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The Role of Nitrogen in Inducing Salt Stress Tolerance in *Crocus sativus* L.: Assessment Based on Plant Growth and Ions Distribution in Leaves

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Abstract: The role of nitrogen (N) in inducing salt stress tolerance in plants is not well understood, and the question is more complicated in saffron (*Crocus sativus* L.), which is sensitive to both nitrogen rates and salinity. The present study was conducted to investigate the effects of different N (0, 50 and 150 kg ha^{-1}) supplies on saffron growth and ions concentration in shoots under several salt stress levels (0, 3, 6 and 9 dS m^{-1}). Salinity negatively affected plant growth assessed by leaves number, leaves length, shoot dry weight, corms number and corms weight. Moreover, there was a clear direct correlation between higher salinity value and less plant growth. Different effects due to salinity and nitrogen were evident in terms of the number and length of leaves during the growing season from day 60 after first irrigation (DAF) and achieved a peak after 90 DAF. Salt stress also affected the ions balance, as Na^+, Cl^- and Ca^{2+} were enhanced and K^+ was reduced, thereby damaging the plants. Nitrogen partially mitigated the negative impacts of salinity on plant growth and ions balance, although this compensatory effect was observed when nitrogen supply was set at 50 kg N ha^{-1}. For example, in 2019–2020, the losses in shoot dry weight due to 9 dS m^{-1} salinity amounted to 47%, 44% and 54%, at 0, 50 and 100 kg N ha^{-1} respectively, thus indicating a less negative effect of salinity at 50 kg N ha^{-1}. Moreover, at 100 kg N ha^{-1} the negative effect of salinity was stronger for six and nine dS m^{-1}. Our findings suggested that the optimum N supply (50 kg N ha^{-1}) strengthened the plant under non-saline and moderately saline (6 dS m^{-1}) conditions, and consequently improved salt tolerance.

Keywords: chlorine; corm; leaf; potassium; salt; sodium

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1. Introduction

Saffron (*Crocus sativus* L.) stigma is the most expensive spice in the world. It is obtained from saffron plants that are cultivated in arid and semi-arid climates [1]. As saffron needs small amounts of water, it could be suitable for sustainable agriculture in regions with limited high-quality water resources. Plant growth and yield, as well as the spice quantity and quality decrease after approximately four–five years. This reduction is due to an increasing competition for water and nutrients as a result of saffron intensive cultivation [2]. The water sources used to irrigate saffron fields recently have experienced increasing electrical conductivity, and, consequently, salinity [3].

Soil salinity affects irrigated agricultural land by 20–50%, with an approximate annual economic loss of more than 12.6 billion USD. The impact of salt stress on plants can be categorized into two phases: osmotic stress and ion toxicity. As NaCl is the most prevalent salt, plants prefer its accumulation compared to other elements available in low concentrations, such as potassium ions. Moreover, most of the plants are capable of excluding sodium and chloride ions from roots when water is absorbed from the soil. Ion toxicity occurs when an over-accumulation of salt occurs in the plant cytoplasm, thereby inhibiting photosynthetic activity, protein synthesis, and other developmental processes. While osmotic stress is dealt with osmotic tolerance, on the other hand, ionic stress is dealt with Na^{+} exclusion and tissue tolerance [4,5]. Although it is believed that saffron is a salt-sensitive crop [6], there is some evidence that this plant is suitable for growth in lightly or moderately saline soil, whereas carbonate deficiency can be a limiting growth factor [7–10]. Sepaskhah and Kamgar [11] proved that the vegetative growth of saffron is not sensitive to water salinity and found a low vegetative growth reduction even at a salinity value of 4 dS m^{-1}. Some researchers consider that saffron is capable of producing acceptable yields under severe salt stress conditions [9].

Some approaches can be explored for reducing the negative impact of salinity on saffron growth and yield, such as: (i) selecting a salinity tolerant population; (ii) leaching the soil to prevent salts accumulation; and (iii) managing nutrients supply. The most important nutrient for the better growth of saffron and higher yield and quality is nitrogen [12], and almost 20–80% of saffron productivity depends on soil fertility, and, in particular, nitrogen availability [13]. Saffron is a low nutrient-demanding crop; nevertheless, about 10 kg of N are subtracted from the soil for each kilogram of harvested dry biomass [14]. A supply of about 100 kg N ha^{-1} is considered the optimum amount to increase saffron yields; however, in some fields up to 100 kg ha^{-1} ammonium phosphate with the first irrigation, and 100 kg ha^{-1} urea as a split, are used [7]. The optimum nitrogen supply is more important in saffron compared with other plants, as a higher N rate promotes vegetative growth and reduces stigma yield. Ensuring the balance between vegetative and reproductive growth is one of the most important aims in saffron fertilization [15]. Amiri [15] stated that N uptake is high in the first year and markedly increases in the second year; and consequently, increases the flowering rate as well as the length of the flowering period.

Some experimental evidence has demonstrated that saffron can be cultivated with brackish or semi-saline water, however, this feature has been neglected in the literature, which contains little information about the salinity tolerance of saffron. On the other hand, there is no general consensus about the effect of N in saline conditions, and it is not totally understood whether N improves salinity tolerance or intensifies the negative effect of salt stress. Therefore, our research was designed to answer these two questions: (1) What is the salt tolerance threshold of saffron? and (2) Can increasing nitrogen levels improve salinity tolerance of saffron? To fill this gap in the research was the main aim of this study, where the response of *Crocus sativus* L. to various salt stress conditions at different N supply in a controlled environment was evaluated.

2. Materials and Methods

2.1. Experimental Design and Procedures

This research aimed at evaluating the effects of nitrogen and salinity on *Crocus sativus* L. was conducted in the Research Greenhouse of the National Salinity Research Center at Yazd during the plant growing seasons of 2018–2019 and 2019–2020. Yazd, as one of the driest cities in Iran, has a hot desert climate (BWh in Köppen climate classification), with a yearly precipitation amount of 49 mm and 23 days of precipitation [16]. In this region, summer temperatures are very frequently above 40 °C in blazing sunshine with no humidity (Figure 1). The study investigated four different salinities of irrigation water: 0 (as control), 3, 6 and 9 dS m^{-1}; and three nitrogen supply rates: 0 (as control), 50 and 100 kg N ha^{-1}. The treatments were set in a factorial experiment based on a completely randomized design (CRD) with three replications.

