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Exogenous Jasmonic Acid Alleviates Adverse Effects of Drought Stress in *Allium cepa* L.

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Abstract: ***Drought stress inhibits plant growth and production and it has been reported that JA minimize the adverse effects of many environmental stresses including drought. The present investigation was carried out to evaluate the role of jasmonic acid in alleviating the adversity of drought stress in A. cepa var. Aggregatum. In this study, plants were grown in pot culture and were raised under normal conditions. After seven weeks of sowing, drought stress was imposed in the form of five days interval up to harvesting and JA (25, 50 and 100 µM) was given through the foliar application prior fifteen days each sampling. Drought stress caused a considerable reduction in growth parameters, photosynthetic pigments and relative water content. JA application enhanced these parameters in drought stressed A. cepa. There was a significant increase in compatible solute accumulation under drought stress, but it was highly significant in presence of JA and drought. Therefore, it is concluded that JA treatment improved plant growth by enhancing the pigments and RWC and trigger the compatible solute concentration which help the plant to maintain its tissue water content under stressful conditions. In other words, JA application mitigated the adverse effects of drought stress in A. cepa var. Aggregatum.***

Keywords: Compatible solutes, Drought, *Allium cepa*, Jasmonic acid.

1. Introduction

Environmental stress disrupt agriculture and food supply worldwide with final consequence-famine. Among the stress factors, drought is the most important factor responsible for disruption of annual agricultural production [1]. Drought stress alters the normal equilibrium and result in a series of morphological, physiological, biochemical and molecular changes in plants and hence affects their growth and yield. Drought stress caused a reduction in cell water potential and turgor, which elevates inter- and intracellular solute concentrations [2]. Drought stress affects stomatal closure, restricts gaseous exchange, declines transpiration rate and arrests carbon assimilation rates. It also affects mineral nutrition and metabolism which in turn causes decrease in leaf area and alteration in assimilate portioning among the organs. Drought stress causes a decline in plant growth by affecting cell expansion and cell division. However, the former one is influenced more than the later one [3], [4].

Chlorophyll, a chloroplast component for photosynthesis is greatly affected under drought stress [5]. Pigment photo-oxidation and chlorophyll degradation under drought stress results in a decrease in chlorophyll content [6]. Drought stress creates alterations in the ratio of chlorophyll 'a' and 'b' and carotenoids [7]. There is a positive relationship between chlorophyll content and photosynthetic rate which is directly linked with biomass production and grain yield [8].

Osmolytes play an important role in drought stress tolerance of plants. Accumulation of compatible solutes such as, free amino acids, proline, glycinebetaine and soluble sugars maintain high relative water content and water potential [9]. The primary function of these osmolytes is to prevent water loss to maintain cell turgor and water gradient uptake into the cell. Besides osmoregulation function, compatible solutes protect enzymes, scavenge free oxygen radicals and protect membrane structures and integrity [10].

Allium cepa L. is one of the most important commercial vegetable crops in India, cultivated in an area of 756,000 ha. India is the second largest producer of onion in the world, next to China and ranks third in export of onions, next to Netherlands and Spain. Two main types of onions, bulb onion (*Allium cepa* var. *cepa*) and shallot or multiplier onion (*Allium cepa* var. *Aggregatum*), are cultivated in India with the production and productivity of 12.16 million tons and 16.10 tons/ha, respectively. In the state of Tamil Nadu, the southernmost part of India, onion is cultivated in an area of 30,255 ha with a production of 286,000 tons. *Allium cepa* var. *Aggregatum* is the most common type of onion cultivated in Tamil Nadu and it is commonly propagated through bulbs. The average productivity of onion in Tamil Nadu is 9.45 tons/ha [11]. *Allium cepa* var. *Aggregatum* are preferred for their shorter growth cycle, better tolerance to disease and longer storage life than the common onion and for their distinct flavor that persists after cooking [12], [13].

Jasmonic acid and methyl jasmonate, ester of JA, are naturally occurring plant growth regulators responsible for regulation of many physiological and metabolic processes in plants [14], [15]. In response to many stress factors, jasmonic acid is involved in signal transduction pathways [16]. Foliar application of jasmonates regulates several physiological responses leading to improved resistance against abiotic stresses [17].

Protecting plants from various abiotic stresses, the role of jasmonic acid/methyl jasmonate, however, has been controversial. For instance, methyl jasmonate has been found to improve resistance against drought in many crop plants such as in rice [18], strawberry [19] and tomato [20]. Likewise, in maize, methyl jasmonate mediated the accumulation of organic solutes and growth hormones and further induced antioxidant activity [21]. On the other hand, foliar application of methyl jasmonate initiated lipid peroxidation in peanut plants which is an indication of oxidative stress mediated damage [22], whereas it caused substantial yield reduction in rice [23].

