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Melatonin and methyl jasmonate-mediated morpho-physiological and biochemical adaptations to drought stress in French bean

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ABSTRACT

Although melatonin (MEL) and methyl jasmonate (MeJA) have been implicated for drought tolerance in plants, the effect of those has yet to be clarified in French bean. Therefore, the French bean plants were treated with exogenous MEL and MeJA under drought (D)-stress conditions to see their morphological and physiological alterations. The drought-stressed French bean plants treated with the combined application of MEL and MeJA showed partial recovery of root and shoot growth and pod yield. Total chlorophylls, carotenoids, and relative water contents were increased by 225.0 %, 109.6 % and 32.0 %, respectively in plants treated with D+ M 150 µM+ J 20 µM as compared to sole drought-stressed plants. Accumulation of osmolyte proline was also increased by 14.5 % in that combination. In contrast, the non-stress control plants showed the best performance in all the above-mentioned parameters. Percent electrolyte leakage (EL) was reduced by 266.0 % by the treatment combination of D+ M 150 µM+ J 20 µM, reflecting the combined role of MEL and MeJA in mitigating tissue damage caused by drought stress. However, the accumulation of flavonoids and phenolics was found to be reduced in drought-affected plants treated with MEL and MeJA that was comparable to non-stress control plants. The study suggests a collective role of MEL and MeJA in mitigating drought effects in French bean. Thus, the findings will contribute to sustainable growth of French bean in climate resilient agriculture.

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1. Introduction

Legume crops are very good source of nutrients and antioxidants, which are beneficial to human health. Among them, the French bean or common bean (*Phaseolus vulgaris* L.), also named Forashi sheem or Jhar sheem has been considered one of the most important legume crops in the world (Beebe *et al.,* 2013). It is one of the dominating pulse crops and staple foods in America and Africa for over 300 million people (Hummel *et al.,* 2020). In Bangladesh, the overall bean cultivation area was estimated as 55076 acres in 2020-2021, where total production of beans, including French beans, was recorded as 169,655 metric tonnes (Begum *et al*., 2023). The crop is grown in many parts of the country, such as Jessore, Rangpur, Comilla and the hilly areas, including Sylhet, Chittagong, and the Chittagong Hill Tracts. The French bean has been gaining popularity in the Asian countries, including Bangladesh, for its nutritive and exporting values. Although having such potential, it has been a matter of concern that the crop yields are greatly affected by various abiotic factors, particularly drought or water deficit. As a major constraint of abiotic stresses, drought is extensively responsible for decreasing crop productivity worldwide to a great extent (Lesk *et al.,* 2016). A lot of plant attributes, such as growth, photosynthetic pigments, water and nutrient use efficiency, cellular and biochemical changes, including enzymatic activities, are adversely affected by drought stress (Chen *et al.,* 2019). Drought causes oxidative damage by enhancing accumulation of reactive oxygen species (ROS) in plants. To counter the effects of drought, plants use different growth regulators that have tremendous roles in enhancing tolerance to various environmental stresses (Hasanuzzaman *et al.,* 2017). About 60% of the French bean production is greatly affected by prolonged drought periods worldwide (Beebe *et al.,* 2013). Most importantly, drought has been postulated as the most influential abiotic factor for reducing common bean yield, leading to the destabilization of the sustainable production system in developing countries. Drought prevailing for a short time negatively affects both the quality and yield of French beans (Ramirez-Vallejo & Kelly *et al.,* 1998). More specifically, flowering, podding and number of seeds are greatly affected by water shortage (Lesznyák *et al*., 2008). In Bangladesh, although the French bean cultivation is arresting to the country people, the dry climate in the winter season is major hindrance to bean production. Along with this, due to gradual exhaustion of the groundwater level, the irrigation facility is going to confront a big challenge in the near future. Therefore, exploration of drought tolerance mechanisms and development of tolerant species of French bean might resolve the problems and increase productivity.

MEL (N-acetyl-5-methoxytryptamine), an emerging growth regulator, ubiquitous to all organisms, has been denoted as the potential modulator of plant growth and development. MEL regulates various morpho-physiological aspects such as growth, flowering, senescence etc. (Park *et al*., 2013). Along with the developmental process, MEL has the potential to enhance tolerance to a variety of abiotic stresses including drought (Kabiri *et al*., 2018). MEL acts as the antioxidant itself and could alleviate the harmful effects of ROS by regulating other enzymatic and non-enzymatic antioxidants (Li *et al.,* 2011). Exogenous application of MEL has been found to be very effective to mitigate drought-induced oxidative damage in crop species such as soybean (Wei *et al.,* 2015) and maize (Huang *et al.,* 2019). Another class of phytohormone, methyl jasmonate (MeJA), a derivative of jasmonates, is also universal to the plant kingdom, affecting various processes of plant growth and development including germination, growth, ripening, and senescence (Abdelgawad *et al.,* 2014). MeJA has been reported to mitigate drought stress by regulating physiological and biochemical processes such as enhancing osmolyte proline, total protein, sugar content and antioxidant activities (Abdelgawad *et al.,* 2014).

