

## Exogenous application of agmatine improves water stress and salinity stress tolerance in turnip (*Brassica rapa* L.)

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### Abstract

This study was carried out to determine the consequence of foliar application of agmatine (0 and 0.5 mM), on growth, physiological and biochemical traits, and yield of turnip (*Brassica rapa* L.) plants grown under water stress or salt stress conditions. The effect of three irrigation regimes (100%, 80% and 40% of field capacity) and three salt concentrations (0, 100 and 200 mM NaCl) on turnip plants grown in pots under greenhouse conditions were studied. Water deficit developed at 40% Field capacity (FC) and salinity stress, especially at 200 mM, resulted in significant decreases in all growth parameters when compared to control plants (100% FC) including root length and diameter as well as shoot weights per plant. Water stress and high salt stress negatively affected most physiological and biochemical characteristics such as total chlorophyll, photosynthetic rate, stomatal conductance, and transpiration rate. Water use efficiency (WUE) increased under 80% FC or 100 mM NaCl. Antioxidant enzymes activity, catalase and peroxidase and glutathione reductase, increased with water stress and salt stress. Foliar application of agmatine seemed to alleviate the adverse effects of water stress and salt stress on turnip. Alleviating harmful effects of salt stress and enhancing water stress tolerance by agmatine was associated with improving leaf gas exchange, antioxidant enzymes and protein profile.

**Keywords:** antioxidant enzymes; protein banding; salinity; turnip; water stress

### Introduction

Turnip (*Brassica rapa* L.) is an important vegetable crop cultivated worldwide because of its unique taste and valuable phytochemical composition (Kaveh and Amiri Chayjan 2017). Its tuberous roots have considerable amounts of vitamins, minerals (Ca, K, Fe, Cu, Mg and Zn) and antioxidants (Dehdari, 2013; Mourato *et al.*, 2015) and are considered as a famous nutritious source and have several pharmaceutical aspects against hepatic injury in diabetics. In Saudi Arabia, the turnip has been used as traditional medicine for several diseases such as constipation, chronic gastritis, and liver diseases (Ramirez *et al.*, 2020). In addition, since turnip grows in winter season, a period of fodder scarcity, it can supply green fodder for animals and livestock during the scarcity periods (Ukai *et al.*, 2016; Javed *et al.*, 2019). In nature, turnip is opposed by a diversity of environmental factors, affecting plant growth and productivity. Water stress and salinity stress are considered

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restraints that alter normal growth and metabolism of turnip in such arid and semi-arid regions (Ashraf and Salam, 2012; Kamran *et al.*, 2021).

Drought is usually developed when the amount of water available to the plant in the root zone is less than that required for maximum growth and productivity (Parvin *et al.*, 2019; Seleiman *et al.*, 2021). Water stress was found to alter many physiological features, such as reducing water potential, CO<sub>2</sub> intake, photosynthetic and transpiration rate as well as stomatal conductance, consequently declining plant growth and crop productivity (Hussein *et al.*, 2019; Darwesh *et al.*, 2019). Plant roots are always crucial in regulating crop productivity when there is Water stress since plants under such conditions always have longer roots to absorb more water and nutrition from deeper layers of stressed soil (Zhang *et al.*, 2020).

Salinity is another vital limiting factor that reduces plant growth and productivity (Negrão *et al.*, 2017; Taha *et al.*, 2021; Alkharabsheh *et al.*, 2021). Stress was reported to affect plants in two ways, 1) dehydration and toxicity due to excess salt accumulating inside plant cells. 2) High salt concentration in the root zone makes it difficult to absorb water and has a direct effect on cell growth and related metabolic processes (Hernandez *et al.*, 2019). It was found that high salinity caused a disturbance of main cell structures such as, cell membranes, and proteins, as well as nucleic acids (Hashem *et al.*, 2019). The formation of toxic radicals (ROS) often alters the normal growth of plants via extra production of free radicals (Kamran *et al.*, 2021).

In modern farming, enhancing the efficient use of water and the improvement of salt stress resistance is essential for effective growth performance and productivity. This can be achieved using anti-stress compounds that cause osmotic adjustment and stability of cell structure. In Saudi Arabia's, excessive attention has been paid to find natural and safe materials that can alleviate stress and protect plants from destruction. One of these materials is agmatine which is a precursor of polyamines (PAs) which are formed in higher plants to regulate plant growth and development (Hussein *et al.*, 2019). In addition, it is an intermediary in the biosynthesis of arginine pathway, which leads to the production of compounds that function as osmo-protective substances, including proline and glutamine (Ali *et al.*, 2016). Polyamines were found to enhance protein biosynthesis, plant growth and development and they can clean free radicals (ROS) in plants under stress, thus protecting plant cells from oxidative damage (Liu *et al.*, 2018). Two problems have existed with the use of PAs; first, they are expensive materials, and their costs are relatively high; secondly, their metabolism often costs plants a relatively high amount of energy than their precursors.