This controversy needs to be resolved by evaluating the effect of jasmonic acid on morphological, anatomical, physiological and biochemical parameters and yield components as well. To best of our information, no such study has been conducted on *Allium cepa* var. *Aggregatum* so far. Taking the above debate in view, measures have been taken to conduct a study on effect of JA on *Allium cepa* var. *Aggregatum* under drought stress. Hence, the aim of the present study was to access the interactive effect of jasmonic acid and drought stress on growth, photosynthesis, water relation and compatible solutes of *Allium cepa* var. *Aggregatum*.

2. Materials and Methods

Allium cepa L. var. *Aggregatum*, family Amaryllidaceae, was selected for present investigation. The seeds were obtained from local farmers and were identified by Tamil Nadu Agriculture University (TNAU), Tamil Nadu, India. Jasmonic acid, obtained from Himedia Laboratories Pvt. Ltd., Mumbai, was used exogenously through foliar spray in this experiment. The experiment was conducted in the Botanic Garden and Stress Physiology Laboratory, Department of Botany, Annamalai University, Annamalai Nagar ($11^{\circ}23'59''$N, $79^{\circ}41'37''$E). Seeds were planted 1-2 cm deep in plastic pots of 32 cm diameter and 20 cm depth each filled with 5 Kg sand, farm yard manure (FYM) and garden soil (ratio 2:2:1) mixed with 3 g NPK fertilizer. After three weeks, the seedlings were thinned to 4 plants per pot and pots were divided into five groups and were arranged in a complete randomized block design with 6 replicates. Group I as control (C) received 0 µM JA (Jasmonic acid) and regular irrigation, group II (D) received 0 µM JA and 5 DID (day interval drought- irrigation with a gap of 5 days interval), group III (DJ1) received 25 µM JA and 5 DID, group IV (DJ2) received 50 µM JA and 5 DID and group V (DJ3) received 100 µM JA and 5 DID. Control plants were irrigated regularly (light water spray). Drought stress was imposed on 35^{th} DAS (day after sowing) onwards up to 95^{th} DAS in the form of 5 DID (days interval drought) and JA treatments were imposed through foliar spray prior 15 days each sampling i.e., on 35^{th}, 50^{th}, 65^{th} and 80^{th} DAS. The *A. cepa* var. *Aggregatum* crop matures between 90 to 100 days that is why sampling days were fixed up to 95 DAS. The plants were harvested for analysis on 50^{th}, 65^{th}, 80^{th} and 95^{th} DAS. Plants were selected randomly from each group, uprooted and washed carefully and then separated into root and shoot for estimating morphological, physiological and biochemical parameters.

2.1. Measurement of growth parameters and relative water content

The length between shoot tip and point of the root-shoot transition region was taken as shoot length. Root length was recorded by measuring below the point of root-shoot transition to the fibrous root and the length of lateral roots was taken as total root length. The shoot and root length are expressed in centimetres per plant. Whole plant fresh weight was determined, using electronic weighing balance (Citizen Scales Pvt. Ltd., Model-CTG602-600), after gently rinsing the plants few times with distilled water. Dry weight of the whole plant was then determined after drying the samples to a constant weight in an oven at 70°C.

The relative water content (RWC) of leaves was measured according to [24]. Immediately after sampling, leaves were weighed and then immersed in distilled water for 4 h at room temperature. The leaves were then blotted dry and weighed prior to oven drying at 80°C for 48 h. The leaf relative content was calculated using the following formula: RWC = (FW – DW)/(TW – DW) ×100, where FW is the fresh weight, DW the dry weight, and TW is the turgid weight (weight after the leaf was kept immersed in distilled water for 4 h).

2.2. Extraction and estimation of photosynthetic pigments and compatible solutes

Chlorophyll contents were measured from the *A. cepa* leaves according to [25] method and Carotenoid content was calculated using the formula of [26]. Fresh leaves (1g) were extracted with 80 % acetone (v/v) and chlorophyll contents were estimated spectrophotometrically at 480, 645 and 663 nm using Hitachi U-2000 spectrophotometer and were expressed in terms of mg g^{-1} fresh mass.

Sucrose content was estimated by the method of [27]. 1 ml of invertase was added to 1 ml sugar extract and incubated at 37˚C for 1 hour and, thereafter, the reaction was stopped by keeping the tubes in boiling water bath for 10 min. Under these conditions, sucrose was completely hydrolyzed. Glucose was determined by the glucose oxidase and peroxidase reaction (sigma) [28] before and after invertase hydrolysis and the difference between these values was taken as the actual amount of sucrose in the sample.

Total soluble sugars (TSS) were quantified following the phenolsulfuric acid method [29]. 100 mg dry weight of shoots was extracted in 80% (v/v) methanol heated to 70°C in a water bath. The extract was then centrifuged at 5,000 × g for 10 min. The supernatant was used for the estimation of soluble sugar concentrations. The reaction mixture consisted of 5% phenol and 98% sulfuric acid. Once the extract had cooled, its absorbance was determined at 490 nm using D-glucose as standard.