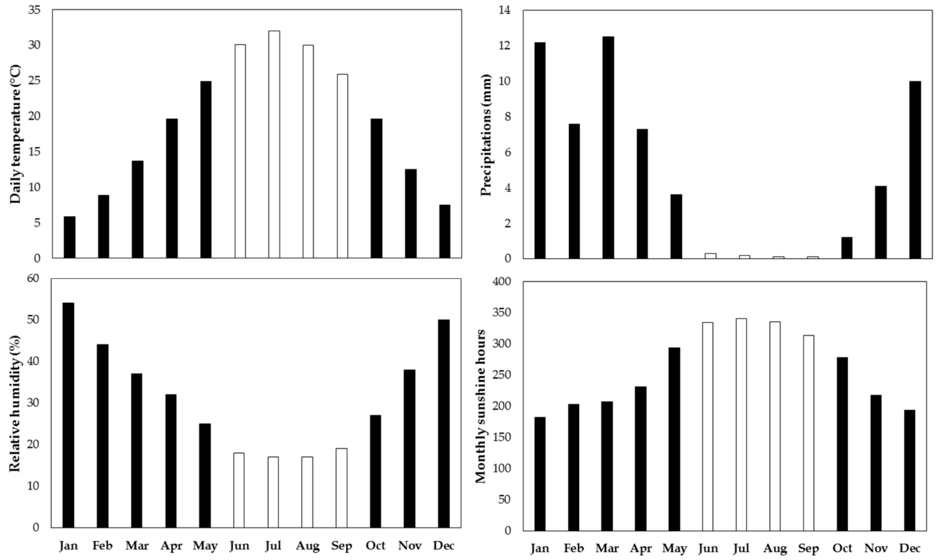

Figure 1. Long-term average (1950–2020) of climatic properties including daily mean temperature, precipitation, relative humidity and mean monthly sunshine hours in Yazd. The black columns show the life cycle of saffron in this experiment.

Saffron corms are obtained through asexual reproduction [17], and the corms needed for this experiment were Tortbat ecotypes obtained from Tortbat Heydarieh, one of the most important saffron production regions in the world. Five uniform corms were sown in 15 L pots on 20 October 2018, and filled with soil, washed sand and cow manure with 2:1:1 weight ratio. Prior to any experiment, soil samples (Table 1) and cow manure (Table 2) were tested, and their physico-chemical properties were evaluated. In the growing season of the second year, the first irrigation was performed on 18 October 2019 with the related salinity treatments.

Table 1. The physico-chemical properties of the soil used for the experiments.

EC_e (dS m^{-1})	Texture	FC (%)	PWP (%)	pH	K (mg kg^{-1})	P (mg kg^{-1})	N (%)	OM (%)
1.23	Sandy loam	22.40	7.60	7.13	180.10	12.23	0.06	0.44

EC_e: Electrical conductivity of saturated soil extract, FC: field capacity, PWP: permanent wilting point, pH: soil reaction, K: potassium, P: phosphorous, N: nitrogen, OM: organic matter.

Table 2. The chemical analysis of the cow manure used to fill the pots.

EC	pH	OC	N	P	K	Ca	Fe	Mn	Zn	Cu
(dS m^{-1})		(%)	(mg kg^{-1})							
2.90	7.41	48.32	11212.0	467.21	945.50	46.12	2546.30	285.22	218.60	26.83

EC: Electrical conductivity, pH: soil reaction, OC: organic carbon.

Different salinity values were obtained by diluting the water of a natural saline well with distilled water. The chemical properties of the well water are presented in Table 3. The electrical conductivity (EC) of the obtained solutions was checked with a portable EC-meter. Salinity treatments were applied gradually to avoid sudden shock to the plants. The EC of drainage was monitored during the experiment to control the soil salinity. About 40% of nitrogen supply (46% N) was added to the pots in terms of urea with the first irrigation for each year of investigation (20 October 2018 and 18 October 2019). The remaining N was applied during the experiment in two steps: the first addition of 30% after flowering (20–22 days after sowing) and the second addition of a further 30% at the maximum leaf growth (135–138 days after sowing). Irrigation was performed at 100% field capacity (FC) according to a 25% leaching fraction. For this purpose, the amount of water to reach 100% of the field capacity (22.4%) was calculated by continuous weighing of the pots.

Table 3. The chemical properties of the used saline water for preparing the salinity treatments.

EC	pH	HCO_3^-	Cl^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	Na^+	K^+
(dS m^{-1})		(meq L^{-1})						
14.79	7.77	3.44	103.22	30.11	11.30	30.43	95.66	0.66

EC: Electrical conductivity.

2.2. Samplings and Measurements

The length and number of leaves were measured four times during the growing seasons. The length and number of the leaves of all five plants in 2018 and five randomly selected plants in 2019 were measured. Saffron plants were cultivated after flowering as long as the leaves dried out (i.e., 25 April 2018 and 23 April 2019). At the end of the experiments in both years, the dried leaves were harvested to measure the dry weight and ions concentration (sodium, potassium, chlorine, calcium, and magnesium). Dry weight was obtained after keeping the samples in the oven at 70 ± 2 °C for 48 h. The concentration of sodium (Na^+) and potassium (K^+) were measured with a flame photometer [18], concentration of chlorine (Cl^-) was obtained with titration method [19] and spectrophotometry was used for measuring calcium (Ca^{2+}) and magnesium (Mg^{2+}) concentrations [20]. In the second year, the corms were removed from the soil and their weight and number were calculated.

2.3. Statistical Analyses

Data in a factorial set were subjected to analysis of variance (two-way ANOVA), and the means were separated using the least significant difference (LSD) test at a 5% probability level, or the standard error values (±SE). The statistical analyses were conducted by SAS software version 9.1.

3. Results

3.1. Leaf Growth

Although the leaf number in all the salinity, as well as nitrogen supply, conditions was increased over the time in both years, significant differences were noticed by comparing the two investigated conditions (Figure 2). Actually, no significant difference was evident, except for 9 dS m^{-1} of salinity and all nitrogen supply rates 30 days after the first irrigation (DAF) in 2018–2019, whereas, differences were significant from day 60 after DAF, and remarkable at day 90 after DAF. High salinity levels were significantly responsible for decreasing the number of leaves, so the highest leaf number was obtained by the control test with the lowest amount of salinity (9 dS m^{-1} level). Nitrogen supply had less of an effect on leaf growth; however, the leaf number per plant was significantly higher at a 50 kg ha^{-1} nitrogen supply rate compared to 0 and 100 kg ha^{-1} for almost all the salinity conditions (Figure 2). A similar trend of the effects due to salinity level and nitrogen supply on leaf number was also observed in the following year, 2019–2020 (Figure 3); therefore, trends were almost the same for the two years. The highest leaf number (5.3 $plant^{-1}$) in 2018–2019 was obtained at day 120 after DAF with 50 and 100 kg N ha^{-1} supply rates and in non-saline conditions (Figure 2). The highest leaf number (5.7 leaf $plant^{-1}$) in 2019–2020, was obtained at day 120 after DAF with 50 and 100 kg N ha^{-1} nitrogen supply and at 3 dS m^{-1} salinity level (Figure 3).