We hypothesized that agmatine treatment will alleviate the adverse effects of both water stress and salt stress on turnip plants. This can be achieved through the regulation of several physiological and the biochemical characteristics including chlorophyll, photosynthetic rate, stomatal conductance, transpiration rate, increasing activity of the activity of particular antioxidant enzymes, and synthesis of new significant polypeptides. Therefore, the goal of this study was to investigate the capability of agmatine to mitigate the negative effects of water stress and salinity stress on turnip plants.

## Materials and Methods

### *Plant material*

Two separate pot experiments were performed in the Department of Biological Science, College of Science, King AbdulAziz University, Jeddah during winter season of 2019/2020 to determine the effect of foliar application of agmatine on turnip plants grown under water stress or salinity stress.

### *Plant growth*

At 15 of November, 2020 seeds of turnip (*Brassica rapa* L., cv. 'Purple Top') obtained from a certified center for seed production in Jeddah, were surface sterilized with 0.5% sodium hypochlorite solution for 3 min and were washed thoroughly with distilled water. Six seeds were sown in each pot (30×30 cm) containing 2.5 kg of soil comprised of sand–clay–peat moss (50%:25%:25%) in completely randomized block design (CRBD)

under a greenhouse condition (day/night temperatures of 25 °C /18 °C, RH 60-70%, 14 h photoperiod, light sources were fluorescent-incandescent lamps with PAR of 355  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ). In order to ensure proper establishment, the plants were at first irrigated with non-saline water for 15 days, then plants were thinned, and 2 uniform seedlings were kept in each pot as one replicate. About 3 g of NPK (20-20-20) fertilizer (Sangral manufacture-AgroSupplies & Development Co (ASDCO), Riyadh, Saudi Arabia) was applied/pot fortnightly as recommended by the manufacturer.

#### *Stress and agmatine treatments*

Pots were then divided into two sets of 30 pots each, which were arranged into 6 rows of 5 pots each. After 21 d of sowing (DAS), pots of the first set were subjected to different irrigation regimes (100%, 80% and 40% of field capacity, FC), each treatment consisted of 5 pots. within the same time, salinity treatments (0, 100 and 200 mM NaCl) were applied to the second set (30 pots) which were arranged in the same manner of the first set. Application of salt solutions started gradually in increment pattern and repeated 2 times in 3-week intervals. For the non-salinity treatment, the plants were irrigated with tap water to reach FC.

Two days prior to water stress and salt treatments, plants were sprayed with 0.0 or 0.5 mM agmatine which was applied 3 times in 10-day intervals. Plants were sprayed until both sides of leaves were completely wet. Tween-20 (1%) was added to agmatine and control as a surfactant. Every 2 days, pots were weighted and tap water was added to replace water loss to reach the assigned FC and amounts of water added were recorded. Each treatment was conducted in triplicate and means of measurements were recorded.

At 60 DAS, a representative sample was taken from each treatment to determine the following physiological and biochemical attributes.

#### *Biochemical analysis*

##### Total chlorophyll

Total chlorophyll was extracted from fresh leaves (500 mg) in 80% acetone in the dark and was determined spectrophotometrically according to (DI, MB, and FR 1956) .

##### Photosynthetic parameters

The middle leaf was used to determine photosynthetic rate (P-max), stomatal conductance ( $g_s$ ), intercellular CO<sub>2</sub> concentration ( $C_i$ ) and transpiration rate (Tr) using Li-6800 Portable Photosynthesis System (Li-Cor. Inc.) as recommended by (Zhang *et al.*, 2020) . During the measurements, leaves were illuminated at 1500  $\mu\text{mol m}^{-2}\text{s}^{-1}$  using the LED light system.

##### Leaf water use efficiency

Leaf water use efficiency (WUE<sub>-leaf</sub>) was calculated according to (Niu *et al.* 2011) following the equation:  $\text{WUE}_{\text{-leaf}} = P_{\text{-max}} \div \text{Tr}$

##### Enzyme extraction and assay

Samples of fresh leaves were frozen in liquid nitrogen and finely ground by pestle in a chilled mortar, then 1g of the frozen powder was used to extract the antioxidant enzymes in 5 ml of extraction buffer containing (50 mM K-phosphate buffer, pH 7.6, and 0.1 mM Na<sub>2</sub>-EDTA). The mixture was then centrifuged at 20,000 g at 4°C for 15 min, and the supernatant fraction was extracted and used to analyse the enzymes, catalase (CAT), peroxidase (POX) and glutathione reductase (GR) according to (Taïbi *et al.*, 2016) .

##### Electrophoresis and protein pattern

Protein was extracted from plant leaves, then proteins profiling of samples was performed using SDS-polyacrylamide gel (SDS-PAGE) as described by (Laemmli, 1970). At the end of electrophoresis, gels were stained with 0.5% Coomassie Brilliant Blue G-250 for 45 min. Then gels were fixed in mixture solution of

Acetic acid and Ethanol (10%: 40% v/v) overnight on a shaker. After fixing, gel was washed with distilled water (Barakat, Hassan, and Darwesh 2017). Banding patterns were scored from each treatment.