Since drought has been a major issue throughout the world for reducing crop production, exploring more drought tolerance strategies might be very practical for the mitigation of drought stress. Although plant growth regulators such as MEL and MeJA have been widely reported to be involved in mitigating drought stress of crop plants, implication of those in growing legumes is very few. For instance, MEL was reported to enhance drought tolerance of mung bean (Kuppusamy *et al.,* 2023). MeJA was reported to enhance drought tolerance of cowpea by improving different physiological parameters (Sadeghipour *et al*., 2018). Alongside, salicylic acid and abscisic acid were found to be effective for improving physiological traits of chickpea (Farjam *et al.,* 2015). Nevertheless, the effects of MEL and MeJA, either sole or in combination, on the drought tolerance of French bean are still to be examined. Therefore, the present investigation was set to elucidate the role of these two important growth regulators in enhancing drought tolerance in French bean by analyzing morphological, physiological and biochemical parameters.

2. Methodology

2.1. Experimental site, plant materials and growing conditions

The experiment was conducted in the department of Crop Botany, Gazipur Agricultural University, Bangladesh. The French bean seeds (BARI Jharsheem-1) were procured from the Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. Ten healthy seeds were sown in plastic pots (25 cm height × 20 cm diameter), containing 9.5 kg of soil mixed with the required dozes of fertilizers, and cow dung to ensure optimum fertility. The pots were allowed to ambient condition by providing a shed to stop the interference of rainfall. Four uniform and healthy plants were retained in each pot. Proper insecticides and fungicides were applied to protect plants from insect and disease attacks.

2.2. Treatments details and their imposition

When the plant attained three trifoliate leaves (21-day-old), those were foliar sprayed with 20 µM MeJA (J), 100 µM MEL (M) and different concentrations of M (50, 100 and 150 µM) with 20 µM J, except the plants supposed to be non-stress control and drought, which were sprayed with the same amount of distilled water. We used only 20 µM J, because the concentration showed drought-recovery in French bean previously (Mohi-Ud-Din *et al*., 2021). After first foliar spray, the plants (except non-stress control) were exposed to drought stress by stopping normal irrigation and maintaining 50% field capacity (FC). The 50% FC was maintained by regular monitoring and adjusting irrigation level in the pots. The irrigation requirement was calculated by following the method used in our previous study (Nazran *et al*., 2019). Irrigation requirement was calculated by using the formula; IR= {(MFC - MBI) ¸ 100} ´ A´ D. Where, IR= irrigation requirement (cm), MFC= Soil moisture (%) at field capacity, MBI= Soil moisture (%) before irrigation monitored by using soil moisture meter, A= Soil bulk density in g cm^{-3}, D= Rooting depth (cm). The second spray was applied after 7 days of first application. The control and only drought-stressed plants were sprayed with same amount of distilled water. The treated plants were kept in drought-stressed condition until harvest. The control plants were provided with normal irrigation throughout the periods. The treatment combinations were like T_1; control; C, T_2; drought; D (50 % FC), T_3; D + J 20 µM; T_4; D+ M 100 µM, T_5; D + M 50 µM + J 20 µM, T_6; D + M 100 µM + J 20 µM, and T_7; D + M 150 µM + J 20 µM.

2.3. Measurement of relative water and proline content

Relative water content (RWC) of leaves was determined by following procedure used by Shivakrishna *et al.* (2018). Briefly, leaves were collected from each treatment and weighed immediately to record the fresh weight (FW) and then placed in petri dishes containing distilled water at room temperature for 4 h to record the turgid weight (TW). The leaves were dried in an oven at 80°C for 24 h to obtain dry weight (DW). Then RWC was calculated using the formula; RWC (%) = [(FW - DW) / (TW - DW)] × 100. Where, FW = Fresh weight of the leaf disks, DW = Dry weight of the leaf disks and TW = Turgid weight of the leaf disks. Proline extraction was done by using the methods described by Bates *et al.* (1973). Briefly, leaf tissue (0.1 g) was homogenized in 2.5 mL of 6% aqueous sulfosalicylic acid. After centrifugation at 4,000 rpm for 20 minutes, 1 mL of supernatant of each was mixed with 1 mL of acid ninhydrin and 1 mL of glacial acetic acid. Then, those were allowed to heating in a boiling water bath for 60 min and the tubes were immediately transferred to an ice bath to terminate the reaction. Then, 2 mL of toluene was added to the reaction mixture and kept at room temperature for 10 min. Finally, the absorbance was taken spectrophotometrically at 520 nm using toluene as a blank. The level of proline in the samples was calculated from a standard curve developed with different concentrations of proline. The proline content was determined as μmolg^{-1} fresh weight.

2.4. *Determination of percent electrolyte leakage (% EL)*

Electrolyte leakage of the damaged tissue in the leaf was measured by following the protocols as followed by Shi *et al*. (2006). The leaf segments were soaked in deionized water for 24h at room temperature. After recording the solution's electrical conductivity (EC_1), the samples were incubated for 20 minutes at 95^{0}C and then the electrical conductivity (EC_2) was again measured after cooling. The % EL was calculated by the equation; EL (%) = (EC_1/EC_2) x 100.

2.5. *Measurement of total phenolics and flavonoids content*

Phenolics content of the methanolic extracts was determined spectrophotometrically according to the Folin-Ciocalteu method (Ainsworth & Gillespie, 2007). The measurement was as micrograms of gallic acid equivalents per gram of biomass (μg g^{-1}). The methanolic extract of plant materials was used for the determination of flavonoids content using the aluminium-chloride colorimetric assay (John *et al.,* 2014). The results were expressed as micrograms of quercetin equivalents (QE) per gram of dry mass (μg g^{-1}).