Total free amino acids (AA) were extracted and estimated by following the method of [30]. Five hundred milligrams of fresh plant material was homogenized in a mortar and pestle with 80% boiled ethanol. The extract was centrifuged at 800 g for 15 minutes and the supernatant was made up to 10 ml with 80% ethanol. In 25 ml test tube, ethanol extract was taken and neutralized with 0.1 N NaOH using the methyl red indicator to which ninhydrin reagent was added. The contents were boiled in a boiling water bath for 20 minutes, and then 5ml of diluting solution was added, cooled and made up to 25 ml with distilled water. The absorbance was read at 570 nm.

Protein content was determined according to the method of [31] using Bovine serum albumin as standard. One gram of fresh plant material was ground in with mortar and pestle with 20 ml of 20 per cent trichloro acetic acid (TCA). The homogenate was centrifuged for 15 minutes at 800 rpm. The supernatant was discarded and to pellet, 5 ml of 0.1 N NaOH was added to solubilize the protein and the solution was centrifuged at 800 rpm for 15 mins. The supernatants was made up to 10 ml with 0.1 N NaOH and used for the estimation of protein content. The absorbance was measured at 595 nm.

Glycine-betaine (GB) was estimated by the method of [32]. Briefly, finely ground dried plant tissue (0.5 g) was stirred with 20 cm^3 distilled water for 24 h and filtered. The filtrate was diluted with equal volume of 1 M H_2SO_4, made into aliquots of 0.5 cm^3 in microcentrifuge tubes, cooled over ice for 1 h and to each of these were added 0.2 cm^3 cold KI-I_2 reagent. The reactants were gently stirred, stored at 4 °C overnight and centrifuged at 12000 *g* for 15 min at 4 °C to get the precipitated periodide crystals. The crystals were dissolved in

1,2-dichloroethane, and absorbance was measured at 365 nm after 2 h. Glycine-betaine dissolved in 1 M H_2SO_4 served as standard.

Free proline (P) was assayed spectrophotometrically by the ninhydrin method [33]. The plant material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 14,000 rpm. The supernatant was used for the estimation of the proline concentration. The reaction mixture consisted of acid ninhydrin and glacial acetic acid, which was boiled at 100°C for 1 h. After termination of reaction in ice bath, the reaction mixture was extracted with toluene, and absorbance was read at 520 nm using L-proline as standard.

3. Statistical data analysis

Experiments were undertaken in complete randomized block design with six replications. Statistical analysis was carried out using SPSS- 16.0 version (SPSS Inc., Chicago- II. USA). The values are mean of six replicates and are expressed in Mean ± SE at $P \leq 0.05$.

4. Results

4.1. Growth parameters

The data obtained, clearly showed that drought stress affected root and shoot length and number of leaves per plant as compared to control (Table 1 and 3). In contrast to drought stress, exogenous application of JA enhanced the above growth parameters to a great extent but does not exceed the control. Drought stress also affected the whole plant fresh and dry weight whereas, foliar application of JA improved fresh and dry weight in drought stressed plants (Table 2). The overall growth vigor of drought stressed *A. cepa* plants under JA application was higher at 100 µM JA as compared to 25 and 50 µM JA, hence DJ3 showed better growth among the treated groups.

Table 1: Interactive effects of drought stress and JA on root and shoot length of *Allium cepa* var. *Aggregatum*.

Growth stages	50 DAS	65 DAS	80 DAS	95 DAS
Root length (cm)				
Control	5.5±0.75	8.4±0.99	11.6±1.12	15.1±1.04
D	4.1±0.76	6.3±1.31	8.8±0.94	11.2±0.87
DJ1	6.0±0.75	9.1±1.23	12.6±1.41	16.8±1.39
DJ2	7.5±0.92	10.0±1.15	13.7±1.26	17.5±1.52
DJ3	7.7±0.78	10.0±1.05	13.4±1.17	17.2±1.21
Shoot length (cm)				
Control	25.0±1.32	31.6±1.41	38.1±1.40	44.7±1.24
D	17.2±1.53	21.9±1.38	27.4±1.31	32.3±1.52
DJ1	19.0±1.13	25.2±1.19	30.9±1.20	36.7±1.55
DJ2	20.1±1.25	27.5±1.11	31.7±1.50	38.5±1.34
DJ3	22.0±1.03	28.3±1.21	32.5±1.46	38.9±1.49

Table 2: Interactive effects of drought stress and JA on the whole plant fresh and dry weight of *Allium cepa* var. *Aggregatum*.