#### Growth parameters and yield

At 70 DAS, plants were harvested with their roots remaining intact and were then rinsed thoroughly with water. The plants of each pot were then placed in labelled polythene bags and were brought into the laboratory to determine the growth parameters such as number of leaves/plants, leaf area/plant (using Planimeter, LI-3000C Portable Leaf Area meter, LI-COR), root length (cm) and root diameter (using Vernier Caliber), root fresh weight (g).

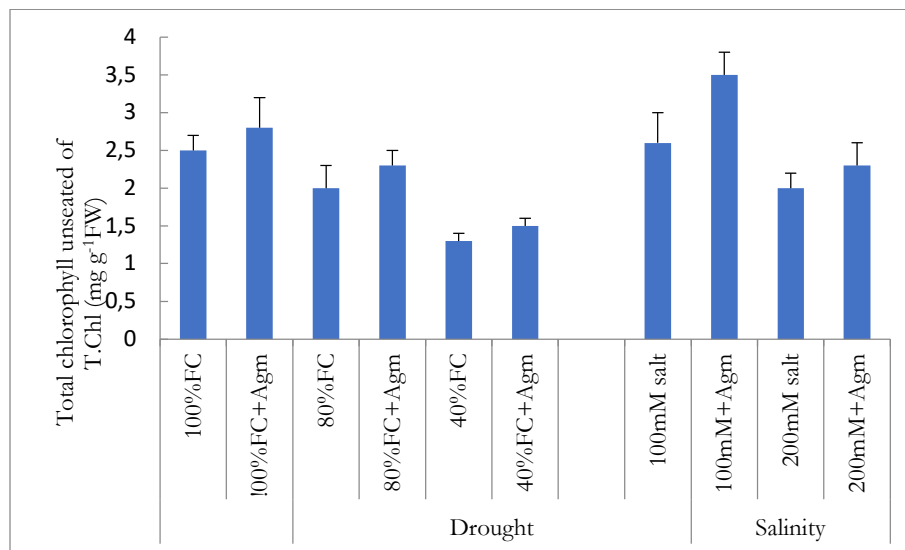
#### *Statistical analysis*

The collected data were statistically analysed using analysis of variance (ANOVA) as described by (Snedecor and Cochran 1980) with the aid of COSTAT computer software. Means were compared by least significant differences (LSD) at 5% levels of probability according to (Duncan, 1955).

### **Results**

#### *Total chlorophyll*

Data represented in Figure 1 showed that total chlorophyll in turnip plants significantly decreased under water stress or salt stress conditions. The water stress treatment was more deleterious on total chlorophyll content than salt stress treatments. In this concern, watering regime of 40% caused a reduction of about 40% in total chlorophyll as compared to 100% FC regime. The highest salt stress treatment (200 mM) caused a decline in total chlorophyll content by 20% as compared to control treatment. Agmatine improved the content of total chlorophyll either in stressed or non-stressed plants. The increase in total chlorophyll produced by agmatine treatment in non-stressed plants was about 12%. In 40% FC stressed plants, agmatine improved total chlorophyll by about 20% as compared with 40% FC stressed plants without agmatine. While total chlorophyll content of high salt (200 mM) stressed plants increased by about 15% in agmatine treated leaves as compared with salt stressed plants in the absence of agmatine.



**Figure 1.** Effect of agmatine on total chlorophyll content of turnip leaves grown under different watering regimes or salt concentrations.

Vertical bars indicate  $\pm$ SD.

*Leaf gas exchange*

All parameters of leaf gas exchange including  $P_{\max}$ ,  $g_s$ ,  $C_i$  and  $Tr$  were significantly decreased by water stress and salinity stress, while agmatine application improved all photosynthetic parameters (Table 1). The irrigation regime of 80% FC and 40% FC decreased leaf photosynthetic rate ( $P_{\max}$ ) by about 12% and 38%, stomatal conductance ( $g_s$ ) by about 11% and 29%, intercellular  $CO_2$  ( $C_i$ ) by 12% and 42% and transpiration rate ( $Tr$ ) by 35% and 56%, respectively, as compared to control treatment (100% FC). On the other hand, WUE increased significantly under both irrigation regimes (80% and 40% of FC) as compared with 100% FC. Moreover, salinity stress at 100 mM NaCl reduced  $P_{\max}$ ,  $g_s$ ,  $C_i$  and  $Tr$  by about 9%, 11%, 8% and 23%, respectively, relative to non-salinized plants. The high salt stress (200 mM NaCl) decreased all gas exchange parameters by about 26%, 29%, 27% and 28%, respectively. Agmatine treatments, on the other hand, caused an observed improvement in leaf gas exchange parameters of water stress and salt stressed and non-stressed plants.

