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RESEARCH ARTICLE

Optimising Kinnow mandarin productivity under rainfed conditions through foliar application of plant growth bioregulators

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Abstract

Citrus, particularly Kinnow mandarin, holds a prominent position in the fruit industry worldwide. The study evaluated the effects of plant bioregulators on Kinnow mandarin at different stages of fruit development. The experiment was laid out in a factorial randomised block design with 14 treatment combinations and three replications. The 7 levels of hormonal applications were as follows: H_1: naphthalene acetic acid (NAA) at 50 µM, H_2: NAA at 100 µM, H_3: gibberellic acid (GA_3) at 150 µM, H_4: GA_3 at 300 µM, H_5: methyl jasmonate (MeJA) at 200 µM, H_6: MeJA at 400 µM, H_7: Control and two stages of hormonal application [S1= at full spray flowering stage (FSFS) and cell division stage and S2 = at full spray flowering stage and cell elongation stage]. Among the treatment combinations evaluated, H_4S_2 (GA_3 at 300 µM applied at full-spray flowering stage (FSFS) and cell elongation stages) demonstrated the most pronounced improvement in reproductive attributes, notably enhancing FSFS, number of fruits per plant, total fruit yield (FY) and yield efficiency (YE). Additionally, this treatment also resulted in the lowest fruit drop (FD) percentage, indicating improved fruit retention. It was also superior in improving the physico-chemical properties of fruit, total soluble solids (TSS), acidity (Ac) balance, ascorbic acid (AA) content, sugars (total, reducing, non-reducing), phenolic compounds, carotenoids and antioxidant activity. Moreover, physiological responses such as relative water content (RWC), chlorophyll concentration and proline accumulation were also maximised under the H_4S_2 treatment regime, signifying improved plant health and tolerance to stress. In contrast, untreated control plants recorded the lowest values across all parameters. Overall, the study demonstrates that foliar application of GA_3 at critical growth stages, particularly FSFS and cell elongation, enhances both the yield and quality of Kinnow mandarin fruits while also improving the physiological resilience of the plant. These findings provide a practical framework for hormone-based spray scheduling aimed at sustainable citrus production in the rainfed region.

Keywords: fruit quality; leaf chlorophyll; PCA; plant hormone; proline content; rainfed conditions

Introduction

Citrus belongs to the family Rutaceae and is widely cultivated in tropical and subtropical regions worldwide, between 30 °N and 30 ° S, in over 137 countries across six continents (1, 2). The Kinnow mandarin, which is renowned worldwide, is produced in northwestern India. Growing mandarins benefits the agricultural community by yielding higher financial returns than other fruit crops (2). It is the primary mandarin orange, holding a prominent place in the citrus sector (3). Punjab holds the prime share of the Kinnow mandarin-producing area of the country, including the states of Rajasthan, Haryana, Madhya Pradesh, Himachal Pradesh and Jammu and Kashmir (2, 4)

In India, Kinnow mandarin was cultivated in an area of 446 hectares with 6170 million tonnes of production and in Punjab, it has over 53 thousand hectares with an annual production of 10,49,977 tonnes (5). In northwestern India, Kinnow cultivation has gained importance due to its early bearing and high yield potential. After gaining popularity among farmers and common people, the Kinnow mandarin has become one of the most desirable citrus fruits in Punjab. Farmers' interest in cultivating Kinnow mandarin in the Punjab region has made it the king of fruits (6).

Several factors reduce overall productivity and quality including limited canal water supply and a lack of understanding of irrigation requirements at crucial stages. One major problem affecting orchards is the insufficient quality of water for irrigating citrus orchards. Farmers have reported a consistent drop in productivity due to water delivery delays during crucial months (7, 8). Farmers are increasingly confronting heat stress, with temperatures rising beyond optimal levels for Kinnow growth. The climatic conditions, prolonged dry spells followed by heavy rains, disrupt the growth cycles of Kinnow trees and lead to significant crop losses (2, 9).

