increased photosynthetic pigments were recorded in the foliar application of ABA increasing concentrations. Moreover, the abscisic acid stimulated the biosynthesis of chlorophyll pigments, significantly reducing drought stress inhibitory effect by improving the photosynthetic pigments (40).

Chlorophyll fluorescence

The measurement of chlorophyll fluorescence in plant leaves is commonly used to investigate photosynthetic responses. Physiological changes in pigment-protein complexes, excitation energy transfer, primary photochemistry and the operating quantum efficiency of electron transport through PSII are all useful information. When compared to the other concentrations, the high peak indicated the carotenoid content range at 480 nm in drought treated leaves. While an increase in carotenoid content may be related to their role as antioxidants, which demonstrated to be implicated in the plant response to drought stress conditions, this carotenoid content also removes ROS and free radicals, allowing the plant to maintain the redox balance. With increasing concentrations of abscisic acid, the concentration of chlorophyll gradually increased, reaching a maximum level of photosynthetic pigment 75 µM ABA treated plants. The results showed an increase in chl a and chl b, contrast a significant decrease in carotenoid content noted in 75 µM ABA concentration treated plants. The photosynthetic peak range at 663 nm indicates chl a, 645 nm represents chl b, and 480 nm reveals carotenoids (Fig. 3).

Previously reported that, water deficit stress induced rice plants affected and significantly decreased non-photochemical fluorescence quenching compared to control plants (41). On the other hand, found that despite a

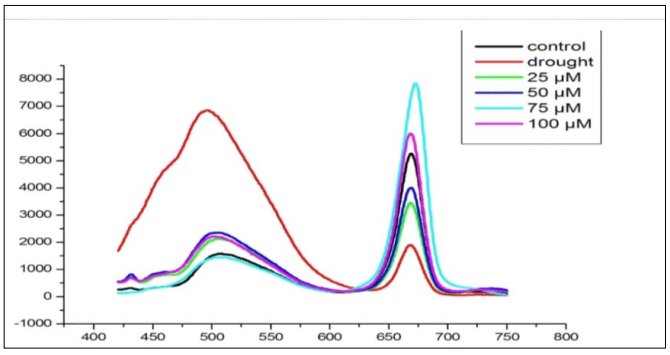

Fig. 3. The Effect of different concentrations of ABA under drought stress chlorophyll fluorescence spectroscopy to detect the maize leaves in photosynthetic pigments at 76th day plant.

significant reduction in photochemistry in drought-stressed cowpea plants, the overall photosynthetic mechanism for foliar plant growth regulators was affected and water stress resulted in greater accumulation of the QB-non-reducing PS-II reaction centres in comparison with control plants. As the key characteristics of non-reducing PS II centres is the inhibition of the electron transport from QA to QB a greater accumulation of the QB-non-reducing PS-II reaction centers may indicate a greater inhibition of electron transport from QA to QB in water-stressed plants (42). These final alterations are also linked to improved photoprotective and antioxidant activities and pathways (43). PS-II is more resistant to water deficits than PSI, therefore negative effects are limited to severe drought (44). Drought stress appears to promote PSII and PSI photochemistry protection by altering the energy allocation between photo systems and activating alternate electron sinks, as seen by the removal of the K-band (45).

Stomata analysis

Stomata opening and closing is an essential survival strategy for maintaining water in plants. The current findings revealed that the experimental plants stomatal opening sizes were significantly varied. In treated plants showed the number of stomata per area smaller size and shape was more irregular and closed than in the drought, the number of stomata per area presented in the foliar application of ABA in 75 µM (Fig. 4).

