Interactive effects of Foliar 24-Epibrassinolide and selenium applications on yield, reduce nitrate accumulation and selenium enrichment in potato tuber in field

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Abstract: The Individual Influence Of Brassinosteroids Or Selenium Has Been Broadly Investigated In Many Plant And Crop Species. However, Least Reports Are Available On The Combined Effects Of Those Two On Plants. Therefore, Present Study Investigated The Impact Of Foliar Application Of 24- Epibrassinolide (EBL) (0, 0.07, 0.1 And 0.14 µm) And Selenium (Se) (0, 15, 30 And 60 Mg L⁻¹) On Plant Growth, Tuber Number And Yield, Antioxidant Enzyme Activity And Also On The Contents Of Nitrate, Selenium, Starch And Amino Acids Of Potato Tubers (Cv. Sante) In Field In Iran (Jiroft), During Two Growing Seasons (2015–2016). The Interaction Of The Growing Season And EBL And Se Was Not Significant For Parameters Recorded And Therefore, The Combined Results Of Two Growing Seasons Are Presented. EBL Or Se Improved Parameters Recorded And Their Interaction Was Significant For Most Parameters, Except For Alanine And Methionine. However, The Combined Effects Of EBL (0.14 µm) And Se (60 Mg L⁻¹) Was Most Effective And Compared With Control, This Combination Increased Shoot Fresh Weight (21%), Tuber Yield (24%), Nitrate

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I am Aboumoslem Bideshki born in Iran, I am and graduated from Tehran University of Science and Research with a degree in Horticulture. So far I have published more than 35 scientific articles in Iranian and international journals ISI and ISC. Most of his research has been in the field of horticulture and vegetable science.

PUBLIC INTEREST STATEMENT
The potato (Solanum tuberosum L.) tuber is the third most important food crop in the world after rice and wheat for human consumption. Excessive use of nitrogen fertilizers increases the nitrate concentration in consumable organs of crops, especially vegetables. Many vegetables, including potato, accumulate nitrate in their tubers. Nitrate nitrogen is not toxic to plants but harmful to consumers. As there are very limited reports on the combined effects of epibrassinolide (EBL) and Selenium (Se) on plant growth and productivity, Due to the high consumption of potatoes in Iran and the world and the lack of selenium in human food and the urgent need of the human body for this element, the present experiment was conducted in Iran, to study the impact of foliar EBL and Se application on growth, yield and tuber nitrate, leaf nitrate reductase, selenium enrichment and antioxidant enzymes activities in potato leaves.
Reductase Activity (47%), Tuber Selenium (5.4 Fold), Tuber Starch (42%), Isoleucine (34%), Superoxide Dismutase (37%), Leaf Total Chlorophyll (23%) And Decreased Tuber Nitrate Reduction (39%) And Malondialdehyde (29%). Thus, It Can Be Concluded That Foliar Application Of EBL And Se As Two Promising And Environment-Friendly Natural Substances Can Profoundly Improve Tuber Yield And Quality And Can Have Commercial Applications.

Subjects: Agriculture; Horticulture; Agriculture And Food; Food Chemistry

Keywords: amino acids; nitrate; nitrate reductase; starch; tuber yield

1. Introduction

The potato (Solanum tuberosum L.) tuber is the third most important food crop in the world after rice and wheat for human consumption and makes an important contribution to the diet of people worldwide (Haverkort, Struik, Visser, & Jacobsen, 2009). Global cultivation area of about 19.35 million hectares in 2014 and annual production of nearly 382 million tons and nearly 5 million tons has been reported in Iran (FAO, 2016).

Brassinosteroids (BRs) are widely accepted as new phytohormones with significant growth-promoting effects (Bajguz & Piotrowska-Niczyporuk, 2014; Vardhini, 2013). Brassinolide, 28-homobrassinolide and 24 epibrassinolide are the most active type of BRs which are broadly used in physiological and experimental studies (Vardhini & Anjum, 2015). BRs are classified as C27, C28 and C29 BRs based on the number of carbons in their structure (Vardhini & Anjum, 2015) and have profound impact on plant physiology, growth and development including; seed germination (Syed Ali Fathima, Johnson, & Lingakumar, 2011), cell division (Ho, 2003; Nakaya et al., 2002), leaf expansion (Zhiponova et al., 2013), source/sink relationships and photosynthesis (Denget, 2007). They also have a role in the control of excess reactive oxygen species (ROS) produced in cells under unfavorable conditions (Bailly et al., 2008). The uncontrolled accumulation of ROS leads to oxidative damage toward a wide range of biomolecules. Moreover, the involvement of BRs in the regulation of ROS metabolism is evident as they can promote the activity of enzymatic and non-enzymatic antioxidants.

