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Plant Growth and Fruit Quality Response of Strawberry is Improved After Exogenous Application of 24-Epibrassinolide

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Abstract

Foliar application of mineral nutrients, proteins, and plant growth regulators is frequently practiced on strawberries for better yield and extended harvest period. Here, we evaluated the influence of 24-epibrassinolide, a brassinosteroid, on strawberry plant growth, yield, and fruit quality. Healthy strawberry plants were foliar sprayed with 1, 2 and 3 µM 24-epibrassinolide after 100, 115, and 130 days of runner transplantation. Foliar application of 24-epibrassinolide enhanced specific leaf area and root-to-shoot ratio in concentration-dependent manner. Though, root weight ratio and leaf area ratio were also positively influenced with 24-epibrassinolide applications, but plants treated with 2 µM 24-epibrassinolide exhibited maximum response. Overall, better vegetative growth of plants exogenously treated with 3 µM 24-epibrassinolide led to threefold increase in flowering and better fruit harvest index than control plants. Principal component analysis was further employed to delineate concentration-dependent effects of 24-epibrassinolide. Though, foliar application of 1 µM 24-epibrassinolide was positively correlated with fruit pH and 2 µM 24-epibrassinolide application had a promotive impact on leaf area, plant dry weight, and fruit sugars but foliar spray of 3 µM 24-epibrassinolide was most influential in inducing plant vegetative growth (leaf area ratio, specific leaf area, root-to-shoot ratio, root weight ratio and shoot weight ratio), yield (flowers and fruits per plant and harvest index), and quality attributes (sugar–acid ratio, ascorbic acid, and organoleptic characteristics) of strawberry fruits. Conclusively, results suggest that foliar application of 3 µM 24-epibrassinolide favours vegetative growth, enhances yield and improves quality of strawberry fruit.

Keywords Brassinosteroids · *Fragaria × ananassa* · Vegetative growth · Yield · Harvest index · Ascorbic acid

Introduction

Strawberry is believed to be originated from Northeast, Europe, and North America and is now cultivated all over the world in hilly tropics and temperate regions as a perennial fruit crop (Rubinstein 2015). Its accessory fruit is an aggregate of achenes attached on the surface of edible flesh developed from receptacle (Hassan et al. 2000). Modern strawberry cultivars are characterized by their large size and bright red colour (Whitaker et al. 2013). Being non-climacteric, strawberry fruit is picked at red ripe stage when fruit attains scrumptious blend of sugars and organic acids (Furio et al. 2019). In addition to its excellent mouthfeel, strawberry also contains significant amount of antioxidants like vitamin E, anthocyanin, *β*-carotene, and ascorbic acid contents (Van De Velde et al. 2013). According to FAO, China holds the largest share (35.4%) in global production of strawberry followed by USA (15.5%), Mexico (8%), Turkey (5%), and Egypt (4.3%) (FAOSTAT 2018).

Brassinosteroids are polyhydroxylated steroidal hormones ubiquitously found in plants (Zullo and Bajguz 2019). Biosynthesis and catabolism of brassinosteroids and their sensing and signalling cascades in plants have also been well elucidated (Clouse 2011; Gruszka 2019;

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Hohmann and Hothorn 2018). As broad-spectrum key regulator, brassinosteroids are involved in plethora of plant growth and developmental processes including rhizogenesis, seed germination, cell elongation, vascular differentiation, leaf formation, flowering, maturation, senescence, abscission, and responses to abiotic and biotic stresses (Anwar et al. 2018a; Hola 2019; Kaur and Pati 2019; Kohli et al. 2019; Krishna et al. 2018; Nolan et al. 2020; Sağlam Çağ 2019; Siddiqui et al. 2019b; Yusuf et al. 2019). Brassinosteroids also interact and crosstalk with other phytohormones like abscisic acid, auxin, ethylene, gibberellins, jasmonic acid, polyamines, salicylic acid, and strigolactones to regulate these physiological and developmental processes in plants (Anwar et al. 2015; Gruszka 2019; Ohri et al. 2019). Among 60 brassinosteroid analogues characterized so far (Bartwal et al. 2013), epibrassinolide, homobrassinolide, and brassinolide are the most stable brassinosteroids (Ali 2017).

Being naturally occurring and eco-friendly, use of brassinosteroids on crop plants holds great potential as an alternative to hazardous chemicals (Ali 2017). Considering the importance of brassinosteroids in plant growth and stress mitigation, use of brassinosteroids on food crops has emerged as a promising strategy to influence traits of agronomic importance and boost yield of economically important agronomic crops (Vriet et al. 2012). Likewise, brassinosteroids have also been reported to enhance plant vigour, and improve productivity and fruit quality of horticultural crops (Ali 2019). Though, few studies on exogenous applications of brassinosteroids and their inhibitors convincingly suggest role of brassinosteroids in enhancing leaf area (Furio et al. 2019; Pipattanawong et al. 1996) and leaf chlorophyll (Asghari and Zahedipour 2016; Furio et al. 2019) but a detailed study to comprehend various aspects of strawberry plant growth regulated by brassinosteroids and impact of these modulations on yield and fruit quality is still needed. Similarly, the promotive effect of brassinosteroids on flowering, yield (Pipattanawong et al. 1996; Symons et al. 2012; Zahedipour-Sheshglani and Asghari 2020), fruit ripening (Bombarely, 2010; Chai et al. 2013; Zahedipour-Sheshglani and Asghari 2020) and sugar–acid ratio in strawberry fruit (Furio et al. 2019; Zahedipour-Sheshglani and Asghari 2020) has been reported. However, effectiveness of brassinosteroid may vary with geographical locations, harvesting years, nature of the crop (Khripach et al. 2000), and applied concentration (Zahedipour-Sheshglani and Asghari 2020).

Thus, it may be hypothesized that foliar application of 24-epibrassinolide affects strawberry plant growth and fruit quality in a concentration-dependent manner. To test this hypothesis and segregate concentration-dependent variations in plant growth and fruit quality, strawberry plants were foliar sprayed with 1 µM, 2 µM, and 3 µM 24-epibrassinolide, and changes in plant vegetative growth, flowering, yield, biochemical and sensorial attributes of strawberry fruit were examined.

Material and Methods

Plant Source, Experimental Design and Treatments

Healthy runners of strawberry cv. Chandler were sourced from Mingora, District Swat, Khyber Pakhtunkhwa and transplanted at Experimental Research Area (31°26′03.5″N 73°04′31.3″E), University of Agriculture, Faisalabad. Runners were transplanted on both sides of ridges with 8-inch plant-to-plant, 9-inch row-to-row, and 18-inch ridge-to-ridge distance. The distribution of plants for treatments was assigned according to randomized complete block design (RCBD). In each of the three blocks, three replications were assigned to each treatment and each replication contained 30 healthy plants. Strawberry production followed standard cultural practices including drip irrigation, mineral nutrition, weed management, and insect/pest and disease control. This field had been under strawberry cultivation followed by bottle gourd (*Lagenaria siceraria*) or zucchini (*Cucurbita pepo*) production for the last five years. One hundred days after transplanting, strawberry plants were foliar sprayed with either only water (control) or with 1 µM, 2 µM or 3 µM 24-epibrassinolide (CAS No. 78821-43-9, ≥ 85% purity, Sigma Aldrich, USA) and repeated three times at 15 days interval. The 24-epibrassinolide was dissolved in 0.1% dimethyl sulfoxide and the final volume of solution was maintained by adding water pre-mixed with 0.1% (v/v) Tween 20 as a surfactant (Babalık et al. 2020). Strawberry plants were foliar sprayed with 24-epibrassinolide early in the morning using 1 L electronic sprayer operated at constant speed. Plant vegetative and reproductive growth data was recorded at 7 days interval until the end of the harvest season.

