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Original Article

Impact of Water Deficit on Growth and Seed Quality of *Cucurbita pepo* L. under Separate Selenium and Calcium Applications

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ABSTRACT

Water scarcity is a global issue, and agriculture in Iraq, is one of the most affected sectors by rising temperatures and reduced water availability. Pumpkin (*Cucurbita pepo* L.) yield, both fruit and seed, is impacted by irrigation deficits and varying water regimes. Therefore, to assess suitability to low water availability, a local cultivar of pumpkin was planted in an experimental farm in Kalar, Iraq. The plants during flowering were exposed to two water regimes: full irrigation to maintain the soil at 100% field capacity (FC) and 50% FC. Selenium and calcium at concentrations of 1.5 mg/L and 5 mg/L, respectively, were applied separately and weekly during the flowering and fruit establishment. A split experiment with a complete randomized block design was implemented with three replicates. Results revealed that selenium application statistically improved the total number of branches, the number of pistillate flowers, and fruit yield per plant compared to the control treatment under 50% FC and 100% FC respectively. Seed traits, including seed weight per fruit, the number of seeds per fruit, the weight of 1000 seeds, and seed yield, enhanced slightly but not significantly when compared with the calcium and control treatments under reduced water usage. Calcium content in seeds increased significantly under 50% FC with the application of selenium and calcium. Similarly, the highest activity of catalase (CAT) and ascorbate peroxidase (APX) enzymes was achieved using calcium in 50% FC. These findings emphasize that selenium demonstrates effectiveness in enhancing certain traits of pumpkin morphology, physiology, and productivity, while calcium was more effective in improving biochemical contents. Further studies are recommended to identify a sustainable and natural alternative to selenium with a similar function and emphasis the role of selenium at the molecular level in pistillate flower development in pumpkin.

Keywords: Reduce Water Utilization, Seed Quality, Selenium, Calcium, Pumpkin.

1 Introduction

Water scarcity became a global focus, specifically in arid and semi-arid regions. Iraq is one of the most impacted countries by unstable environment, particularly reduced precipitation. In northern Iraq, the groundwater had been falling nearly by half, and the supply only satisfied 55% of water demand [1,2].

Maintaining yield and crop quality requires the formulation of novel ways to enhance water use efficiency to face the water scarcity challenge. In water-limited environments, selecting a suitable crop to study sustainable farming practices, such as the application of plant nutrition, is essential. Therefore, pumpkin (*Cucurbita pepo* L.) is chosen due to its multiuse and nutritional, and economic values of its plant parts, particularly, fruits and seeds.

Pumpkins belong to the family Cucurbitaceae and are the most popular crop during the autumn season and Thanksgiving. Nutritionally, pumpkin fruits and seeds contains linoleic acid, antioxidant, as well as carotenoids, ascorbic acid, vitamins, minerals, dietary fibres, zeaxanthin, and vitamin E [3,4]. The pumpkin research has increased dramatically for the last decade. A web search resulted in 1,010 findings related to pumpkin production in Iraq for the period 2023–2024 and 575 results for the period 2013–2014. In Iraq, the cultivated area for pumpkins, squash, and gourds went from 9,726 to 10,035 hectares, with a production rise from 10,035 to 10,719.8 kg/ha. Similarly, in the European Union, the cultivated area increased from 47,288 to 82,499 hectares, with a yield rise of 31,622.7 to 31,651.3 kg/ha [5]. The statistical data on cultivated area and yield, particularly in Iraq, necessitate the development of improvement strategies to maintain and enhance production, specifically seed quality.

The growth of pumpkins and the quality of their seeds are affected by certain abiotic stressors, such as drought.

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Morphological, physiological, and plant productivity, including flowering, fruiting and seeds inhabit dramatically during the decrease of water availability [6,7]. As a result, various methods followed to reduce the impact of water scarcity, including the application of micronutrients, are becoming increasingly significant.

Selenium (Se) as a non-essential nutrients and calcium (Ca) as an essential nutrient play crucial role in mitigating the damage caused by abiotic stress including drought [8]. Selenium enhances the enzymatic and non- enzymatic antioxidant activities by reducing the accumulation of reactive oxygen species, enhances water uptake, and sustains photosynthesis and chlorophyll levels [9]. Alsamadany *et al.* [10] found that selenium application to pumpkins plants during osmotic and ionic stress greatly lowered the amounts of O_2•–, H_2O_2, and Na^+. Foliar application of $SeO3^{2-}$ on pumpkin plant in the field increased anthocyanin slightly and fruit and seed yield were not affected [11]. On the other hand, calcium as essential element for plant growth and development, contributed in plant abiotic stress through stabilize cell walls and membranes; regulate Na+ and K+ ratios; regulate hormone levels; maintain photosynthesis; regulate respiratory metabolism and root activities; induce stress-resistant gene and protein expressions [12]. Feng also demonstrated that under drought conditions, plants are less capable of absorbing and translocating calcium [12]. Colombari *et al.* [13] found that foliar application of 0.2% calcium chloride during flowering stage was increased the amount of pollen per flower and also the number of seeds per fruit of zucchini (*Cucurbita pepo* L.).

This research examines the independent effects of selenium and calcium in alleviating the impact of decreased water utilization on a local pumpkin, *Cucurbita pepo* L. The aim is to provide insights into which elements are most effective in mitigating the impact of water scarcity and optimizing pumpkin cultivation. Therefore, plant vegetative growth, flowering parameters, seed yield, and seed quality traits were evaluated.

2 Material and Methods

2. 1 Experimental Site and Agronomic Practices

A field experiment was conducted during the summer growing season from April to September of 2024 at the experimental farm at 34°36’29.30 N, 45°18’04.04 E, and an elevation of 200 m above sea level. The soil at the experimental site was characterized as sandy clay throughout the profile; see supplementary table 1 for some soil physical and chemical properties of the experimental site, and supplementary table 2 shows some water properties of irrigation being used in the experimental side. Monthly meteorological data, including temperatures, humidity, and wind speed, are shown in supplementary table 3.

Pumpkin seeds (*Cucurbita pepo* L.), a local variety, were sown on April 5, 2024, in seedling trays filled with commercial peatmoss, and seedlings were transplanted to the field one month later. Each plot (4.3 × 2 m) contained 10 plants grown in two rows in the middle of the bed. Seedlings were transplanted by hand with 1m row spacing and 0.8 m plant spacing within each plot.

A split-plot experiment following a completely randomized design with three replications was implemented. Irrigation regimes were allocated within the main plots, while the foliar application of selenium, calcium and control was assigned to the subplots. The irrigation regimes were determined by calculating the time of water flow, with the flow volume measured per minute (36.67 mL/min). Two irrigation regimes were implemented: 100% and 50% of field capacity (FC), corresponding to irrigation durations of 2 hours and 1 hour, respectively. 100 % FC is the point that soil capacity to hold maximum water [14]. FC was achieved through the application of the required water to maintain the FC in both watering regimes. The total water applied under the full irrigation regime (100% FC) for (2h) during the growing season was 5569.62 m^3/ha per irrigation, while the total water applied for the reduced irrigation regime (50% FC) for (1h) was 2784.81 m^3/ha per irrigation. Irrigation regimes commenced three weeks after transplanting, during the flowering stage, and continued for 1 month until the fruits began to colour.

Selenium in the form of sodium selenate (Na_2SeO_4), purchased from Thomas Baker, India, was applied at a concentration of 1.5 mg/L. Calcium in the form of calcium nitrate [$Ca(NO_3)_2 \cdot 4H_2O$], sourced from Casa De Amor, India, was applied at a concentration of 5 mg/L. Applications continued for 2–6 weeks after the first fruits reached 20 mm in diameter, ensuring the fruit surface was thoroughly wetted. Irrigation was discontinued 20 days prior to harvest.

Standard agronomic practices, fertilization NPK (20-20-20), were used twice, one week before transplanting and after transplanting. The complete amount of fertilizer applied each time was 48.45 kg/ha. Pest and disease management were conducted as the manufacturer recommended for Speed SC and Orizon SL from Agrichem, Australia.

2. 2 Growth Traits

During growth and development, various morphological characteristics were recorded for five randomly chosen plants per experimental unit and replicate, including plant height (m), leaf area (cm^2), which was measured using Fiji (ImageJ 1.54f), stem diameter (cm), the number of branches per plant, internode length (cm), leaf fresh weight (g), leaf dry weight (g), root length (cm), root fresh weight (g), and root dry weight (g).