BRs can be applied to plants at various phases of the life cycle; vegetative phase (Asha and Lingakumar, 2015), flowering Phase (Vardhini, 2013) or grain filling phase (Vardhini, 2013) and at various methods; seed soaking (Syed Ali Fathima et al., 2011) or foliar spray (Hemmati, Ebadi, Khomari, & Sadeghi, 2018).

BRs are reported to have significant interaction with other phytohormones for regulating plant growth and metabolism including; auxins, cytokinins and gibberellins (Zhao & Li, 2012).

Foliar application of BRs improves Cd tolerance in Brassica juncea and Phaseolus vulgaris L. via increasing the activity of antioxidant enzymes (Hayat, Ali, Hasan, & Ahmad, 2007; Rady, 2011). Furthermore, the increase of nitrate reductase activity and reduction of nitrate accumulation and also increases in amino acids of cucumber shoot has been reported (Yuan et al., 2012).

Selenium (Se), as an essential part of human nutrition, plays a significant role in a number of metabolic functions and also shelters plants from several abiotic and biotic stresses (Schiavon & Pilon-Smits, 2017). Se can affect the quality of vegetables and fruit. Increased cellular content of linoleic acid and sterols and a decreased oleic acid content have been observed in Camellia oleifera plants treated with Se (Song et al., 2015).

Se spraying has been used to enhance the selenium content in potatoes (Poggi, Arcioni, Filippini, & Pifferi, 2000), rice (Chen et al., 2002), broccoli (Sindelarova et al., 2015), radishes (Schiavon et al., 2016), basil (Mezeyova, Hegedüssova, Andrejiova, Hegedüs, & Golian, 2016), tomatoes (Zhu, Chen,
Zhang, & Li, 2016, grapes (Zhu, Liang, Gao, An, & Kong, 2017) and other crops (Puccinelli, Malorgio, & Pezzarossa, 2017). Plants may be grown on selenium-enriched substrates (Hernandez-Castro et al., 2015) or treated with Se foliar application (Mezeyova et al., 2016). Exogenous application of Se could substantially increase Se concentration in crops, vegetables, and fruits (Cartes, Gianfreda, & Moro, 2005; Hartikainen, 2005). Elevating Se content in plant food can effectively avoid and prevent Se deficiency in humans. Thus, increasing selenium concentration and reducing \( \text{NO}_3^− \) content in crops arouse widespread concern for both researchers and farmers (Bian, Yang, & Liu, 2015; Bian, Cheng, Yang, Wang, & Lu, 2016; Cartes et al., 2005; Hartikainen, 2005) (Also, Se increased vegetative growth and tuber yield, protein content and starch percentage in potato (Yassen, Safia, & Sahar, 2011). Se treatment increased nitrate reductase activity and decreased nitrate accumulation in a leaf of lettuce (Bo et al., 2018).

Previous studies found that exogenous Se could affect nitrogen metabolism in plants (Nowak, Kaklewski, & Ligocki, 2004; Rios et al., 2010) and also affects uptake and translocation of some mineral elements, such as inhibiting cadmium and \( \text{Na}^+ \) accumulation, and promoting \( \text{K}^+ \) uptake (Sun, Wang, Dai, Zhang, & Wu, 2013).

As there are very limited reports on the combined effects of EBL and Se on plant growth and productivity, the present experiment was conducted to study the impact of foliar EBL and Se application on growth, tuber number and yield and also on tuber nitrate, leaf nitrate reductase and antioxidant enzymes activities in potato leaves.

2. Materials and methods
Two field experiments were carried out in Jiroft (57 90 E L. 28 13 N Lat., 625 m above sea level), Kerman, Iran, during 2015–16 (October to Jan) and 2016 (Jan to Apr) with Sante cultivar. In Jiroft Minimum and maximum temperatures over growing season were 8 and 40 °C, respectively in first season and 8 and 43 ºC, respectively in the second. Relative humidity was 55-60% in every two years. The soil had a sandy-loam texture, and the acidity (pH), electrical conductivity (EC), absorbable potassium, absorbable phosphorus, total nitrogen and selenium content of the soil extract were 7.50, 2.41 ds m\(^{-1}\), 159 ppm, 8.80 ppm, 0.03 % and 0.17, respectively.

This study was carried out as a factorial experiment based on randomized complete block design (RCBD) with three replicates. Experimental treatments included foliar application of 24-epibrassinolide (EBL) (0, 0.07, 1.00 and 0.14 \( \mu \text{M} \)) 30 days after plant emergence and selenium (Se) (0, 15, 30 and 60 ppm) as sodium selenate (\( \text{Na}_2\text{SeO}_4 \)) 35 days after plant emergence.