The initial reaction to drought among plants is the closing of stomata, which causes a subsequent decrease in photosynthesis. In control plants, changing the size of the stomata opening is an essential strategy for controlling

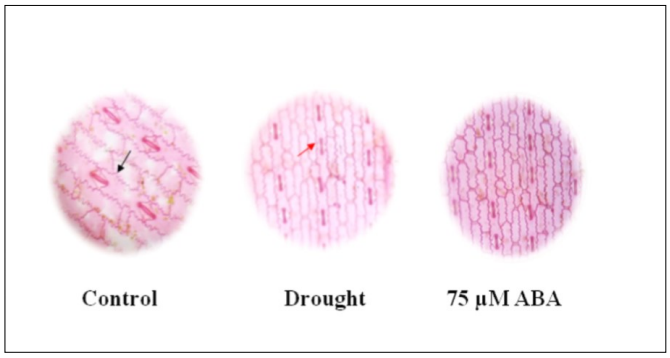

Fig. 4. Effect of abscisic acid after drought on stomata movement for maize control, drought and 75 µM ABA. Black color arrow mark to indicate opening stomata in control leaves and red color is closing stomata in drought leaves through microscope (image analysis) at 40x power.

water loss and survival. The stomatal closing minimises the effects of drought, and the plant responds by modulating its growth pattern up-regulation accumulation of antioxidants and compatible solutes (46). Stomata conductance is the most effective way to reduce the water loss in drought plants and act as a barrier against the CO_2 diffusion to the photosynthetic cells. In parallel, the application of ABA led to significant decreases in stomatal conductance, which regulate the stomata opening and photosynthetic rate (47). In the ABA concentration 75 µM treated maize plants present, the number of stomata per area was higher; the smaller shape was more irregular and closed than in control (48).

Biochemical parameters

The quantitative changes in proline, amino acid, and carbohydrate content may be responsible for adjustment in a metabolic pathway, which adjusts intercellular osmotic potential and the early reaction of the drought period. Drought maize plants triggered the production and accumulation of various osmolyte accumulations. Data demon-

strate (reducing sugar shoot: 4.98; root: 2.29) was decreased in drought plants compared to ABA concentration. The lower (carbohydrate shoot: 0.319; root: 0.295) was oserved in drought plants and (amino acid shoot: 0.643; root: 0.491) significantly decreased under drought stress compared to the control and ABA concentration. On the other hand ABA treatment caused significantly improved in 75 µM of (reducing sugar shoot: 8.20; root: 2.70), (carbohydrate shoot: 0.497; root: 0.398) (amino acid shoot: 0.857; root: 0.598) compared to the drought plants (Fig. 1F, 5A, 5B). While the positive correlation of carbohydrate content in ABA treated plants, the osmolyte accumulation of proline content of root had a p-value of *<0.001.*

Water stress increased reducing sugar, amino acids, and carbohydrates in plants, and ABA applied plants in *Oryza sativa* (49, 50). Accumulation of these osmolytes in stressed plants may serve as a storage form of nitrogen that is re-utilised when the stress is over and plays a part in osmotic adjustments. The concentration of soluble sugars increased while decreasing starch content in *Brassica napus* (51). Amino acid, usually considered an osmoprotectant agent, is also known to reduce oxidative damage by scavenging free radicals (52).

The osmotic accumulation of proline was increased in drought-stressed plants (shoot: 1.116; root: 2.177) than ABA 75 µM concentration treated maize plant (shoot: 1.017; root: 2.135) and the control (Fig. 5C). In contrast, proline was increased in drought plants compared to the foliar ABA *Vigna radiata* plants (36). On the other hand, it found that osmolytes proline is concerned with tolerance mechanisms against ROS, and this primary strategy of plants to avoid detrimental effects of drought. Increasing proline content help to osmotic adjustment, ROS scavenging, and protein stabilisation rather than osmotic regulation to product membranes from oxidation under drought stress (12).

The protein was decreased in drought-stressed plants (shoot: 0.634; root: 0.280) compared to application of ABA showed the maximum value (shoot: 0.766; root: 0.339) at 75 µM and the control (Fig. 5D). Protein content was increased due to the negative correlation among MDA of ABA-treated plants (*p<0.001*). ABA could remain active in some enzymes, regulates the osmotic adjustment. The enhancement of the protein content in maize shoots and roots is dependent on the time of drought stress, whereas the maize plants treated with ABA increased compared to control plants (53).