2. 3 Flowering Traits

When 50% of the plants completed flowering, the total number of staminate and pistillate flowers and their sex ratio (number of staminate / number of pistillates) [15] were recorded. The data were collected from 10 plants per experimental unit and replicate.

2. 4 Fruit Traits

At an interval of 14 weeks after transplanting, when the majority of pumpkin fruits had reached their maturity phase, the following fruit characteristics were recorded: fruit weight (kg), fruit diameter (cm), fruit length (cm), pulp weight (kg), fibrous strands and seed weight (kg), fibrous strands weight (kg), the total number of fruits per plant, and fruit yield (tons per hectare).

2. 5 Seed Traits

The seeds from each pumpkin were removed, washed with water, dried, and then stored for further analysis and measurements as follows: Seed weight per fruit (g), the number of seeds per fruit, weight of 1000 seeds (g), and seed yield (kg per hectare).

Seed size was estimated using SmartGrain version 1.1 (http://phenotyping.image.coocan.jp/) [16]. Images were captured using a phone camera and analyzed using the software. The following measurements were taken: area size (AS) [mm²], perimeter length (PL) [mm], length (L) [mm], width (W) [mm], length-to-width ratio (LWR), circularity (CS), and the distance between the intersection of length and width (IS) and center of gravity (CG) (DS) [mm] (Figure 1). Data were recorded from 5 randomly selected fruits per experimental unit, with 30 seeds per captured photo.

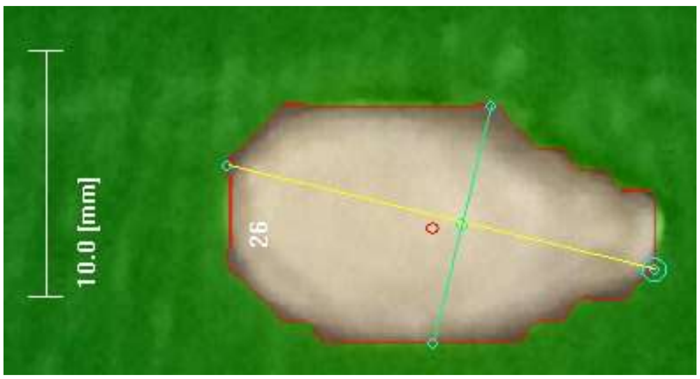

Figure 1: SmartGrain automatically identifies pumpkin seeds within an image and measures their shape profiles automatically.

The whole weight of seeds (achene), kernel, and husk (seed coat) was measured using fifty randomly selected seeds of dried seed. Analytical balance (Akern and sohn Gmbh, Germany), was used for this purpose.

In addition, the production value of fruits and seeds was determined by estimating the cost of fruit and seed production per hectare. The cost was calculated based on a rate of 1 kg = 1000 IQD.

2. 6 Minerals and Biochemical Analysis

For Se and Ca determination in pumpkin seeds, 1 g of dried and ground powder was added to 5 mL of 65% nitric acid and 1 ml of 36.5% hydrochloric acid. The samples were digested using Mars Xpress (CEM Corporation, Matthews, USA) microwave oven in four stages. The minerals in sample solutions were quantified by inductively coupled plasma–optical emission spectrometry (ICP-OES Optima 7000; Perkin Elmer, USA), in accordance with Martinec *et al*., [17]. Additionally, total protein was quantified using Bradford method, the samples were measured at 595 nm by ELISA reader (Thermo Scientific Varioskan Flash Multimode Reader) [18]. The assessment of catalase (CAT), and ascorbate peroxidase (APX) activity was carried out using the procedures described by Najafi *et al.* [18], the activity of CAT and APX were measured using spectrophotometer at 240 nm and 290 nm wavelength, respectively. All mineral and biochemical analyses were executed by the researcher at the University of Tehran, College of Agricultural and Natural Resources.

2. 7 Statistical Analysis

Data was analyzed using the SPSS software. The data was subjected to one-way analysis of variance (ANOVA), and Duncan's multiple range test compared the means of the treatments. Graphs were generated using Microsoft Office Excel 2013 software.

3 Results and Discussion

3. 1 Growth Traits

During the growing season, the pumpkin plants were monitored almost daily. Vegetative growth parameters were recorded at the changing colour stage of the fruit. Pumpkin vine length, which was represented as plant length, the longest average was (3.25 m) when plants were fully irrigated (100% FC) and selenium was applied (Table 1). The shortest plant average (2.34 m) was recorded when plants were not fully irrigated and treated with calcium. Although there are differences in the fully irrigated and reduced irrigation on plant height, generally, there are no statistical differences between the treatments (Table 1). Leaf area was not affected by lowering water utilisation, despite increasing the values of leaf area treated with calcium (Table 1). Fully irrigated plants treated with selenium increased the value of stem diameter, but statistically it was not different from other treatments. Similarly, selenium treatment increased the number of branches per plant, and the fully irrigated was significantly higher when compared with reduced irrigation, calcium, and control treatments. Calcium application statistically increased the length of internode to about 9.18 cm and selenium to 8.22 cm; these values were the highest statistically recorded values when plants were fully irrigated in comparison with half-irrigated. Additionally, during the growing season, when vegetative parameters were collected from April to August, average temperature and humidity fluctuated by nearly 15 degrees, and solar radiation varied by approximately 52 units (Supplementary Table 3). These environmental fluctuations may have contributed to the variability observed in the non-significant findings, although slight improvements were noted with the applied treatments. These results underscore the importance of nutrients such as calcium and selenium in plant growth, suggesting that their availability—and potentially their uptake—may be influenced by water availability and environmental conditions during the vegetative stage of pumpkin. This interpretation is further supported by previous findings discussed below.

A study was shown that waterlogging stress inhibited the growth of 27 Chinese pumpkins, with significant differences in plant length with the average of 0.80 m and minimum 0.448 m, stem diameter average 0.76 cm and minimum 0.46 cm, the average of root length 0.70 m, aboveground and underground fresh and dry weight were reduced [6]. Correspondingly, in this study, the statistical reduction of aboveground growth, including plant height, the number of lateral branches, and internode length, under the reduction of irrigation level, may correlate to the destruction of the metabolism process and unbalanced plant hormones and enzymatic and non-enzymatic antioxidants that negatively impacted cell division and elongation [19]. In addition, selenium showed a positive effect on plant growth whether under stress or optimal growth conditions; particularly, the number of lateral branches when compared with half-irrigated untreated plants (Table 1). Fowmina [20] found the highest vine length (214.6 cm), the number of leaves (113.7), and leaf length (15.9 cm) when cucumber plants were foliar treated with selenium (20 µg L^{-1}). Similarly, foliar application of squash plants with calcium 250 and 500 mg/L and nano calcium 50 and 100 mg/L gave the highest values of stem length, diameter, fresh and dry weights, and the number of leaves and total leaf area; fresh and dry weights; and root length, diameter, and fresh and dry weights; and photosynthetic pigments compared with the control [21]. Under 50% FC, plant length, stem diameter, fresh leaf weight, stem fresh weight, stem dry weight, root fresh weight, root dry weight, and plant dry weight were statistically increased with the foliar application of $CaCl_2$, 50 mg/L, in pearl millet [22]. A breeding study demonstrated that the main stem lengths are positively correlated with yield per plant and have mostly additive genetic variance, as did multiple lateral branching of some cucurbits [23]. The recorded results of the vegetative traits under reduced water usage in this study distinguish that selenium improves plant height, stem diameter, the number of branches, and the root fresh weight. While the application calcium increased the recorded values of leaf area, internode length, leaf fresh weight, and leaf dry weight table (1). These findings indicate the importance of selenium and calcium on the vegetative growth of pumpkins under restricted water availability regions.

Table 1: Effect of reduced water usage and separate applications of selenium and calcium on vegetative growth traits of pumpkin.

FC	Treatment	Plant length (m)		Leaf Area (cm2)		Stem Diameter (cm)		Number of Branches		Internode length (cm)		Leaf Fresh Weight (g)		Leaf Dry Weight(g)	
		Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE
100%	Selenium	3.25 a	0.12	124.35 a	11.84	3.04 a	0.04	7.03 a	0.37	8.22 b	0.33	4.40 a	0.17	0.84 a	0.06
	Calcium	3.13 ab	0.14	133.29 a	9.83	2.79 a	0.46	6.97 a	0.09	9.18 a	0.15	4.46 a	0.23	0.88 a	0.03
	Control	2.75 abc	0.31	108.11 a	9.79	2.41 a	0.19	6.43 ab	0.12	7.24 c	0.16	4.14 a	0.39	0.82 a	0.08
50%	Selenium	2.37 c	0.23	104.72 a	2.10	2.52 a	0.21	6.23 ab	0.61	6.49 c	0.19	3.84 a	0.15	0.84 a	0.05
	Calcium	2.34 c	0.09	115.95 a	12.90	2.73 a	0.07	5.43 bc	0.26	6.64 c	0.24	4.23 a	0.40	0.96 a	0.11
	Control	2.49 bc	0.24	110.64 a	2.52	2.48 a	0.15	5.17 c	0.15	6.72 c	0.49	3.97 a	0.20	0.94 a	0.09

Means in the same column with different letters are significantly different at α = 0.05.