The seed tuber was planted at 25 cm inter-row spacing producing a population of 53,000 plants per hectare. The soil was fertilized by the applications of 650 kg ha\(^{-1}\) urea (half before planting and half 10 weeks after) and also 60 kg Potassium phosphate and 100 kg potassium sulfate per hectare, applied before planting.

Foliar application of EBL and Se was applied using a hand sprayer until the solution began to drip off leaves at sunset to ensure its complete uptake by the leaves. It was applied on three consecutive days. The plants not receiving EBL treatment similarly sprayed with an equivalent amount of water. Weeding was done manually throughout the growing season.

Superoxide dismutase (SOD), catalase (CAT), proline (Pro), malondialdehyde (MDA) and chlorophyll content (Chlo) in potato leaves were measured on the fully expanded leaves for each treatment at 30–45 day before final harvest. Finally, tuber was harvested when the leaves turned yellowish and data of tuber number and yield were collected from the middle three rows avoiding 1 meter from the end of rows. Furthermore, the content of nitrate, selenium accumulation, starch content, alanine, glycine, isoleucine and methionine in tuber of potato and also leaf nitrate reductase were measured.
2.1. **Determination of total chlorophyll content**

Chlorophyll a and b as well as carotenoids were measured as reported by Lichtenthaler and Wellburn (1987). One-hundred mg of fresh leaf material was taken from the leaves and extracted with 95% methanol. Absorption was read using a spectrophotometer at wavelengths of 653 and 666 nm for chlorophyll a and b, respectively. The chlorophyll concentrations were calculated according to the following equations:

\[
\text{Chl a} = (12.25 \times A_{663} - 2.79 \times A_{646})
\]

\[
\text{Chl b} = (21.21 \times A_{646} - 5.1 \times A_{663})
\]

\[\text{Chl Total} = \text{Chl a} + \text{Chl b}\]

2.1.1. **Determination of superoxide dismutase (SOD) and catalase (CAT) activities**

The fully expanded leaves were harvested, simultaneously frozen in liquid N$_2$. The leaf samples were homogenized in ice-cold 1 ml of 0.1 M potassium phosphate buffer (pH 7.8) containing 1mM ethylene di-amine-tetra-acetic acid (EDTA) and 2% (w/v) PVP. The mixture was centrifuged for 20 min at 12,000 g at 4°C and the supernatant was used for enzyme activity determinations. The SOD activity assay was determined using the modified protocol of Dhindsa, Plumb-Dhindsa, and Thorpe (1981). The activity of CAT was appraised following the protocol described by Aebi (1984).

2.2. **Determination of proline (pro) and malondialdehyde (MDA)**

Leaves from each treatment were taken and immersed in double-distilled water. Dry leaf samples were placed in boiling distilled water contained in a water bath for 30 min. The mixture was subjected for 10 min to centrifugation at 8000 g. To an aliquot of 0.5 ml of the supernatant 1.5 ml of distilled water and 0.5 ml of 5% phenol and 5 ml of conc. H$_2$SO$_4$ were added. After vigorously shaking the mixture it was placed for one min in a boiling water bath. After cooling the mixture at room temperature its color change was noted at 485 nm using a spectrophotometer. The protocol described by Bates, Waldren, and Teare (1973) was employed for determining leaf free Pro. Lipid peroxidation was determined by estimating the MDA content following the method of Heath and Packer (1968). One gram of leaves was macerated in 5 ml of 0.1% (w/v) trichloroacetic acid (TCA). The resulting homogenate was centrifuged for 5 minutes at 10,000 X g. 4 ml of 20 % TCA containing 0.5% TBA was added per 1 ml of the aliquot of the supernatant,. The mixture was heated at 95º C for 30 minutes and cooled quickly in an ice bath. The contents were centrifuged at 10,000 X g for 10 minutes and the absorbance was measured at 532 nm and the value for the non-specific absorbance at 600 nm was subtracted. The concentration of MDA was calculated by using an extinction coefficient of 155 mM$^{-1}$ cm$^{-1}$. MDA content was expressed as nM g$^{-1}$ fresh weight.

2.2.1. **Determination of alanine (ala), glycine (gly), isoleucine (ile) and methionine (met)**

The precolumn derivatization method of amino acid analysis using high-performance liquid chromatography (HPLC) was used (Biddlingmeyer, Cohen, & Tarvin, 1984). The derivatization reagent consisted of ethanol-triethylamine (TEA)-water-phenyl isothiocyanate (PITC) in the ratio of 71: 1: 1. The free amino acid pool was extracted with 2 mL of distilled water, and 20 PL of the supernatant was derivatized. The derivatized volume was reconstituted to 200 PL, and 20 PL of this volume was injected into the HPLC. The total amino acid content was assayed by hydrolyzing the aqueous extract. Hydrolysis was carried out by adding 0.1 M HCl to the extract and heating the mixture to 150 °C for 95 min. The hydrolyzed sample was derivatized, reconstituted, and injected into the chromatograph. Selenocysteine and selenomethionine were used as standards in addition to the standard amino acids.