SDS-PAGE protein profiling

The effect of exogenous ABA in the maize leaves showed protein expression, identified by performing drought and control leaves on the 76th day. In general, accumulation of dehydrin-like proteins was not detected in ABA leaves, but several proteins are detected in severe drought leaves; this means that induced drought tolerant proteins. These changes in protein expression strongly suggest that the induced proteins play a role in plant response to drought. The residual bands, either structural drought stress-induced proteins, were not visible in the control plants. However, some dominant protein bands are presented in drought leaves compared to the ABA concentration a differential protein expression pattern was observed under different treatments. Some specific polypeptides were induced only in drought stress conditions, while few were expressed only in control and ABA treatments. New bands at 29.4 kDa, 20.1, 14.4. 10.5 kDa appeared respectively on drought leaves and 6.5 bands presented in all treated leaves. However, at the control and ABA concentration leaves alone, the band at 29.4 kDa, 20.1, 14.4 and 10.5 kDa were disappeared (Fig. 6).

In the early stages of drought stress, it seems that new stress responsive proteins were produced, nevertheless, to a severe drought. The loss of polypeptides due to drought is offset by increased synthesis (54). Total Protein content significantly increased in peanut drought leaves compared to non treated plants and foliar ABA (55). ABA on the positive role of protein accumulation and changes protein pattern induced drought stress plants (56). Drought and exogenous ABA were increased in protein content. Protein content was enhanced in response to drought and exogenous ABA. Shoot protein analysis revealed the highest percentage of polypeptides band in drought-stressed leaves (56, 57).

Fourier Transform Infrared Spectroscopy

The basic tenet of FT-IR relies on vibration of chemical bonds in the IR region. In the IR region, chemical bonds absorb radiation between 4000 and 400 cm^{-1}, each functional group in a molecule has its own characteristic absorption frequency in the IR Spectrum. In this study, FT-IR profiling of leaves was done so as to confirm physiological response of maize to induced drought treatment. All the samples were scanned from 500 - 4000 cm^{-1} wave number and 3000 - 2000 cm^{-1} region was assigned for lipids, 1800 - 1500 cm^{-1} for proteins, 1500-1200 cm^{-1} for carbohydrates and 1000 - 600 cm^{-1} for cell wall components (58).

The FT-IR spectra peaks and their probable functional groups are showed in control; drought and foliar ABA treated leaves. The FTIR spectra peaks and their lipid area, the leaves exhibited different spectra. In drought treatments, the peak at 2981 cm^{-1} confirmed malondialdehyde accumulation pattern in maize under drought stress. MDA is an oxidative damage molecule and an indication of oxidative stress. It was proportional to the magnitude of the stress. Whereas, the FTIR drought peaks of 3471 cm^{-1}, 2879 cm^{-1} and 1290 cm^{-1} corresponding to lipids, amides and carbohydrates respectively. The bandwidth range of the control samples was 2910 cm^{-1}, 1602 cm^{-1}, 1121 cm^{-1}, 479 cm^{-1} and 617 cm^{-1} corresponding to primary amine , hemicelluloses, polysaccharides and halogen compounds together. The maximum number of characteristics band presented in 75 µM of ABA 3976 cm^{-1}, 3433 cm^{-1}, 2935 cm^{-1}, corresponding to lipids, alkenes, amide I protein, 1741 cm^{-1} showed increase peak area with increment in ABA treatment. These peak characteristics of C=O ketones, C=C benzenes, C- N amino group these functional groups. 1553 cm^{-1} amide II protein cm^{-1}, 1649 cm^{-1} amino acid, 1454 cm^{-1} sulphate, 1259 cm^{-1} amide III, 1184 cm^{-1} lignin, 1060 cm^{-1} polysaccharides, 912 cm^{-1} carbohydrate are presented (Fig. 7).