Nitrogen, Phosphorus, and Potassium Determination in Leaf Tissues

Fully expanded, mature, and healthy trifoliate leaves along with petiole were collected 3–5 leaves back from the growing point following standard leaf sampling procedure (Haifa 2017). The first sampling was done one week before start of foliar applications and the second sampling was done during peak harvest season. Leaf washing, drying, digestion, and estimation of nitrogen, phosphorus and potassium in leaf tissues were carried out as described by Estefan et al. (2013).

Plant Vegetative Growth Attributes

The relative greenness index of *on-planta* leaves was measured with chlorophyll SPAD meter (CCM-200 plus, Opti-Sciences, USA) and presented as SPAD value. After final fruit harvest, whole plants were taken out and fresh weight of each plant was measured using digital weighing balance (MJ-W176P, Panasonic, Japan). Each plant was then separated into leaves, roots, and crown (shoot) parts. Leaf area was measured with the help of automated digital image analysing software 'Easy Leaf Area' version 1.02 (Easlon and Bloom 2014). For dry weight determination, individual plant sections were oven-dried at 65 °C until constant dry mass was attained (Kuisma et al. 2014). Dry weight percentage was calculated on fresh weight bases. Root weight ratio, shoot weight ratio and leaf weight ratio were calculated by dividing dry weight of roots, shoot (crown) and leaves to dry weight of whole plant, respectively (Butler et al. 2002; Chiariello et al. 1989; Martínez-Ferri et al. 2016). Root-to-shoot ratio was calculated by dividing dry weight of below-ground parts to dry weight of above-ground parts of the plant (Butler et al. 2002). Leaf area was divided with dry weight of leaves to get specific leaf area (Chiariello et al. 1989; Fernandez et al. 2001). Leaf area ratio was estimated by dividing leaf area to dry weight of the whole plant (Butler et al. 2002; Fernandez et al. 2001).

Number of Flowers, Fruits and Harvest Index

Number of flowers, total number of red ripe fruits (> 75% red) per plant were recorded every week until final harvest. Harvest index was calculated at the time of harvest by dividing fresh weight of fruits to fresh weight of the whole plant (Martínez-Ferri et al. 2016).

Fruit Fresh Weight, Length, Diameter, Fruit Shape Index and Dry Weight

Fruit fresh weight, length (from maximum vertical point), diameter (from maximum horizontal point) was calculated by taking average of five fruit batches where each batch contained ten strawberries from same treatment. Fruit weight was measured with digital weighing balance (MJ-W176P, Panasonic, Japan), whereas length and diameter were measured with digital Vernier callipers (DR-MV0100NG, Ningbo Dongrun Imp. & Exp. Co., Ltd., China). Length was divided by diameter of each fruit to calculate length-to-width ratio, hereafter called as fruit shape index. Fresh fruits were dried in hot air dehydrator (Ultimate 4000, Fowlers Vacola Australia Pty Ltd) until complete loss of moisture contents in fruit tissues (Kuisma et al. 2014). Dry weight percentage was calculated on fresh weight bases.

Ion Leakage, Total Titratable Acids, Total Soluble Solids and Sugar–Acid Ratio

Electrolyte/ion leakage in strawberry fruits was determined by cutting ten small pieces of fruit in 20-mL of double-distilled water. After 30 min, initial reading was recorded with EC meter (HI-98304, Hanna Instruments Inc., Mauritius). Then, samples were heated at 100 °C for 15 min. After 60 min, volume was made up with double-distilled water and final reading was recorded. Relative ion leakage with respect to initial and total amounts of ions present in fruit tissues was calculated and presented in percentage (Lutts et al. 1995). A supernatant from homogenized mixture of ten healthy fruits centrifuged at 10,000×*g* for 15 min was used for further biochemical analyses. Total titratable acids were determined with NaOH-based titrimetric method (Hortwitz 1960) and expressed as percent citric acid. Total soluble solids were determined with hand-held digital refractometer (Atago, Hybrid PAL-BXIACID F5, Japan). The sugar–acid ratio was calculated by dividing total soluble solids to total titratable acids within the same sample (Hasan 2020).

Determination of Sugars, Ascorbic Acid Contents, and pH

Fruit juice pH was recorded with digital pH meter (Hanna, HI-98107, Mauritius), whereas 2,6-dichlorophenol indophenol dye was used as chemical reagent for the determination of ascorbic acid (Shahzad et al. 2020). The method earlier detailed by Anwar et al. (2018b) was used to determine sugar content. Briefly, an aliquot of fruit pulp, treated with 25% lead acetate and 20% potassium oxalate, was hydrolysed with HCl, and kept overnight to convert non-reducing sugars into reducing sugars. Then, HCl-hydrolysed aliquot was neutralized with 0.1 N NaOH and titrated against Fehling solutions.

Organoleptic Evaluation

Organoleptic evaluation of strawberries was done by panellists (aged between 22 and 26 years) using pre-defined sensory scales (Jouquand et al. 2008; Resende et al. 2008; Schwieterman, 2014). Strawberries samples were randomly arranged and blindly labelled for presentation to panel of judges. Texture, aroma, appearance and flavour were evaluated with 1–9 hedonic scale (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely), whereas sweetness and tartness/sourness were evaluated with five-point just-right scale (1 = dislike extremely; 3 = neither like nor dislike; 5 = like extremely).

Statistical Analysis

The experiment was conducted under the randomized complete block design (RCBD) with three blocks. Each block contained one replication of each treatment. Collected data was analysed for analysis of variance (ANOVA) and Fisher's least significance difference (LSD) method for pair-wise comparison of mean values at 5% significance level using analytical software package 'Statistix 8.1'. Variables having statistically significant impact of treatments ($P \leq 0.05$) were further subjected to principal component analysis using XLSTAT ver. 2018. Correlation coefficient values were determined with Pearson (n) method. Clustering of variables with associated treatments was determined with their highest squared cosine values corresponding to factor, F1, F2 or F3.

Results

Nitrogen, Phosphorus and Potassium Levels in Leaf Tissues

Before foliar application of 24-epibrassinolide, levels of nitrogen and potassium in strawberry leaf tissues were statistically similar among all treatments, whereas strawberries grown for application of 2 μM 24-epibrassinolide had slightly high level of phosphorus (Fig. 1, Online Resource 1). Foliar application of 24-epibrassinolide, regardless of concentration applied, caused 30% reduction in nitrogen levels in strawberry leaves as compared with control (Fig. 1a). In contrast, foliar application of 2 μM 24-epibrassinolide elevated phosphorus levels (1.9-fold higher than control) in strawberry leaves, whereas potassium levels remained unaffected with 24-epibrassinolide application (Online Resource 1).