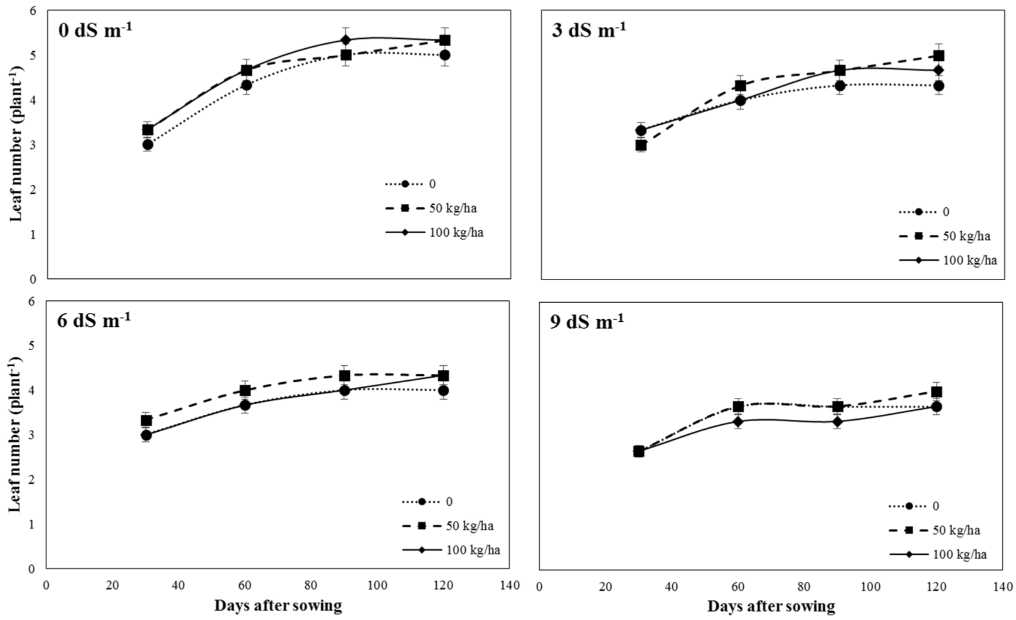

Figure 2. The effect of different nitrogen supply rates (0–100 kg N ha^{-1}) on leaf number of saffron (*Crocus sativus* L.) under varied salinities (0–9 dS m^{-1}) in 2018–2019. The average with overlap had no significant difference based on standard error (±SE).

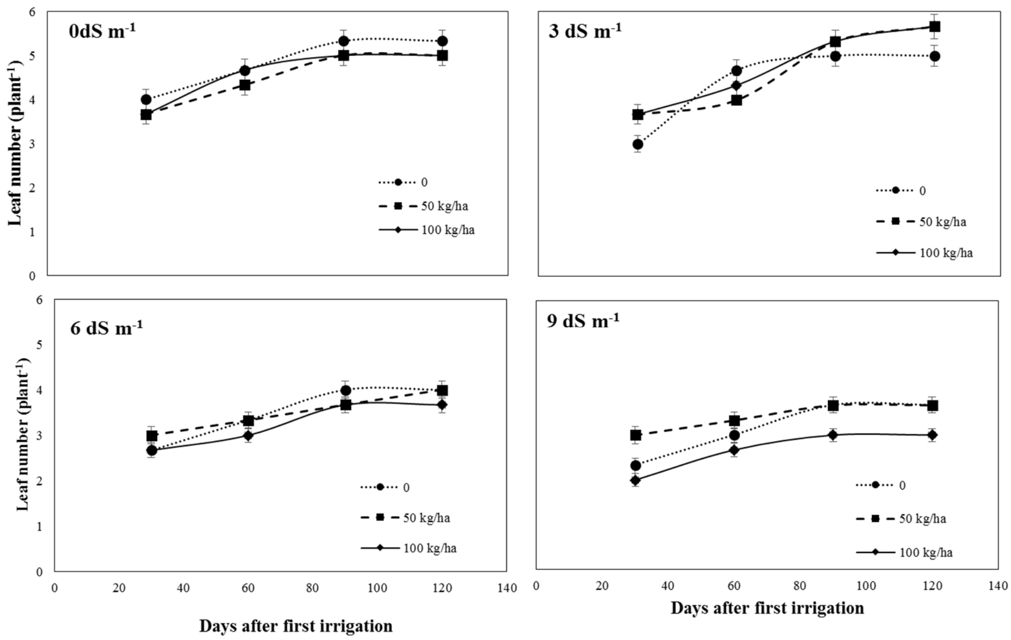

Figure 3. The effect of different nitrogen rates supply (0–100 kg N ha^{-1}) on leaf number of saffron (*Crocus sativus* L.) under varied salinities (0–9 dS m^{-1}) in 2019–2020. The average with overlap had no significant difference based on standard error (±SE).

Compared to the leaf number, the effect of salinity and nitrogen supply rates was more pronounced on leaf length (Figures 4 and 5). In 2018–2019, leaf length did not change remarkably from 30 to 60 days after DAF in all the tests, whereas, a significant increase in leaf length was evident at days 90 and 120 after DAF (Figure 4). In 2019–2020, the fast leaf growth phase started earlier and a significant increase in leaf length was observed at day 60 after DAF (Figure 5). The leaf length increased with increasing nitrogen supply rates in all salinity conditions, except in 9 dS m^{-1}. Maximum and minimum leaf lengths were obtained with 100 and 0 kg N ha^{-1} nitrogen supply rates, respectively. At 9 dS m^{-1}, the effect of nitrogen supply was less evident when compared to other salinity levels. In fact, salinity levels of 6 and 9 dS m^{-1} caused a significant decrease in leaf length. On average, leaf length under 6 and 9 dS m^{-1} salinity levels were shorter compared to non-saline conditions by 27.7% and 43.6% in 2018–2019 and by 33.4% and 61.8% in 2019–2020, respectively.

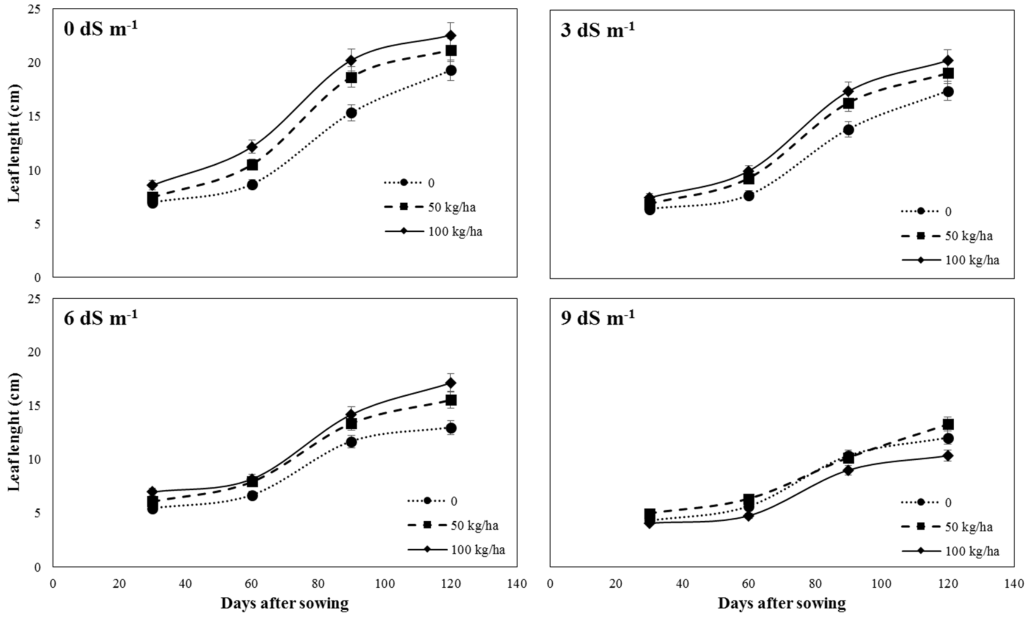

Figure 4. The effect of different nitrogen supply rates (0–100 kg N ha^{-1}) on leaf length of saffron (*Crocus sativus* L.) under varied salinities (0–9 dS m^{-1}) in 2018–2019. The average with overlap had no significant difference based on standard error (±SE).