Despite bearing 40000–80000 flowers, only a small number (400–1200) of Kinnow mandarins mature because of an abundance of blooms and fruit droop at different stages of fruit development (10, 11). This issue caught the interest of several researchers who sought to employ plant growth regulators (PGRs) and other substances to identify effective measures to control fruit droop and

improve quality and yield (11). The development of appropriate methods necessary for the cost-effective production of citrus fruits is hampered by the lack of understanding of the critical physiological mechanisms underpinning fruit growth and development (12). In light of the already published information, a study was conducted to standardise PGR doses to reduce FD and enhance Kinnow quality and output (13, 14). Gibberellic acid is a vital growth hormone that regulates a number of physiological processes, including ion transport, growth, blooming and the acclimation of plant leaf canopy (14). Furthermore, it promotes the growth, elongation and osmoregulation of internodes as well as the formation of dry matter and biomass. Methyl jasmonate (MeJA) is used to regulate plant growth and response to environmental stresses and it is a natural compound that provides tolerance against biotic and abiotic stress in plants such as salinity, drought and pest resistance (15). By reducing transpiration and membrane lipid peroxidation, MeJA treatments were shown to change plant metabolism, making them more resilient to drought and water stress. A chemical substance called naphthalene acetic acid (NAA) increases fruit size and quality (16). By increasing total soluble solids (TSS) and juice percentage and reducing reducing and non-reducing sugars in Kinnow mandarin trees, preharvest NAA spray enhanced fruit quality features (17). Plants are vegetatively propagated from stem and leaf cuttings using NAA, a rooting agent. In a variety of species and cultivars, the application of NAA around June drop has been successful in lowering crop load, decreasing alternate bearing and improving fruit size and quality. Time of application, concentration, tree and environmental conditions and cultivar are some of the variables that affect it (18). Exogenous application of GA_3 at water-deficit growth stages such as full-spray flowering stage (FSFS) and cell elongation can notably improve flowering, yield, fruit quality and physiological performance in Kinnow mandarin (2, 19).

Most previous studies have focused on irrigated environments or generalised citrus cultivation systems, with limited emphasis on Kinnow mandarin grown under moisture stress conditions. Furthermore, the optimum type, concentration and stage of application of these PGRs for improving productivity and fruit quality under the specific rainfed stress conditions of central Punjab are not yet clearly established. Therefore, this study was designed to systematically evaluate different PGRs and their application schedules to identify suitable management strategies for enhancing Kinnow performance under water-limited conditions.

Materials and Methods

Experimental site

The current study was conducted in an experimental field in Kharorha (Fatehgarh Sahib), Tehsil Sirhind, Fatehgarh Sahib, Punjab, which is an arid rainfed zone. The experimental site (30°46 ′ N and 76° 04 ′ -76° 38 ′ E) falls in the subtropical plains of Punjab at an elevation of 247 meters above mean sea level. The meteorological data of the orchard are presented in Fig. 1.

Plant material and experimental details

The investigation was carried out on 4-year-old Kinnow mandarin plants, plantedat 4.5 x 4.5 m spacing. Four-year-old Kinnow mandarin plants were spaced 4.5 x 4.5 m apart in the study. Throughout the 2024–2025 growth seasons, a factorial randomised complete block design (FRBD) was used. A total of 42 plants, with 14 treatment combinations and three replications for each treatment combination, were selected for the experiment. Fruits were randomly harvested from the selected plants using citrus cutters and other quality parameters were measured at the Fruit Science laboratory of Mata Gujri College, Fatehgarh Sahib, Punjab.

Treatment details and application

The hormonal concentrations and their application schedules are displayed in Table 1. Treatment solutions were applied to the foliage using a battery-operated knapsack sprayer until a runoff condition occurred. The treatments were sprayed during the growing season at specific stages: once at the fruit stage, at FSFS and 30 days after FS (DAFS) for cell division and at FSFS and 60 DAFS for cell elongation in 2024. Each treatment was replicated thrice to ensure reliable results. All spraying activities were conducted in the morning, specifically between 7 AM and 9 AM, to minimise the potential impact of environmental factors such as temperature and wind on the effectiveness of the foliar applications.

Estimation of relative water content

Relative water content (RWC) was measured using a standard protocol (20). Fresh leaf samples were weighed and floated in distilled water in a closed Petri dish at room temperature for 24 hr.