3. 2 Flowering Traits

Flowering stage was initiated at a very early stage of growth. The pumpkin plants show a monoecious phenomenon. At node 12, the fully irrigated and selenium-treated plants produced their first pistillate flower. They continued to alternately produce pistillate and staminate flowers until node 35, when they again produced staminate flowers (Figure 2A). The fully irrigated plants with calcium treatment produced the first pistillate flowers at node 10, then the second at node 15; from node 17, the plant produced pistillate flowers alternately with staminate flowers until node 36 (Figure 2B). The control treatment, which received full irrigation, produced the first pistillate flower at node 15, the second at node 17, and then alternated with staminate flowers until node 36 (Figure 2C). The half-irrigated plot with selenium treatment led to the production of the first pistillate flowers at node 10, the second at node 17, the third at 19, and then, alternatively, staminate flowers until node 32 (Figure 2D). When half the irrigated plot received the calcium treatment, node 18 produced the first pistillate flowers, node 22 produced the second, and node 43 recorded the last pistillate flower (Figure 2E). Under the control treatment, node 7 produced the first pistillate flower, node 12 produced the second, node 17 produced the third, and node 38 produced the last pistillate flower (Figure 2F). Generally, the results showed that the first pistillate flower at node 7 with lowered water utilisation and untreated plant (control), and the last one at node 43 with calcium treatment. The appearance of the first flower in fewer days than expected suggests an abnormal phenomenon known as early or stress-induced flowering. Although the genetic makeup of the local pumpkin variety plays a role, environmental fluctuations may have disrupted hormonal balance during the early growth stages, leading to what is commonly referred to as stress-escape flowering [24].

Plants half-irrigated and treated with calcium produced the highest number of staminate flowers (145.8), but statistically, this value did not differ from other values recorded from other treatments. The highest number of pistillate flowers, 13.20, was compared to the lowest number, 7.73, recorded with half-irrigated land, plants treated with selenium, and fully irrigated with the control treatment, respectively. Consequently, the fully irrigated and control treatment recorded the highest sex ratio of staminates to pistillates (18.92), while the half irrigation and selenium treatment recorded the lowest (11.34) (Figure 3). These results indicate that treating pumpkin plants with selenium or calcium stimulates pistillate flower production in comparison to the control treatment. In general, reduced water use had no statistical effect on the number of pistillate flowers.

The flowering stage in pumpkin was continued almost eight weeks after planting, starting from May to mid of August; with the highest flowering pick occurring in June and July (data not shown). Pumpkin flowering is sensitive to drought [25]. In this study, the plants were exposed to reduced water usage to determine the local pumpkin in Iraq to drought tolerance, in addition, selenium and calcium were applied weekly during flowering stage to evaluate their impact on mitigating the effect of lower usage of water and the flowering dynamics. Figure (2) shows the higher number of staminate and pistillate flowers at fully irrigated plot than half irrigated plots. Pistillate flowers represented by red dots impacted dramatically by reducing water usage, the number of staminate flowers were higher than the pistillate flowers under both conditions (Figure 2). This result aggresses with previous study demonstrate that during the flowering period, the staminate flowers were higher than pistillate flower bud production, and drought led to reduction of pistillate flowers by threefold and staminate by almost onefold [25]. However, most of the pistillate flowers were aborted before fruit set and the flower color was brownish- yellow; this may be the consequences of the high temperature (supplementary table 3) in the open field experiment and lead to the pistillate bud not developing to anthesis [26]. Therefore, in this study, selenium and calcium improved the formation of pistillate buds (Figure 2) compared with the control plants. The highest number of pistillate buds developed to anthesis was found on plants treated with selenium (Figure 3). This results in agreement with previous finding by Abedi *et al.* [27], which showed that the application of selenium nanoparticles increased the number of flowers in chicory (*Cichorium intybus* L.). These results may also correlate to the impact of the foliar application of selenium on its level in the pistil and stamen and also pollinator visitor. A study was shown that the higher level of selenium in hyperaccumulated selenium plant specifically in reproductive organs elevate the selenium in nectar and increase the pollinator visitor in *Stanleya pinnata* [28]. On the other hand, calcium was demonstrated as an essential macronutrient for pollen grain germination, fertilization and flower and fruit set of pumpkin [13]. The percentage differences under full irrigated plot and treated plants with calcium was 31.88% pistillate and 1.66% staminate flowers when compared with control. Under half irrigated plots with calcium treatment was 6.76% pistillate and 2.59% staminate flowers, respectively when compared with control (Figure 3). This indicate that calcium increased the number of pistillate flower set under fully and half irrigated plots, although statistically the data show non-significant differences. As a compression between selenium and calcium effect on mitigate the impact of reduced water on pistillate flowers, the result conclude that selenium is more effective on increasing the set of pistillate flowers of cultivated local pumpkin and the percentage increased differences was 25.64% and 32.26% of pistillate flowers under half irrigated plot compared with the calcium and control treatments, respectively.

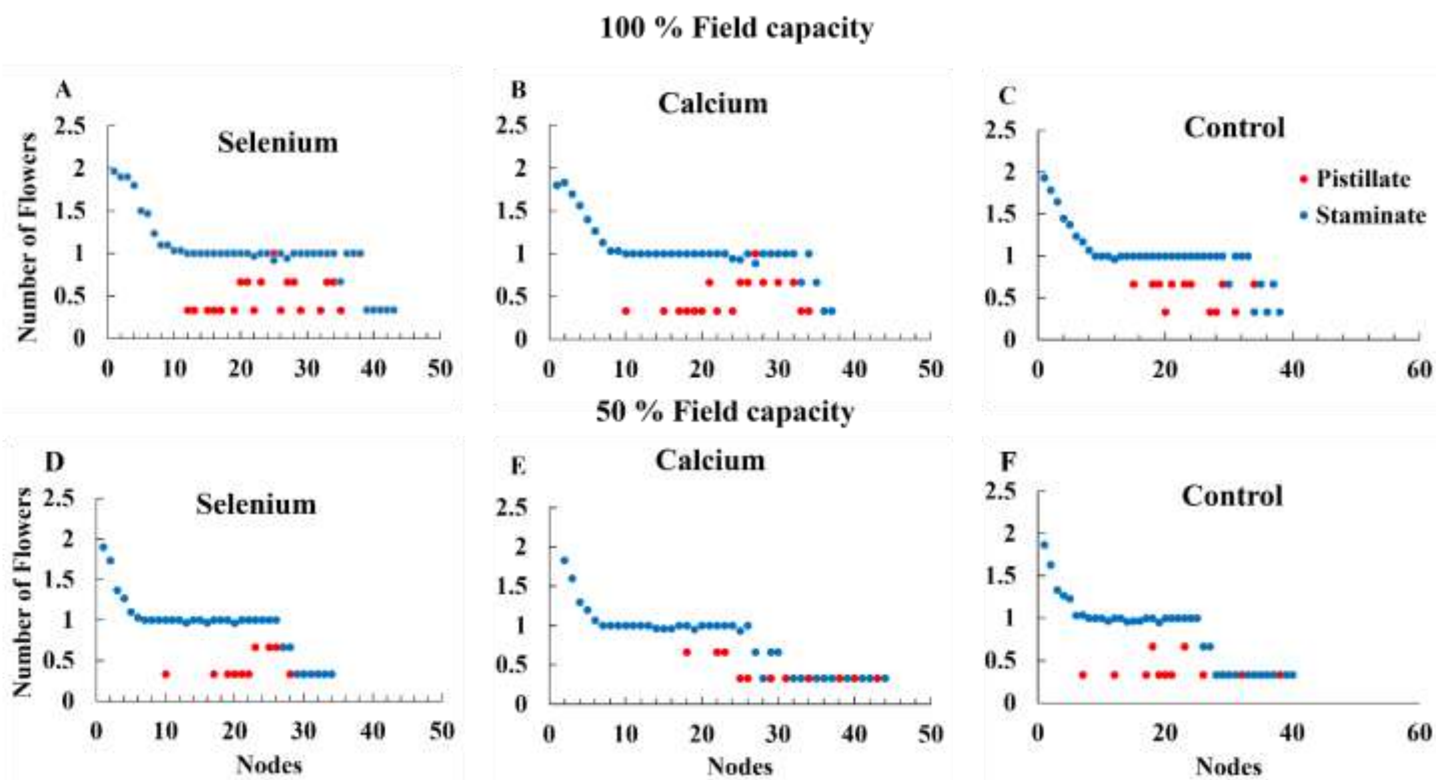

Figure 2: Effect of reduced water usage and separate applications of selenium and calcium on the number of pistillate and staminate flowers per node of pumpkin. A, B and C represent the values of flowers under fully-irrigated area and plant treated with selenium.