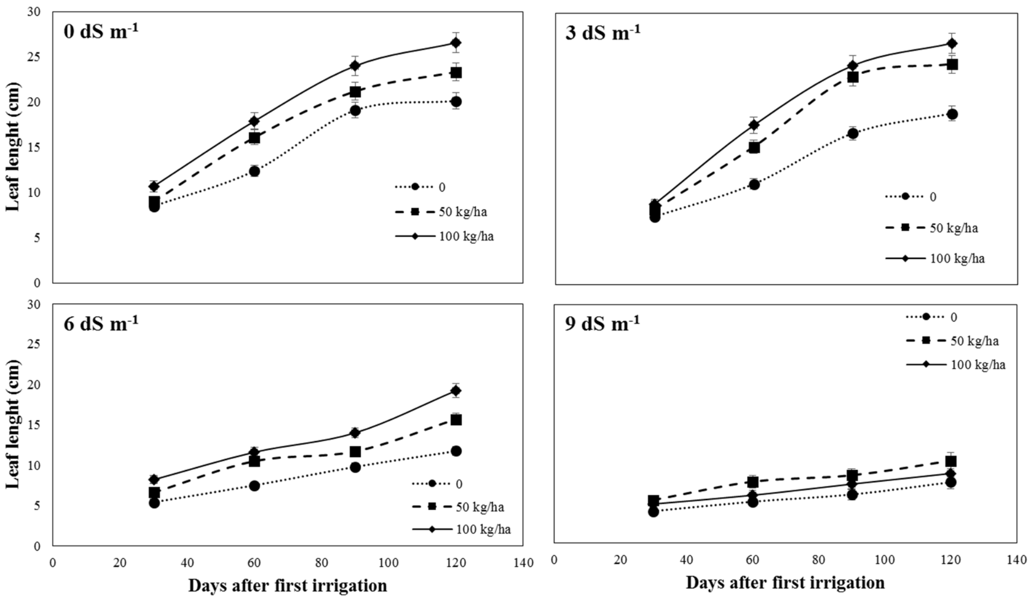

Figure 5. The effect of different nitrogen rates (0–100 kg N ha^{-1}) on leaf length of saffron (*Crocus sativus* L.) under varied salinities (0–9 dS m^{-1}) in 2019–2020. The average with overlap had no significant difference based on standard error (±SE).

3.2. Shoot and Corm Growth

In both years, saffron shoot dry weight was significantly affected by nitrogen supply rates and salinity conditions (Figure 6). Salinity was associated with a significant decrease in shoot dry weight, whereas nitrogen was associated with a significant increase. The loss in shoot dry weight due to salinity depended on plant growth stress. Salinity levels of 3, 6 and 9 dS m^{-1} were responsible for a decrease in shoot dry weight of 27.3%, 50.2% and 71.6% in 2018–2019 and of 13.0%, 34.0% and 50.8% in 2019–2020, respectively. The opposite effect due to nitrogen supply resulted in percentages not closely related to those obtained under salinity conditions. In 2018–2019, for example, shoot dry weight in 50 and 100 kg N ha^{-1} supply rates was significantly higher compared to non-N supply, as the increase amounted for 35.8% and 36.0%, respectively (Figure 6). The use of a moderate nitrogen supply rate (50 kg N ha^{-1}) mitigated the negative effect due to salinity stress on shoot dry weight, thus saffron tolerance was significantly higher under a moderate nitrogen supply rate. The amount of shoot dry weight loss due to 9 dS m^{-1} salinity in 0, 50 and 100 kg N ha^{-1} supply rates in 2019–2020 was 47.0%, 44.8% and 54.4%, respectively, thus proving a less negative effect of salinity in 50 kg N ha^{-1}.

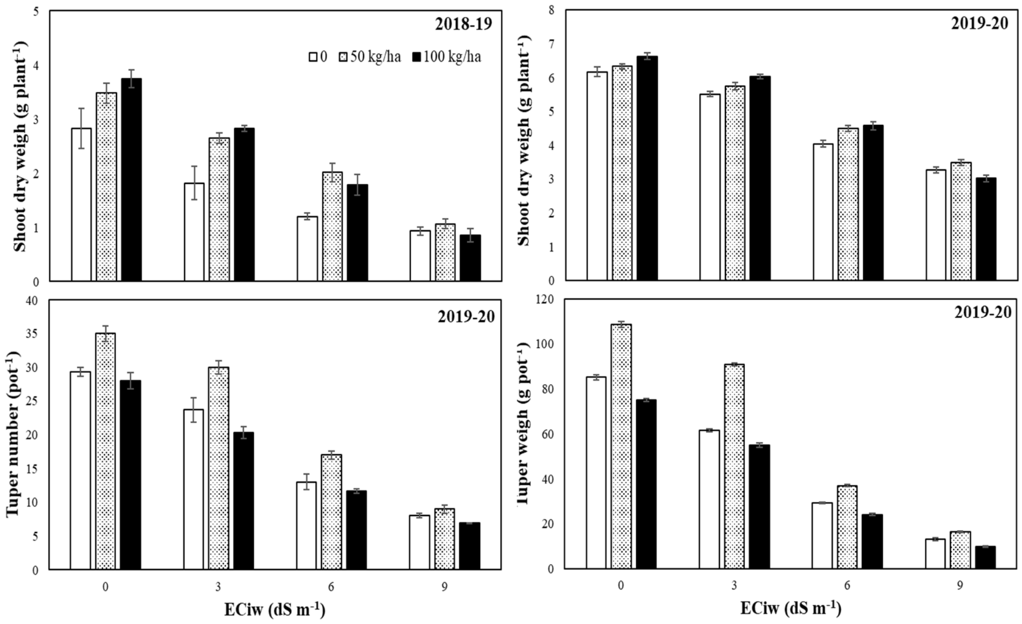

Figure 6. The effect of different nitrogen supply rates (0–100 kg N h^{-1}) on shoot dry weight in 2018–2019 and 2019–2020, and on corm number and weight (±SE) of saffron (*Crocus sativus* L.) in 2019–2020 under varied salinities (0–9 dS m^{-1}). The average with overlap had no significant difference based on standard error (±SE).

Salinity had a negative effect on the number and dry weight of corms (Figure 6), as 3, 6 and 9 dS m^{-1} salinity levels were responsible for a decrease in corms numbers of 19.9%, 54.9% and 74.0%, and in corms dry weight of 22.8%, 66.3% and 85.3%, respectively, when the results were compared with non-saline conditions. Nitrogen showed a conflictual effect on saffron corms (Figure 6), as the corms number and dry weight increased at 50 kg N ha^{-1} supply rate and decreased at 100 kg N ha^{-1} supply rate compared to non-N supply conditions. On average, 50 kg N ha^{-1} increased the corms number and dry weight by 23.0% and 33.7%, respectively, and 100 kg N ha^{-1} decreased the corms number and dry weight by 9.5% and 13.4%, respectively. A positive effect due to a moderate nitrogen supply was observed in both non-saline and saline conditions. The effect of nitrogen supply under non-saline and mild salinity conditions was more pronounced than under severe salinity conditions. Actually, nitrogen supply did not significantly increase the corms growth in severe salinity conditions (Figure 6).