2.3. **Determination of nitrate and nitrate reductase**

Nitrate measurement with an ion selective electrode by the Swiss Mtrvhm manufactured (Jones, 2001). The activity of leaf nitrate reductase enzyme was measured according to the method proposed by Stewart, Lee, and Orebamja (1972).
2.4. **Determination of selenium accumulation**

Selenium in tuber samples was analyzed by an electrothermal atomic absorption spectrometry method (Ekholm, 1997).

2.5. **Determination of starch content**

Tuber cubes in a blender with distilled water. The homogenate was filtered through three layers of a thin membrane of cotton and the filtrate was collected in a beaker. The residue retained on the membrane was discarded and the filtrate containing the starch was re-suspended in distilled water and left to rest for 4 h/4ºC until the starch settled. Subsequently, the starch was separated from the supernatant and suspended in water. This step was repeated five times to acquire a white starch and a semi-transparent supernatant. The extraction of potato starch required the use of the antioxidant sodium sulfite at a concentration of 0.03%, in agreement with established food legislation (Brasil, 2005) to avoid enzymatic degradation, mainly by peroxidases and phenolases. This antioxidant was only employed during the first extraction phase, during tuber milling.

2.6. **Data analysis**

The collected data for each attribute were subjected to analysis of variance using SAS v.9.1 software. The Duncan's Multiple Range test (P ≤ 0.05) was used to determine the significant difference between treatment means.

3. **Results**

3.1. **Plant growth and tuber yield**

The results for the effect of treatments on plant growth and tuber yield are presented in Table 1. All levels of 24-epibrassinolide (EBL) or selenium (Se) at 60 mg l⁻¹ increased shoot fresh weight (SFW) by 15% whereas, combined EBL (0.1 or 0.14 µM) and Se (60 mg l⁻¹) further increased it by 22%, compared with control treatment. Se had little effect on tuber number, tuber yield or leaf chlorophyll whereas, EBL (0.14 µM) increased tuber number by 32%, tuber yield by 21% and leaf chlorophyll by 14%, relative to control. However, the combined effects of EBL (0.14 µM) with Se (30 mg l⁻¹) was most effective and further increased tuber number (37%), tuber yield (26%) and leaf chlorophyll contents (29%).

3.2. **Antioxidant enzymes activity, proline and malondialdehyde**

Catalase was not affected by EBL or Se whereas; SOD was increased by both treatments. The combined effect of EBL (0.14 µM) and Se (60 mg l⁻¹) increased the SOD activity by 37%, relative to control treatment (Table 2).

The results of this research showed that, malondialdehyde (MDA) was not significantly affected by the application of Se and 0.07 and 0.1 µM EBL concentrations. Although, application of 0.14 µM EBL significantly reduced (27%) the MDA in potato leaves. Indeed, an applied combination of Se and EBL (0.1 and 0.14 µM) could reduce the malondialdehyde (Table 2). The leaf free proline was enhanced markedly under treatments of 30 and 60 ppm Se, by 13 and 9% respectively. Externally applied EBL caused a marked increase in leaf free proline content in the plants of potato. In addition, maximum proline content (2.50 mg g⁻¹ dw⁻¹) was observed under an applied combination of Se (60 ppm) and EBL (0.1 and 0.14 µM) (Table 2).

3.3. **Tuber amino acids**

EBL or Se had a similar promotive impact on tuber contents of alanine, methionine and glycine. Se (30 mg l⁻¹) increased the contents of alanine, methionine and glycine by 48%, 34% and 25% and EBL (0.14µM) increased those by 56%, 46% and 70%, respectively, compared with the control treatment (Table 2). However, the effect of Se or EBL on isolenine was different, such that, Se had little effect and EBL (0.14 µM) increased it by 21% (Table 2). Meanwhile, the combined EBL (0.14 µM) and Se (30 mg l⁻¹) increased it by 37% (Table 2).
Table 1. Effect of 24-epiassinosteroid (EBL) and selenium (Se) foliar application on shoot fresh weight, leaf chlorophyll content, tuber number and yield of potato. RC: Percent change