2.6. *Statistical analysis*

Randomized Complete Block Design (RCBD) was followed by maintaining at least three replications. Statistix 10 software was used for analysis of variance (ANOVA). The least significant difference (LSD) was used to see significant differences among the treatments at 5% level of significance.

3. Results and Discussion

3.1. *Effect of MEL and MeJA on the growth of French bean plant under drought*

The best phenotypic appearance of French bean was observed in irrigated control conditions (C). Among the drought-stressed plants, better phenotypic appearances were observed when the plants were treated with the combined application of MEL (M) and MeJA (J) (Fig. 1A). The root and shoot growth of French bean were greatly affected after 15 days of drought imposition. As compared to non-stress control, root length was significantly increased in other treatment combinations either drought or drought with MEL and MeJA. The root length was found to be lowest (13.13 cm) in control plant as compared to drought-stressed plants or drought-stressed plants treated with different concentrations and combinations of MEL and MeJA (Fig. 1B). Root length was found to be triggered in drought-stressed plants treated with MEL and MeJA. The number of leaflets per plant was found to be highest (22.00) in control plants followed by 15.66, 15.33 and 14.00 by the treatment combinations of D+M 50 μM+ J 20 μM, D+M 100 μM and D+M

100µM+ J 20 µM respectively. The lowest number of leaflets (8.66) was recorded in the drought affected plants only (Fig. 1C). The highest shoot length (33.6 cm) was recorded in control condition, whereas as compared to drought-stressed plants only, shoot length was significantly increased by the plants when treated with D+M 150 µM+ J 20 µM followed by D+M 100 µM+ J 20 µM (Fig. 1D). Although fresh weight of shoot was found to be highest (19.22 g) in control, D+M 150 µM+ J 20 µM significantly increased fresh weight (12.2 g) as compared to drought-stressed plants only (4.07 g) (Fig. 1E). Drought causes reduction in plant growth and development by inhibiting cell division and enlargement due to loss of tissue water content and turgor pressure (Alghamdi, 2024). Foliar spray of MeJA was reported to increase plant's growth parameters in drought-stressed soybean plants (Mohamed and Latif *et al.,* 2017). Similarly, the growth of maize plants was enhanced under drought stress by exogenous application of 50 µM MeJA (Abdelgawad *et al.,* 2014).

The enhancement of growth by MeJA during drought stress was due to recovery of the cell division and permeability of the plasmamembrane (Kaur *et al.,* 2013). Along with MeJA, MEL is also crucial to reduce the detrimental effects of drought for enhancing plant's growth by triggering cell elongation, expansion and differentiation (Huang & Liu *et al.,* 2017). MEL was found to enhance seed germination and lateral root formation in cucumber under cold and drought stresses. (Zhang *et al*., 2015). However, it could stimulate root and shoot growth by means of protecting sub-cellular structure and photosynthetic apparatus (Arnao & Hernández-Ruiz, 2017) and by improving the level of indole-3-acetic acid (IAA) in plant (Wen *et al.,* 2016).

The enhancement of biomass and leaf area was recorded in abiotic-stressed plants by exogenous application of MEL (Ren *et al.,* 2019). MEL was also reported to improve shoot weight and leaf size of corn seedlings (Ye *et al.,* 2016). Although single application of MeJA or MEL was investigated under drought but neither of those two alone produced any noticeable improvement of French bean in this study (Fig. 1). Rather combined application of MEL and MeJA (D+M 150 µM+ J 20 µM) was found to enhance root and shoot growth significantly as compared with drought-stressed plants only (Fig. 1). Our findings were supported by the results of (Mohi-Ud-Din *et al.,* 2021), where combined application of MeJA and salicylic acid (SA) was found to enhance shoot and root length of French bean plant rather than their sole application. Therefore, our findings suggest the combined application of MEL and MeJA is crucial for restoring the growth and development under drought stress.

3.2. Effect of MEL and MeJA on the development of flowers and pods under drought

The number of flowers and pods, and fresh weight of pods were obviously highest in control condition but those were enhanced in plants under drought stress treated with either sole and combined application of MEL and MeJA (Fig. 2). Surprisingly,