*Leaf water use efficiency*

Data recorded in Table 1 illustrated that leaf WUE improved under water or salt stress as compared to unstressed plants. In this concern, at severe water stress (40% FC) and high salt stress (200 mM) WUE<sub>leaf</sub> increased by about 7% and 4%, respectively, as compared to non-stressed plants. While at moderate stress (80% FC and 100 mM NaCl) WUE increased by about 36.8 and 19.7%, respectively, as compared with non-stressed plants. Agmatine was found to improve WUE<sub>leaf</sub> of non-stressed plants, while in stressed plants agmatine didn't affect significantly the WUE<sub>leaf</sub>.

**Table 1.** Effect of agmatine on  $P_{\max}$ , Cond,  $C_i$ ,  $Tr$  and WUE-leaf of turnip plants grown under different watering regimes or salt concentrations

Traits		$P_n$ $\mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$	$G_s$ $\text{mol } H_2O \text{ m}^{-2} \text{ s}^{-1}$	$C_i$ $\mu\text{mol } CO_2 \text{ mol}^{-1}$	$Tr$ $\text{mmol } H_2O \text{ m}^{-2} \text{ s}^{-1}$	WUE <sub>leaf</sub> $\mu\text{mol mmol}^{-1}$
Drought (%FC)	Agmatine (mM)					
100	0	11.22±2.11	0.51±0.22	346±77	14.66±3.12	0.76±0.23
	0.5	12.31±3.24	0.62±0.28	348±79	14.97±3.56	0.82±0.27
80	0	09.81±2.66	0.40±0.17	303±68	09.45±2.11	1.04±0.36
	0.5	10.33±2.80	0.44±0.18	326±72	11.22±2.55	0.92±0.28
40	0	05.21±1.65	0.21±0.06	198±42	06.45±1.85	0.81±0.34
	0.5	06.84±1.87	0.33±0.10	226±64	07.56±2.11	0.90±0.27
Salt (mM)	Agmatine (mM)					
0	0	11.22±2.11	0.51±0.22	346±77	14.66±3.12	0.76±0.23
	0.5	12.31±3.24	0.62±0.28	348±79	14.97±3.56	0.82±0.27
100	0	10.14±2.34	0.45±0.17	318±82	11.15±4.12	0.91±0.23
	0.5	10.86±2.54	0.47±0.19	321±92	12.35±4.76	0.85±0.28
200	0	08.05±2.65	0.36±0.14	252±71	10.62±3.05	0.79±0.22
	0.5	08.68±2.73	0.40±0.15	273±86	11.13±3.43	0.78±0.21

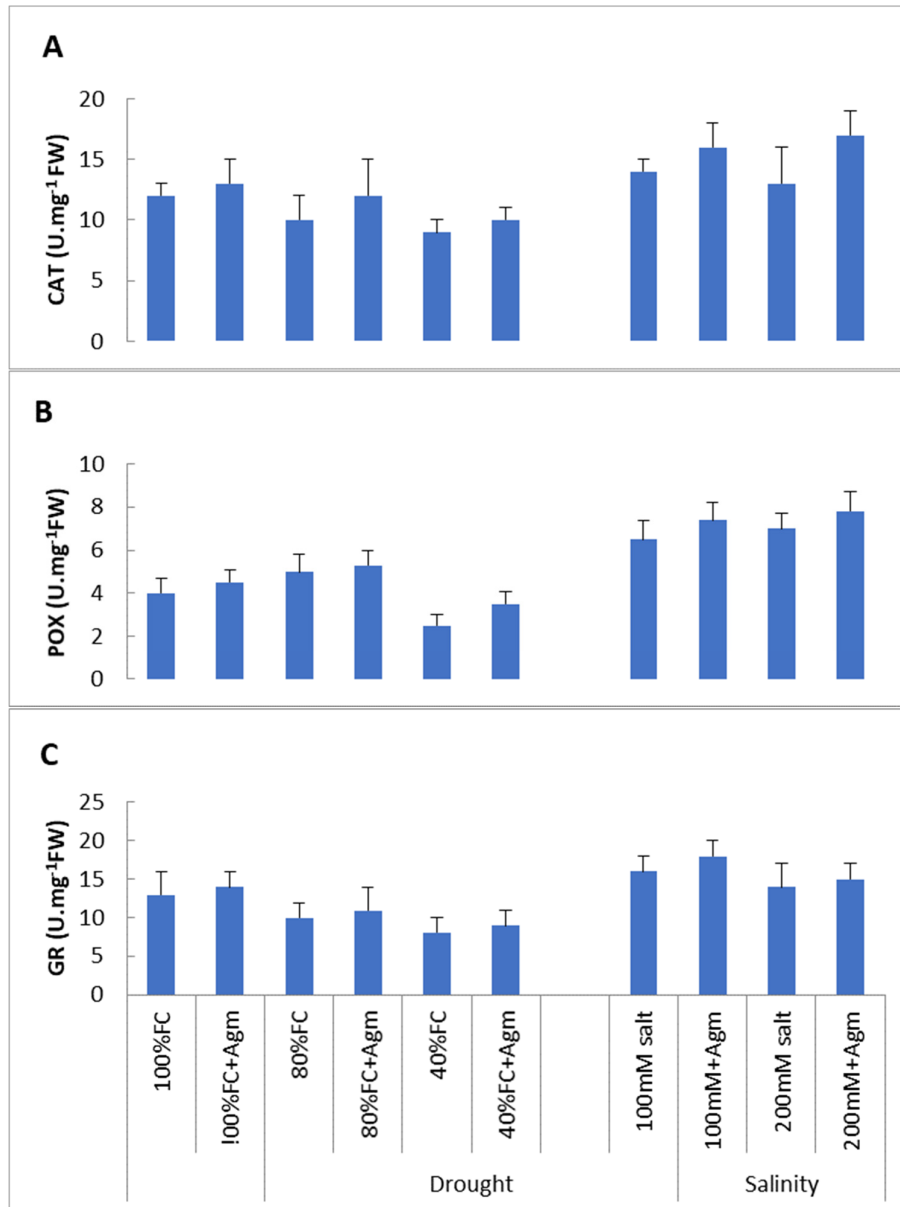
Each value is a mean of three replicates ± standard error (SE).  $P_n$ = Net photosynthesis;  $G_s$ = stomatal conductance;  $C_i$ = d intercellular  $CO_2$  concentration;  $Tr$  = Transpiration rate; WUE<sub>leaf</sub> = Water use efficiency