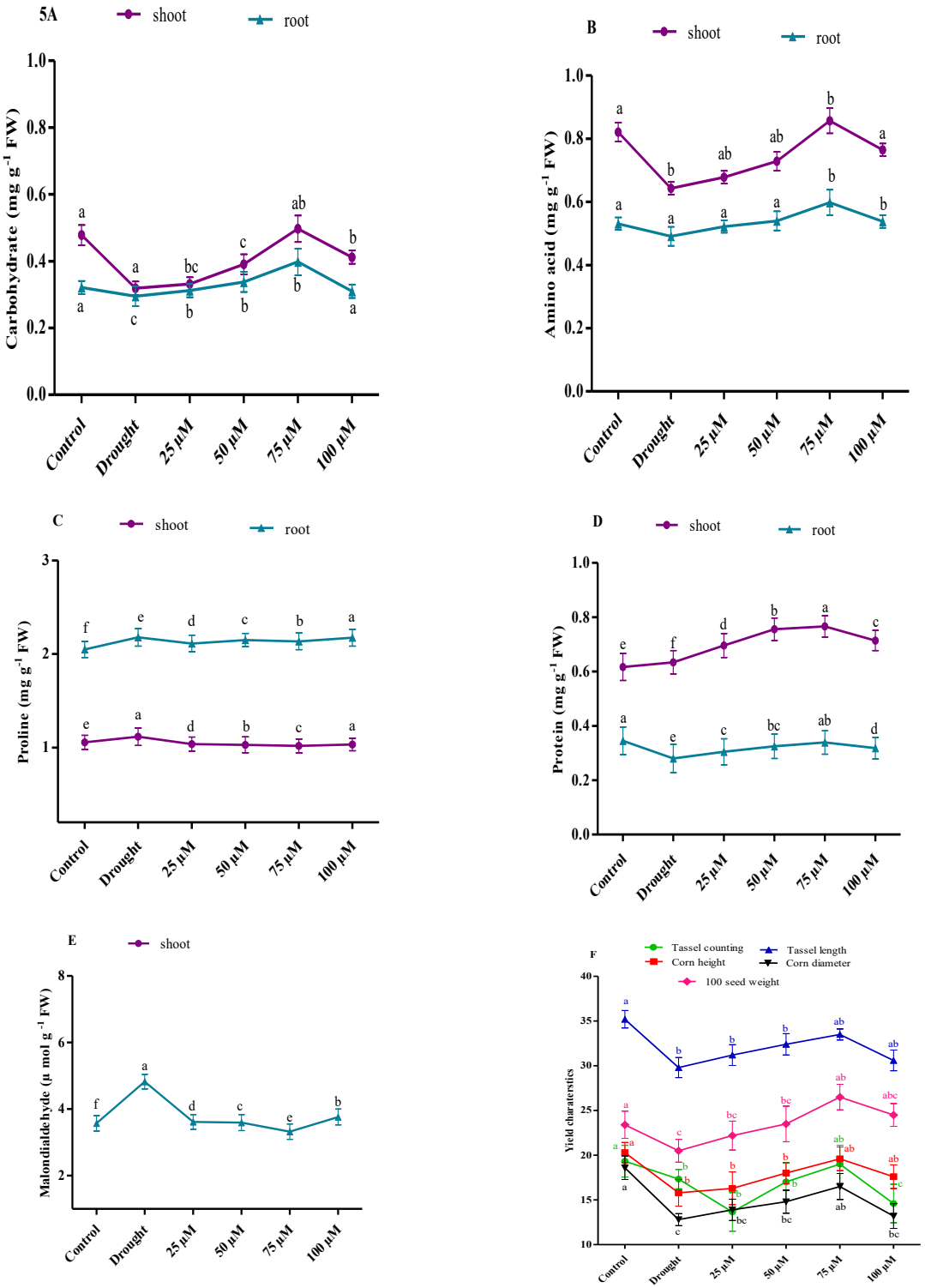

Fig. 5. The Effect of different concentration of ABA under drought stress (A) Carbohydrate (B) Amino acid (C) Proline (D) Protein (E) Melondialdehyde (F) Yield parameters at 76th day plant. The same subset letters indicate no significant different ($p< 0.05$) according to Tukey multiple range tests.