| EBL (µM) | Se (mg l\(^{-1}\)) | Plant fresh weight (gr) | RC | Chlorophyll content (mg/gfw) | RC | Tuber number | RC | Yield (ton/ha) | RC |
|----------|---------------------|-------------------------|----|-----------------------------|----|---------------|----|---------------|----|
| 0        | 0                   | 679.60\(^{d}\)          | 100| 10.14\(^{ef}\)             | 100| 6.80\(^{d}\) | 100| 32.60\(^{b}\) | 100|
|          | 15                  | 680.55\(^{cd}\)         | 112| 10.38\(^{ef}\)             | 102| 7.44\(^{cd}\) | 109| 33.62\(^{a}\) | 103|
|          | 30                  | 674.22\(^{c}\)          | 111| 9.82\(^{f}\)               | 97 | 7.41\(^{d}\) | 109| 34.48\(^{a}\) | 106|
|          | 60                  | 699.05\(^{bc}\)         | 115| 10.07\(^{d}\)              | 99 | 7.55\(^{d}\) | 111| 34.44\(^{a}\) | 106|
| 0.07     | 0                   | 701.55\(^{bc}\)         | 115| 10.32\(^{de}\)             | 102| 7.55\(^{d}\) | 111| 33.88\(^{b}\) | 104|
|          | 15                  | 697.82\(^{bc}\)         | 114| 11.44\(^{bcde}\)           | 113| 8.41\(^{bc}\) | 124| 36.14\(^{f}\) | 111|
|          | 30                  | 704.44\(^{b}\)          | 116| 12.07\(^{bc}\)             | 119| 8.66\(^{bc}\) | 127| 37.33\(^{a}\) | 115|
|          | 60                  | 713.55\(^{bc}\)         | 117| 11.56\(^{bcde}\)           | 114| 8.69\(^{bc}\) | 128| 36.71\(^{ef}\) | 113|
| 0.1      | 0                   | 700.55\(^{bc}\)         | 115| 11.01\(^{cde}\)            | 109| 7.95\(^{bc}\) | 117| 36.11\(^{f}\) | 111|
|          | 15                  | 699.99\(^{bc}\)         | 115| 11.62\(^{bcd}\)            | 115| 8.16\(^{bc}\) | 120| 37.01\(^{ef}\) | 114|
|          | 30                  | 706.77\(^{b}\)          | 116| 12.41\(^{b}\)              | 122| 8.63\(^{bc}\) | 127| 38.70\(^{d}\) | 119|
|          | 60                  | 743.53\(^{c}\)          | 122| 11.44\(^{bcde}\)           | 113| 8.69\(^{bc}\) | 128| 38.76\(^{ad}\) | 119|
| 0.14     | 0                   | 698.88\(^{c}\)          | 115| 11.56\(^{bcd}\)            | 114| 8.96\(^{bc}\) | 132| 39.41\(^{cd}\) | 121|
|          | 15                  | 712.99\(^{b}\)          | 117| 12.28\(^{bc}\)             | 121| 9.30\(^{a}\)  | 137| 41.40\(^{a}\) | 127|
|          | 30                  | 743.39\(^{d}\)          | 122| 13.09\(^{c}\)              | 129| 9.29\(^{a}\)  | 137| 41.06\(^{ac}\) | 126|
|          | 60                  | 734.76\(^{c}\)          | 121| 12.50\(^{b}\)              | 123| 9.02\(^{b}\)  | 133| 40.29\(^{bc}\) | 124|

Means following same letters within each column do not differ significantly using DMRT at \(P \leq 0.05\).
Table 2. Effect of 24-epiassinosteroid (EBL) and selenium (Se) foliar application on leaf catalase and superoxide dismutase enzymes, proline, malondialdehyde and tuber alanine, methionine, isoleucine and glycine. RC: Percent change