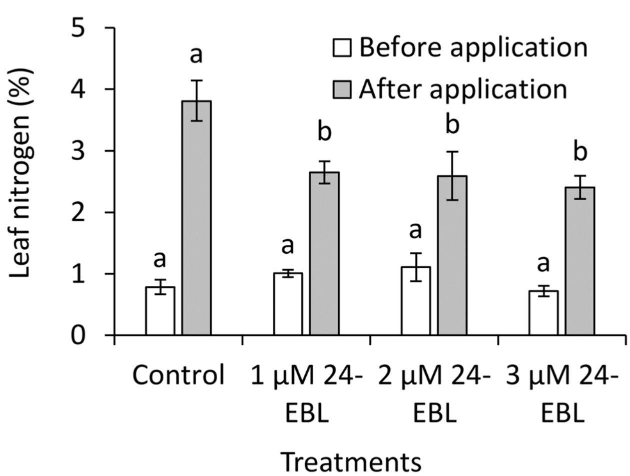

Fig. 1 Effect of foliar application of 24-epibrassinolide (EBL) on nitrogen content in strawberry leaves. Strawberry plants were foliar sprayed with 24-epibrassinolide 100, 115 and 130 days after transplantation. Similar letters at a single time-point (either before or after fertilizer application followed by foliar spray of 24-epibrassinosteroid) indicate non-significant difference among treatments ($\alpha = 0.05$). Vertical bars indicate mean ± standard error ($n = 3$, 3-block RCBD arrangement)

Determining the Plant Growth and Development

Overall, greenness index in strawberry leaves did not change with application of 24-epibrassinolide except slight but statistically significantly decrease was observed in plants treated with 1 μM 24-epibrassinolide when compared with control (Online Resource 2a). Foliar application of 2 μM 24-epibrassinolide slightly increased dry mass of strawberry plants, whereas no change in whole plant dry weight was observed in plants treated with 1 μM and 3 μM 24-epibrassinolide as compared to control (Online Resource 2b). Foliar application of 2 μM 24-epibrassinolide resulted in 1.5-fold increase in leaf area and 4.6-fold increase in leaf area ratio (leaf area divided by plant dry weight) of strawberry plants when compared with control (Fig. 2a, b). This positive effect of 2 μM 24-epibrassinolide was followed by 3 μM 24-epibrassinolide application, whereas lowest values were recorded in control plants (143.27 cm^2 and 0.09 $m^2\ g^{-1}$, respectively). Interestingly, specific leaf area (leaf area to leaf dry weight ratio) increased in a concentration-dependent manner (Fig. 2c). Foliar application of 1, 2, and 3 μM 24-epibrassinolide resulted in fourfold, ninefold, and 11-fold increase in specific leaf area, respectively, compared to control plants.

Leaf weight ratio (leaf dry weight-to-plant dry weight ratio) of strawberry plants was negatively influenced by treatment with 24-epibrassinolide (Fig. 3a). Overall, foliar application of 24-epibrassinolide induced 41–48% reduction in leaf weight ratio compared to control. In contrast to leaf weight ratio, 24-epibrassinolide positively influenced shoot weight ratio (shoot dry weight-to-plant dry weight ratio) (Fig. 3b). Shoot weight ratio of strawberry plants, foliar applied with 1 μM and 3 μM 24-epibrassinolide, was 2.2-fold higher than control, whereas strawberry plants treated with 2 μM 24-epibrassinolide exhibited 1.6-fold increase in shoot weight ratio when compared with control.

Like increase in specific leaf area, root-to-shoot ratio also increased with the application of 24-epibrassinolide in dose dependant manner (Fig. 3c). Foliar application of 1 μM, 2 μM and 3 μM 24-epibrassinolide resulted in fourfold, five-fold and 12-fold increase in root-to-shoot ratio than control. Root weight ratio (root dry weight-to-plant dry weight ratio) was positively influenced by foliar application of 24-epibrassinolide (Fig. 3d). Strawberry plants supplemented with 2 μM 24-epibrassinolide exhibited 2.4-fold increase in root weight ratio than control, whereas those supplied with 1 μM and 3 μM 24-epibrassinolide exhibited statistically similar but twofold increase in root weight ratio than control.

Number of Flowers, Fruits and Harvest Index

Foliar application of 24-epibrassinolide enhanced flowering and yield on strawberry plants (Fig. 4). Until final harvest,

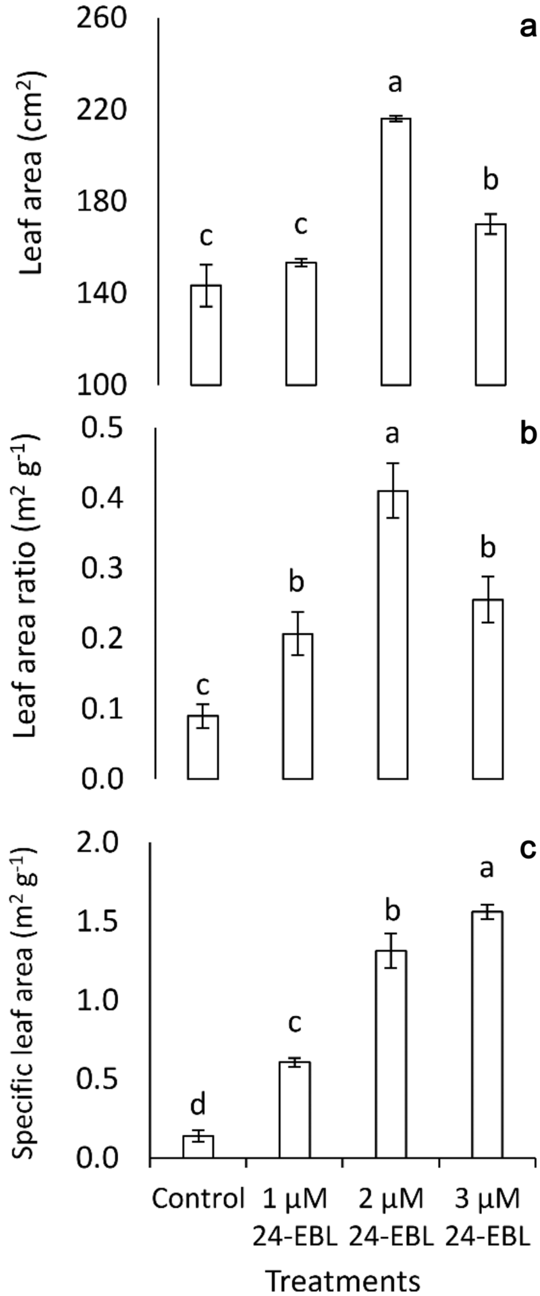

Fig. 2 Effect of foliar application of 24-epibrassinolide (EBL) on leaf area, leaf area ratio and specific leaf area of strawberry plants. Strawberry plants were foliar sprayed with 24-epibrassinolide 100, 115 and 130 days after transplantation. Leaf area ratio is the leaf area to whole plant dry weight ratio, whereas specific leaf area is leaf area to leaf dry weight ratio. Similar letters indicate non-significant difference among treatments ($\alpha = 0.05$). Vertical bars indicate mean ± standard error ($n = 3$, 30 plants per replicate under 3-block RCBD arrangement)

59 days after first foliar spray, plants treated with 3 µM 24-epibrassinolide produced 3.7-fold more flowers and 4.3-fold more ripe fruits than control, whereas increase in flowering and yield due to 1 µM and 2 µM 24-epibrassinolide application was statistically comparable with each other but still significantly higher than control, i.e. 1.7-fold increase in flowering, twofold increase in yield than control.