3.3. Ions

Salinity conditions were responsible for the increase of sodium (Na^+) and chlorine (Cl^-) ions concentration and the decrease of that of potassium (K^+) ion in saffron leaves in both years (Figure 7). The Na^+ and Cl^- concentrations were significantly higher in

2019–2020 than 2018–2019, whereas K^+ concentrations was higher in 2018–2019 than 2019–2020. Salinity levels of 3, 6 and 9 dS m^{-1} increased the Na^+ concentration by 1.92, 2.91 and 4.21 holds in 2018–2019 and 1.67, 2.92 and 3.58 holds in 2019–2020, respectively (Figure 7). The Cl^- concentrations in saffron plants under 3, 6 and 9 dS m^{-1} salinity levels were higher than in non-saline conditions by 1.75, 3.73 and 3.76 holds in 2018–2019 and by 1.74, 3.23 and 3.96 holds in 2019–2020, respectively. Salinity levels higher than 3 dS m^{-1} caused a decrease in K^+ concentration (Figure 7), for example 6 dS m^{-1} salinity was responsible for a decrease in K^+ concentration of 60.0% and 89.3% in 2018–2019 and 2019–2020, respectively. Furthermore, 9 dS m^{-1} induced a decrease in K^+ concentration of 63.7% and 90.8% in 2018–2019 and 2019–2020, respectively.

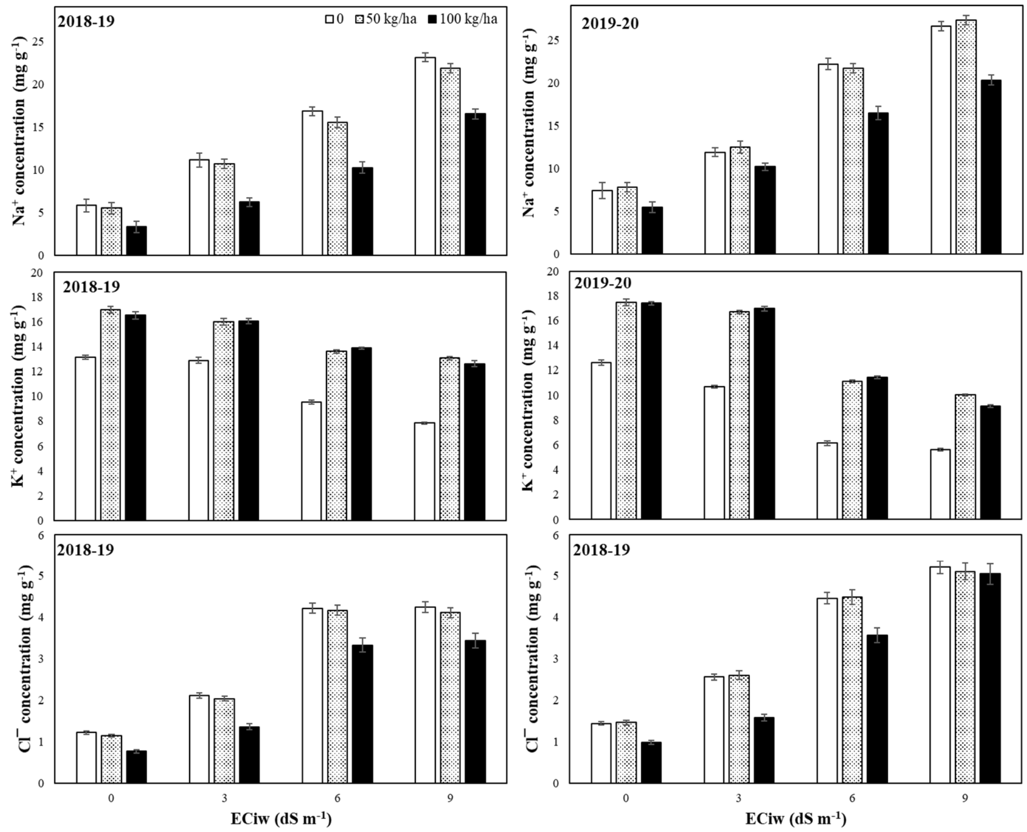

Figure 7. The effect of different N supply rates (0–100 kg N h^{-1}) on sodium (Na^+), potassium (K^+) and chlorine (Cl^-) of saffron (*Crocus sativus* L.) in 2019–2020 and 2019–2020 under in varied salinities (0–9 dS m^{-1}). The average with overlap had no significant difference based on standard error (±SE).

A supply rate of 50 kg N ha^{-1} had no effect on the Na^+ and Cl^- concentrations (Figure 7), while that of 100 kg N ha^{-1} was responsible for a decrease of Na^+ and Cl^- concentrations. Moreover, supply rates of 50 and 100 kg N ha^{-1} increased K^+ concentration with no significant difference between the different rates. A supply rate of 50 kg N ha^{-1} induced a decrease by 36.3% and 22.9% of Na^+ concentration and by 24.6% and 18.2% of Cl^- concentration in 2018–2019 and 2019–2020, respectively (Figure 7). The ratio between Na^+ and K^+ (Na/K) was affected by salinity and nitrogen supply in accordance with their effect on Na^+ and K^+ concentrations (Table 4). Salinity significantly increased the Na/K ratio in both years, while the effect of 3 dS m^{-1} salinity on this ratio was not significant in 2019–2020. In both years, nitrogen supply was responsible for a decrease of Na/K ratio: the highest and lowest Na/K ratio were obtained with no-N supply and 100 kg N ha^{-1} supply rate, respectively (Table 4).

Table 4. The effect of different N supply rates (0–100 kg ha^{-1}) and different salinity conditions (EC_{iw}, 0–9 dS m^{-1}) on Na/K ratio, as well as Ca^{2+} and Mg^{2+} concentration in saffron (*Crocus sativus* L.) shoot in 2019–2020 and 2019–2020.

N (kg ha^{-1})	Na/K				Ca (mg kg^{-1})				Mg (mg kg^{-1})			
	2018–2019		2019–2020		2018–2019		2019–2020		2018–2019		2019–2020	
0	1.51	a	2.51	a	65.9	a	72.1	a	30.1	a	36.1	a
50	0.95	b	1.47	b	68.9	a	74.5	a	33.7	a	32.9	a
100	0.66	c	1.15	c	62.6	a	76.3	a	35.6	a	40.8	a
ECiw (dS m^{-1})												
0	0.32	D	0.45	C	60.4	B	66.8	B	32.7	A	37.2	A
3	0.64	C	0.82	C	62.6	B	71.0	B	33.3	A	35.0	A
6	1.21	B	2.34	B	61.4	B	71.1	B	33.7	A	36.5	A
9	1.98	A	3.23	A	78.8	A	88.3	A	32.7	A	37.7	A

The means with similar letter in each column have no significant differences based on LSD 0.05.