*Antioxidant enzyme activities*

Data recorded in Figure 2A showed that catalase (CAT) activity was decreased at 80% and 40% FC by about 20% and 25% as compared with 100% FC (control). In the contrary, peroxidase (POX) activity Figure 2B increased by about 25% under 80% FC treatment, while 40% FC caused a decline of about 35% in POX activity control treatment. Similarly, glutathione reductase (GR) activity Figure 2C decreased substantially by all watering regimes less than 100%. In this concern, 80% and 40% FC reduced GR activity by about 23% and

38%, respectively, relative to control. On the other hand, all salinity stress treatments caused an observed increase in all the tested antioxidant enzymes. The highest increase was observed at 100 mM NaCl treatment.

Agmatine treatments enhanced the activity of CAT, POX and GR in stressed and non-stressed plants. In non-stressed plants agmatine enhanced antioxidant enzyme activities by about 8-13% as compared to non-stressed plants. Data in the Figure 2A-2C showed that the effect of agmatine on antioxidant enzyme activities of salinized plants was more pronounced than its effect on water stressed plants.



**Figure 2.** Effect of agmatine on catalase (CAT), peroxidase (POX) and glutathione reductase (GR) of turnip plants grown under different watering regimes or salt concentrations  
Vertical bars indicate  $\pm$ SD.

#### *Protein electrophoresis*

Protein banding patterns of turnip leaves were revealed by SDS-PAGE and were presented in Table 2 and Figure 3 for the response to agmatine at different watering regimes and in Table 3 and Figure 4 for the

response to agmatine at different NaCl concentrations. According to the analysis, there were a total of 25 bands of turnip protein, with molecular weight (MW) ranging from 250 to 10 KDa. There were some differences in the number of bands obtained in response to irrigation regime or salt stress.

Data showed also that majority of polypeptides were found in all agmatine treated water deficit or regular irrigated turnip plants. However, under 80% FC polypeptides with 200, 180, 160, 150, 80 and 50 kDa were missed in all tested plants compared to control plants. While at 40% FC, polypeptide with polypeptides with 250, 190 and 60 kDa besides those missed under 80% FC disappeared in agmatine-untreated plants as compared to unstressed control plants. In contrast, polypeptides that disappeared at high salt concentration (100 mM NaCl) were much less than disappeared at high salinity stress (200 mM NaCl), at which only polypeptides with 200, 150 and 130 disappeared, but they reappeared by agmatine treatments.

**Table 2.** Effect of agmatine on protein profile of turnip leaves grown under different watering regimes

No	MW	A	B	C	D	E	F
1	250	+	+	+	+	-	+
2	240	+	+	+	+	+	+
3	230	-	-	+	-	+	+
4	220	+	+	+	+	+	+
5	210	+	+	+	+	+	+
6	200	+	+	-	-	-	-
7	190	+	+	+	-	-	-
8	180	-	-	-	-	-	-
9	170	-	-	+	+	+	+
10	160	+	+	-	-	+	-
11	150	+	-	-	-	+	+
12	140	+	+	+	+	+	+
13	130	-	-	+	+	+	+
14	120	+	-	+	+	+	-
15	110	+	-	+	+	+	-
16	100	-	+	+	+	+	-
17	90	+	+	+	+	+	+
18	80	+	+	-	+	-	-
19	70	+	+	+	+	+	+
20	60	+	+	+	+	-	-
21	50	+	+	-	-	+	+
22	40	+	+	+	+	+	+
23	30	+	+	+	+	+	+
24	20	+	+	+	+	+	+
25	10	+	+	+	+	+	+