Harvest index was calculated at the time of harvest by dividing fresh weight of fruits to fresh weight of whole plant (Fig. 5). Increase in harvest index was also positively correlated with the applied concentration of 24-epibrassinolide. Compared to control, foliar application of 1 µM, 2 µM and 3 µM 24-epibrassinolide led to threefold, 9.2-fold and 11.9-fold increase in harvest index, respectively.

Fresh Weight, Dry Weight and Fruit Shape Index

Fresh weight, dry weight, and shape index (length-to-width ratio) of fruits from strawberry plants treated with 24-epibrassinolide was statistically comparable to control (Online Resource 3) suggesting that foliar application of 24-epibrassinolide within the studied range did not influence these characteristics of strawberry fruits.

Ion Leakage, Titratable Acids, Soluble Solids and Sugar–Acid Ratio

Ion leakage, an indicator of membrane permeability, from tissues of red ripe strawberries was also determined. Results showed that ion leakage increased in dose dependant manner (Fig. 6a). Strawberry plants treated with 3 µM 24-epibrassinolide produced fruits with highest membrane permeability, i.e. 1.7-fold higher than control. Fruit from strawberry plants receiving 1 µM or 2 µM 24-epibrassinolide foliar sprays also showed slight but significant increase in pH level, whereas effect of 3 µM 24-epibrassinolide on flesh pH was non-significantly different than control (Fig. 6b). A reciprocal effect of 24-epibrassinolide on total titratable acids and total soluble solid contents in strawberries tissues was observed (Fig. 6c, d). Foliar application of 1 µM and 3 µM 24-epibrassinolide decreased total titratable acids, whereas increased total soluble solids in strawberry flesh when compared with control. Consequently, strawberry fruits harvested from 1 µM and 3 µM 24-epibrassinolide-treated plants exhibited 1.4-fold and twofold increase in sugar–acid ratio (TSS/TTA ratio) than control, respectively (Fig. 6e). In contrast to relatively low or high doses, foliar application of 2 µM 24-epibrassinolide did not affect total titratable acids, total soluble solids and sugar–acid ratio as compared to control.

Higher values of pulp ascorbic acid contents were measured in 1 µM 24-epibrassinolide-treated plants (117.5 mg 100 g^{-1}) followed by 3 µM 24-epibrassinolide-treated plants (Fig. 6f). These values were significantly different than control but non-significantly different from each other. In contrast, lowest ascorbic acid content (83.56 mg 100 g^{-1}) was recorded in control plants. Exogenous application of

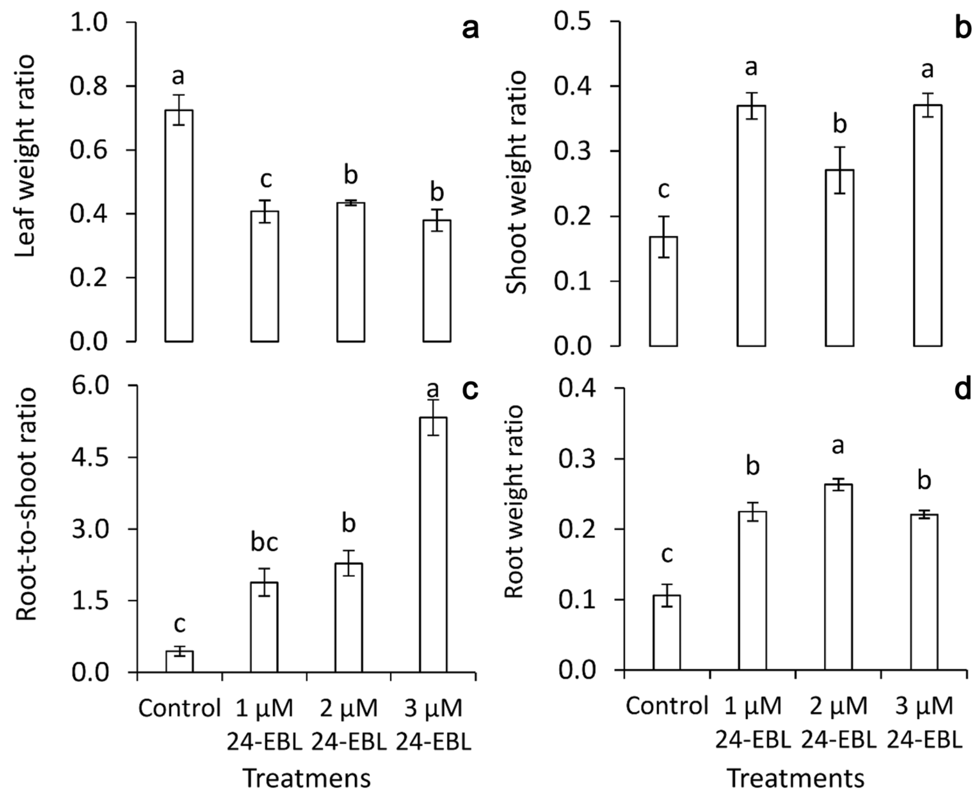

Fig. 3 Effect of foliar application of 24-epibrassinolide (EBL) on leaf weight ratio, shoot weight ratio, root-to-shoot ratio, and root weight ratio of strawberry plants. Strawberry plants were foliar sprayed with 24-epibrassinolide 100, 115 and 130 days after transplantation. Leaf, shoot, or root weight ratio was calculated by dividing dry weight of leaf, shoot (crown) or root to dry weight of whole plant, respectively. Root-to-shoot ratio was calculated by dividing dry weight of below-ground parts to dry weight of above-ground parts of the plant. Similar letters indicate non-significant difference among treatments ($\alpha = 0.05$). Vertical bars indicate mean ± standard error ($n = 3$, 30 plants per replicate under 3-block RCBD arrangement)

24-epibrassinolide positively influenced accumulation of ascorbic acids in strawberry fruit tissues (Fig. 6f). Overall, foliar application of 1–3 µM 24-epibrassinolide resulted in about 1.4-fold increase in ascorbic acid content when compared with control.