Growth stages	50 DAS	65 DAS	80 DAS	95 DAS
Whole plant fresh weight (g)				
Control	21.9±1.15	28.5 ±1.19	37.4 ±1.10	42.8±1.23
D	13.6±1.22	17.0 ±1.41	23.4 ±1.32	28.0±1.08
DJ1	16.3±1.12	22.6 ±1.53	28.4 ±1.01	34.2±1.32
DJ2	18.2±1.41	22.9 ±1.14	30.2 ±1.57	36.3±1.09
DJ3	19.8±1.04	24.3±1.43	31.9 ±1.91	37.8±1.44
Whole plant dry weight (g)				
Control	2.25±0.31	2.81 ±0.12	3.74 ±0.61	4.49±0.43
D	1.37±0.61	1.61 ±0.52	2.29 ±0.14	2.72±0.32
DJ1	1.63±0.71	2.26 ±0.38	2.84 ±0.90	3.42±0.61
DJ2	1.85±0.22	2.39 ±0.41	3.08 ±0.23	3.71±0.19
DJ3	1.91±0.53	2.55 ±0.28	3.35 ±0.47	3.97±0.33

4.2. Relative water content

Table 3 depicts a major reduction in RWC was observed in drought stressed *A. cepa* plants as compared to unstressed plants (control). However, foliar application of JA improved RWC in leaves of drought stressed plants.

Table 3: Interactive effects of drought stress and JA on number of leaves per plant and leaf relative content of *Allium cepa* var. *Aggregatum*.

Growth stages	50 DAS	65 DAS	80 DAS	95 DAS
Number of leaves per plant				
Control	9.3±0.81	12.1±1.45	19.2±1.21	29±1.04
D	5.1±0.62	8.3±1.01	13.1±1.81	15.8±2.10
DJ1	6.7±0.74	9.3±1.30	14.6±2.17	20.3±2.02
DJ2	7.9±1.12	9.2±1.00	14.9±1.52	21.1±0.97
DJ3	8.2±0.92	10.4±1.20	17±1.25	23.2±2.40
Leaf relative water content (%)				
Control	89.4±0.84	90.6±0.89	90.9±0.65	91.3±1.21
D	54.8±0.23	56.3±0.48	57.2±0.32	57.8±0.29
DJ1	62.4±1.31	62.7±1.48	63.3±1.32	64.0±0.85
DJ2	67.2±1.20	68.1±1.11	68.5±0.95	69.2±0.88
DJ3	73.6±1.72	74.3±1.81	74.9±0.99	75.6±1.05

4.3. Photosynthetic pigments

Drought stress caused a significant reduction in chlorophyll 'a', 'b' and carotenoid contents as compared to control. However JA treatment enhanced these pigment contents in drought stressed plants (Table 4). 100 µM JA showed better enhancement as compared to 25 and 50 µM JA.

Table 4: Interactive effects of drought stress and JA on chlorophyll and carotenoid contents of *Allium cepa* var. *Aggregatum*.

Growth stages	50 DAS	65 DAS	80 DAS	95 DAS
Chlorophyll 'a'				
Control	0.813±0.03	0.898±0.07	0.946±0.10	1.019±0.05
D	0.409±0.11	0.458±0.08	0.492±0.02	0.537±0.04
DJ1	0.472±0.05	0.530±0.02	0.569±0.10	0.618±0.05
DJ2	0.565±0.02	0.629±0.08	0.666±0.06	0.725±0.10
DJ3	0.634±0.01	0.706±0.04	0.751±0.02	0.820±0.05
Chlorophyll 'b'				
Control	0.451±0.01	0.542±0.04	0.595±0.01	0.667±0.02
D	0.219±0.02	0.271±0.01	0.301±0.02	0.335±0.05
DJ1	0.260±0.03	0.319±0.02	0.351±0.01	0.390±0.02
DJ2	0.309±0.01	0.373±0.03	0.415±0.09	0.462±0.11
DJ3	0.347±0.08	0.421±0.02	0.465±0.02	0.527±0.06
Carotenoid				
Control	0.632±0.01	0.697±0.02	0.768±0.01	0.803±0.04
D	0.430±0.02	0.483±0.01	0.512±0.05	0.564±0.01
DJ1	0.481±0.08	0.540±0.01	0.590±0.05	0.629±0.01
DJ2	0.512 0.03	0.574±0.09	0.642±0.07	0.684±0.10
DJ3	0.548±0.11	0.607±0.03	0.688±0.04	0.723±0.06

4.4. Compatible solutes and protein content

Data presented in figure 1 shows accumulation of sucrose in control, drought and JA treated groups. There was a linear increase in sucrose content in root and shoot of *A. cepa* under drought stress as compared control. However, exogenous application of JA further enhanced the sucrose content in drought stressed plants.