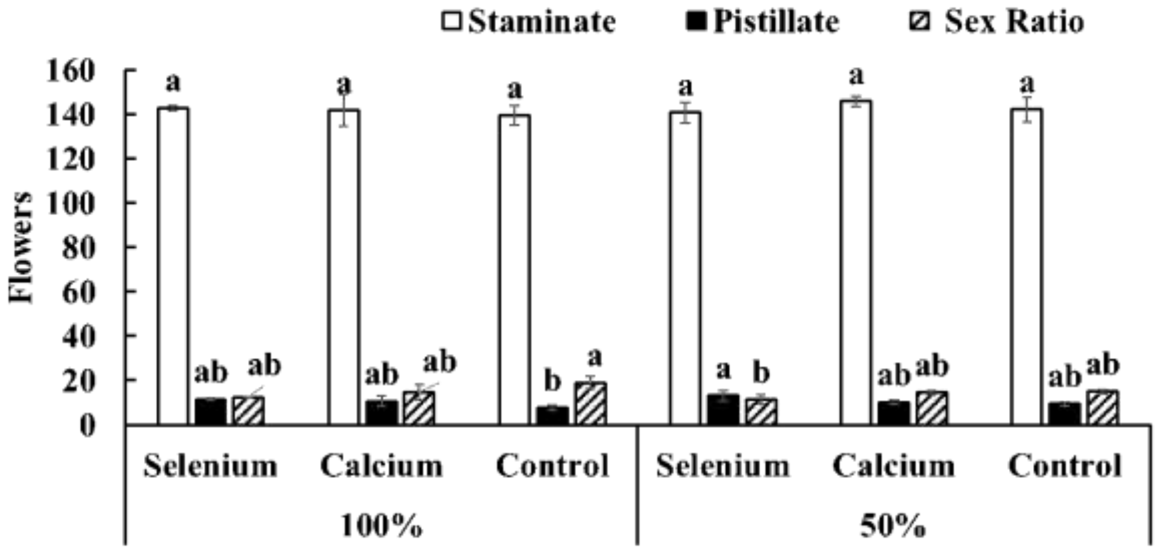

Figure 3: **Effect of reduced water usage and separate applications of selenium and calcium on the number of pistillate and staminate flowers and sex ratio per plant of pumpkin.** Means in the same bar with different letters are significantly different at α = 0.05.

3. 3 Fruit Traits

The data related to fruit characteristics showed no statistical differences between the full and half-irrigated areas and selenium and calcium when compared with the control (Table 3). However, the fruit parameters including; fruit weight (kg), fruit diameter (cm), fruit length (cm), pulp weight (kg), fibrous strands and seeds weight (g), and fibrous strands weight (g) show the higher values when the plants were fully irrigated compared with lower or half-irrigated and treated plants with selenium, calcium, and control.

The average fruit weight obtained under fully irrigated plots and plants treated with selenium and calcium was 1.78 kg and 1.69 kg, respectively. The average fruit weight under reduced irrigation time and selenium and calcium treatments was 1.53 kg and 1.29 kg. The increased percentage differences between selenium and calcium under fully irrigated conditions were 5.68% and under reduced irrigation conditions were 17.40% (Table 2). These results indicate that despite the non-significant impact of drought on fruit characteristics, selenium was much more effective at reducing the impact of reduced water usage than calcium.

Furthermore, the total number of mature fruits per plant was recorded when plants were fully irrigated and treated with selenium; the average was 2.34 fruits per plant (Figure 4 A). This value was statistically higher than the control in fully irrigated and half-irrigated plants treated with selenium calcium and control, respectively. These values reflected the total yield, which ranged from 12.50 to 48.79 tonnes per hectare from fully irrigated with selenium treatment and half-irrigated with control, respectively (Figure 4 B).

Fruit set was improved statistically when pumpkin plants were treated with selenium and calcium and grown under fully irrigated and reduced irrigation conditions compared with the control treatment, respectively. The flower abortion was increased dramatically with the reduced water usage and the control treatment, respectively (Figures 2 and 3). Under the fully irrigated conditions, the highest fruit set percentage (20.43%) was recorded with the treatment of selenium, with the average value of the total number of fruits being 2.37 fruits out of 11.60 pistillate flowers. Under reduced water conditions, the lowest fruit set percentage (6.61%) was recorded with the treatment of control, with the average value of the total number of fruits being 0.63 fruits out of 9.53 pistillate flowers. Our results indicate an enormous reduction of fruit set when compared with a previous study, which shows that under field conditions the average of fruit set was 50.9% among six studied cultivars of pumpkin in Ithaca, New York [29]. However, this data may agree with previous studies that indicate that selenium improves the pollinator visitors and calcium stimulates the egg cell fertilisation [28,13]. Correspondingly with the number of fruits per plant, the total fruit yield (tonne/ha) was reduced with the lowered water usage conditions (Figure 4 B). A comparison study among twenty genotypes of pumpkin under drought stress recorded the lowest and highest fruit weight of 0.54 kg and 1.80 kg, fruit length 8.13 cm and 20.87 cm, and fruit width 9.30 cm and 14.53 cm, and demonstrated that drought reduced all fruit characteristics statistically when compared with the plant grown under irrigated conditions [7]. In this study, under lowered water usage conditions, the range of fruit weight was from 1.29 to 1.79 kg, fruit diameter 51.03 to 55.63 cm, and fruit length 21.81 to 23.35 cm (Table 2). This indicates that the local cultivar from Iraq used in this study recorded slightly higher values of fruit-related features in comparison to the recorded fruit-related features by Seymen *et al.* [7]. Contrary to the non-statistical differences in fruit characteristics of pumpkins among fully irrigated and reduced or half-irrigated conditions, previous studies have shown a significant statistical reduction in fruit weight specifically under deficit irrigation conditions when compared with irrigated conditions [30,31].

Table 2: Effect of reduced water usage and separate applications of selenium and calcium on fruit traits of pumpkin.

FC	Treatment	FW (kg)		FD (cm)		FL (cm)		PW (kg)		FS+S W (g)		FS W (g)	
		Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	± SE	Mean	± SE
100%	Selenium	**1.78 a**	0.10	**55.30 a**	1.49	**24.06 a**	0.48	**1.84 a**	0.13	**68.15 a**	3.89	**46.32 a**	5.35
	Calcium	**1.69 a**	0.07	**54.92 a**	2.27	**23.65 a**	0.46	**1.68 a**	0.07	**80.13 a**	13.52	**63.01 a**	14.07
	Control	**1.77 a**	0.17	**56.94 a**	1.40	**24.00 a**	1.00	**1.85 a**	0.22	**78.87 a**	10.39	**56.51 a**	5.31
50%	Selenium	**1.53 a**	0.20	**54.62 a**	1.95	**22.35 a**	1.23	**1.56 a**	0.31	**63.65 a**	20.84	**49.82 a**	12.73
	Calcium	**1.29 a**	0.23	**51.03 a**	4.21	**21.81 a**	0.77	**1.50 a**	0.15	**60.11 a**	21.17	**52.52 a**	17.64
	Control	**1.79 a**	0.41	**55.63 a**	7.09	**23.35 a**	5.11	**1.95 a**	0.42	**61.84 a**	13.88	**50.70 a**	8.90

(FW)Fruit Weight (kg), (FD) Fruit Diameter (cm), (FL) Fruit Length (cm), (PW) Pulp Weight (kg), (FS+S W) Fibrous Strands and Seeds Weight (g), and (FS W) Fibrous Strands Weight (g). Means in the same column with different letters are significantly different at α = 0.05.