Non-reducing, Reducing and Total Sugars

Sugar profiles of strawberry fruits responded differentially to different concentrations of 24-epibrassinolide (Fig. 7). Foliar application of 1 µM 24-epibrassinolide enhanced non-reducing sugars, whereas foliar application of 2 µM 24-epibrassinolide enhanced reducing sugars in strawberry fruit when compared with control. Overall, strawberry plants supplemented with 2 µM 24-epibrassinolide exhibited 8% increase in total sugars in fruit tissues as compared to control.

Organoleptic Evaluation

Organoleptic evaluation of strawberry fruits harvested during peak harvest from understudy plants showed highest score of texture, aroma, appearance, flavour and sweetness of ripe fruits due to treatment with 3 µM 24-epibrassinolide followed by 2 µM 24-epibrassinolide treatment (Table 1). Lowest score was recorded in control plants. Plants foliar sprayed with 1 µM 24-epibrassinolide showed non-significant difference in texture as compare to control. According to just-right scale, highest score of tartness/sourness was recorded in control plants (3.8 score) followed by 1 µM 24-epibrassinolide-treated plants (two score), while lowest score was recorded in fruits from plants treated with 2 µM and 3 µM 24-epibrassinolide. Overall, strawberry fruits harvested from plants receiving 3 µM 24-epibrassinolide were awarded highest scores during organoleptic evaluation (Table 1).

Discussion

Strawberry production has become a high-income generating business world over as exceptional flavour, attractive red colour and easy-to-serve attributes have made strawberries an important component of consumer's nutritional basket. To maximize crop yield and improve quality attributes, exogenous application of growth-promoting phytohormones has been widely investigated (Asghari 2019; Asghari and Aghdam 2010; Asghari and Zahedipour 2016; Mir et al. 2019; Rademacher 2015) as an eco-friendly alternative to hazardous chemicals. Among them, brassinosteroids are pleiotropic phytohormones that regulate diverse pomological attributes including cell division, cell expansion, vegetative growth, shoot architecture, flowering, fruit set, and ripening (Ali et al. 2019; Baghel et al. 2019). Epibrassinolide, homobrassinolide and brassinolide are among the

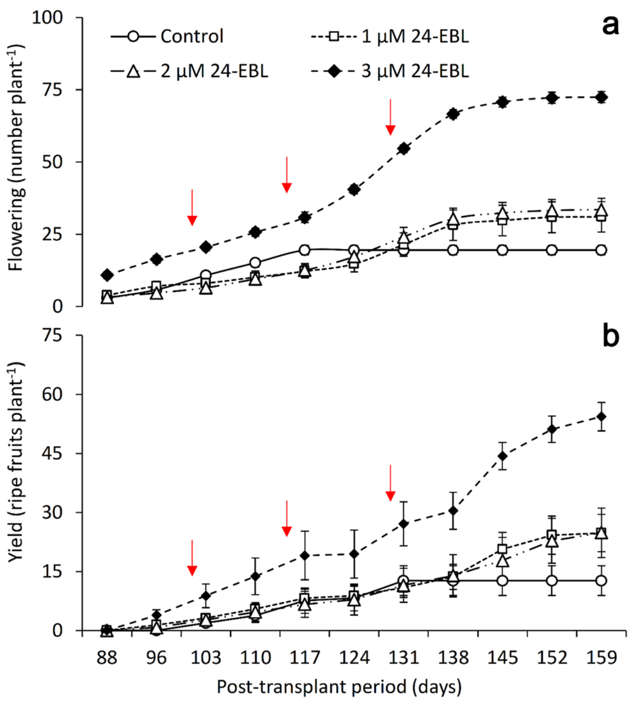

Fig. 4 Effect of foliar application of 24-epibrassinolide (EBL) on flowering and fruit yield from strawberry plants. Strawberry plants were foliar sprayed with 24-epibrassinolide 100, 115 and 130 days after transplantation. Vertical bars indicate mean ± standard error (n = 3, 30 plants per replicate under 3-block RCBD arrangement). LSD (α = 0.05) for flowering: treatment (T) = 4.384, post-transplant period (P) = 7.269 and $T \times P$ = 14.539; yield: T = 6.147, P = 10.194 and $T \times P$ = 20.389

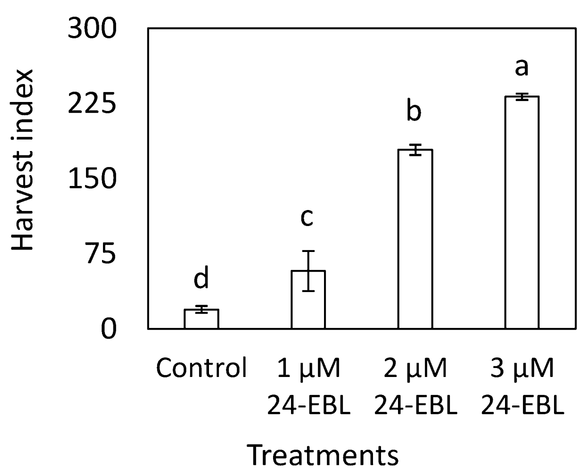

Fig. 5 Effect of foliar application of 24-epibrassinolide (EBL) on harvest index of strawberry plant. Strawberry plants were foliar sprayed with 24-epibrassinolide 100, 115 and 130 days after transplantation. Harvest index was calculated by dividing fresh weight of fruits to fresh weight of whole plant. Similar letters indicate non-significant difference among treatments at α = 0.05. Vertical bars indicate mean ± standard error (n = 3, 30 plants per replicate under 3-block RCBD arrangement)

most stable brassinosteroid analogues (Ali 2017). This study shows a positive influence of exogenously sprayed 24-epibrassinolide on some of the important growth, yield, and quality-related parameters of strawberry plants.

Leaf area is one of the important plant growth parameters influencing plant growth. Increase in leaf area enhances leaf photosynthetic capacity and net carbon assimilation into plant tissues (Siddiqui et al. 2019a). Results showed that foliar application of 2 μM and 3 μM 24-epibrassinolide enhanced strawberry leaf area (Fig. 2a). In corroboration, Furio et al. (2019) also reported 24% increase in leaf area when strawberry plants were foliar sprayed with 0.1 mg L^{-1} 24-epibrassinolide. In another study, application of 0.01 ppm TS303, a brassinosteroid-related substance, also increased total leaf area of strawberry plants by 150–180% (Pipattanawong et al. 1996). Jiang et al. (2012) also reported 22.6% increase in leaf area and 20.6% increase in shoot biomass in of cucumber plants applied with 0.1 M 24-epibrassinolide.

Strawberry plants treated with 24-epibrassinolide did not show any change in whole plant dry weight except slight increase in those treated with 2 μM 24-epibrassinolide (Online Resource 2b). Though, Furio et al. (2019) also reported no change in whole plant dry weight with foliar application of 0.1 mg L^{-1} 24-epibrassinolide but foliar application of 0.1 mg L^{-1} DI-31, a brassinosteroid spirostanic analogue, enhanced both root and whole plant dry weight of strawberry plants. This suggests that the effect of brassinosteroids on plant biomass accumulation seems to depend on brassinosteroid analogue used.