| EBL (µM) | Catalase (ΔOD/g fw min) | Superoxide dismutase (U/mg protein) | RC Proline (mg/g gdw) | RC malondialdehyde (nmol/cm) | RC Alanine (mg/g) | RC Methionine (mg/g) | RC Isoleucine (mg/g) | RC Glycine (mg/g) | RC |
|----------|------------------------|------------------------------------|-----------------------|-----------------------------|----------------|---------------------|---------------------|----------------|---|
| 0        | 12.59<sup>abc</sup>   | 100                                | 8.03<sup>e</sup>      | 100                         | 4.96<sup>*</sup>  | 100                 | 1.92<sup>e</sup>  | 100            | 2.80<sup>m</sup>  | 100 | 199<sup>h</sup>  |
| 15       | 12.28<sup>ac</sup>    | 98                                 | 8.13<sup>e</sup>      | 101                         | 2.08<sup>*</sup>  | 102                 | 4.24<sup>e</sup>  | 85             | 2.66<sup>g</sup>  | 139 | 120<sup>c</sup>  |
| 30       | 11.41<sup>h</sup>     | 91                                 | 8.24<sup>e</sup>      | 103                         | 2.30<sup>*</sup>  | 113                 | 4.59<sup>e</sup>  | 93             | 2.85<sup>cd</sup> | 148 | 134<sup>c</sup>  |
| 60       | 12.27<sup>ac</sup>    | 97                                 | 8.71<sup>e</sup>      | 109                         | 2.20<sup>*</sup>  | 109                 | 4.42<sup>e</sup>  | 89             | 2.82<sup>cd</sup> | 147 | 127<sup>c</sup>  |
|          | 0.07                   |                                    |                       |                             |                |                     |                     |                |                        |     |                       |
| 0        | 12.15<sup>bc</sup>    | 97                                 | 8.48<sup>e</sup>      | 106                         | 2.13<sup>*</sup>  | 105                 | 4.73<sup>bc</sup> | 95             | 2.94<sup>cd</sup> | 153 | 124<sup>c</sup>  |
| 15       | 12.99<sup>ac</sup>    | 103                                | 8.65<sup>e</sup>      | 108                         | 2.37<sup>*</sup>  | 117                 | 4.41<sup>bc</sup> | 89             | 2.77<sup>ef</sup> | 144 | 125<sup>c</sup>  |
| 30       | 12.44<sup>ac</sup>    | 99                                 | 8.85<sup>e</sup>      | 110                         | 2.43<sup>*</sup>  | 120                 | 4.12<sup>bc</sup> | 83             | 2.85<sup>def</sup> | 148 | 127<sup>c</sup>  |
| 60       | 13.41<sup>bc</sup>    | 107                                | 8.42<sup>e</sup>      | 105                         | 2.29<sup>*</sup>  | 113                 | 4.06<sup>bc</sup> | 82             | 2.88<sup>def</sup> | 150 | 134<sup>c</sup>  |
|          | 0.1                    |                                    |                       |                             |                |                     |                     |                |                        |     |                       |
| 0        | 13.43<sup>bc</sup>    | 107                                | 8.95<sup>e</sup>      | 111                         | 2.24<sup>*</sup>  | 110                 | 3.96<sup>bc</sup> | 80             | 2.91<sup>def</sup> | 151 | 134<sup>c</sup>  |
| 15       | 12.09<sup>bc</sup>    | 96                                 | 9.29<sup>e</sup>      | 116                         | 2.31<sup>*</sup>  | 114                 | 3.83<sup>bc</sup> | 77             | 2.78<sup>bc</sup> | 145 | 132<sup>c</sup>  |
| 30       | 12.22<sup>bc</sup>    | 97                                 | 10.05<sup>e</sup>     | 125                         | 2.40<sup>*</sup>  | 118                 | 3.71<sup>bc</sup> | 75             | 2.96<sup>def</sup> | 154 | 127<sup>c</sup>  |
| 60       | 12.69<sup>bc</sup>    | 101                                | 10.41<sup>e</sup>     | 130                         | 2.50<sup>*</sup>  | 123                 | 3.57<sup>bc</sup> | 72             | 3.03<sup>bcd</sup> | 158 | 139<sup>c</sup>  |
|          | 0.14                   |                                    |                       |                             |                |                     |                     |                |                        |     |                       |
| 0        | 13.14<sup>bc</sup>    | 104                                | 8.97<sup>e</sup>      | 111                         | 2.29<sup>*</sup>  | 113                 | 3.62<sup>bc</sup> | 73             | 3.00<sup>bc</sup> | 156 | 146<sup>c</sup>  |
| 15       | 13.21<sup>bc</sup>    | 105                                | 9.12<sup>e</sup>      | 114                         | 2.37<sup>*</sup>  | 117                 | 3.67<sup>bc</sup> | 74             | 2.86<sup>def</sup> | 149 | 125<sup>c</sup>  |
| 30       | 13.91<sup>h</sup>     | 110                                | 10.42<sup>e</sup>     | 130                         | 2.46<sup>*</sup>  | 121                 | 3.54<sup>bc</sup> | 71             | 2.93<sup>cde</sup> | 153 | 129<sup>bc</sup> |
| 60       | 13.41<sup>bc</sup>    | 106                                | 10.98<sup>e</sup>     | 137                         | 2.54<sup>*</sup>  | 125                 | 3.55<sup>bc</sup> | 72             | 3.06<sup>bc</sup> | 158 | 134<sup>c</sup>  |

Means following same letters within each column do not differ significantly using DMRT at P ≤ 0.05.
3.4. Tuber nitrate, selenium, starch and leaf nitrate reductase activity