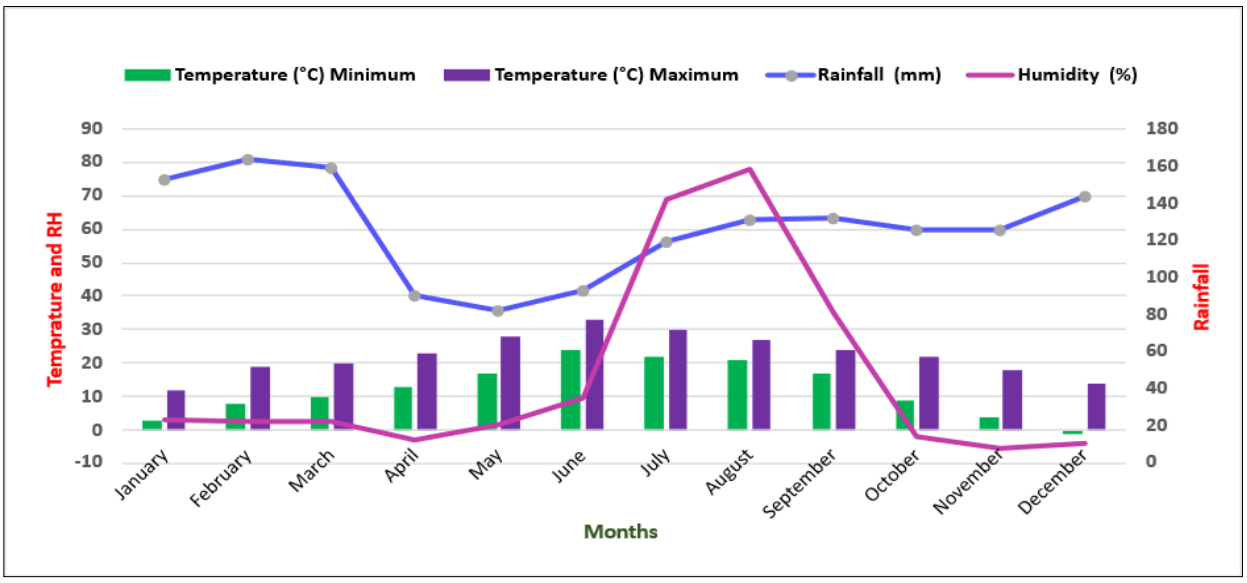


Fig. 1. Monthly meteorological data of the experimental location of the rainfed zone of Shri Fatehgarh Sahib Punjab (2024).

Table 1. Plant hormones on spray scheduling of kinnow mandarin under the rainfed condition

Hormones	Factor 1 (hormonal application)	Factor 2 (Stages of hormonal application)
H_1	Naphthalene acetic acid at 50 µM	
H_2	Naphthalene acetic acid at 100 µM	**S_1**: At FS and cell division stage (at FS and 30 days after FS)
H_3	Gibberellic acid at 150 µM	
H_4	Gibberellic acid at 300 µM	**S_2**: At the FS and cell elongation stage (at FS and 60 days after FS)
H_5	Methyl jasmonate at 200 µM	
H_6	Methyl jasmonate at 400 µM	
H_7	Control	

DAFS: days after fruit set; H: plant hormones; S_1: at FS and 30 DAFS (cell division); S_2: at FS and 60 DAFS (cell elongation)

After being gently dried with blotting paper, the turgid weight (TW) was recorded. The leaves were then dried in a pre-heated oven at 65 °C for 48 hr to obtain the dry weight (DW). The percentage of RWC was calculated using the formula:

$$\text{Relative water content (\%)} = \frac{\text{Fresh weight - dry weight}}{\text{turgid weight - dry weight}} \times 100 \quad \text{(Eqn. 1)}$$

Where; FW= Fresh weight, DW=Dry weight and TW= Turgid weight

Estimation of chlorophyll content

In the first week of July, ten fully grown leaves from each experimental tree were collected during the morning and transported in an ice box to the laboratory (21). To prevent chlorophyll degradation, the samples were stored in a freezer at below 0 °C.

Extraction

The leaves were washed and chopped into fine pieces under subdued light. A 100 mg portion was placed in a vial with 7 mL of dimethyl sulfoxide (DMSO) and incubated at 65 °C for 30 min. Afterwards, the extract was transferred to a graduated test tube and diluted to 10 mL with DMSO (21).