Further breakup of biomass accumulation into different plant tissues revealed that 24-epibrassinolide induced increase in shoot weight ratio and root weight ratio but reduction in leaf weight ratio (Fig. 3). This suggests that foliar application of 24-epibrassinolide shifted biomass distribution pattern more towards shoot and roots. And, higher root-to-shoot ratio (Fig. 3c) indicates that biomass accumulation in roots was higher than biomass accumulation in shoot. So, the exogenous application of 24-epibrassinolide seems to reorient biomass distribution pattern in strawberry plants in the order of shoot > roots > leaves. Though, previous studies have also shown ≥ 1.5-fold increase in shoot and root mass in response to foliar application of 10^{-8} M 24-epibrassinolide or its analogue 28-homobrassinolide in tomatoes (Hayat et al. 2012) and 0.1 or 1 μM DI-31 (a brassinosteroid analogue) in salt-stressed lettuce plants (Serna et al. 2015) but, this is the first report, indicating possible role of 24-epibrassinolide in reorienting carbon partitioning in vegetative tissues of strawberry plant. Since, nitrogen plays key role in regulating strawberry plant growth (Acuña-Maldonado and Pritts 2008; Anwar et al. 2018c), decrease in leaf weight ratio may also be correlated with lower total nitrogen contents in 24-epibrassinolide-treated strawberry leaves (Fig. 1). It is plausible that 24-epibrassinolide mediates

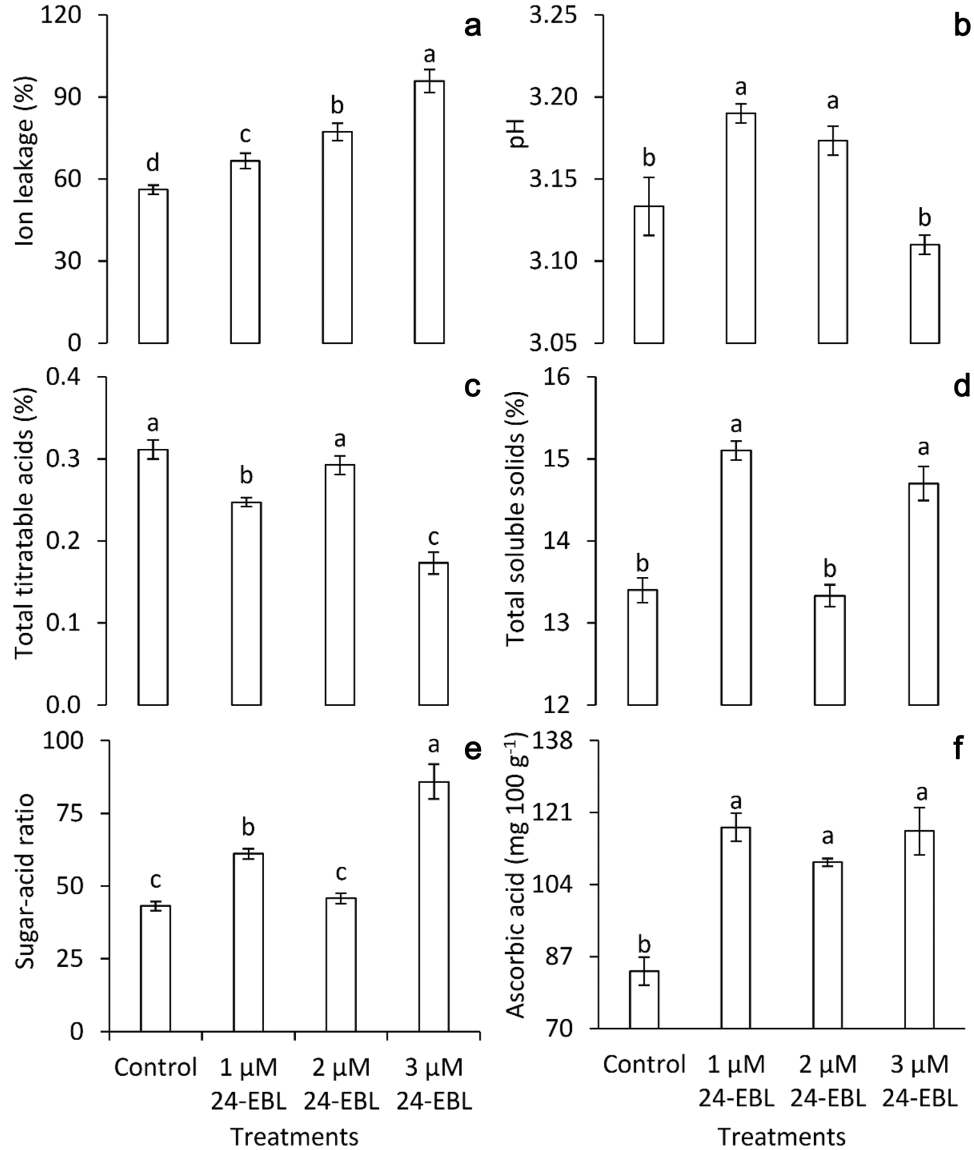

Fig. 6 Effect of foliar application of 24-epibrassinolide (EBL) on ion leakage, pH, total titratable acids, total soluble solids, sugar–acid ratio, and ascorbic acid contents in strawberry fruit. Strawberry plants were foliar sprayed with 24-epibrassinolide 100, 115 and 130 days after transplantation. Similar letters indicate non-significant difference among treatments ($\alpha = 0.05$). Vertical bars indicate mean ± standard error ($n = 3$, 3-block RCBD arrangement)

uptake and metabolization of nitrogen, but not phosphorus and potassium (Online Resource 1), to regulate strawberry plant growth.

Higher leaf area ratio and specific leaf area of 24-epibrassinolide-applied plants indicate that increase in leaf area was even higher than accumulation of biomass in leaves and whole plant, respectively (Fig. 2b, c). Though, recent reports have modelled a non-linear relationship between leaf area and plant biomass in *Arabidopsis thaliana* (Weraduwage et al. 2015), this study suggests 24-epibrassinolide as an important factor regulating this relationship curve. Here in this study, increase in leaf surface area without concomitant increase in leaf biomass (dry weight) might explain slight decrease in leaf greenness index in strawberries leaves sprayed with 1 µM 24-epibrassinolide (Online Resource 2a). Decrease in greenness index contradicts with earlier report where foliar application of 24-epibrassinolide increased leaf chlorophyll content with respect to control (Asghari and Zahedipour 2016; Furio et al. 2019) which may be due to difference in plant growth stage, growth conditions, frequency of application and concentration of 24-epibrassinolide.