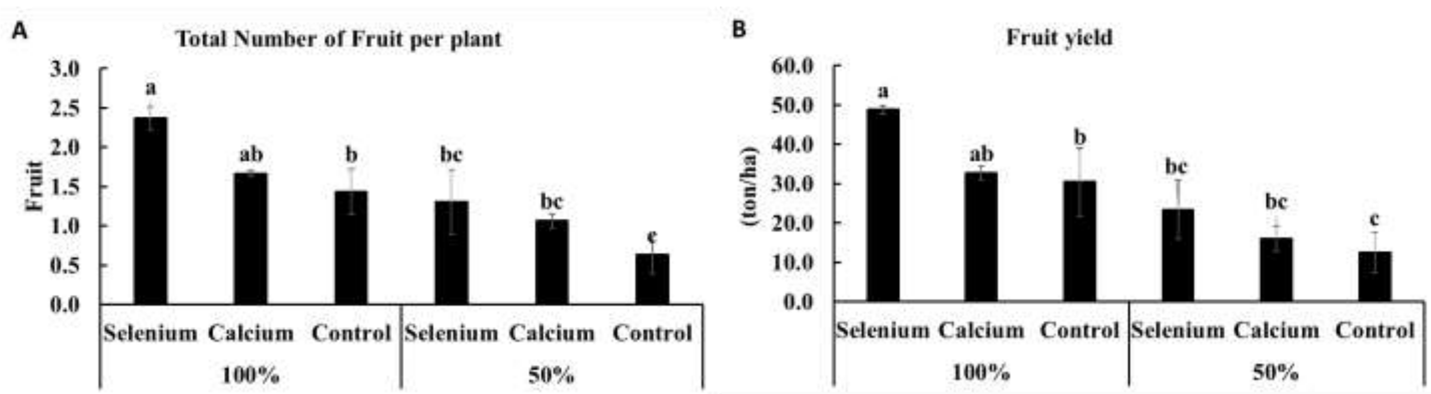

Figure 4: Effect of reduced water usage and separate applications of selenium and calcium on fruit productive traits of pumpkin. A. the total number of fruits per plant, B. the fruit yield ton per ha. Means in the same bar with different letters are different at α = 0.05.

3. 4 Seed Traits

The study focuses on seeds and their potential involvement in pumpkin plantation and productivity value. Due to the high number of replicates and uncontrolled open field environments in this study, the error bar fluctuated, making it difficult to clearly represent the statistical differences in Figure 5. In fully and half-irrigated areas, the highest seed weight (g per fruit) values with untreated (22.37), (11.14) and selenium-treated (21.82), (11.14), compared to the calcium treatments (17.12), (7.56), respectively, were recorded (Figure 5 A). The number of seeds per fruit ranged between 104.26 and 170.72; the two values were recorded from plants treated with calcium with half and fully irrigated areas, respectively. However, statistically there were no differences between the treatments (Figure 5B). The weight of 1000 seeds were statistically higher when plants were fully irrigated and control treatment (160.04 g), and the lowest value was recorded when plants were half irrigated and treated with calcium (72.81 g) (Figure 5 C). Using selenium in a fully irrigated area yielded the highest seed yield, while selenium also increased in a half-irrigated area when compared to calcium and control treatments (Figure 5 D).

In general, there were percentage differences between selenium and calcium, as well as selenium and control, in both fully and half-irrigated areas. In fully irrigated areas, the seed weight per fruit with selenium application was 24% higher than with calcium and 2.5% higher than the control, and in half-irrigated areas, 38% and 0%, respectively (Figure 5 A). The number of seeds per fruit was 1.4%, 2.5%, 6.8%, and 3.1%, respectively (Figure 5B). The weights of 1000 seeds were 25%, 11.5%, 43%, and 20.3%, respectively (Figure 5 C). Seed yield (kg per hectare) was 56%, 37%, 60%, and 91%, respectively (Figure 5 D). The percentage differences indicate that selenium was much more impactful than calcium in enhancing the seed traits under fully and reduced water usage conditions.

The seed area size (AS) (mm^2), seed perimeter length (PL) (mm), seed length (L) (mm), seed width (W) (mm), seed length-to-width ratio (LWR), seed circularity (CS), and seed distance between intersection of length and width (IS), centre of gravity (CG) (DS) (mm) for thirty randomly selected seeds from fruits were calculated. The analysis revealed no significant differences between the treatments; however, the selenium treatment resulted in the largest seed size under full irrigation (Table 3).

Contrary, the statistical analysis demonstrates a clear, significant difference when seeds were dried and fifty seeds were randomly selected. The whole seeds and kernel weight were recorded as the highest values for selenium and calcium treatment under fully and reduced water usage conditions, respectively (Table 4). The highest fifty kernel weight (5.76 gm) was recorded with selenium treatment under half irrigated conditions. Statistically, there were no significant differences between the treatments in husk weight (Table 4).

Selenium and fully irrigated plants reached the highest production value of 48792144.70 IQD and 593644.57 IQD for fruits and seeds, respectively. Control treatment under reduced water usage conditions recorded the lowest values, which were 12500144.8 IQD and 67875.19 IQD for fruits and seeds, respectively (Figure 6).

Water is the main element that controls plant growth and development. Pumpkin plants are known as a resistant plant to low soil moisture; however, there are differences in drought tolerance between genotypes and the time of exposure to water stress [7]. In this study the pumpkin plants were exposed to drought during flowering; this treatment shows a dramatic decrease growth and development in control treatment under reduced water usage in comparison to fully irrigated conditions. The reduction percentage of seed weight (g/fruit) (67%), seed count per fruit (35%), weight of 1000 seeds (g) (54%), and seed yield (Kg/ha) (143%). This reduction may be a consequence of the inhibition of translocation and not adequate nutrition and growth to produce seeds, as also the effect of lowering water usage conditions was shown to reduce morphological vegetative, flowering, and fruit traits (Table 1, 2) (Figure 1, 2, 3). Previous study showed a loss of approximately 80% in average seed yield when pumpkin plants of different genotypes were exposed to drought during the flowering period [7]. The lowest weight of 1000 seeds were (92 g) under controlled lowered water usage conditions (Figure 4 C); simultaneously, deficit irrigation during fruiting statistically reduced the weight of 1000 seeds (128.5 g) and seed yield (816 kg/ha) when compared with deficit irrigation during flowering (137.5 g) (788.6 kg/ha) and control (175.5 g) (817.1, respectively) [30]. Statistical analysis of seed-related traits shows non-significant differences among pumpkin plants treated with selenium and calcium under fully irrigated and reduced water usage conditions (Figure 5). In line with the present results, no significant improvement in fruit and seed yield was reported for different biofortification treatments with SeO_3^{2-} and SeO_4^{2-} [11]. Contrary, whole dried seed and kernel significantly increased with selenium compared to the control (Table 4). Golob *et al.* indicated that selenium can have positive effects on different plants when applied at the correct concentrations by their simultaneous addition on plant physiology and biochemistry [11]. This statement may align with the result of whole seed and kernel weight and the ability of selenium mobility within the plant, and antioxidant properties may affect translocation and nutrient accumulation in the seeds. On the other side, calcium improved the whole seed and kernel weight under fully irrigated conditions (Table 4), but its effect was not significant under reduced water usage conditions. This indicates that calcium effect was less effective compared with selenium. However, the main effect of calcium shows improvement of seed-related traits compared to control; this is in agreement with Colombari *et al.* [13], who demonstrate that calcium has a low mobility in plants, and calcium improves the number of seeds per fruit due to its impact on the number of pollen grains of pumpkin flowers.