Estimation

The optical density (OD) of the extract was measured using a Spectronic-20 D at 645 nm and 663 nm, with DMSO as a blank, to determine the total chlorophyll content (CC). was calculated by using the following formula:

$$\text{Total chlorophyll (mg/g)} = 20.2\,(A645) - 8.02\,(A663) \times V/1000 \times W \quad \text{Eqn. 2)}$$

Where V = Volume of extract used

a = Length of light path in cell (1cm)

W = Weight of the sample (g)

A (645) = Absorbance at 645 nm

A (663) = Absorbance at 663 nm

The results thus obtained were expressed as mg of total chlorophyll per gram of fresh weight.

Estimation of proline content

The proline content (PC) in leaf samples was measured using a standard protocol (22). A 0.5 g sample of fully expanded, sunlit leaves was ground in 10 mL of 3 % sulfosalicylic acid and filtered. Then, 2.0 mL of acid ninhydrin and glacial acetic acid were added and the mixture was incubated in a boiling water bath for 1 hr. After cooling, the mixture was extracted with 4.0 mL of toluene and the absorbance was measured at 520 nm. Proline content was calculated using the formula

$$\text{Proline} \left(\frac{\mu\text{mol}}{\text{g fresh wt.}}\right) = \frac{\mu g \frac{proline}{ml} \times toluene\ in\ ml}{115.5 \frac{\mu g}{\mu mole}} \Big/ \frac{gram\ sample}{5} \quad \text{(Eqn. 3)}$$

Estimation of antioxidant content

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the pulp was evaluated using a standard protocol (23). Fresh pulp tissue (3.0 g) was mixed with 30 mL of ethanol for 45 min, then centrifuged at 4 °C at 18000 g for 20 min. The supernatant (0.1 mL) was combined with 2.9 mL of 0.1 mM DPPH dissolved in methanol and incubated for 30 min at 25 °C in the dark. A control without the sample served as the blank and absorbance was measured at 517 nm with a spectrophotometer (Shimadzu UV–VIS, 04834, India). The DPPH radical scavenging activity (%) of the sample was calculated as

$$\text{Antioxidant (µmol L}^{-1}) = (1\text{-absorbance of sample/absorbance of control}) \times 100 \ldots\ldots\ldots\ldots \text{(Eqn. 4)}$$

Evaluation of flowering and yield parameters

Fruit set

Fruit set was determined using a standard protocol (24). The total number of flowers on the tagged branches was counted and the number of fruits that developed was recorded 20 days after full bloom. The formula used for determining the FSFS was:

$$\text{FS \%} = \frac{\text{Total number of FS x 100}}{\text{Total number of flowers}} \quad \text{(Eqn. 5)}$$

Fruit drop

Fruits on the selected branches were counted initially and again at harvest. Percent fruit retention was calculated as follows:

$$\text{Fruit retention (\%)} = \frac{\text{Total number of fruits set-fruits retained at maturity x 100}}{\text{Total number of fruits set}} \quad \text{(Eqn. 6)}$$

Fruit yield

The total number of fruits per treatment was counted at harvest and expressed as fruits per plant. Mature fruits were periodically harvested and their weights were recorded to calculate total yield (kg plant^{-1}). Yield efficiency (kg cm^{-2}) was determined for each treatment using a standard protocol (24), based on the trunk's cross-sectional area (kg cm^{-2} TCA) using the formula:

$$\text{Yield efficiency (kg cm}^{-2}\text{ TCA)} = \frac{\text{Fruit yield}}{\text{Trunk cross dectional area}} \quad \text{(Eqn. 7)}$$

Fruit quality parameters

Estimation of total soluble solids

Juice was extracted from the fruits using a muslin cloth and TSS were measured with a digital refractometer (Model: PAL-1; Atago Co., Ltd, Tokyo, Japan) with a range of 0–53°Brix.

Estimation of titratable acidity

The fruit Ac was measured as per standard protocol (25). Dilute 10 mL of fruit juice with distilled water to a total volume of 100 mL. Add 2-3 drops of phenolphthalein indicator to an aliquot and titrate with 0.1 N sodium hydroxide (NaOH) until a faint pink colour appears. Record the volume of NaOH used, then calculate the percentage of titratable Ac:

$$\text{Titratable acidity \%} = \frac{\text{1x Eq. Wt, of acid x 0.1N NaOH used (mL) x 100}}{\text{Volume of juice taken (mL)}} \quad \text{(Eqn. 8)}$$

Estimation of ascorbic acid

The AA content in the fruit juice was measured using 2, 6-dichloroindophenol dye, expressed as milligrams per 100 mL of the sample (26). For extraction, 10 g of fruit juice was mixed with 70 mL of 3 % metaphosphoric acid (HPO_3) and diluted to 100 mL. After filtration, a 10-mL aliquot was titrated until a persistent rose-pink colour appeared for 15 to 20 seconds.