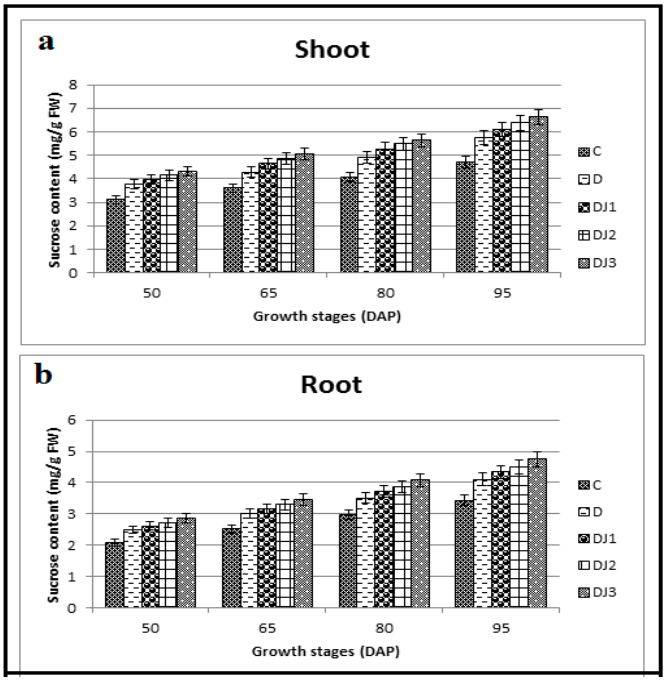

Figure 1: Interactive effects of drought stress and JA on sucrose content of *Allium cepa* var. *Aggregatum*.

Data shown in figure 2 depicts that soluble sugar content increased in both root and shoot of *A. cepa* on all growth stages as compared to control. However, JA treatment further enhanced the soluble sugar accumulation as compared to drought stress alone. Group DJ3 exhibited highest soluble sugar content as compared to other groups.

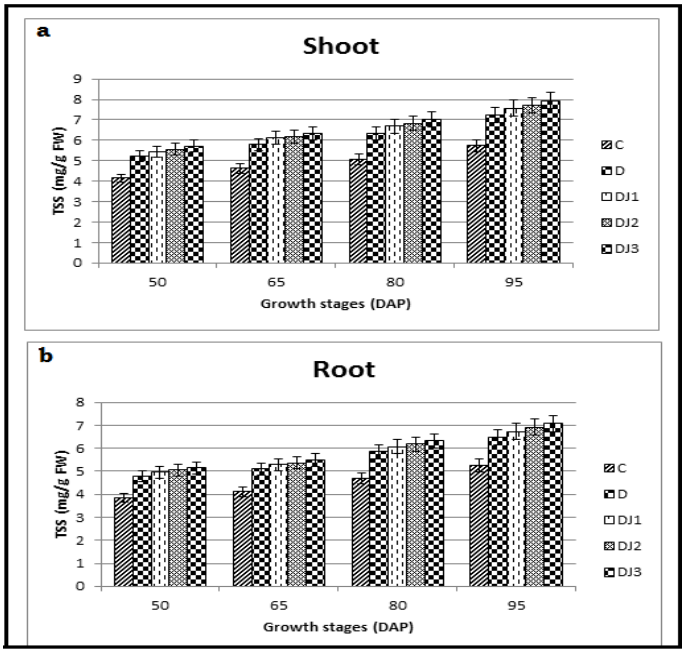

Figure 2: Interactive effects of drought stress and JA on total soluble sugar (TSS) content of *Allium cepa* var. *Aggregatum*

Drought stress caused a significant increase in root and shoot amino acid content as compared to control (Figure 3). However, foliar application of JA in presence of drought stress further increased amino acid content in both organs on all growth stages.

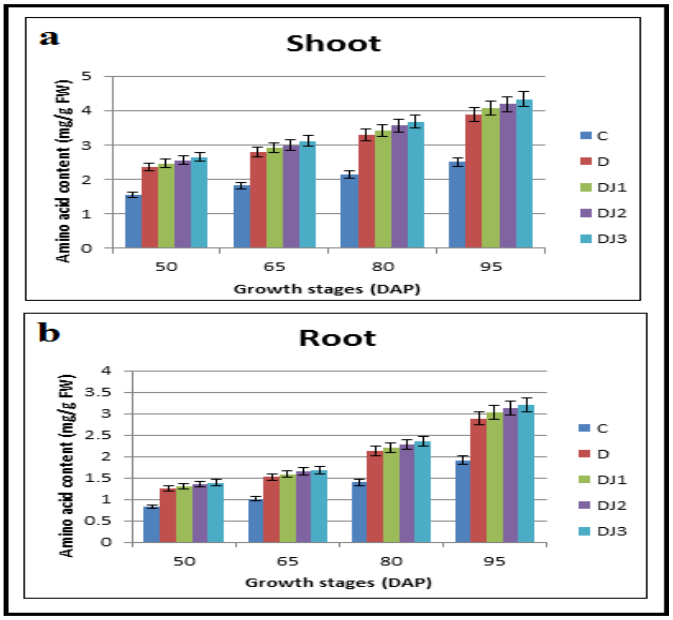

Figure 3: Interactive effects of drought stress and JA on amino acid content of *Allium cepa* var. *Aggregatum*.