However, there was a differential response in the intensities observed at amide region with drought .The absorption bands from the spectra in this study suggest that the same metabolites detected in sorghum during prolonged drought are also involved in progressive drought in maize. In addition, reduction in the intensities of the bands detected with increasing drought indicated a reduction in lipid and carbohydrate content and also changes in the composition of the proteome (59). Three protein absorption bands located around 75 µM of ABA 1700 (C=O), 1,553 (N–H) and 1,259 (C– N) cm^{-1} were assigned as amide I, II and III bands respectively (60). ABA treatment, which suggests

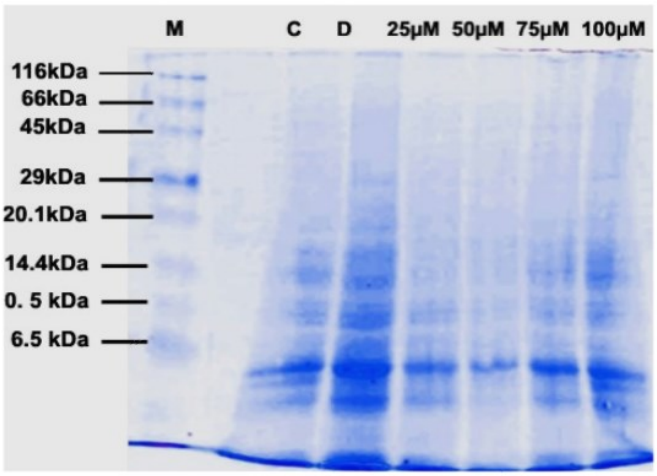

Fig. 6. SDS- PAGE (Sodium Dodecyl Sulfate protein profile at 76th day. M - Marker, C- Control, D- Drought and foliar ABA treated maize plant.

that protein synthesis, was enhanced, the most frequently used IR spectral range in carbohydrate analysis region at 950 to 750 cm^{-1} where it is possible to drought and ABA treated leaves, the wave number 650 - 400 cm^{-1} is presented in halogen compound in wheat, maize and kodo millet multivariate analysis enabled the identification of biochemical variables under abiotic stress (61).

Lipid peroxidation

Drought stress caused a dramatic increase in MDA content in leaves, indicating the presence of oxidation. It is evident that lipid peroxidation, significantly increased in the (shoot: 4.826) drought plants compared to the control and ABA-treated shoot. However, the application of ABA decreased the concentration of MDA in the shoot of maize plant. A plant treated with 75 µM ABA showed decreased content of MDA (3.324) under drought shoot. It indicates that ABA mitigates the damage of membranous lipids of the cell un-