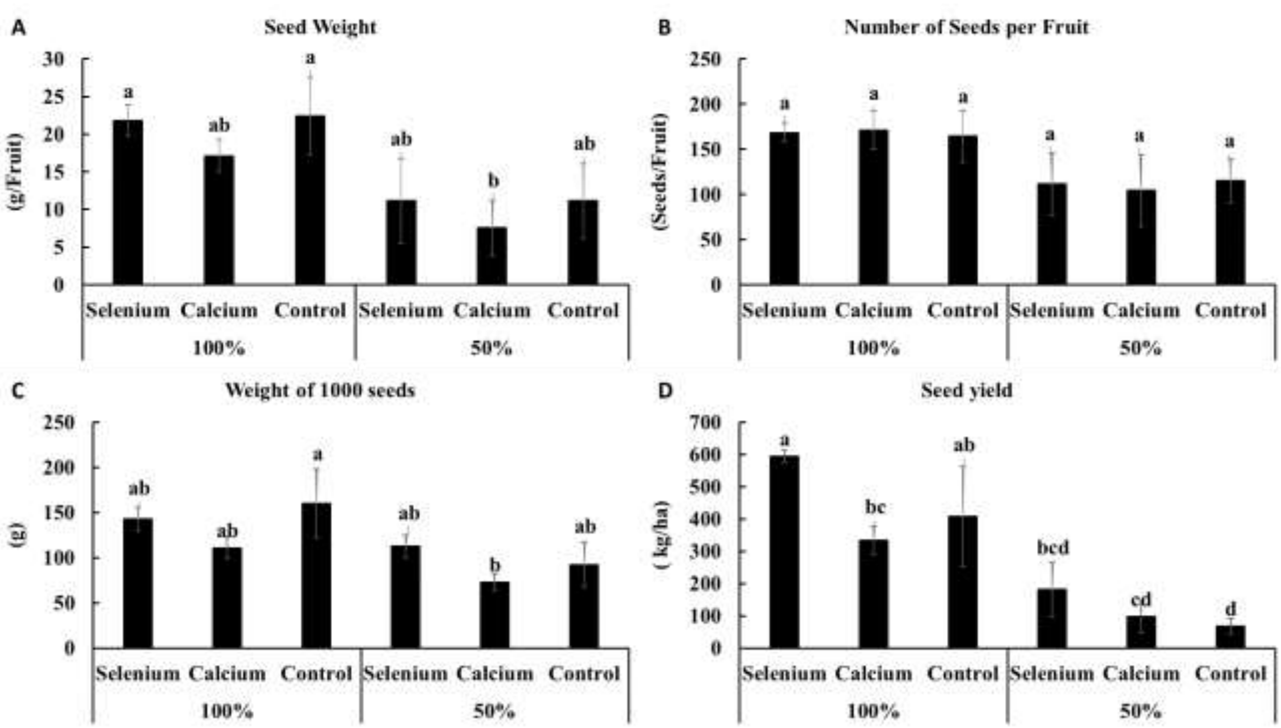

Figure 5: Figure 5. Effect of reduced water usage and separate applications of selenium and calcium on seed traits of pumpkin. A seed weight g per fruits, B the number of seeds per fruits, weight of 1000 seeds g, D seed yield kg per ha. Means in the same bar with different letters are significantly different at α = 0.05.

Table 3: Effect of reduced water usage and separate applications of selenium and calcium on seed size of pumpkin.

FC	Treatment	(AS)[mm^2]		(PL)[mm]		(L)[mm]		(W)[mm]		(LWR)		(CS)		(DS)[mm]	
		Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE
100%	Selenium	**147.31 a**	6.39	**49.87 a**	0.95	**18.22 a**	0.27	**11.29 a**	0.32	**1.62 a**	0.02	**0.74 a**	0.00	**1.45 a**	0.04
	Calcium	**146.96 a**	13.16	**49.61 a**	1.89	**18.15 a**	0.55	**11.21 a**	0.61	**1.63 a**	0.04	**0.74 a**	0.01	**1.39 a**	0.04
	Control	**159.06 a**	2.94	**51.64 a**	0.51	**18.71 a**	0.19	**11.74 a**	0.13	**1.60 a**	0.02	**0.74 a**	0.00	**1.46 a**	0.05
50%	Selenium	**126.66 a**	7.09	**46.10 a**	1.37	**16.84 a**	0.62	**10.52 a**	0.19	**1.6 a**	0.04	**0.74 a**	0.01	**1.43 a**	0.07
	Calcium	**137.10 a**	20.30	**47.60 a**	3.49	**17.33 a**	1.24	**10.90 a**	0.85	**1.60 a**	0.06	**0.75 a**	0.01	**1.29 a**	0.18
	Control	**145.35 a**	4.69	**49.63 a**	0.78	**18.18 a**	0.29	**11.19 a**	0.22	**1.63 a**	0.02	**0.74 a**	0.01	**1.54 a**	0.06

Seed Area size (AS)[mm2], Seed Perimeter length (PL)[mm], Seed Length(L)[mm], Seed Width(W)[mm], Seed Length-to-width ratio (LWR), Seed Circularity (CS)and Seed Distance between IS and CG (DS)[mm]. Means in the same column with different letters are significantly different at α = 0.05.

Table 4: Effect of reduced water usage and separate applications of selenium and calcium on seed size of pumpkin.

FC	Treatment	Whole seed (gm)		Kernel (gm)		Husk (gm)	
		Mean	± SE	Mean	± SE	Mean	± SE
100%	Selenium	**7.56 a**	0.31	**5.76 a**	0.13	**1.80 a**	0.18
	Calcium	**7.29 a**	0.34	**5.30 a**	0.27	**1.99 a**	0.08
	Control	**6.03 b**	0.20	**4.17 b**	0.23	**1.86 a**	0.04
50%	Selenium	**5.96 b**	0.00	**4.46 b**	0.06	**1.51 a**	0.06
	Calcium	**4.97 c**	0.03	**3.33 c**	0.01	**1.63 a**	0.02
	Control	**5.08 c**	0.21	**3.42 c**	0.11	**1.66 a**	0.32

Fifty dried whole seeds were weighted and the kernel were extracted to measure whole seed weight (gm), kernel weight (gm) and husk (gm). Means in the same column with different letters are significantly different at α = 0.05.

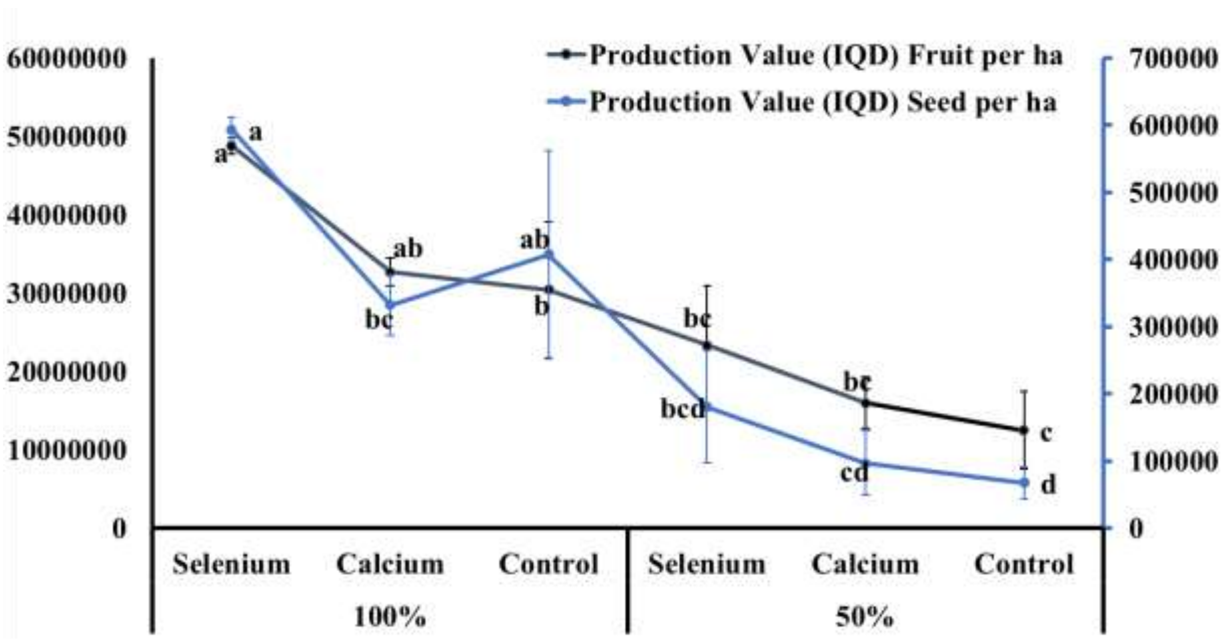

Figure 6: Effect of reduced water usage and separate applications of selenium and calcium on production value of pumpkin. Solid black line (Primary axis): Fruit production value IQD per hectare, and solid blue line (secondary axis): Seed production value IQD per hectare. Means in the same line with different letters are significantly different at α = 0.05.

3. 5 Biochemical analysis

The mineral contents of the seeds varied significantly across different treatments and field capacity (FC) levels. The highest value of selenium in pumpkin seeds were recorded when plants were fully irrigated (100% FC) and treated with calcium (2.66 ppm), while the lowest selenium level was found when plants were half irrigated (50% FC) and control treatment (Figure 7 A Solid black line). Plants treated with calcium under fully irrigation and half irrigation, statistically, resulted in the highest level of calcium in seeds (2389.96 ppm) and (2247.13 ppm) when compared with selenium and control treatments, respectively (Figure 7 A Solid blue line).