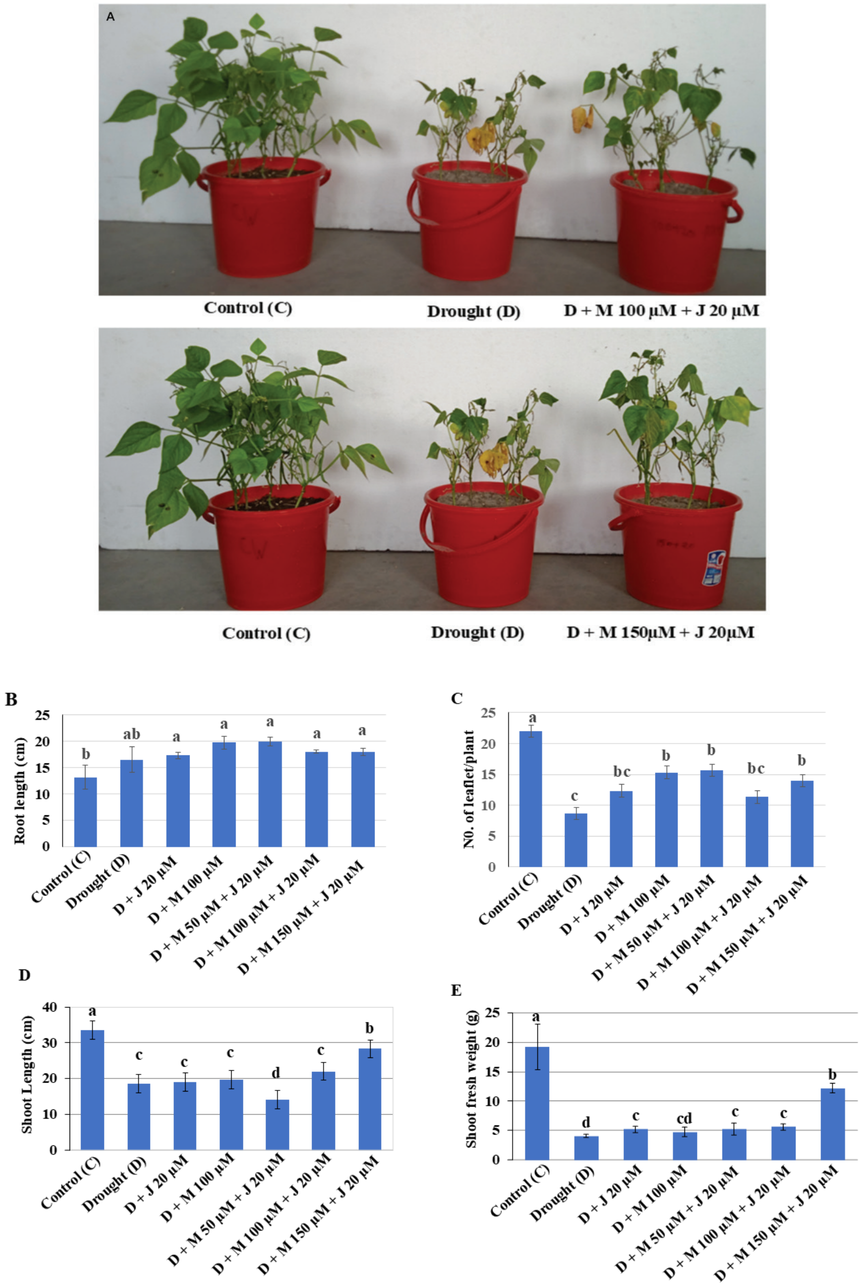

Fig. 1. Phenotypic appearance of French bean plants subjected to non-stress control (C), drought (D) and drought with combined applications of MEL (M) and MeJA (J) after 15 days of drought imposition (A). Effect of sole and combined applications of MEL and MeJA on root length (B), number of leaflets (C), shoot length (D) and shoot fresh weight (E) of French bean. Error bars indicate standard error. Different alphabetical letters on the bars show significant differences among

number of flowers per plant was more or less similar in both control (C) and the treatment combination of D+M 150 µM+ J 20 µM (Fig. 2A). Accordingly, number of pods was found to be similar (15.33) in control (C) and the treatment combinations of D+M 150 µM+ J 20 µM (15.0). The lowest number of pods (5.33) was recorded in only drought (D) stress (Fig. 2B). Consistently fresh weight of pods (14.83g) was found to be highest in control plant which was followed by 8.13g under D+M 150 µM+ J 20 µM (Fig. 2C). Only drought affected plants showed lowest pod fresh weight (1.90g) (Fig. 2C). Drought has tremendous impacts on growth and yield performances of plants (Sanchita *et al.* 2015). However, the mitigation of drought effects has been accelerated by using different growth regulators including MeJA (Javadipour *et al.* 2019). The growth and yield of two varieties of bread wheat were triggered under different irrigation regimes by exogenous application of MeJA (Javadipour *et al.* 2019). However, exogenous application of MeJA was found to enhance the yield and grain quality of rice under drought stress (Meng *et al.* 2023). Likewise, MEL also found to have tremendous impacts on flowering and fruit settings of the plant. For instance, exogenous MEL enhanced the yield of cotton under drought stress (Zhu *et al.* 2024). Along with sole application of growth regulators, the combined application