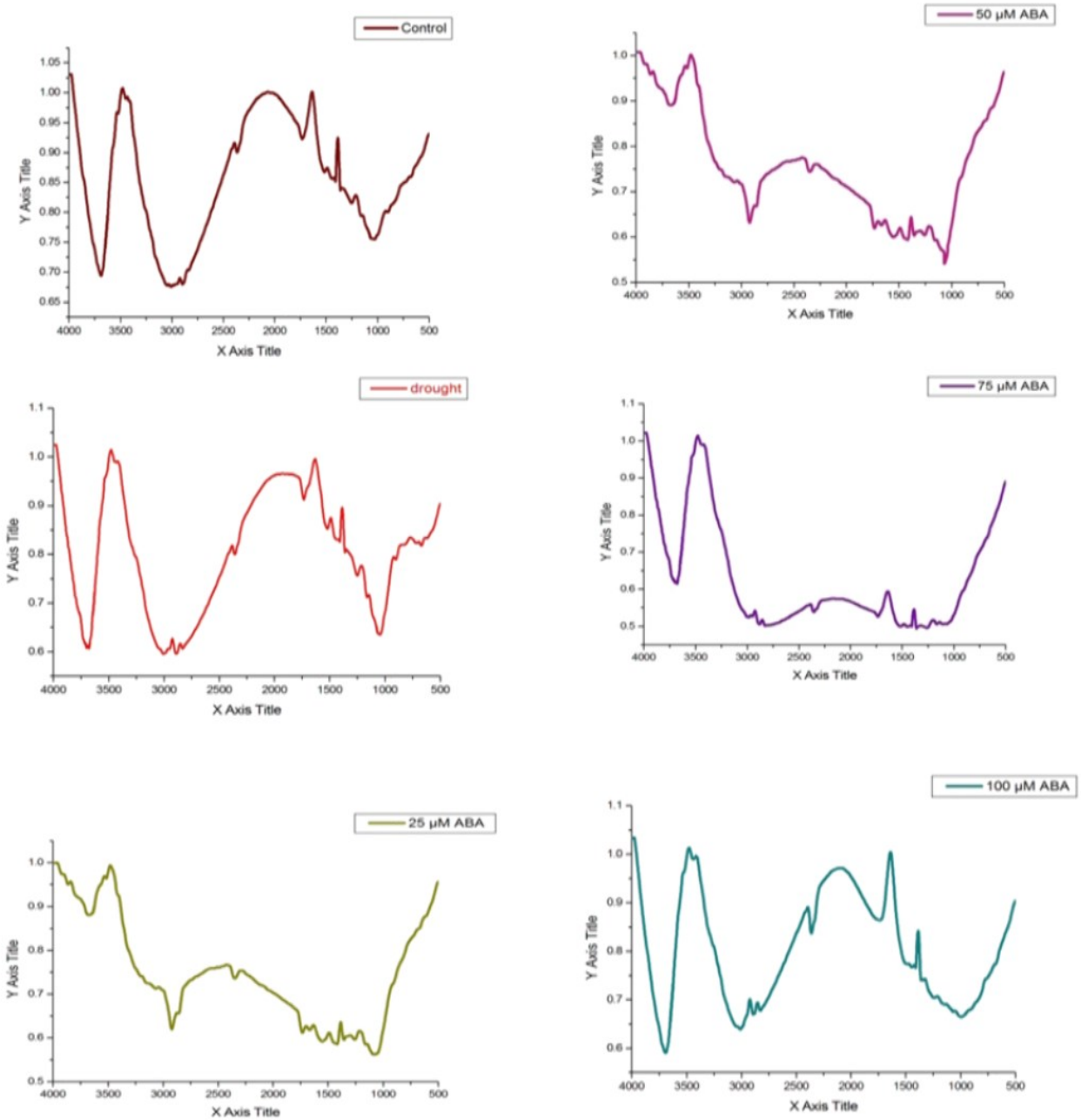

Fig. 7. Fourier Transform Infrared Spectroscopy control, drought and different foliar ABA on maize leaves at 76th day.

der drought. Water deficit stress causes 75 µM treated maize plants to show lower lipid peroxidation levels than those exposed to ABA (Fig. 5E). The MDA content showed a significant negative correlation between growth characteristics, chlorophyll and biochemical content (F=0.18, $p<0.01$) drought-induced after ABA treated plants (62). According to earlier reports, the MDA concentration of stressed plants rose. It was shown that the MDA content of faba bean under drought conditions was substantially decreased when proline and ABA were applied in combination (63). Under drought stress, persistent foliar ABA efficiently decreased leaves of MDA and H_2O_2, reducing electrical conductivity in leaves, while drought and exogenous ABA also improves plant ability to retain membrane integrity (64).