It is clear from the data presented in figure 4 that drought stress caused a considerable decline in levels of protein content as compared to control from 50 to 95 DAS. On the other hand, exogenous application of JA improved protein content in both organs of *A. cepa* when compared with drought stress alone. 100 µM JA showed better enhancement than other two concentrations.

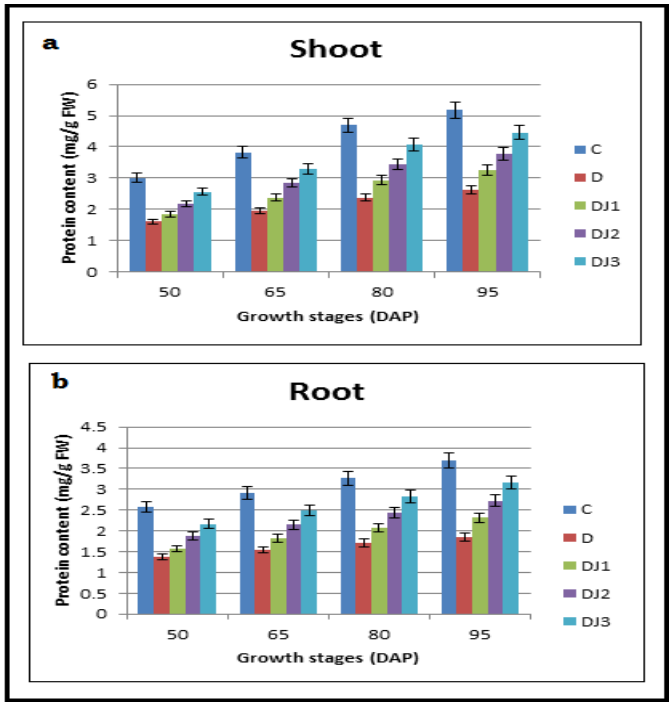

Figure 4: Interactive effects of drought stress and JA on protein content of *Allium cepa* var. *Aggregatum*.

Figure 5 depicts that drought stress caused a significant increase in root and shoot glycinebetaine content of *A. cepa* as compared to control. However, JA application to drought stressed plants further triggered glycinebetaine accumulation on all growth stages. Highest glycinebetaine accumulation was recorded in DJ3 group treated with drought stress and 100 µM JA.

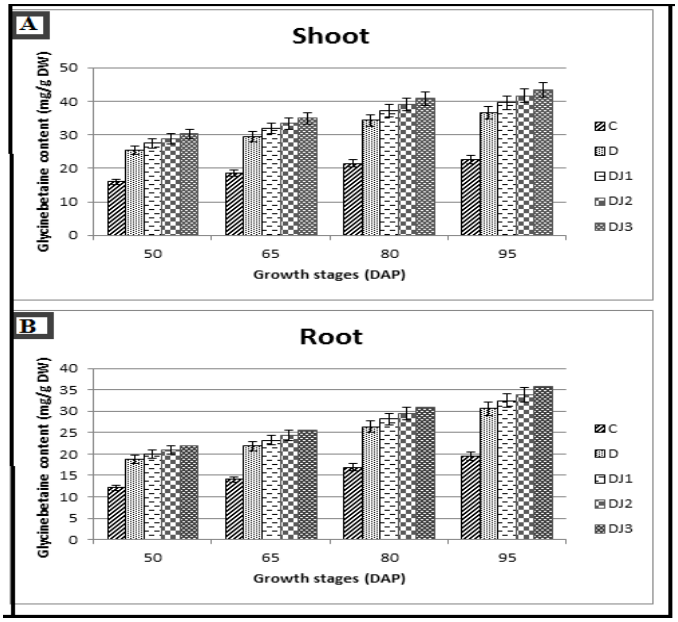

Figure 5: Interactive effects of drought stress and JA on glycinebetaine content of *Allium cepa* var. *Aggregatum*.

Drought stress triggered proline accumulation in both root as well as shoot of *A. cepa* when compared with normal plants (control). On the other hand, foliar application of JA further increased proline content in drought stressed plants to a significant level. There was recorded almost two fold increase in proline content in plants drought stressed plants treated with 100 µM JA (figure 6).