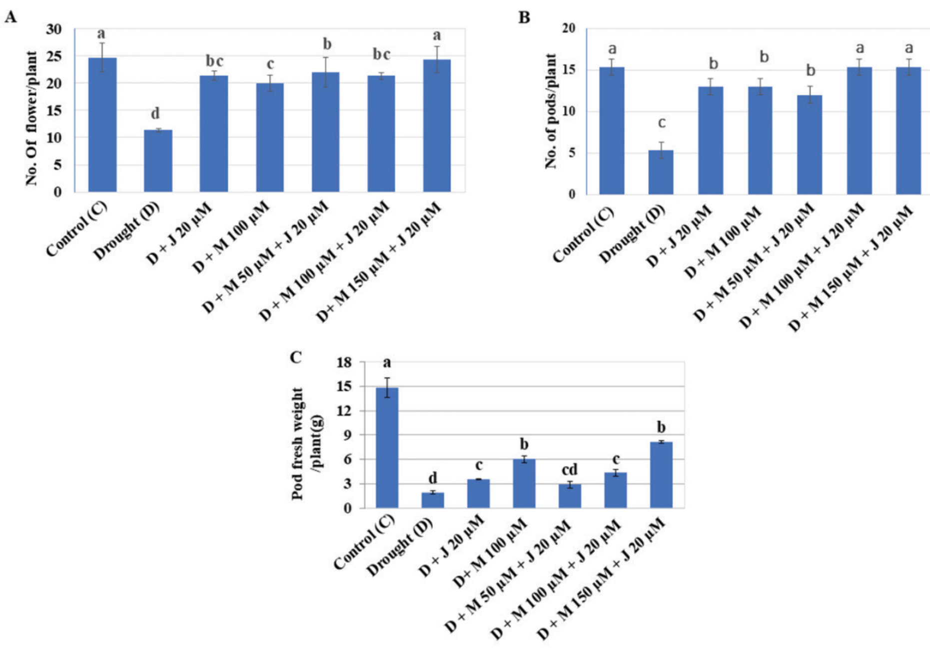

Fig. 2. Effect of sole and combined applications of MEL (M) and MeJA (J) on the number of flowers per plant (A), number of pods per plant (B) and pod fresh weight (C) per plant recorded during harvest. Error bars indicate standard error. Different alphabetical letters on the bars show significant differences among the treatments (p < 0.05)

of different growth regulators was also found to be effective to maintain better yield under drought stress. For instance, SA and jasmonic acid were reported to enhance grain yield in wheat (Anjum *et al.* 2011). Along with those, our results regarding higher initiation of flowers and pods, and improvement of pod fresh weight of French bean by the application of MEL and MeJA either as sole or combined application (Fig. 2) suggesting that those two growth regulators are vital for mitigating drought effects in French bean. However, combined application specifically D+ M 150 µM + J 20 µM showed best performances to restore and maintain crop yield as compared to drought-stressed plants only (Fig. 2).

3.3. Effects of MEL and MeJA on the photosynthetic pigments under drought

Photosynthetic pigments specially Chl *a*, Chl *b* and total chlorophyll were greatly damaged in French bean under drought condition (Fig. 3). The Chl *a* content was found to be highest (0.45 mg g^{-1}) in control plants followed by 0.39 and 0.27 mg g^{-1} led by the treatment combinations of D+M 150 µM+ J 20 µM and D+M 100µM+ J 20 µM respectively (Fig. 3A). The lowest amount of Chl *a* (0.14 mgg^{-1} FW) was recorded in drought-stressed plants only. The Chl *b* was also found to be lowest in drought affected plants only (Fig. 3B). The total chlorophyll content was found to be the highest (0.79 mg g^{-1}) in the treatment combination of D+ M 100 µM + J 20 µM and lowest (0.023 mg g^{-1}) in only drought affected plants (Fig. 3C). SPAD value was found to be more or less similar in both control (32.9 mg g^{-1}) and at D+ M 150 µM+ J 20 µM (32.46 mg g^{-1}) (Fig. 3D). Carotenoids content was recorded to be highest (0.16 mg g^{-1}) in controlled plants followed by 0.14 mg g^{-1} led by the treatment of D+ M 150 µM+ J 20 µM (Fig. 3E). Among the abiotic stresses, drought is most the tremendous one having negative consequences on photosynthetic pigments, such as chlorophylls and carotenoids (Pandey *et al.* 2012). Drought stress led to cause reduced synthesis and formation of the PSI and PSII light-harvesting complexes and suppress biosynthesis of chlorophyll (Pandey *et al.* 2012). While acclimating to abiotic stresses, plants deploy MEL to regulate chlorophyll degradation by enhancing overall chlorophyll content (Chen *et al.* 2019). The negative consequences of drought on the photosynthetic pigments were partially complemented by the application of MeJA and SA either as sole or combined applications (Mohi-Ud-Din *et al.,* 2021).

Previous investigations also reported the implications of different growth regulators including MeJA in ameliorating photosynthetic pigments in several crops (Mohamed & Latif 2017, Tayyeb *et al.* 2020). Along with those, our findings indicates both MEL and MeJA are crucial for stabilizing photosynthetic pigment molecules while facing to drought stress (Fig. 3). This was due to positive impacts of exogenous MEL on the MeJA-mediated abiotic stress tolerance in plants (Sehar *et al.* 2023). However, the fluctuating effects of Chl *b* in this study was due to the differential responses of this pigment molecule to drought stress. During abiotic stress, along with Chl *a*, plants also regulate synthesis of Chl *b* for proper maintenance of photosynthetic efficacy and thereby preventing damage to photosynthetic apparatus (Challabathula *et al*. 2016). Exogenous MEL sufficiently increased total chlorophyll content in both control and drought affected *Rosa centifolia* L. plant (Ahsan *et al*. 2025) and MeJA drammatically enhanced Chl *a* and Chl *b* in drought affected soybean plant (Mohamed and Latif 2017) reflecting their potential in photosynthesis under drought stress. Increased rate of photosynthesis by MEL and MeJA in heat-stressed wheat plant was due to the enhanced expression of PSII-related genes like *psbA* and *psbB* under drought stress (Sehar *et al*. 2023). Likely, the combined application (D+M 150 µM+ J 20 µM) showed better potential for chlorophyll synthesis reflecting its better potential to confront drought-effects in French bean plants. Alongside, photosynthetic pigment carotenoids showed crucial performances in scavenging of ROS under water stress (Gul *et al.* 2022) and the results of which are in agreement to our findings where carotenoids content was remarkably suppressed under water stress (Fig. 3 E). Thus, partial recovery of growth and yield in