Endogenous ABA level

HPLC spectra showed endogenous ABA levels in control, drought, and ABA treated plant leaves which elucidated the endogenous ABA was increased in drought leaves compared to control. However, various concentrations (25, 50, 75 and 100 µM ABA) of exogenous application of ABA on drought shoot showed alteration of endogenous ABA level. The endogenous ABA level was significantly increased at 75 µM concentrations which optimised the drought and closing the stomata to escape endogenous ABA improves drought plant drought tolerance throughout development due to metabolic control of ABA production (Fig. 8). An HPLC method for analysing endogenous ABA in cowpea was effectively established. We observed that exogenous application of ABA increased endogenous ABA levels and prevented a decrease of endogenous ABA levels (65). Considering that ABA is a crucial hormone in controlling

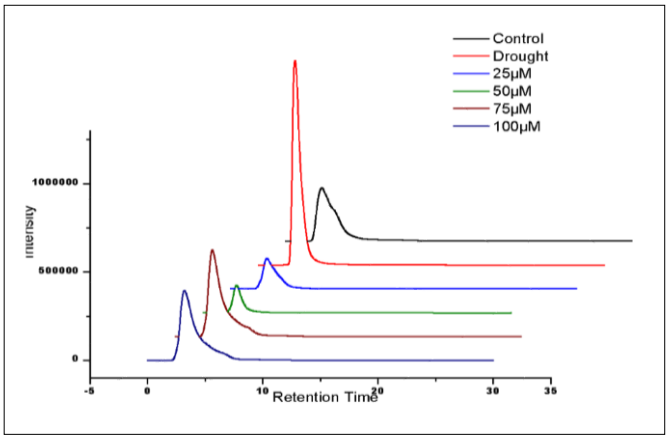

Fig. 8. High performance liquid chromatography to detect endogenous abscisic acid level in maize leaves, under drought and exogenous abscisic acid.

drought stress responses, abiotic stress has been shown to cause an increase in ABA levels in drought induced *Brassica* spp. (66).

Histochemical detection of ROS accumulation

The impact of drought stress on ROS accumulation as H_2O_2 and $O_2^{\cdot-}O_2^{\cdot-}$content in maize treated leaves was observed. Significantly the ROS level was discernible detect the staining method for DAB and NBT. Histochemical staining was performed to localise H_2O_2 and $O_2^{\cdot-}O_2^{\cdot-}$ in the drought and foliar ABA leaves. A significant increase of hydrogen peroxide (H_2O_2) in drought leaves indicated brown spots by diaminobenzodine and accumulation of singlet oxygen ($O_2^{\cdot-}O_2^{\cdot-}$) indicated by dark blue spots by nitro blue tetrazolium (Fig. 9).

Histochemical detection generation of $O_2^{\cdot-}O_2^{\cdot-}$is induced in drought-affected plant cells through unpaired electron transport in the chloroplasts. This $O_2^{\cdot-}O_2^{\cdot-}$ can be converted into H_2O_2, which triggers various symptoms in water-stressed plants (67). Similar, visual identification of H_2O_2 and $O_2^{\cdot-}O_2^{\cdot-}$ was reported previously as brown patches and dark blue spots respectively, in drought-affected plants (68). Drought caused a noticeable increase in the $O_2^{\cdot-}O_2^{\cdot-}$ generation rate and H_2O_2 level with a marked increase in lipid peroxidation (indicated by higher MDA level) (69). ABA is proved to be an inducer of H_2O_2 production in rice seedlings under drought conditions. ABA has also been shown to effectively induce H_2O_2 production in guard cells in *Vicia faba* in the leaves of maize seedling exposed to drought (70).