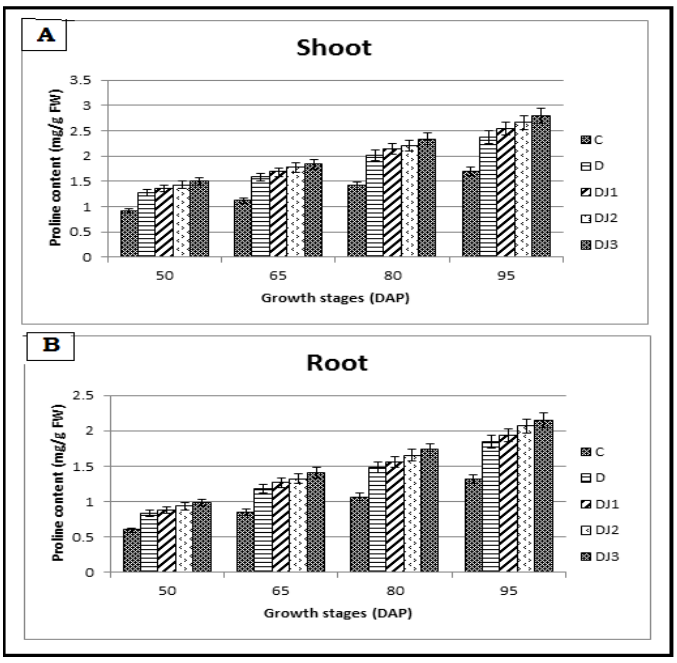

Figure 6: Interactive effects of drought stress and JA on proline content of *Allium cepa* var. *Aggregatum*.

5. Discussion

Drought stress affected the growth of *Allium cepa* var. *Aggregatum*, as evidenced by reduced root and shoot length, reduction in total biomass and arrest of photosynthesis due to chlorophyll degradation and loss of water content within cells. However, these negative effects of drought stress were ameliorated by exogenous application of jasmonic acid (JA).

Drought, one of the most important environmental stresses, severely hinders plant growth and development, limits production and the performance of crop plants than any other abiotic stress [21]. The role of jasmonic acid or methyl jasmonate in plants against abiotic stresses received considerable attention [34] and can act as a true plant hormone, which regulates various development and stress responses [6]. Jasmonic acid induces a wide variety of morphological, physiological and biochemical responses in plants exposed to stress [35]. The present investigation indicated that drought stress led to severe decline in morphological traits, such as, root and shoot length, fresh and dry weight and number of leaves per plant (Table 1-3) possibly by oxidative stress as indicated by noticeable decline in tissue water status (Table 3) caused due to decreased water absorption in stressed *A. cepa* plants. The reason for reduced growth under drought stress might be the root damage which led to decreased number of lateral roots and decline in cell enlargement resulted from more turgor and leaf senescence. On the other hand, foliar application of JA to drought stressed plants improved these morphological traits to a significant level. Similar reports were observed in soybean [36], *Cajanus cajan* [37] and *A. sativum* [38].

Relative water content (RWC) is an important marker to measure the impact of drought stress in plants. Drought stress caused a significant loss in leaf RWC, whereas, JA enhanced RWC when applied to drought stressed plants. A decrease RWC was reported in mungbean [39] and *Paspalum scrobiculatum* [40] under drought stress condition. A sharp reduction in RWC was observed in drought stressed soybean plants; however, methyl jasmonate application recovered this loss to a marked level [41].

Maximizing efficiency of photosynthesis is an important point of debate in plant research [42]. The rate of photosynthesis is directly proportional to the chlorophyll bearing surface area, irradiance and its potential to utilize CO_2 [43]. Indeed, photosynthesis is a key metabolic pathway in plants and maintaining of photosynthetic rate leads to better growth and development under water stress [44]. Our results indicate that drought stress caused a noticeable reduction in chlorophyll-a, chlorophyll-b, and carotenoid contents of *Allium cepa* var, *Aggregatum*. However, JA application enhanced the pigment content in presence of drought stress. These results are in accordance with the observation that treatment with JA improved chlorophyll content in sweet basal under different water regimes [45] and pigeon pea under copper mediated oxidative stress [37]. El-Bassiouny [46] suggested that, in cowpea, reduction in chlorophyll content under drought stress is mainly due to stomatal closure caused by increased levels of ABA during stress. It has been suggested that JA treatment increases active cytokinin concentration which enhanced chlorophyll accumulation in potato plants [47]. Drought might arrest net photosynthetic assimilation by stomatal as well as metabolic limitations [48]. Many researchers reported that moderate stress causes stomatal damages, whereas, severe stress causes biochemical limitations [49].

The concentration of osmolytes within the cells often increase in species resistant to water deficit, which not only helps in maintenance of tissue water status but also are involved in osmoregulation [7]. In our study, the osmolyte concentration substantially increased in the plants exposed to drought stress; however, upon JA application osmolyte concentration further increased in drought stressed plants. The compatible solute accumulation might have helped in maintaining tissue water content which is evidenced by higher values of RWC (Table 3) possessed by plants treated with JA in presence of drought stress.