The interaction of salinity and nitrogen supply on Na^+ and Cl^- concentrations of saffron leaves showed that the effect of salinity was more evident under the highest N supply rates (Figure 7). In 2018–2019, for example, an increase in Na^+ concentration due to 9 dS m^{-1} salinity was associated with an increase of about four-fold and five-fold in Na^+ concentration under non-N and 100 kg N ha^{-1} supply rates, respectively. These values were 2.6- and 4.2-fold higher for Cl^- concentrations in 2019–2020, respectively. In all salt stress conditions, no significant difference was observed between non-N and 50 kg N ha^{-1} supply rates (Figure 7), thus showing that salinity reduced the positive effect of N supply on Na^+ and Cl^- concentrations. The supply rates of 50 and 100 kg N ha^{-1} increased K^+ concentration in all the saline conditions as well as non-saline (Figure 7), however, no significant difference was observed between the two N supply rates. The highest K^+ concentrations in 2018–2019 and 2019–2020 were 16.95 and 17.43 mg g^{-1}, respectively, observed for 50 kg N ha^{-1} under non-saline condition.

Nitrogen had no significant effect on Ca^{2+} and Mg^{2+} concentrations in saffron leaves in both years (Table 4). The effect of salinity on Mg^{2+} concentration was not significant, whereas affected the Ca^{2+} concentration. The highest and the lowest Ca^{2+} concentrations in both years were observed in non-saline and 9 dS m^{-1} conditions, respectively. However, no significant differences were found between 0, 3 and 6 dS m^{-1} in both years. The Ca^{2+} concentration in 9 dS m^{-1} was significantly higher compared to non-saline condition, thus accounting for an increase by 30.5% in 2018–2019 and 32.2% in 2019–2020 (Table 4).

4. Discussion

Although both parameters, leaf number and leaf length of saffron, were affected by salinity and nitrogen supply, leaf length was more sensitive. The leaf number did not change considerably and varied between three to six leaves per plant. It seems that the leaf number is genetically influenced and almost constant, having no direct relationship with environmental conditions. Behnia et al. [13] observed a positive trend between leaf growth and fresh flower weight as well as stigma yield and concluded that an optimum number of leaves has a positive effect on flower production, however, beyond that might have little or no effect.

However, both parameters showed lower numbers in salt-stressed plants. Leaf length coupled with the leaf number determine the final leaf area. In plants such as saffron that do not present a true stem, the role of the leaves is more prominent. The daughter corms of saffron formed on mother corms without roots, and so saffron plants rely on the leaves through photosynthesis and the absorption of nutrients from rainfall for vital activities during March and April [21]. A high number of leaves, and significantly long leaves, provide a large leaf area and consequently affect the plant's ability to photosynthesize. Therefore, in addition to directly reducing photosynthesis, salinity can also reduce the photosynthetic assimilations in saffron by reducing leaf area.

The effect of N supply on leaf length was evident: leaf length increased with increases in N supply rates. The effect of N on vegetative growth in plants is well demonstrated in the literature. Importantly, high N supply rates at 9 dS m^{-1} were responsible for a decrease in leaf number and a reduction in the positive effect of N on leaf length. Such results indicate that in high salinity conditions, high N supply rates not only do not result in a positive effect on leaf growth but can also have a detrimental effect. Although excessive N rates in general increase plant biomass and soil acidification, plant litterfall with high N/P ratios which can aggravate ecosystem-level P limitation [22].

Shoot dry weight decreased under salt stress conditions and increased with an increase in the N supply rate. It was previously discussed that salinity reduced leaf number and length, which in turn directly reduced shoot dry weight, although salt stress conditions may also reduce single leaf weight. In one research study, a decrease in the number, length and area of leaves of *Crocus sativus* L. [23] was associated with a salt stress level of 3 g NaCl L^{-1} and higher. In this research, 1 g NaCl L^{-1} salinity not only had no significant effect on leaf growth, but also increased leaf number, length, and area.

According to the previous results of this research, N supply increased leaf length, which could also result in an increase of the dry weight of saffron shoot. However, N supply also was responsible for enhancing the growth of a single leaf directly by increasing the cell number and elongation. Behnia et al. [13] reported relevant saffron growth as a result of N supply rates of 100 kg N ha^{-1} at Birjand and 50 kg N ha^{-1} at Ghaen. Unal and Cavusoglu [24] also reported that the highest and the lowest leaf growth rates were obtained from calcium ammonium nitrate and no fertilizers additions, respectively.

Nitrogen was more effective at low and medium salinity levels than at high salinity levels. The N effect decreased with increasing salinity; thus 100 kg N ha^{-1} had a negative effect on shoot dry weight in 9 dS m^{-1}. Furthermore, the effect of 50 kg N ha^{-1} in severe salinity conditions had no significant effect on shoot dry weight. By managing N supply and salinity, the growth of saffron shoots can be maximized, as an organ of the saffron that has been neglected. A considerable number of dry leaves are left in the saffron field at the end of each growing season, about twice the number of fresh flowers [10,13]. Saffron leaves as forage are comparable with other crops in terms of crude fiber, N-free extract, ether extract, ash, and total digestible nutrients [8,10,13]. Saffron shoots are mainly composed of leaves and do not have a real stem.

Salt stress significantly reduced the corms number and weight, and this decrease was dependent on the increase of salinity levels. Even the lowest level of salt stress (i.e., 3 dS m^{-1}) significantly decreased the corms number and weight. Mzabria et al. [23] showed that 1 g NaCl L^{-1} (about 2 dS m^{-1}) had no significant effect on corm growth, however, 3 and 5 g NaCl L^{-1} (about 6 & 10 dS m^{-1}) significantly decreased the corms number and weight. Interestingly their results showed that salinity level increased the number of small diameter corms and decreased the diameter of corms. Research has proved that large corms have a strong relationship with large yields [25]. In other research it was shown that 1, 2, and 3 dS m^{-1} salinity levels reduced saffron corm growth. The decrease in the diameter of daughter corms in salt-stressed plants could be explained by the small amount of reserves stored in corms at the vegetative stage due to a reduction in photosynthesis [23]. The large corms are associated with enhanced precocity, flowering density, and large daughter corms for the following season [25].

At all levels of salinity stress, the corms number and weight were the highest at 50 kg N ha^{-1}. The balanced supply of nutrients, especially nitrogen, for saffron, plays a direct role in increasing the growth of daughter corms during the current growing season and the following year's yield [26,27]. Nitrogen is one of the necessary elements to increase the saffron corm growth and flower yield, and as a mobile element, it can be transferred from the shoot to the corms, especially at the end of the season [22,28]. Therefore, the amount of nitrogen uptake in saffron in response to fertilizer applications is important [27].