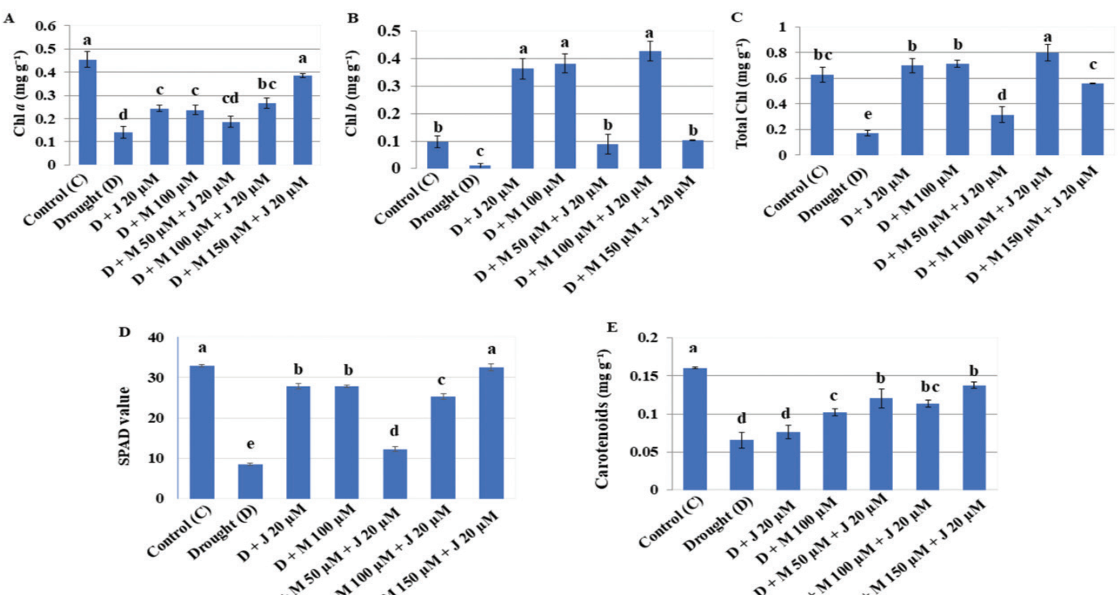

Fig. 3. Effect of sole and combined applications of MEL (M) and MeJA (J) on the photosynthetic pigments. Chlorophyll *a* (Chl *a*) (A), Chlorophyll *b* (Chl *b*) (B), total chlorophyll (C), SPAD value (D), and carotenoids contents (E) determined in the leaf of French bean after 15 days of drought imposition. Error bars indicate standard error. Different alphabetical letters on the bars show significant differences among the treatments ($p < 0.05$)

drought stressed-French bean was led by the recovery of photosynthetic pigments including chlorophylls and carotenoids diminished by drought stress in this study (Fig. 3, Fig. 2 and Fig. 1).

3.4. *Effects of MEL and MeJA on leaf relative water content and accumulation of osmolyte proline in French bean*

Leaf relative water content (LRWC) was found to be the highest (86.18%) in irrigated control plants followed by 85.26% in the treatment combination of D+ M 150 µM+ MeJA 20 µM (Fig. 4A). LRWC was found to be enhanced by 32.0% in plants treated with D+ M 150 µM+ MeJA 20 µM when compared to sole drought-stressed plants (Fig. 4A). Exogenous application of MeJA either as alone or in combination showed significant increment of relative water content in soybean as compared to drought-stressed plants alone (Mohamed and Latif, 2017). More likely, exogenous application of growth regulators such as MeJA and SA either sole or combined application was shown to improve LRWC by previous investigations (Mohi-Ud-Din *et al.* 2021). In contrast to those findings, our findings indicated that the sole application of MEL or MeJA was not sufficient to enhance relative water content in French bean plant whereas the combined application (D+ M 150 µM+ MeJA 20) improved tissue water content significantly (Fig. 4A). The finding was supported by Mohi-Ud-Din *et al.* (2021), who claimed that combined application of MeJA and SA showed better performances than sole applications regarding tissue water content during drought stress. The variation was due to the variation of crop species, stages and tenure of drought imposition.