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titre x dye equivalent x dilution}}{\text{weight of sample}} \text{ x 100} \quad \text{(Eqn. 9)}$$

Estimation of carotenoid content

Hundred milligrams of fresh fruit sample was crushed with 3.0 mL of 80 % acetone and centrifuged at 3000 rpm for 10 min. The supernatant was collected, mixed with a second extraction from the pellet and adjusted to 10 mL with 80 % acetone. Absorbance was measured at 645, 665 and 480 nm.

Total carotenoids were measured. Five grams of fruit pulp were crushed in acetone, transferred to a separating funnel and mixed with petroleum ether and sodium sulphate. After separation, the coloured layer was collected, adjusted to volume and then the absorbance was measured at 452 nm. Results were expressed as mg/100 g FW (27).

$$\text{Carotenoid (µ/g)} = \text{A480 + 0.114 A6665 - 0.638A645 X V / 1000 XW} \quad \text{(Eqn. 10)}$$

$$\text{Total Carotenoid content} = \frac{OD_{452} \times 3.87 \times \text{volume made up}}{\text{weight of sample x 1000}} \text{ x 100} \quad \text{(Eqn. 11)}$$

Estimation of total phenolic content

Total phenolic content (TPC) was determined by the Folin–Ciocalteu method (28). In a 2 mL centrifuge tube, 10 mL of distilled water, 0.2 mL of extract and 0.1 mL of Folin–Ciocalteu reagent were mixed. After 1 min, 2 mL of sodium carbonate (Na_2CO_3) was added and the mixture was kept in the dark for 2 h. Absorbance was then measured at 765 nm using a Spectronic 20D+ (Thermo Scientific, USA) against a blank. A gallic acid standard curve was used to calculate the gallic acid equivalent (GAE), which was expressed as mg/kg fresh weight.

Estimation of total sugar

A 200 mL filtered stock solution was prepared. Then, 10 mL of 45 % lead acetate was added and the solution was filtered. Subsequently, 10 mL of 22 % potassium oxalate was added, the mixture was filtered again and the final volume was adjusted to 250 mL (29).

100 mL of this filtrate was mixed with 5 mL of concentrated hydrochloric acid (HCl) and allowed to hydrolyse overnight. After neutralising excess HCl with NaOH, the volume was adjusted to 250 mL.

Total sugar was determined by titrating with Fehling solutions and using methylene blue as an indicator, with results expressed as a percentage of fresh fruit weight.

Total sugar (%) = (Factor × Dilution × 100) / (Titre value × Weight of sample taken) (Eqn. 12)

% Sucrose = (% total invert sugars - % reducing sugars) × 0.95 (Eqn. 13)

% Total sugars = (% reducing sugars + % sucrose) (Eqn. 14)

Estimation of reducing sugar

The clarified solution from the total sugar estimation was titrated with a boiling mixture of 5 mL each of Fehling A and B, using methylene blue as the indicator (29). Reducing sugar content was expressed as a percentage of the fresh berry weight.

Reducing sugar (%) = (Factor × Dilution × 100) / (Titre value × Weight of sample taken) (Eqn. 15)

Factor of solution (g of invert sugar) = (Titre value × 2.5 × 100) / 1000 = 0.05 (Eqn. 16)

Statistical analysis

All statistical analyses were performed using RStudio *version* 4.4.2 (30). The ANOVA procedure in Factorial Randomised Block Designs was performed using the package *Agricole*. Principal Component Analysis (PCA) was carried out for multivariate analyses using the FactoMineR and factoextra packages. The ggplot2 and GGally packages were used to create graphical representations, such as trait variations and PCA biplots, which allowed for a thorough interpretation of treatment effects and trait interrelationships.