The effect of N on the corms growth decreased with increasing salinity level, thus differences among N supply rates were not significant in severe salinity conditions (i.e., 9 dS m^{-1}). Ghoreishi et al. [29] found that N foliar application at low salinity levels and solid N in high salinity conditions increase saffron yield. Nitrogen has a double effect on the saffron growth and yield. On the one hand, the use of a low N supply rate inhibits saffron growth and reduces the corm growth as well as stigma yield. On the other hand, excessive N supply rates can increase vegetative growth, delay maturation, increase disease susceptibility, reduce storage capability, and also lead to reduced flower and corm yield [29,30]. Saffron production in saline and semi-saline conditions complicates this equation: does nitrogen increase yield or suppress stigma yield by stimulating vegetative growth? Saffron reacts very differently to N supply in these situations [17]. Since the uptake of N by roots can be hampered at high salinity conditions, a great attention is needed in finding the optimal nitrogen supply rates under salt stressed saffron plants, especially under high saline conditions [1].

The results of this research showed that salt stress enhanced the concentration of Na^+ and Cl^- in leaves and decreased the concentration of K^+. These changes were correlated with salinity stress levels, thus the increase in Na^+ and Cl^- concentrations and the reduction in K^+ concentrations were intensified with increasing salinity levels. Our findings are in accordance with those from other authors [3,6,10,31,32]: for example, Yarami and Sepaskhah [32] showed that increasing salinity levels from 0.5 to 3 dS m^{-1} significantly enhanced the saffron leaf concentration of Na^+, Cl^- and decreased K^+ concentration by about 4.0-, 1.5- and 1.3-fold, respectively. Toxicity due to an accumulation of Na^+ and Cl^- decreased the uptake of essential nutrients like N, P and K from soil [33].

High Na^+ and low K^+ concentrations in saline conditions resulted in high Na/K ratio in plant tissues. Furthermore, Na/K ratio in plants increased when sodium adsorption ratio (SAR) of soil increased [32,34]. A K^+ reduction uptake due to Na^+ increase is a competitive mechanism and occurs regardless of whether the soil solution is dominated by Na^+ salts of Cl^- or $SO_4{}^{2-}$ [33].

The effects of salinity on Ca^{2+} and Mg^{2+} concentrations were less evident than those of other ions: only 9 dS m^{-1} salinity level significantly increased Ca^{2+} and salinity had no significant effect on Mg^{2+} concentration. Yarami and Sepaskhah [32] also reported an increase by 1.4 holds in Ca^{2+} concentration of saffron leaf in response to the highest salinity level. Pirasteh-Anosheh et al. [35] showed that 10 dS m^{-1} salinity reduced Ca^{2+} concentration in roots and had no significant effect on Ca^{2+} concentration in shoots. Their results agreed with those found from this study about the effect of salinity on Mg^{2+} concentration: no significant effect of salt stress on Mg^{2+} concentration of shoot and root occurred.

N application decreased Na^+ and Cl^- concentrations and increased K^+ concentrations, whereas had no effect on the concentration of Ca^{2+} and Mg^{2+}. Under saline conditions, the effect of N was less pronounced, and differences among different rates of N supply were minimum. Enhanced N, P and K concentrations as a result of N applications in saffron fields were reported by Kirmani et al. [36]. Maintaining the balance between vegetative and reproductive growth is one of the most important aims of saffron fertilizer management. One of the main measures for increasing the cultivation efficiency is the use of existing sources, and for increasing the yield per area, the use of integrated nutrient supply and management practices that also help to restore and sustain soil fertility [36,37].

5. Conclusions

Saffron is much more sensitive to N supply rates than other plants, because high N rates in the first season can stimulate vegetative growth and as a result, the yield is reduced or even close to zero. The sensitivity of saffron to N is more complicated in saline conditions. Salt stress had a negative effect on the saffron growth and worsened the ions balance to the detriment of the plant. The role of Na^+, Cl^- and K^+ were more important than other ions. N supply at optimum rate (50 kg ha^{-1}) improved the growth; however, higher rates had a negative effect. Under moderate salinity conditions (up to 6 dS m^{-1}), 50 kg N ha^{-1} strengthened the plant and thus increased salinity tolerance, whereas, at higher salinity rates (i.e., 9 dS m^{-1}), medium and high nitrogen rates (i.e., 50 and 100 kg ha^{-1}) either had no effect or negative effects on saffron. Therefore, according to the results from this research work, high amounts of N supply should be avoided in saline conditions and a supply of 50 kg N ha^{-1} approximately is recommended. More research is needed to clarify the molecular and physiological mechanisms of the nitrogen–salinity interaction, to examine the effect of other nutrients on improving the salt tolerance of saffron, and to monitor the status of nitrogen and other elements in saline soils.

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References

1. Bicharanloo, B.; Moghaddam, P.R.; Asadi, G. Does summer irrigation alter nitrogen uptake and utilisation efficiency of saffron (*Crocus sativus* L.) for different organic and chemical fertilisers? *Arch. Agron. Soil Sci.* **2021**, *67*, 1754–1769. [CrossRef]
2. Gresta, F.; Santonoceto, C.; Avola, G. Crop rotation as an effective strategy for saffron (*Crocus sativus* L.) cultivation. *Sci. Hortic.* **2016**, *211*, 34–39. [CrossRef]
3. Yarami, N.; Sepaskhah, A.R. Physiological growth and gas exchange response of saffron (*Crocus sativus* L.) to irrigation water salinity, manure application and planting method. *Agric. Water Manag.* **2015**, *154*, 43–51. [CrossRef]
4. Pandey, A.; Khan, M.K.; Hakki, E.E.; Gezgin, S.; Hamurcu, M. combined boron toxicity and salinity stress—An insight into its interaction in plants. *Plants* **2019**, *8*, 364. [CrossRef]
5. Liu, X.; Luo, M.; Chen, J.; Ye, R.; Tan, J.; Zhai, Z.; Yang, Y.; Huang, J. Root iron plaque abundance as an indicator of carbon decomposition rates in a tidal freshwater wetland in response to salinity and flooding. *Soil Biol. Biochem.* **2021**, *162*, 108403. [CrossRef]
6. Yarami, N.; Sepaskhah, A.R. Saffron response to irrigation water salinity, cow manure and planting method. *Agric. Water Manag.* **2015**, *150*, 57–66. [CrossRef]
7. Mollafilabi, A. Experimental findings of production and echo physiological aspects of saffron (*Crocus sativus* L.). *Acta Hortic.* **2003**, *650*, 195–200. [CrossRef]
8. Pirasteh-Anosheh, H.; Kamgar, H.A. Using Brackish Water for Saffron Production in Arid and Semi-Arid Regions. In Proceedings of the 2nd International Conference on Water Saving and Plant Production Strategies: Constraints and Implications for Sustainable Agriculture, Faisalabad, Pakistan, 26–28 March 2019.
9. Feizi, H.; Moradi, R.; Pourghasemian, N.; Sahabi, H. Assessing saffron response to salinity stress and alleviating potential of gamma amino butyric acid, salicylic acid and vermicompost extract on salt damage. *S. Afr. J. Bot.* **2021**, *141*, 330–343. [CrossRef]
10. Pirasteh-Anosheh, H.; Hashemi, S.E.; Del Borghi, A.; Spasiano, D.; Rad, M.; Race, M. Feasibility study of saffron cultivation using a semi-saline water by managing planting date, a new statement. *Environ. Res.* **2022**, *203*, 111853. [CrossRef]
11. Sepaskhah, A.R.; Kamgar, H.A. Saffron irrigation regime. *Int. J. Plant Prod.* **2009**, *3*, 1–16.
12. Gresta, F.; Lombardo, G.M.; Siracusa, L.; Ruberto, G. Saffron, an alternative crop for sustainable agricultural systems. A review. *Agron. Sustain. Dev.* **2008**, *28*, 95–112. [CrossRef]
13. Behnia, M.R.; Stilai, A.; Ehdaie, B. Application of fertilizers for increased saffron yield. *J. Agron. Crop Sci.* **1999**, *182*, 9–15. [CrossRef]
14. Naseer, S.; Nehvi, F.A.; Nagoo, S.A.; Samad, S.S.; Iqbal, A.M.; Dar, N.A. Effect of organic and inorganic sources of fertilizers on growth and yield of saffron (*Crocus sativus* L.). In *IV International Symposium on Saffron Biology and Technology*; ISHS: Leuven, Belgium, 2012.
15. Amiri, M.E. Impact of animal manures and chemical fertilizers on yield components of saffron (*Crocus sativus* L.). *Am.-Eurasian J. Agric. Environ. Sci.* **2008**, *4*, 274–279.
16. Akbari, H.; Cherati, S.M.; Monazam, N.H.; Noguchi, M. Effect of courtyards' geometrical parameters on climate adaptability and shading performance in hot-arid climate of Yazd (Iran). *Sustain. Energy Technol. Assess.* **2021**, *48*, 101594. [CrossRef]
17. Kafi, M.; Koocheki, A.; Rashed, M.H.; Nassiri, M. *Saffron Production and Processing*; Science Publisher: New York, NY, USA, 2006; pp. 126–150.
18. Havre, G.N. The flame photometric determination of sodium, potassium and calcium in plant extracts with special reference to interference effects. *Anal. Chim. Acta* **1961**, *25*, 557–566. [CrossRef]