In comparison with the half-irrigated area, the 100% field capacity increased the soluble protein content in seeds by 14.81% and 6.90 % when selenium and calcium were applied (Figure 7 B solid blue line). The application of selenium and calcium, under 50% field capacity increased the values of CAT by (37.5%, 51.43%) and APX by 51.04 %, 62.85%) when compared with the control treatment, respectively (Figure 7 B).

The value of calcium and selenium determined in the seeds in this study are higher from values found in previous studies [11, 32, 33]. The variability of the recorded values maybe is due to the particular local cultivar and the environment sever fluctuation during growing session which the minimum temperature increased almost by half from April to August (Supplement table 3). In addition, the selenium and calcium content in soil and irrigation water (Supplement table 1,2), may had crucial involvement in cation and anion exchange rate in the rhizosphere region. This suggestion agrees with previous studies found that "selenium content in tea plant organs is indicated to be strongly associated with the selenium content in the main roots" [34]. Furthermore, researchers found that the protein contents on seeds of some species of Cucurbitaceae varieties ranged 30 to 36 %, within the amino acids, glutamic acid ranged from 33.03 to 34.76 g per 100 g of protein [33, 35, 36]. In this study, irrigation level show non-significant effect on soluble protein content of pumpkin seeds (Figure 7 B) and this in agreement with previous results show the depleted moisture from 20 to 100% reduced the protein content from 34.7 to 32.3 % [37]. This explains that irrigation level may have no or moderate impact on protein level of pumpkin seeds. The half-irrigated plants and treated with selenium and calcium increased the CAT and APX values significantly. Increasing the activity of antioxidant may have greater resistant against oxidative damage and improve the growth and development of the plant and consequently improve the nutritious quality of seeds [18]. Correspondingly, it was found that waterlogging stress increased the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) and the contents of proline, pyruvate, total chlorophyll, and carotenoids in pumpkins, which were higher in waterlogging-tolerant pumpkins than in waterlogging-sensitive ones [6].

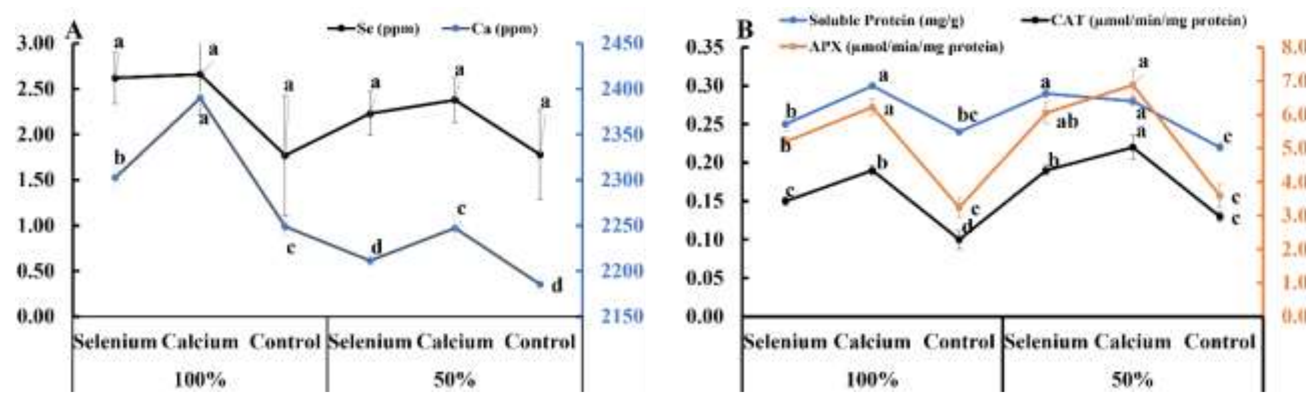

Figure 7: Figure 7: Effect of reduced water usage and separate applications of selenium and calcium on biochemical contents in seed traits of pumpkin. (A) Selenium (primary axis) and calcium (secondary axis) content. (B) The soluble protein, CAT (primary axis) and APX (secondary axis) in seeds. Means in the same line with different letters are significantly different at α = 0.05.

In general, selenium and calcium play crucial roles in physiological and biochemical processes in plants; their impact on pumpkin productivity and seed yield under irrigated and reduced usage of water conditions may vary due to the experimental environmental conditions, and this experiment was conducted in an open field with the very hot summer temperature. Therefore, the time of application and research performance and the concentration of the elements may have a direct contribution to the results.

4 Conclusion

Water scarcity is impacting the cultivation practices in the Kurdistan region. Pumpkin, as a summer-based vegetable crop, is exposed to the longest fluctuating environment in the open field. The reduction of water usage and the application of selenium and calcium partially enhanced vegetative growth, flowering, fruit set, seed traits, and biochemical contents under both 100% FC and 50% FC. The yield and nutritious quality of seeds were improved by upgrading the production values of fruits and seeds and the nutritious values and antioxidant values of seeds. The results have considerable significance for sustainable agriculture, tackling environmental concerns and economic prospects globally, and particularly for Iraqi pumpkin production. However, further research is needed to address critical challenges such as water scarcity and to develop optimal agricultural practices that enhance productivity of local vegetable crops in Kurdistan region, Iraq.

References

[1] Nanekely, M., Scholz, M. & Aziz, S. Towards sustainable management of groundwater: A case study of semi- arid area. *Eur. Water* **57**, 451–457 (2017).

[2] Mustafa, J. S. & Mawlood, D. K. Assessment of the Groundwater in Erbil Basin with Support of Visual MODFLOW. *J. Ecol. Eng.* **25**, 203–227, doi: 10.12911/22998993/184184, (2024).

[3] Chuwa, C. & Dhiman, A. K. Nutrition and Health Benefits of Ripe Pumpkin Fruit, Pulp and Powder. in *Recent Progress in Science and Technology 4,* 123–133 (B P International, doi:10.9734/bpi/rpst/v4/9411F, 2023).

[4] Polyzos, N., Fernandes, Â., Calhelha, R. C., Petrović, J., Soković, M., Ferreira, I. C., Barros, L., & Petropoulos, S. A. Biochemical Composition of Pumpkin Seeds and Seed By-Products. *Plants* **13**, 2395, doi: 10.3390/plants13172395, (2024).

[5] FAOSTAT, "Crops and livestock products." Retrieved from https://www.fao.org/faostat/en/#data/QCL

[6] Liu, Z., Liu, Z., Wang, P., Guo, L., Sun, L., Chen, B., & Li, X. Physiological and biochemical responses of the waterlogging-tolerant pumpkin resources to waterlogging stress. *Sci. Hortic. (Amsterdam).* **336**, 113347, doi: 10.1016/j.scienta.2024.113347, (2024).

[7] Seymen, M., Yavuz, D., Dursun, A., Kurtar, E. S. & Türkmen, Ö. Identification of drought-tolerant pumpkin (Cucurbita pepo L.) genotypes associated with certain fruit characteristics, seed yield, and quality. *Agric. Water Manag.* **221**, 150–159, doi: 10.1016/j.agwat.2019.05.009, (2019).

[8] Lanza, M. G. D. B. & Reis, A. R. dos. Roles of selenium in mineral plant nutrition: ROS scavenging responses against abiotic stresses. *Plant Physiol. Biochem.* **164**, 27–43, doi: 10.1016/j.plaphy.2021.04.026, (2021).

[9] Liu, H. , Xiao, C., Qiu, T., Deng, J., Cheng, H., Cong, X., Cheng, S., Rao, S., & Zhang, *Y.* Selenium Regulates Antioxidant, Photosynthesis, and Cell Permeability in Plants under Various Abiotic Stresses: A Review. *Plants* **12**, 44 doi: 10.3390/plants12010044, (2022).

[10] Alsamadany, H. , Alharby, H. F., Al-Zahrani, H. S., Kuşvuran, A., Kuşvuran, S., & Rady, M. M. Selenium fortification stimulates antioxidant- and enzyme gene expression-related defense mechanisms in response to saline stress in Cucurbita pepo. *Sci. Hortic. (Amsterdam)* **312**, 111886, doi: 10.3390/plants9070899, (2023).

[11] Golob, A., Kroflič, A., Jerše, A., Kacjan Maršić, N., Šircelj, H., Stibilj, V., & Germ, M. Response of Pumpkin to Different Concentrations and Forms of Selenium and Iodine, and their Combinations. *Plants*, **9**(7), 899, doi: 10.3390/plants9070899, (2020).