Role of brassinosteroid in enhancing crop yield and affecting traits of agronomic importance is well acknowledged (Liu et al. 2017; Tadayon and Moafpourian 2019; Vriet et al. 2012). Brassinosteroids have also been reported to improve yield and fruit quality even when plants are challenged to saline and drought stresses (Coll et al. 2015; Nie et al. 2019; Shahid 2014; Wang 2019; Yuan et al. 2010; Zeng et al. 2010). Molecular studies in *Arabidopsis* led to unveil role of brassinosteroids in promotion of floral induction (Li et al. 1996). In this study, in addition to promoting plant vegetative growth, 24-epibrassinolide application also enhanced strawberry flowering and yield (Fig. 4). Strawberry plants

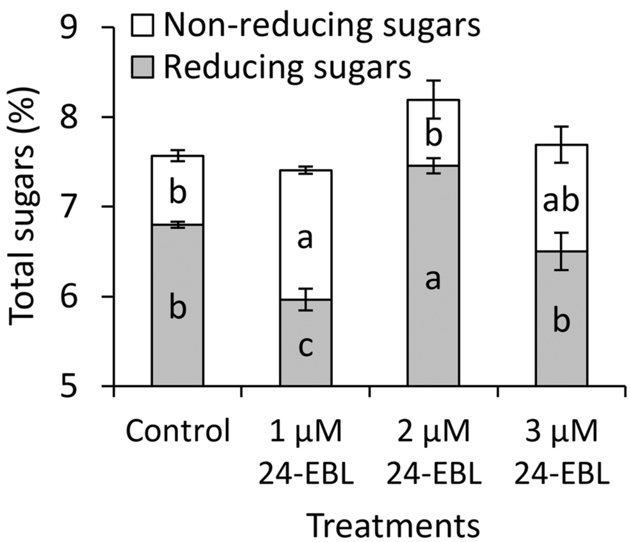

Fig. 7 Effect of foliar application of 24-epibrassinolide (EBL) on reducing, non-reducing and total sugars in strawberry fruit. Strawberry plants were foliar sprayed with 24-epibrassinolide 100, 115 and 130 days after transplantation. Similar letters indicate non-significant difference among treatments ($\alpha = 0.05$). Vertical bars indicate mean ± standard error ($n = 3$, 3-block RCBD arrangement)

receiving 3 µM 24-epibrassinolide exhibited highest number of flowers per plant, whereas the promotive effect of 1 µM and 2 µM 24-epibrassinolide on reproductive growth could only be observed in later half of the harvest season. Overall, foliar application of 24-epibrassinolide increased strawberry plant's harvest index (fruit fresh weight-to-plant fresh weight ratio) in a concentration-dependent manner (Fig. 5). Foliar application of 3 µM 24-epibrassinolide lead to 11.9-fold increase in harvest index compared to control suggesting a crucial implication of 24-epibrassinolide in commercial strawberry production, i.e. higher yield and better income. Strawberry flowers contain higher amount of endogenous castasterone, a bioactive brassinosteroid, compared to later stages of fruit development which also supports a promotive effect of brassinolide on flowering (Symons et al. 2012). Likewise, exogenous application of brassinosteroid has also been reported to promisingly increase number of flowers per plant and yield in strawberry (Pipattanawong et al. 1996; Zahedipour-Sheshglani and Asghari 2020). However, the effectiveness of brassinosteroids may vary with geographical locations, harvesting years, and nature of crops (Khripach et al. 2000).

Foliar application of 2 µM or 3 µM 24-epibrassinolide enhanced organoleptic quality of strawberries, i.e. texture, aroma, appearance, flavour, sweetness, and sourness (Table 1). Positive influence of 24-epibrassinolide on sensorial attributes of strawberries could partly be related to changes in sugars and organic acids. Soluble solids and organic acids are considered important constituents of taste and flavour (Cordenunsi et al. 2003). Higher total soluble solids, whereas lower titratable acids in strawberries harvested from 1 µM or 3 µM 24-epibrassinolide-supplemented plants resulted in twofold higher sugar–acid ratio (Fig. 6) and comparatively better fruit flavour than control (Table 1). Increase in total soluble solids was also positively correlated with increase in reducing sugars in strawberry fruits from 24-epibrassinolide-treated plants (Fig. 7). The nutritional value of strawberries is often evaluated with reference to the amount of ascorbic acid as strawberries are one of the rich sources of ascorbic acid (Derossi et al. 2010). Foliar applications of 24-epibrassinolide elevated ascorbic acid in strawberries by 1.3-fold (Fig. 6f). These findings suggest a promotive effect of brassinosteroid on physical and nutritional quality of strawberry fruit. In corroboration, recent studies have also reported decrease in titratable acids and increase in soluble solids in strawberries cv. Pájaro foliar sprayed with 0.1 mg L^{-1} 24-epibrassinolide or brassinosteroid spirostanic analogue DI-31 (Furio et al. 2019). Similarly, foliar application of 4 µM 24-epibrassinolide 15 days after anthesis and then before ripening, also increased soluble sugars in strawberries (Zahedipour-Sheshglani and Asghari 2020). In grape seedlings cv. Thompson also, foliar application of 0.6 mg L^{-1} 24-epibrassinolide at véraison stage increased sugar–acid ratio by 17% (Ghorbani et al. 2017). In another study, exogenous application of 0.4 mg L^{-1} 24-epibrassinolide reduced titratable acids but increased reducing sugars in grape berries cv. Cabernet Sauvignon (Xu et al. 2015). Recently, foliar application of 0.4 mg L^{-1} 28-epibrassinolide has been reported to reduce total titratable acidity and increase juice pH, soluble solids, and sugar–acid ratio in

Table 1 Effect of foliar application of 24-epibrassinolide (EBL) on organoleptic attributes of red ripe strawberries cv. Chandler

Treatments	Texture	Aroma	Appearance	Flavour	Sweetness	Tartness/sourness
Control	6.0^{c}	6.0^{c}	5.0^{c}	5.0^{c}	2.0^{c}	3.8^{a}
1 µM 24-EBL	6.1^{c}	6.1^{c}	5.13^{c}	5.13^{c}	2.1^{c}	2.0^{b}
2 µM 24-EBL	7.0^{b}	7.0^{b}	6.0^{b}	6.0^{b}	3.0^{b}	1.3^{c}
3 µM 24-EBL	8.8^{a}	8.8^{a}	7.8^{a}	7.8^{a}	4.8^{a}	1.4^{c}

Strawberry plants were foliar sprayed with 24-epibrassinolide 100, 115 and 130 days after transplantation. Values sharing the same letter are non-significantly different within an organoleptic attribute at $\alpha = 0.05$ ($n = 3$, 3-block RCBD arrangement) grapes cv. 'Khalili' (Tadayon and Moafpourian 2019). The seedless grapes treated with 24-epibrassinolide at three different stages (4 weeks after full bloom, at véraison stage and one day before harvest) exhibited substantial enhancement in soluble solids content and ascorbic acid level (Asghari and Rezaei-Rad 2018). During fruit ripening, the application of brassinolide was also effective in increasing soluble sugars and ascorbic acid content of tomato compared with the control (Zhu et al. 2015). Zhu et al. (2010) also reported increase in soluble solids and ascorbic acid in brassinosteroid-treated jujube fruits compared to control fruits (Zhu et al. 2010). Radish roots also exhibited elevation in ascorbic acid and reducing sugars following foliar application of two brassinosteroids, 28-Homobrassinolide and 24-epibrassinolide (Vardhini et al. 2011). Foliar application of 28-homobrassinolide enhanced sugar accumulation in sweet cherries cultivars, i.e. Tulare, Bing, and Rainier (Mandava and Wang 2016). Molecular genetic studies have elucidated a possible mechanism in brassinosteroid signalling cascade that regulates these fruit quality attributes. *Brassinazole resistant 1* (*BZR1*) is a transcription factor and is likely to be regulated by brassinosteroid signalling through protein phosphorylation (He et al. 2002; Wang 2002). Transgenic tomato lines overexpressing *Arabidopsis BZR1-1D* exhibited enhanced soluble solids, soluble sugars and ascorbic acid during fruit ripening (Liu 2014). Foliar spray of 4 µM 24-epibrassinolide has been reported to decrease firmness and enhance early ripening of strawberries (Zahedipour-Sheshglani and Asghari 2020). Similarly, strawberry plants treated with 1 µM 24-epibrassinolide exhibited improved fruit quality and nutritional value as compared to untreated fruits (Sun et al. 2020). Loss in textural integrity in 24-epibrassinolide-treated strawberry tissues may be correlated with loss of membrane permeability and consequent increase in electrolyte leakage (Fig. 6a).