Since relative tissue water content greatly depends on the accumulation of osmolyte proline, we determined accumulation proline in French bean plants either treated and non-treated with drought, and MEL and MeJA. Proline content was dramatically increased by 530.5 % in drought affected plants compared to non-stress control. However, as compared to only drought stressed plants, proline accumulation was found to be triggered by 14.5% in D + M 150 µM + J 20 µM treatment combination (Fig. 4B). Proline has been considered as the vital osmo-regulator to protect and maintain membrane stability in plants under abiotic stresses including drought (Abdelgawad *et al.,* 2014; Ramadan *et al.,* 2023). MEL-induced proline accumulation was found to be involved in enhancing drought tolerance of rice seedling (Luo *et al.* 2022). Along with MEL, MeJA is also vital for mitigating the negative consequences of drought stress. For instance, combined application of MeJA and SA was found to enhance proline accumulation in drought-stressed maize plants (Tayyab *et al.* 2020). Our findings regarding the enhanced level of proline accumulation in drought-stressed French bean plant by the combined application of MEL and MeJA (Fig. 4B) is consistent to that finding. Thus, higher proline accumulation by combined application of those growth regulators could perform in osmotic adjustment, higher relative water content, destabilizing ROS and partial recovery of plant growth (Fig. 4A and Fig. 1).

3.5. Effects of MEL and MeJA in mitigating drought-induced tissue damage

We measured percent electrolyte leakage (% EL) to evaluate tissue damage under drought stress. Percent EL of the plants of control (C), drought (D), D + J 20 µM, D + M 100 µM, D+ M 50 µM + J 20 µM, D+ M 100 µM + J 20 µM, D+ M 150 µM+ J 20 µM were 29.87%, 87.47%, 34.90%, 46.749%, 41.56%, 47.96%, 23.23% respectively (Fig. 5B). Abiotic stresses including drought causes detrimental effects on cell membrane stability by enhancing lipid peroxidation resulting to higher electrolyte leakage from the damaged tissue (Ghosh *et al.* 2021). Higher electrolyte leakage indicates instability in cell membrane permeability. The previous efforts investigated that combined application of MeJA and SA could produce lower level of tissue damage in drought-stressed maize plant (Tayeeb *et al.* 2020). Another recent investigation showed that combined application of MeJA and SA led to the lowering of cell membrane stability in French bean plant under drought stress (Mohi-Ud-Din *et al*. 2021). Both of the above-mentioned findings corroborated to the results of this finding where combined application of MEL an MeJA (D+ M 150 µM+ J 20 µM) produced lowest EL in drought-stressed French bean plants (Fig. 5) indicating that exogenous application of both MEL and MeJA is crucial for drought stress acclimation of French bean.

3.6. Effects of MEL and MeJA on the accumulation of non-enzymatic antioxidants

Since phenolic acid is an important non-enzymatic antioxidant, we determined total phenolics in the French bean plants in this study. Total phenolics content was found to be lowest (269.58 µg g^{-1}) in control growing condition whereas it was highest (1016.50 µg g^{-1}) in drought affected plants (Fig. 6 A).

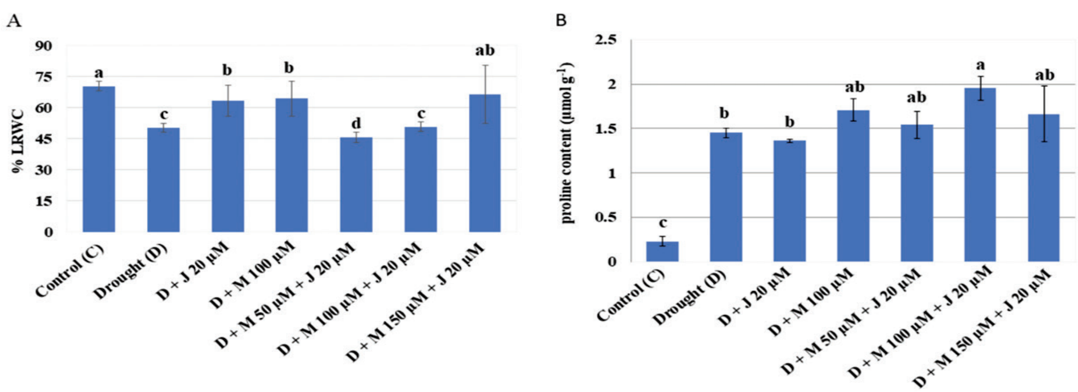

Fig. 4. Effect of sole and combined applications of MEL (M) and MeJA (J) on the leaf relative water content (% LRWC) (A) and accumulation of osmolyte proline (B). Error bars indicate standard error. Different alphabetical letters on the bars show significant differences among the treatments ($p < 0.05$)

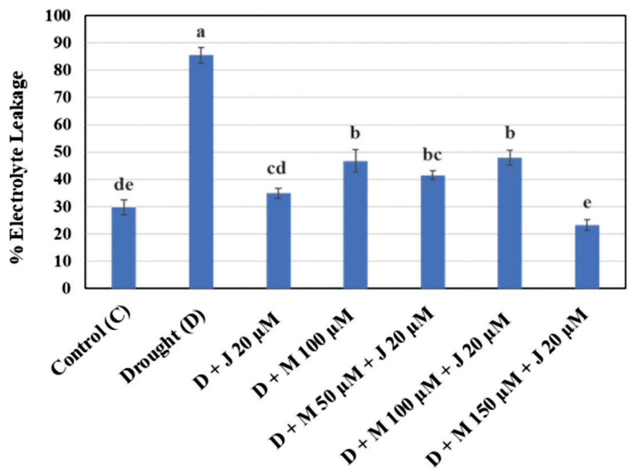

Fig. 5. Effect of sole and combined applications of MEL (M) and MeJA (J) on percent electrolyte leakage (% EL) of the damaged tissue under drought stress. Error bars indicate standard error. Different alphabetical letters on the bars show significant differences among the treatments ($p < 0.05$)