The results indicate that sucrose and total soluble sugar contents were higher in plants exposed to drought stress. On the other hand, JA application considerably enhanced sucrose and soluble sugar accumulation in root as well as shoot organs of drought stressed *A. cepa*. These results are in accordance with [50] and [51], who suggested that sucrose and total soluble sugar concentrations were higher soybean and *Zea mays* respectively during drought stress exposure. Similar results were found in epiphytic orchid [52] under drought stress. Sucrose might be playing a non-nutritive role as a regulator of gene expression [53]. The data of our study revealed that JA application during drought period stimulated the accumulation of sucrose and total soluble sugar. The increase in the levels of sucrose and soluble sugar concentrations may be due to starch degradation [54]. Starch reduction may occur in response to drought stress and may play a vital role in soluble sugar accumulation [55]. This may be supported by the evidence that a simultaneous increase in soluble sugar and decrease in starch concentration was witnessed in maize [56]. There is a strong relation between osmoprotectant accumulation (such as sucrose and soluble sugars) and drought tolerance mechanism. Sucrose and soluble sugar accumulation is strongly correlated with attainment of drought tolerance in plants [57].

The results obtained that amino acid accumulation increased in drought stressed *A.* cepa as compared to untreated control plants. At the same time, there was a gradual decrease in protein content under drought stress. Data presented in figure 2 shows that JA application substantially increased amino acid and protein contents in drought stressed *A. cepa*. Drought stress exposure might have induced the accumulation of free amino acids and degradation of proteins by triggering protease activity. Our results are in agreement with those reported by [58] and [59]. Similarly foliar application of JA markedly enhanced free amino acid and protein content in salt stressed soybean plants [36]. JA treated pea plants exhibited higher accumulation of amino acid content [60]. In another report it was suggested that JA treatment, both in presence or absence of oxidative stress, significantly increased protein content in *Cajanus cajan* [37]. Gzik [61] attributed that the amino acid accumulation might be due to the hydrolysis of proteins or/and it may be occurring in response to the change in osmotic adjustment of the cellular contents [62]. Amino acid accumulation plays a very significant role in drought tolerance, possibly through osmotic adjustment in different plant species, such as *Radix astragali* [63].

The results revealed that glycine betaine accumulation increased under drought stress in both root and shoot of *Allium cepa*. These results are in accordance with the observations of [63] which indicate that glycine betaine content increased under drought stress in *Radix astragali*. There are similar evidences that glycine betaine content increased under drought stress in barley [64]. Glycine betaine is considered to be one of the most abundant quaternary ammonium compounds produced in higher plants under stressful environment [65]. Foliar application of JA further boosted the glycine betaine accumulation in drought stressed *Allium cepa* plants. Gao and co-workers [66] suggested that JA is able to elicit betaine accumulation in pear.

It is clear from the results that proline accumulation increased in plants exposed to drought stress. However, foliar application of JA, to the drought stressed plants, further triggered the proline accumulation to a significant level. Proline accumulation is the first response of plants exposed to water-deficit stress in order to reduce cell injury. These results are consistent with those of [6], who observed that progressive drought stress induced a considerable accumulation of proline in water stressed maize plants. Similar results were observed under drought stress in Sorghum [67]. There was a steady increase in proline content in soybean under drought stress; however, MeJa application further enhanced the proline accumulation in these drought stressed plants [41]. Foliar application of JA significantly increased proline content in salt stressed pea [60] and soybean [36]. There are many reports which suggest increased proline content by foliar application of JA in *Cajanus cajan* [37], pear [66] and barley [68] under various environmental stresses. Proline accumulation in plants might act as a scavenger of ROS and acting as an osmoprotectant to reduce water potential which in turn helps to retain water content inside the cell. Under stressful conditions, proline accumulation supplies energy for the growth and survival and thereby helps the plant to tolerate stress [69]. Proline, as an osmoprotectant compound, plays a major role in osmoregulation and osmotolerance [70]. However, its definite role in exerting stress resistance continues to be a debate [71. Moreover, proline accumulation can be explained by the higher inhibitory rate of proline oxidase. A significant higher elevation in γ-glutamyl kinase activity (proline synthesis) associated with inhibition of proline oxidase activity (proline oxidation) is the reason for the higher level of free proline accumulation [72].

6. Conclusion

From the above investigation, it may be proposed that drought stress caused a considerable reduction in plant growth and total biomass of *A. cepa* which may be caused by low photosynthetic rate and decreased RWC; however, these traits were almost restored to normal by JA application. This may be allied with the increased photosynthetic rate, RWC and compatible solute concentrations in *A. cepa* by JA treatment. It is clear from the results that the applied chemical (JA) performed to ameliorate the plant growth by diminishing the adverse effects of drought stress by decreasing the oxidative damage, possibly by enhancing the compatible solute concentrations to balance osmoregulation. This might have favored better growth and development to the plant under stressful conditions. So, from the above it may be concluded that JA is a very beneficial growth regulator which enhanced growth and total biomass of *A. cepa* var. *Aggregatum* and it may be recommended for better growth and yield both in arid as well as semiarid regions.

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