Se (60 mg l\(^{-1}\)) or EBL (0.14 µM) reduced tuber nitrate by 12% and 27%, respectively, relative to control (Table 3). However, the combined effect of Se and EBL (60 mg l\(^{-1}\) and 0.14 µM) was most effective and the relative reduction was 39% (Table 3). Tuber selenium content was mainly affected by Se application and relative to control, 60 mg l\(^{-1}\) Se increased Se content by 4.14 fold. However, the combined application of Se (60 mg l\(^{-1}\)) and EBL (0.14 µM) further increased the Se content by 5.4 fold (Table 3). The impact of EBL (0.14 µM) or Se (60 mg l\(^{-1}\)) was very similar on tuber starch content and the relative increase was 33% and 27%, respectively (Table 3). However, the combined effect was more effective and the relative increase was 42%. The response of nitrate reductase activity to EBL (0.14 µM) or Se (60 mg l\(^{-1}\)) was very similar and the relative increase was 21%. However, the combined effect was more effective and the relative increase was 47% (Table 3).

4. Discussion

Brassionsteroids (BRs) can have a great impact on different aspects of plant growth and development. Besides, they could increase plant tolerance to many biotic or abiotic stresses in many crop species (Hemmati et al., 2018; Bajguz & Piotrowska-Niczyporuk, 2014; Nakaya et al., 2002; Zhiponova et al., 2013). Also, several reports show that changing the level of endogenous BRs improves the quality of agricultural products (Vrient et al., 2012). However, exogenous application or manipulation of endogenous BRs may lead to great changes in agricultural productivity and product quality (Hemmati et al., 2018).

Improved plants growth, treated with BRs is associated with increased levels of DNA, RNA, soluble proteins and numerous forms of carbohydrates (Vardhini and Rao 1998). Recently, it has been shown that BRs stimulate cell division independently of other growth hormones. However, BRs respond to the anoxic levels of auxin and increase the effect of each other (Arteca, 1996), thereby increasing plant height and increasing plant fresh weight.

Foliar application of EBL on potato plant in the present experiment, increased tuber number and yield, by regulating the number of tubers in each plant, stimulate cell division and proliferation on the entire tuber yield as reported by Arteca (1996). Also, EBL reduced the stress effect imposed on plants by reducing leaf MDA and increasing proline and SOD in the present experiment which is frequently reported in the plant (Vardhini & Anjum, 2015). In terms of agricultural products quality, the contents of amino acids, starch and essential oil in the plant were tremendously improved following EBL application which has been reported in some research (Hemmati et al., 2018; Syed Ali Fathima et al., 2011).

Although Se is not yet confirmed as an essential micronutrient in higher plants (Rani, Dhillon, & Dhillon, 2005; Turakainen, Hartikainen, & Seppanen, 2004), many researchers have already shown that selenium (Se) could increase the tolerance of plants exposed to stressful environments (Aslam, Harbit, & Huffaker, 1990; Djanaguiraman, Devi, Shanker, Sheeba, & Bangarusamy, 2005; Djanaguiraman, Prasad, & Seppanen, 2010). In the present study, potato plants treated with Se resulted in an increase in shoot fresh weight and tuber yield. This outcome is consistent with previously published reports that exogenous Se increased shoot and root biomass production in lettuce (Liu et al., 2017; Simojoki, Xue, Lukkari, Pennanen, & Hartikainen, 2003), cucumber seedlings (Jozwiak et al., 2016) and pepper (Shekari, Kamelmanesh, Mozafariyan, Hasanuzzaman, & Sadeghi, 2017). Moreover, the worthwhile effect of Se on seedling length and dry weight of broccoli sprout was reported by Takeda, Kondo, Ueda, and Iida (2016). However, Saffaryazdi, Lahouti, Ganjeali, and Bayat (2012) reported increasing leaf number and fresh and dry weight of spinach herbs at low levels of Se and stated that the positive effect of Se on fresh weight of the plant due to the synthesis of chlorophyll, carbon stabilization, synthesis and hydrolysis of starch and stimulated cell division caused by the use of Se in meristem cells.