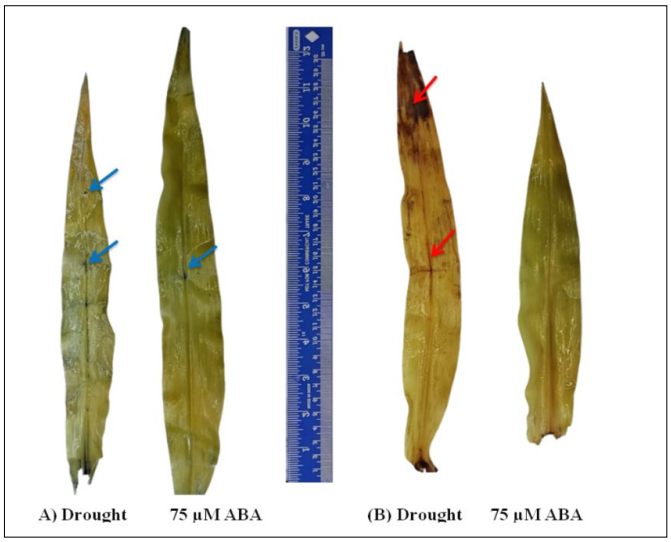

Fig. 9. Histochemical staining was performed to localise H_2O_2 and $O_2^{\cdot-}O_2^{\cdot-}$ in the drought and foliar 75 µM ABA leaves. A significant increase of (A) Accumulation of singlet oxygen ($O_2^{\cdot-}O_2^{\cdot-}$) blue arrow mark indicated by dark blue spots in drought leaves by nitro blue tetrazoliuum. (B) Hydrogen peroxide (H_2O_2) red arrow mark in drought leaves indicated brown spots by diaminobenzodine.

Yield characters analysis

The number of tasseling, tassel length, corn height, corn diameter and 100 seed weight was significantly decreased. The drought stress-induced application of ABA priming completely ameliorated significantly increases in grain yield and 100 seed weight compared to non-treated maize plants. Analysis of variance showed that drought stress conditions, significantly decreased and in the exogenous application of ABA for enhanced yield characters at maximum increasing concentration 75 µM. Number of tasseling (drought: 17.0; 75 µM: 21.0) tassel length (drought: 29.8; 75 µM: 35.5), corn height (drought: 15.8; 75 µM: 22.6), corn diameter (drought: 12.8; 75 µM: 17.5), 100 seed weight (drought: 20.5; 75 µM: 26.5). Corn height was positively correlated with corn diameter ($p<0.01$) (Fig. 5F). Reported that drought-induced and ABA-treated plants with 100 seed weight and grain yield

increased in maize plants (54). Therefore, the foliar application of ABA ameliorated and reduced drought stress on maize plants and promoted the yield characteristics. The researchers reported a positive role of ABA membrane stability, osmotic balance, sugar accumulation, proline content and enhanced yield parameters under drought stress (71).

Conclusion

The foliar application of 75 µM ABA prevents and mitigates drought damage in maize by limiting lipid peroxidation in the cellular membrane. ABA is attributed to its ability to induce the antioxidant system and reduce ROS species. Drought stress limits maize production and yield. Hence, the FTIR results confirm the applicability of biomolecules and prove its further application in finding drought stress adaptation mechanisms in plants. The application of ABA also slightly altered endogenous ABA, which improved drought tolerance in maize under drought stress condition. Exogenous ABA improved the drought resilience of maize plants for a shorter periods of crop management program, particularly a ten-day drought. ABA pre-treatment practical approach to alleviating drought stress in maize plants under field conditions. Overall, the results indicated that ABA treatment could reduce the effects of drought stress on maize plants. Foliar application of ABA effectively ameliorates the adverse effects of drought stress to improve the maize yield. As a present result, provides fundamental insights into the role of ABA and its future application in the production of *Zea mays*. Therefore, this study provides a basic understanding of the role of ABA and its potential use in the production of *Zea mays.* Further studies are needed to identify the expression of stress-responsive genes under drought conditions and the exogenous applications of abscisic acid.

Acknowledgements

This work a part of the research project fund University Grand Commission (F1 Rajiv Gandhi National Fellowship) RGNF-2016-17-TAM-SC/27555 and authors are highly grateful for thanks to Periyar University, Salem, Tamil Nadu, India for providing lab facilities for the conduct of research Mrs. S. Ramya expresses her gratitude to Periyar University, Salem, Tamil Nadu with a fellowship under the scheme.

Authors contributions

SR carried out the analytical experiment, SR and MR collection of data, data analysis and manuscript preparation; DAB designed the experiment, edited the manuscript and supervised the overall work.

Compliance with ethical standards

Conflict of interest: Authors declare no conflict of interest.

Ethical issues: None.

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