[12] Feng, D., Wang, X., Gao, J., Zhang, C., Liu, H., Liu, P., & Sun, X. Exogenous calcium: Its mechanisms and research advances involved in plant stress tolerance. *Front. Plant Sci.* **14**, doi: 10.3389/fpls.2023.1143963, (2023).

[13] Colombari, L. F., Da Silva, G. F., Chamma, L., Chaves, P. P. N., Makita, M. K., Soman, J. M., & Cardoso, A. I. I. Calcium and Boron Foliar Application in the Production and Quality of Zucchini Seeds. *Brazilian Arch. Biol. Technol.* **65**, doi: 10.1590/1678-4324-2022220056, (2022).

[14] de Jong van Lier, Q., Logsdon, S. D., Pinheiro, E. A. R. & Gubiani, P. I. Plant available water. in *Encyclopedia of Soils in the Environment* 509–515, doi:10.1016/B978-0-12-822974-3.00043-4, (Elsevier, 2023).

[15] Willis Chan, D. S. & Raine, N. E. Phenological synchrony between the hoary squash bee (Eucera pruinosa) and cultivated acorn squash (*Cucurbita pepo*) flowering is imperfect at a northern site. *Curr. Res. Insect Sci.* **1**, 100022, doi: 10.1016/j.cris.2021.100022, (2021).

[16] Tanabata, T., Shibaya, T., Hori, K., Ebana, K. & Yano, M. SmartGrain: High-Throughput Phenotyping Software for Measuring Seed Shape through Image Analysis. *Plant Physiol.* **160**, 1871–1880, doi: 10.1104/pp.112.205120, (2012).

[17] Martinec, N., Balbino, S., Dobša, J., Šimunić-Mežnarić, V., & Legen, S. Macro- and microelements in pumpkin seed oils: Effect of processing, crop season, and country of origin. Food Science & Nutrition, **7**(5), 1634–1644, doi: 10.1002/fsn3.995, (2019).

[18] Najafi, S., Nasi, H. N., Tuncturk, R., Tuncturk, M., Sayyed, R. Z., & Amirnia, R. Biofertilizer Application Enhances Drought Stress Tolerance and Alters the Antioxidant Enzymes in Medicinal Pumpkin (*Cucurbita pepo* convar. pepo var. Styriaca). *Horticulturae*, **7**(12), 588, doi: 10.3390/horticulturae7120588, (2021).

[19] Yang, X., Lu, M., Wang, Y., Wang, Y., Liu, Z., & Chen, S. Response Mechanism of Plants to Drought Stress. *Horticulturae*, **7**(3), 50, doi: 10.3390/horticulturae7030050, (2021).

[20] Fowmina, S. Influence of organic manures and biofortification of selenium on growth, yieldand selenium contentof cucumber (*Cucumis sativus* L.). *Ann. Plant Soil Res.* 88–92, doi:10.47815/apsr.2021.10035, (2021).

[21] Abd AlRaof, M., abeer, . H. M. Ismaeil, faten, A.E.Elnabarway, amira & S.A. Eldesouky, heba. Improvement of squash plants growth growing under cold stress conditions by using calcium and phosphorus forms. *Benha J. Appl. Sci.* **9**, 43–53, doi: 10.21608/bjas.2024.268354.1321, (2024).

[22] Yasir, T. A., Azhar, W., Ali, Q., Masood Bhutta, M. U., Ateeq, M., Wasaya, A., Hussain, M., & Riaz, R. Foliar applied calcium chloride alleviated drought stress in pearl millet (*Pennisetum glaucum* L.) by improving growth and yield contributing traits and antioxidant activity. *PLoS One* **19**, e0310207, doi: 10.1371/journal.pone.0310207, (2024).

[23] Neppl, P. & Wehner, C. Vine length of a diverse set of watermelon cultivars.Cucurbit Genetics Cooperative Report **24**, 65–67. (2001).

[24] Zhang, M., Ma, M., Lang, H., & Jiang, M. Research advances and perspectives on early flowering traits in cucumber. Plants **14**(8), 1158, doi: 10.3390/plants14081158, (2025).

[25] Barman, M., Tenhaken, R. & Dötterl, S. Negative and sex-specific effects of drought on flower production, resources and pollinator visitation, but not on floral scent in monoecious Cucurbita pepo. *New Phytol.* **244**, 1013–1023 doi: 10.1111/nph.20016, (2024).

[26] Wien, H. C., Stapleton, S. C., Maynard, D. N., McClurg, C. & Riggs, D. Flowering, Sex Expression, and Fruiting of Pumpkin (*Cucurbita sp.*) Cultivars under Various Temperatures in Greenhouse and Distant Field Trials. *HortScience* **39**, 239–242, doi: 10.21273/HORTSCI.39.2.239, (2004).

[27] Abedi, S., Iranbakhsh, A., Oraghi Ardebili, Z. & Ebadi, M. Nitric oxide and selenium nanoparticles confer changes in growth, metabolism, antioxidant machinery, gene expression, and flowering in chicory (*Cichorium intybus* L.): potential benefits and risk assessment. *Environ. Sci. Pollut. Res.* **28**, 3136–3148, doi: 10.1007/s11356-020-10706-2, (2021).

[28] Quinn, C. F., Prins, C. N., Freeman, J. L., Gross, A. M., Hantzis, L. J., B. Reynolds, R. J., Covey, P. A., Bañuelos, G. S., Pickering, I. J., Fakra, S. C., Marcus, M. A., Arathi, H. S., & H. Pilon-Smits, E. A. Selenium accumulation in flowers and its effects on pollination. *New Phytol.* **192**, 727–737, doi: 10.1111/j.1469-8137.2011.03832.x, (2011).

[29] Stapleton, S. C., Wien, H. C. & Morse, R. A. Flowering and Fruit Set of Pumpkin Cultivars under Field Conditions. *HortScience* **35**, 1074–1077, doi: 10.21273/HORTSCI.35.6.1074, (2000).

[30] Masoodi, S. & Hakimi, L. Medicinal pumpkin responses to Thiobacillus and sulfur under water stress. *Plant Physiol.* 7(4), 2185–2194, doi: 10.22034/ijpp.2017.537983, (2017).

[31] Ertek, A., Şensoy, S., Küçükyumuk, C. & Gedik, İ. Irrigation frequency and amount affect yield components of summer squash (*Cucurbita pepo* L.). *Agric. Water Manag.* **67**, 63–76, doi: 10.1016/j.agwat.2003.12.004, (2004).

[32] Men, X., Choi, S., Han, X., Kwon, H., Jang, G., Choi, Y., Park, S., & Lee, O. Physicochemical, nutritional and functional properties of Cucurbita moschata. *Food Science and Biotechnology* **30**(2), 171–183, doi: 10.1007/s10068-020-00835-2, (2020).

[33] Jahan, F., Islam, M. B., Moulick, S. P., Bashera, M. A., Hasan, M. S., Tasnim, N., Saha, T., Boby, F., Waliullah, M., Saha, A. K., Hossain, A., Ferdousi, L., Rahman, M. M., Saha, B. K., & Bhuiyan, M. N. H. Nutritional characterization and antioxidant properties of various edible portions of Cucurbita maxima: A potential source of nutraceuticals. *Heliyon* **9**(6), e16628, doi: 10.1016/j.heliyon.2023.e16628, (2023).

[34] Wu, H., Zhang, D., Wu, X., Tian, X., Hu, G., Liu, S., Jie, X., & Wang, D. Uptake and transport of selenium in a Soil–Tea Plant–Tea infusion system: A study of typical tea plantations in a Selenium-Rich area of China. *Forests* **15**(6), 914, doi: 10.3390/f15060914, (2024).

[35] Steiner-Asiedu, M., Nuro-Ameyaw, P., Agbemafle, I., H. Hammond, S. & Tano-Debrah, K. Nutrient Composition and Protein Quality of Four Species of the Cucurbitaceae Family. *Adv. J. Food Sci. Technol.* **6**, 843–851, doi: 10.19026/ajfst.6.122, (2014).

[36] Al-Anoos IM, RAH El-dengawy & HA Hasanin. Studies on chemical composition of some Egyptian and Chinese pumpkin (*Cucurbita maxima*) seed varieties. *Plant Sci. Res.* **2**, 1–4 (2015).

[37] Kirnak, H., Irik, H. A., Sipahioglu, O. & Unlukara, A. Variations in oil, protein, fatty acids and vitamin E contents of pumpkin seeds under deficit irrigation. *Grasas y Aceites* **70**, e301, doi: 10.3989/gya.0692181, (2019).