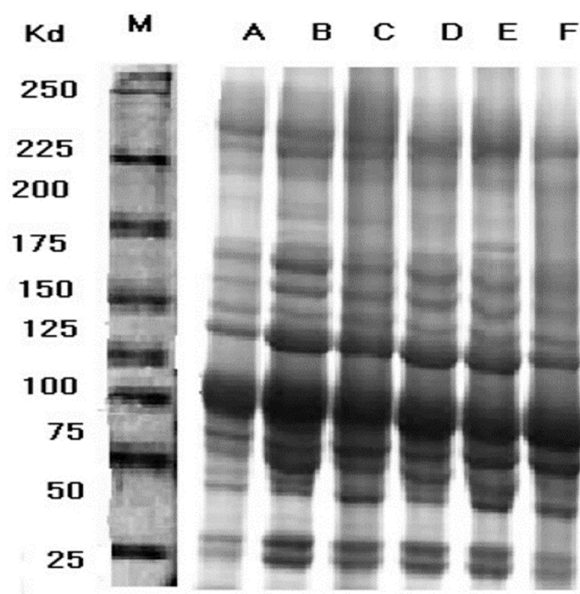
Where A, 100% FC; B, 100% FC + agmatine; C, 80% FC; D, 80% FC + agmatine; E, 40% FC; F=40% FC + agmatine.

**Table 3.** Effect of agmatine on protein profile of turnip leaves under different watering regimes

No	MW	A	B	C	D	E	F
1	250	+	+	+	+	+	+
2	240	+	+	+	+	+	+
3	230	-	-	-	+	+	+
4	220	+	+	+	+	+	+
5	210	+	+	-	+	+	+
6	200	+	+	+	+	-	+
7	190	+	+	+	+	+	+
8	180	-	-	-	-	+	+

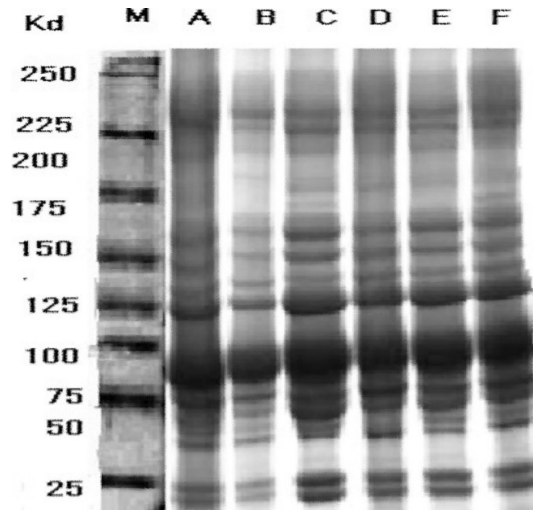
9	170	-	-	-	+	+	+
10	160	+	+	+	+	+	-
11	150	+	-	-	-	-	+
12	140	+	+	+	+	+	+
13	130	-	-	-	-	-	+
14	120	+	-	+	+	+	+
15	110	+	-	+	+	+	+
16	100	-	+	-	-	+	-
17	90	+	+	+	+	+	-
18	80	+	+	+	+	+	+
19	70	+	+	+	+	+	+
20	60	+	+	+	+	+	+
21	50	+	+	+	+	+	+
22	40	+	+	+	+	+	+
23	30	+	+	+	+	+	+
24	20	+	+	+	+	+	+
25	10	+	+	+	+	+	+

Where A,100% FC; B,100% FC + agmatine; C, 80% FC; D, 80% FC + agmatine; E, 40% FC; F=40% FC + agmatine



**Figure 3.** Effect of agmatine on protein pattern of turnip leaves under different watering regimes  
Where A,100% FC; B,100% FC + agmatine; C, 80% FC; D, 80% FC + agmatine; E, 40% FC; F=40% FC + agmatine.





**Figure 4.** Effect of agmatine on protein pattern of turnip leaves under different NaCl salt concentrations Where A, control; B, control + agmatine; C, 100 mM NaCl; D, 100 mM NaCl + agmatine; E, 200 mM NaCl; F=200 mM NaCl + agmatine.

#### *Growth and yield attributes*

Data recorded in Table 4 illustrated that both water stress and salinity stress significantly decreased most growth parameters as compared to the non-stressed plants. It was clear that the negative effect of water stress was more pronounced than that produced by salinity stress. In this concern, leaf area (LA) decreased by about 14% and 60% at 80% and 40% FC, respectively, as compared with non-stressed plants, while under 100 and 200 mM NaCl the LA decreased by about 10% and 30%, respectively.