Efficacy of brassinosteroid to modulate plant physiology depends on its concentration, application method and plant genetics (Asghari and Zahedipour 2016; Bartwal et al. 2013). Results from this study also showed that response of strawberry plant growth and fruit development to 24-epibrassinolide application changed with change in concentration of hormone. So, principal component analysis was conducted to delineate concentration-dependent effects (Fig. 8). Based on the highest squared cosine value corresponding to factors F1, F2 or F3, plant growth, yield, and fruit quality attributes were clustered around 1, 2, or 3 µM 24-epibrassinolide treatments. Factor F1, covering 61.82% variability in data (eigenvalue 16.691), showed clustering of leaf nitrogen level, leaf weight ratio, total titratable acids and sourness with control suggesting negative influence of 24-epibrassinolide application, regardless of concentration, on these parameters. Specific leaf area, leaf area ratio, root weight ratio, shoot weight ratio, root-to-shoot ratio, yield (number of flowers and fruits per plant, and harvest index), fruit sugar–acid ratio, ascorbic acid, ion leakage, and organoleptic attributes (excluding sourness) showed a positive association with 3 µM 24-epibrassinolide application. This cluster was located opposite to control on F1 axis suggesting strong positive influence of 3 µM 24-epibrassinolide application on these parameters compared to control. Second factor, covering 22.35% variability in data (eigenvalue 6.034), showed clustering of leaf area, plant dry weight, fruit total soluble solids, and sugars (reducing, non-reducing and total sugars) with 2 µM 24-epibrassinolide. However, the distribution of clusters in two distinct groups on either side of F2 axis indicated that application of 2 µM 24-epibrassinolide had positive correlation with plant dry weight, total soluble solids and reducing sugars but negative correlation with total soluble solids and non-reducing sugars in fruits. Third factor of principal component analysis, covering 15.84% variability in data (eigenvalue 4.275; not shown), showed clustering of fruit pH value with foliar application of 1 µM 24-epibrassinolide. Thus, principal component analysis helped to delineate individual roles of 24-epibrassinolide concentrations

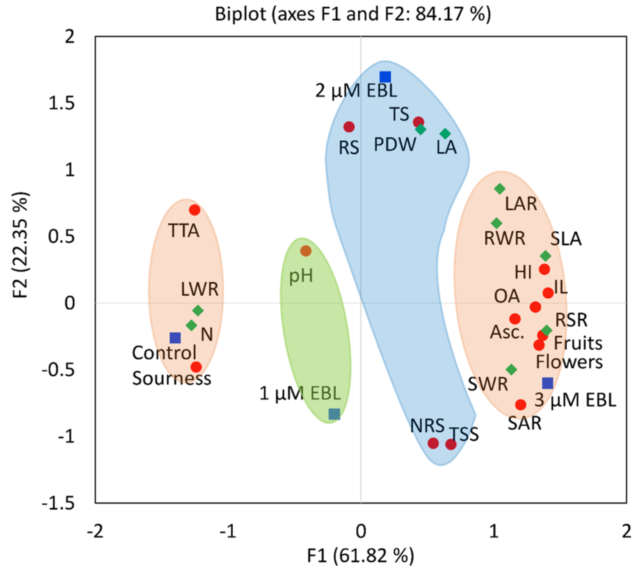

Fig. 8 Principal component analysis among 24-epibrassinolide treatments and various plant growth, yield, and fruit quality attributes of strawberries cv. Chandler. Clustering of observation (treatment) and variables (plant growth, yield, or fruit quality attributes) into groups (coloured shapes) is based on their highest squared cosine values corresponding to the factor, F1 (orange), F2 (blue) or F3 (green). *Asc.* ascorbic acid; *Flowers* number of flowers per plant; *Fruits* number of fruits per plant; *HI* harvest index; *IL* ion Leakage; *LA* leaf area; *LAR* leaf area ratio; *LWR* leaf weight ratio; *N* leaf nitrogen level after EBL application; *NRS* non-reducing sugars; *OA* organoleptic attributes (appearance, texture, aroma, flavour, sweetness, sourness); *PDW* plant dry weight; *pH* pH; *RS* reducing sugars; *RSR* root-to-shoot ratio; *RWR* root weight ratio; *SAR* sugar–acid ratio; *SLA* specific leaf area; *SWR* shoot weight ratio; *TS* total sugars; *TSS* total soluble solids; *TTA* total titratable acidity (Color figure online)

in regulating various aspects plant growth, yield, and fruit quality attributes.

Concerning the future prospects of the study, there is need to investigate 24-epibrassinolide-modulated molecular mechanisms regulating these plant growth and yield-related aspects. Since, foliar application of 24-epibrassinolide under field conditions has been evaluated on only few crops, including strawberry, there is need to expand and evaluate field performance of brassinosteroids on other annual and perennial horticultural crops.

Conclusion

This study suggests that foliar application of 24-epibrassinolide can be used to increase yield and nutritional quality of strawberry, two important factors contributing to economic profit and consumer health, respectively. Since foliar applications of 1 µM, 2 µM or 3 µM 24-epibrassinolide differentially regulate distinct aspects of plant growth and development, specific concentration of 24-epibrassinolide may help achieve specific objective of fruit production. Result suggest that 24-epibrassinolide promoted vegetative and reproductive growth of strawberry plants in a concentration-dependent manner. Foliar application of 1 µM 24-epibrassinolide was positively correlated with change in fruit pH, whereas foliar application on 2 µM 24-epibrassinolide had a promotive impact on leaf area, plant dry weight, and fruit sugars. Foliar spray of 3 µM 24-epibrassinolide not only enhanced plant vegetative growth (leaf area ratio, specific leaf area, root-to-shoot ratio, root weight ratio, and shoot ratio) but also improved yield (flowers and fruits per plant and harvest index) and quality attributes (sugar–acid ratio, ascorbic acid, and organoleptic characteristics) of strawberry fruits.

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Declarations

Conflict of interest The authors have no relevant financial or non-financial interest to disclose.

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