As compared to drought affected plants, total phenolics was found to be significantly reduced in other treatment combinations provided with drought and MEL or MeJA either sole or combined applications (Fig. 6A). Alongside, flavonoid content was found to be higher and more or similar in the plants under drought stress only (1169.1 µg g^{-1}) and plants treated with 20 µM J (1202.1 µg g^{-1}) and 100 µM M (1198.7 µg g-1) and D+ M 50 µM + J 20 µM (1175.2 µg g^{-1}) (Fig. 6B). On the other hand, the lower flavonoid contents were recorded in plants treated with non-stress control (624.15 µg g^{-1}) and plants treated with D+ M 100 + J 20 µM (685.89 µg g^{-1}) and D+ M 150 + J 20 µM (745.34 µg g^{-1}) (Fig. 6B). While acclimating to drought stress, plants followed enhanced accumulation of phenolic acid and flavonoids to reduce the effects of oxidative stress (Gharibi *et al.* 2019). Our findings regarding drought-induced remarkable enhancement of the synthesis of phenolics and flavonoids content are consistent to that finding (Fig. 6). Exogenous application of MeJA triggered accumulation of non-enzymatic antioxidant rutin and qurecetin acids in soybean under water stress (Mohamed and Latif 2017). Likewise, foliar application of MEL on *Moringa oleifera* increased the accumulation of indole acetic acid and phenolics to boost up photosynthesis efficacy under drought stress (Sadak *et al.* 2020). Contrary in our case, as compared to only drought stress, the declined accumulation of phenolics and flavonoids content by sole or combined application of MEL and MeJA under drought stress are not in agreement with those findings (Fig. 6). Interestingly, more or similar performance of irrigated control and D+ M 150 + J 20 µM suggests that combined application of those can mitigate the drought effects which was supported by the reduced level of ROS effects by the combination of D+ M 150 + J 20 µM (Fig.5). Therefore, our findings suggest

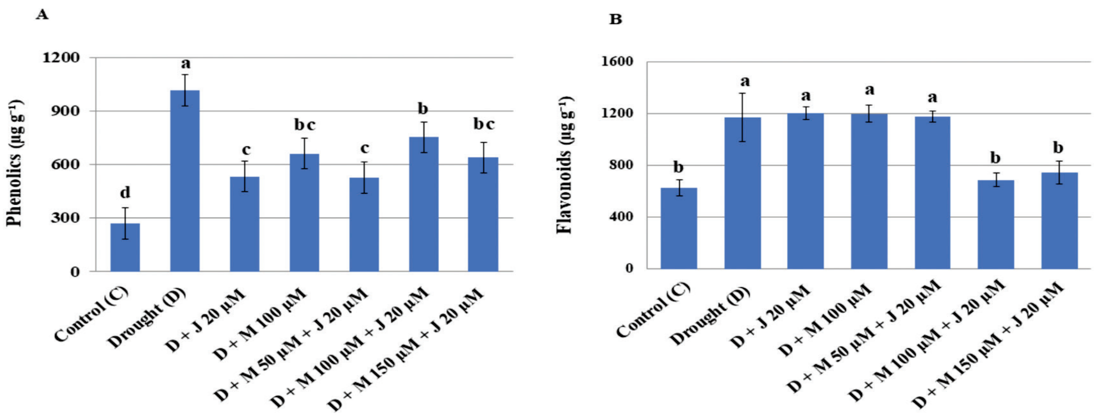

Fig. 6. Effect of sole and combined applications of MEL (M) and MeJA (J) on the accumulation of phenolics (A) and flavonoids (B) content in French bean. Error bars indicate standard error. Different alphabetical letters on the bars show significant differences among the treatments ($p < 0.05$)

that plants treated with D+ M 150 + J 20 µM could lead to repair photosynthetic machinery, induce accumulation of osmolyte proline and tissue water content, and reduce drought induced tissue damage which altogether contributed to the partial recovery of growth and development of French bean plant.

4. Conclusion

French bean plants treated with the combined application of MEL and MeJA (D+ M 150 µM+ J 20 µM) produced better phenotypic responses and accumulated higher photosynthetic pigments, relative water, and proline content as compared to drought-stressed plants only. French bean plants treated with D+ M 150 µM+ J 20 µM showed a significant reduction of EL as compared to drought-stressed plants, indicating the reduction of drought-induced oxidative damage by the combined application of those growth regulators. In contrast, as compared to drought-stressed plants, comparatively lower accumulation of non-enzymatic antioxidants like phenolics and flavonoids in the plants of non-stress control and those treated with D+ M 150 µM+ J 20 µM suggests the partial complementation of drought stress effects by the collective application of MEL and MeJA. Thus, French bean plants pre-treated with exogenous MEL and MeJA partially mitigate the drought effects by improving plants' phenotypes, photosynthetic efficacy, relative water content, proline accumulation, and membrane stability, which altogether improves the growth and development of French bean plant.

The findings of this study provide a basic ground for the future research regarding exploring molecular mechanisms of MEL and MeJA in enhancing drought tolerance in plant.

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