**Table 4.** Effect of agmatine on leaf area (LA), leaf FW (LFW), root length (RL), root diameter (RD), root FW (RFW) and yield water use efficiency (WUE<sub>yield</sub>) of turnip plants grown under different watering regimes or NaCl concentrations

Stress type		Traits	LA (cm <sup>2</sup> ×1000)	LFW (g)	RL (cm)	RD (cm)	RFW (g)
Drought (%FC)	Agmatine (mM)						
100	0		24,0±8.2	125±32	08.55±2.15	06.55±2.11	225±37
	0.5		24,1±7.5	126±27	08.62±2.66	07.25±2.23	254±41
80	0		20,7±7.3	105±22	10.14±2.16	05.12±1.54	204±38
	0.5		22,8±8.1	118±15	11.45±3.11	06.01±2.51	211±40
40	0		09,7±2.6	076±18	09.64±2.66	04.00±1.43	166±43
	0.5		15,1±4.2	088±16	09.55±2.16	04.76±1.16	175±46
Salt (mM)	Agmatine (mM)						
0	0		24,0±8.2	125±32	08.55±2.15	06.55±2.11	225±37
	0.5		24,1±7.5	126±27	08.62±2.66	07.25±2.23	254±41
100	0		21,6±5.9	112±16	07.64±2.15	06.05±2.12	216±38
	0.5		23,8±5.6	121±26	08.44±3.10	06.85±2.15	220±46
200	0		16,5±3.3	086±21	06.11±2.14	04.66±1.34	185±34
	0.5		18,0±3.8	095±25	07.23±2.11	05.14±2.22	197±48

Each value is a mean of three replicates ± standard error (SE).

The fresh weight of leaves (LFW) declined by about 40% and 30% at 40% FC at 100 mM NaCl, and 200 mM NaCl, respectively. Root diameter (RD) declined by about 40% at 40% FC and by 30% at 200 mM NaCl. Similarly, root fresh weight (RFW) decreased by about 26% and 17%, respectively. On the

contrary, water stress significantly increased root length by about 18% and 13% at 80% and 40% FC, respectively, relative to non-stressed plants. Salinity treatments caused a reduction in RL of about 10% and 28% at 100 and 200 mM NaCl, at 100 and 200 mM NaCl, respectively.

Agmatine significantly enhanced all growth parameters of non-stressed and stressed plants. It seemed that agmatine counteracted the negative effects of water stress and salt stress on turnip growth and yield of tuberous roots. The highest values of LFW, RL, RD and RFW were observed in agmatine-treated plants as compared with agmatine-untreated plants.

## Discussion

Severe water stress (40% FC) and high salinity stress (200 mM NaCl) reduced significantly total chlorophyll content as a consequence of either fast breakdown or slow formation of the pigments (Kapoor *et al.*, 2020). To clarify this result, we assume that the stress conditions enhanced the production of toxic compounds such as  $H_2O_2$  leading to thylakoid membrane damage and decreasing chlorophyll content (Taïbi *et al.*, 2016). This reduction may be attributed to a decline in the expression of photosynthesis enzymes under severe water stress or salt stress (Sharma *et al.*, 2020). On the other hand, total chlorophyll content increased with foliar application of agmatine which may enhance the production of certain amino acids involve in chlorophyll biosynthesis (Anjum, 2011). Moreover, agmatine, as a precursor, enhances the formation of polyamine in plant tissue (Majumdar *et al.*, 2013). and reduces the negative effects of water stress and salt stress. In addition, external agmatine application was found to increase arginine intermediate in agmatine-polyamine network and performs as a signaling molecule involved in stress tolerance (Santisree *et al.*, 2020).

Photosynthetic rate ( $P_{max}$ ) was significantly decreased by water stress and salt stress, thus negatively affecting turnip growth and tuberose roots attributes (Table 4). These findings were similar to those observed by (Hussain *et al.*, 2018). The decrease of  $P_{max}$  due to abiotic stress was also reported in turnip plants (Mäkelä *et al.*, 2002), and was attributed to stomatal closure under water stress or salinity stress (Sharma *et al.*, 2020). It is well known that stomatal closure is the first response to water stress in most plant species. This closure is more closely related to soil moisture content, and it is primarily controlled by abscisic acid (ABA) production in dehydrating roots (Takahashi *et al.*, 2020) as under 40% FC treatment in the present study. According to (Zhang *et al.*, 2020), stomatal closure reduces the diffusion of  $CO_2$  in plant leaves and becomes more dominant compared to  $CO_2$  assimilation, potentially lowering the rate of photosynthesis rate and crop productivity. In agreement with earlier studies, the decrease in the photosynthetic rate seen in this study can be attributed to two different mechanisms: First, due to stomatal closure leading to the reduction of  $CO_2$  diffusion within leaves (reduced by about 12 and 42% under low (80% FC) and high (40% FC) water stress, respectively). Second, through reduction or inhibition of the photosynthetic enzyme (Rubisco) activity due to low intercellular  $CO_2$  ( $C_i$ ) concentration (Farooq *et al.*, 2017). Similarly, the concentration of  $C_i$  is also playing an important role in evaluating the effects of salinity on photosynthetic efficiency (Munns and Gilliham, 2015). Soil salinity stress may increase the leaf cellular concentrations of  $Na^+$  and  $Cl^-$ , reducing cell expansion and photosynthetic rate leading to the reduction of crop yield (Mickelbart *et al.*, 2015). In addition, the reactive oxygen species (ROS) produced under stress conditions can affect the integrity of cellular membranes, enzymes activities and photosynthetic apparatus leading to decreased  $P_{max}$  efficiency. Turnip plants exposed to agmatine application showed a better intercellular  $C_i$  concentration in the stressed leaves (Table 1), thus enhanced the leaf photosynthetic rate (Negrão *et al.*, 2017; Taha *et al.*, 2021).