An increase in the yield of other tuber plants has also been reported following the application of Se, and the main reason for this increase is related to the antioxidant effects of Se. Based on the results of previous studies, the growth of plants treated with Se was associated with a decrease in lipid peroxidation associated with increased activity of GSH-Px (Djanaguiraman et al., 2005).
Table 3. Effect of 24-epiassinosteroid (EBL) and selenium (Se) foliar application on tuber nitrate content, leaf nitrate reductase and the content of selenium and starch content of potato tuber. RC: Percent change

| EBL (µM) | Se (mg/l) | Nitrate content (mg/kg) | RC | Nitrate reductase (µg nitrite/gfw hour) | RC | Selenium content (mg/gdw) | RC | Starch content (%) | RC |
|----------|-----------|-------------------------|----|----------------------------------------|----|-------------------------------|----|-----------------------|----|
| 0        | 0         | 654.86a                | 100| 9.12bc                                  | 100| 1.82a                         | 100| 12.68f               | 100|
| 15       | 600.20b   | 609.20b                | 92 | 9.63a                                  | 106| 5.29f                         | 291| 13.68f               | 108|
| 30       | 587.05c   | 591.05c                | 90 | 10.76c                                 | 118| 7.19c                         | 394| 13.74f               | 108|
| 60       | 573.27d   | 569.27d                | 88 | 11.01c                                 | 121| 7.54d                         | 414| 16.08de              | 127|
| 0.07     | 0         | 604.88d                | 92 | 9.99d                                  | 109| 2.06e                         | 113| 15.52e               | 122|
| 15       | 534.35e   | 538.35e                | 82 | 10.87c                                 | 119| 7.32bcd                        | 402| 16.13de              | 127|
| 30       | 493.09f   | 489.09f                | 75 | 11.42d                                 | 125| 9.17e                         | 501| 16.97bcd              | 134|
| 60       | 510.87g   | 506.87g                | 78 | 11.88e                                 | 130| 8.50d                         | 467| 16.45de              | 130|
| 0.1      | 0         | 558.78h                | 85 | 10.19g                                 | 112| 2.32e                         | 128| 17.24bcd              | 136|
| 15       | 470.01i   | 474.01i                | 72 | 12.36h                                 | 136| 5.81cd                        | 319| 17.07bcd              | 135|
| 30       | 407.92j   | 404.92j                | 62 | 12.87i                                 | 141| 8.50e                         | 467| 18.41bd              | 145|
| 60       | 405.40k   | 402.40k                | 62 | 12.75j                                 | 139| 10.17f                        | 559| 16.74cde             | 132|
| 0.14     | 0         | 478.83l                | 73 | 11.04c                                 | 121| 2.48g                         | 136| 16.91bcd              | 133|
| 15       | 417.92m   | 414.92m                | 64 | 13.14d                                 | 144| 7.29bcd                        | 401| 18.06abc             | 142|
| 30       | 403.77n   | 400.77n                | 62 | 13.44d                                 | 147| 7.51bcd                        | 413| 19.07a               | 150|
| 60       | 389.34o   | 386.34o                | 61 | 13.43d                                 | 147| 9.83n                         | 540| 18.01abc             | 142|

Means following same letters within each column do not differ significantly using DMRT at P ≤ 0.05
Turakainen, Hartikainen, Ekholm, and Seppanen (2007) reported that more potato function was achieved in potato plants treated with Se. Se increases the allocation of phytosimulates to grow tubers, so tubers act as a rich source for carbohydrates and selenium accumulation. They also attributed the positive effect of selenium on potatoes to the antioxidant effects of selenium on the plant’s aging delay. They reported that selenium fertilization could improve the nutritional value of potato by increasing the level of organic compounds containing selenium in the tubers.

The obtained results reveal that both EBL and Se can improve most recorded parameters individually as reported by many researchers in many crop species. However, the promotive combined effects of BRs and Se has not been reported in details as in the present study. Indeed, the combined application of EBL and Se at proper concentrations (0.14 µM EBL and 60 mg l⁻¹ Se) very effectively improved most key parameters recorded including tuber yield and the contents of tuber nitrate, starch and selenium by further enhancement of SOD and nitrate reductase activity and proline contents far more effective than individual application.

5. Conclusion
Foliar application of EBL on potato plant in the present experiment, increased tuber number and yield, by regulating the number of tubers in each plant, stimulate cell division and proliferation on the entire tuber yield. In the present study, potato plants treated with Se resulted in an increase in shoot fresh weight and tuber yield. In conclusion, foliar application of Se and EBL especially when applied in combination are effective in promoting potato tuber number and yield, reduction of nitrate content and increasing selenium accumulation. In addition, alleviation of tuber nitrate accumulation by foliar application of Se and EBL was found to be associated partly with enhanced leaf nitrate reductase activity.

Abbreviations

| Abbreviation | Definition |
|--------------|------------|
| Ala | Alanine |
| EBL | 24-Epibrassinolide |
| CAT | Catalase |
| Gly | Glycine |
| Chlo | Chlorophyll |
| Ile | Isoleucine |
| MDA | Malondialdehyde |
| Met | Methionine |
| Pro | Proline |
| Se | Selenium |
| SOD | Superoxide Dismutase |

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