19. VDLUFA. *Handbuch der Landwirtschaftlichen Versuchs-und Untersuchungsmethodik (VDLUFA-Methodenbuch), Bd. III Die chemische Untersuchung von Futtermitteln*, 3rd ed.; VDLUFA-Verlag: Darmstadt, Germany, 2012.
20. Lee, J.; Campbell, C.M. Atomic absorption Spectrophotometric and ethylene diaminetetra acetate-titration methods for calcium and magnesium determinations. *J. Dairy Sci.* **1969**, *52*, 121–124. [CrossRef]
21. Hosseini, M.; Sadeghiand, B.; Aghamiri, S.A. Influence of foliar fertilization on yield of saffron (*Crocus sativus* L.). *Acta Hortic.* **2004**, *650*, 207–209. [CrossRef]
22. Luo, M.; Moorhead, D.L.; Ochoa-Hueso, R.; Mueller, C.W.; Ying, S.C.; Chen, J. Nitrogen loading enhances phosphorus limitation in terrestrial ecosystems with implications for soil carbon cycling. *Funct. Ecol.* **2022**, *36*, 2845–2858. [CrossRef]
23. Mzabri, I.; Legsayer, M.; Aliyat, F.; Maldani, M.; Kouddane, N.; Boukroute, A.; Bekkouch, I.; Berrichi, A. Effect of salt stress on the growth and development of saffron (*Crocus sativus* L.) in eastern Morocco. *Acta Hortic.* **2017**, *1184*, 55–62. [CrossRef]
24. Unal, M.; Cavusoglu, A. The effect of various nitrogen fertilizers on saffron (*Crocus sativus* L.) yield. *J. Akdeniz Uni. Facul. Agric.* **2005**, *18*, 257–260.
25. Koocheki, A.; Ganjeali, A.; Abbassi, F. The effect of duration of incubation and photoperiod on corm and shoot characteristics of saffron plant (*Crocus sativus* L.). *Acta Hortic.* **2007**, *739*, 61–70. [CrossRef]
26. Azari, S.J.; Sorooshzadeh, A.; Nabati, J.; Oskoueian, E. Relationship between fertilization and planting depths on antioxidant activity in saffron (*Crocus sativus* L.). *Indust Crops Prod.* **2023**, *191*, 116004. [CrossRef]
27. Koocheki, A.; Jamshideyni, M.; Seyyedi, S.M. The effects of mother corm size and type of fertilizer on nitrogen use efficiency in saffron. *Saffron Agron. Technol.* **2015**, *2*, 243–254.
28. Itoo, Z.A.; Reshi, Z.A.; Shah, M.A. Characterizing arbuscular mycorrhizas in Saffron: Implications for bridging the yield gaps. *Biologia* **2023**, *78*, 91–100. [CrossRef]
29. Ghoreishi, S.G.; Khashei, A.; Beyki, A. Effects of salt stress and nitrogen application forms on yield of saffron (*Crocus sativus* L.) as a medicinal plant yield under Birjand climatic conditions. *J. Saffron Res.* **2020**, *7*, 343–353. [CrossRef]
30. Ghanbari, J.; Khajoei-Nejad, G. Integrated nutrient management to improve some soil characteristics and biomass production of saffron. *Ind. Crops Prod.* **2021**, *166*, 113447. [CrossRef]
31. Dong, H. Technology and field management for controlling soil salinity effects on cotton. *Aust. J. Crop Sci.* **2012**, *6*, 333–341.
32. Yarami, N.; Sepaskhah, A.R. Effect of irrigation water salinity, manure application and planting method on soil ions variation and ions uptake by saffron (*Crocus sativus* L.). *Int. J. Plant Prod.* **2016**, *10*, 197–219.
33. Al-Farsi, S.M.; Nawaz, A.; Nadaf, S.K.; Al-Sadi, A.M.; Siddique, K.H.; Farooq, M. Effects, tolerance mechanisms and management of salt stress in lucerne (*Medicago sativa*). *Crop. Pasture Sci.* **2020**, *71*, 411–428. [CrossRef]
34. Ashraf, M.; Afzal, M.; Ahmad, R.; Maqsood, M.A.; Shahzad, S.M.; Tahir, M.A.; Akhtar, N.; Aziz, A. Growth response of the salt-sensitive and the salt-tolerant sugarcane genotypes to potassium nutrition under salt stress. *Arch. Agron. Soil Sci.* **2012**, *58*, 385–398. [CrossRef]
35. Pirasteh-Anosheh, H.; Emam, Y.; Rousta, M.J.; Ashraf, M. Salicylic acid induced salinity tolerance through manipulation of ion distribution rather than ion accumulation. *J. Plant Growth Regul.* **2017**, *36*, 227–239. [CrossRef]
36. Kirmani, N.A.; Sofi, J.A.; Bhat, M.A.; Ansar-Ul-Haq, S. Sustainable saffron production as influenced by integrated nitrogen management in Typic Hapludalfs of NW Himalayas. *Commun. Soil Sci. Plant Anal.* **2014**, *45*, 653–668. [CrossRef]
37. Appireddy, G.K.; Saha, S.; Mina, B.L.; Kundu, S.; Selvakumar, G.; Gupta, H.S. Effect of organic manures and integrated nutrient management on yield potential of bell pepper (*Capsicum annuum*) varieties and on soil properties. *Arch. Agron. Soil Sci.* **2008**, *54*, 127–137. [CrossRef]