In arid regions, WUE is a key indicator of crop productivity that connects water and nutrient cycling in agroecosystems (Li *et al.*, 2018). In agreement with the findings of (Zhang *et al.*, 2020), data recorded in the present study showed that water stress positively affected  $WUE_{leaf}$  values, while salinity stress showed a negative effect on WUE values (Table 1). Moreover, water deficit enhanced  $WUE_{leaf}$  much more than salinity stress. Agmatine addition significantly improved WUE of non-stressed plants, while in stressed plants agmatine didn't affect significantly the  $WUE_{leaf}$ . The positive effect of water stress and salinity stress on  $WUE_{leaf}$  could

be attributed mainly to stomatal closure and transpiration rate reduction under stress conditions (Wang *et al.*, 2018), which reduce water evaporation and enhance  $WUE_{leaf}$ . The inversed results were probably caused by root growth responding to water stress and salinity stress in distinct ways, since root length increased significantly under water stress, and obtained water from soil (Uddin *et al.*, 2018), but decreased under salinity stress. Catalase (CAT), peroxidase (POX) and glutathione reductase (GR) are commonly considered as important components of plants' antioxidant defence (Khan, 2020) which play an important role in trapping harmful free radicals. Our results illustrated that water deficit and salinity stress had different effects on the activity of these antioxidant enzymes. There was a linear decline in CAT and GR activities with decreasing irrigation regime to 40% FC, while POX activity increased at 80% FC and decreased significantly at 40% FC. On the contrary, in accordance with (Al Kharusi, 2019) salinity stimulated all the tested antioxidant enzymes. Our data showed that exogenous application of agmatine was found to increase the activity of antioxidant enzymes of turnip plants particularly under stress conditions. Increasing the capability of antioxidant enzymes to remove ROS in response to agmatine treatment under water stress or salt stress could improve plant tolerance and enhance growth parameters. (Pathak, 2014) illustrated that agmatine converts into polyamines, that plays an important role in scavenging ROS. Polyamines, in turn, was reported to play a crucial role in stress signalling to induce tolerance responses (Chen *et al.*, 2019). Furthermore, agmatine may form more efficient complexes with CAT than the isolated single enzyme (Sang *et al.*, 2016).

An interesting result in the current study was that clear differences in protein bands in response to water stress and salt stress. Analysis of the SDS-PAGE of the total proteins extracted from leaves of control and stressed plants showed that water stress was linked to the loss or reduction of certain protein bands (Figure 3). In contrast, high salt stress (200 mM NaCl) stimulated the induction of a new protein bands, while low salt stress (100 mM NaCl) had little effect on the protein pattern (Figure 4). The appearance of some protein bands which disappeared by water stress in response to foliar application of agmatine may predict that some stress responsive genes exist and linked to agmatine action in adaptation to water deficit (Barakat *et al.*, 2015). Protein expression changes in turnip plants subjected to water stress or salt stress and/or treated with agmatine, as indicated by protein profiles, may have had an important role in some physiological processes such as redox regulation.

As usually, water stress or salt stress can postpone plant growth and reduce fresh weight of shoots and roots with decreasing irrigation regime or increasing salt stress (Liu *et al.*, 2014). The present study showed that water stress was more deleterious on growth parameters of turnip than salinity stress effect. Most growth parameters including leaf area, leaf fresh weight, root diameter and root fresh weight were significantly decreased under water deficit conditions. While root length increased in order to search for water in the soil. Water scarcity and salt stress are also affecting a wide range of biochemical components and physiological processes including chlorophyll, photosynthesis and stomatal regulation (Kapoor *et al.*, 2020). Thus, both stress conditions would impair gas exchange and ultimately plant performance.

## Conclusions

The results of foliar application of agmatine on turnip plants grown in pots under water deficit or salt stress conditions. The 40% FC reduced chlorophyll by about 40%, photosynthetic rate by about 38%, stomatal conductance by about 29% and intercellular  $CO_2$  by about 42% as compared with control treatment. The results showed that agmatine treatment improved water stress and salinity tolerance in turnip plants through regulation of photosynthetic rate, stomatal conductance, transpiration rate and increasing the activity of some antioxidant enzymes as well as synthesis of new polypeptides. The appearance of some protein bands may consider an important signal to recognize the alleles associated with the stress tolerance in turnips.

### Authors' Contributions

Both authors read and approved the final manuscript.

### Ethical approval (for researches involving animals or humans)

Not applicable.

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### Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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