Results

Plant physiological parameters

The hormonal application showed a significant effect ($p \leq 0.05$) on physiological parameters of Kinnow Mandarin leaves, including RWC, chlorophyll content and proline content. The maximum RWC of 92.25 % was found in treatment combination H_4S_2 [gibberellic acid (GA_3) at 300 µM+ at FSFS and 60 FS DAFS). The minimum increase in RWC (60.43 %) was observed in H_7S_1 (control + at FSFS and 30 FS DAFS), as shown in Fig. 2(a). The maximum increase in PC (10.56 µmol g^{-1} FW) was significantly recorded ($p \leq 0.05$) under treatment H_4S_2, i.e., GA_3 at 300 µM + at FSFS and 60 FS DAFS, as shown in Fig. 2(b). The minimum increase in PC (1.47 µmol g^{-1} FW) was recorded in H_7S_1 (control + at FSFS and 30 FS DAFS). The plant hormonal application at both stages also revealed a significant effect ($p \leq 0.05$) on leaf CC. However, the maximum CC (4.82 µmol m^{-2}) was found under the treatment H4S2 (GA_3 at 300 µM + at FSFS and 60 FS DAFS). While the minimum increase in the CC (1.21 µmol m^{-2}) was recorded in H_7S_1 (control + at FSFS and 30 FS DAFS as shown in Fig. 2 (c).

Fruiting and yield parameters

The exogenous application of growth regulators at both stages showed a significant effect on fruiting and yield parameters of Kinnow mandarin, with a proven statistical significance ($p \leq 0.05$) as shown in Fig. 3 (a-e). Among the different treatments, H_4S_2 (GA_3 at

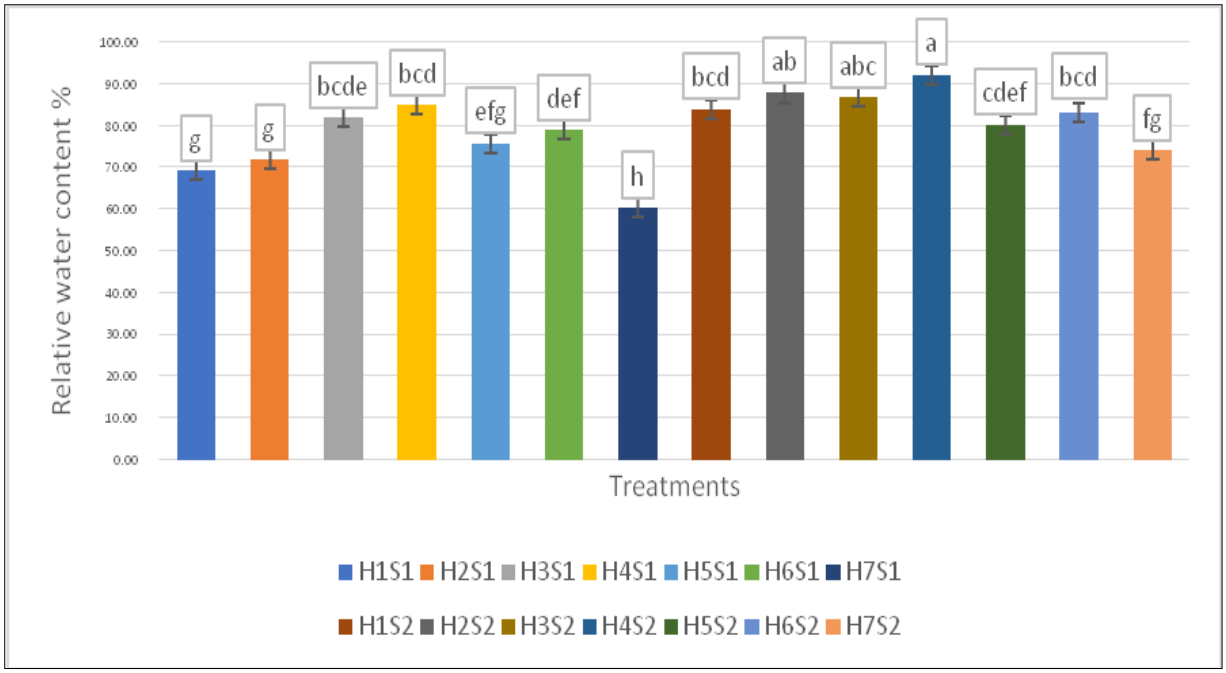


Fig. 2(a)

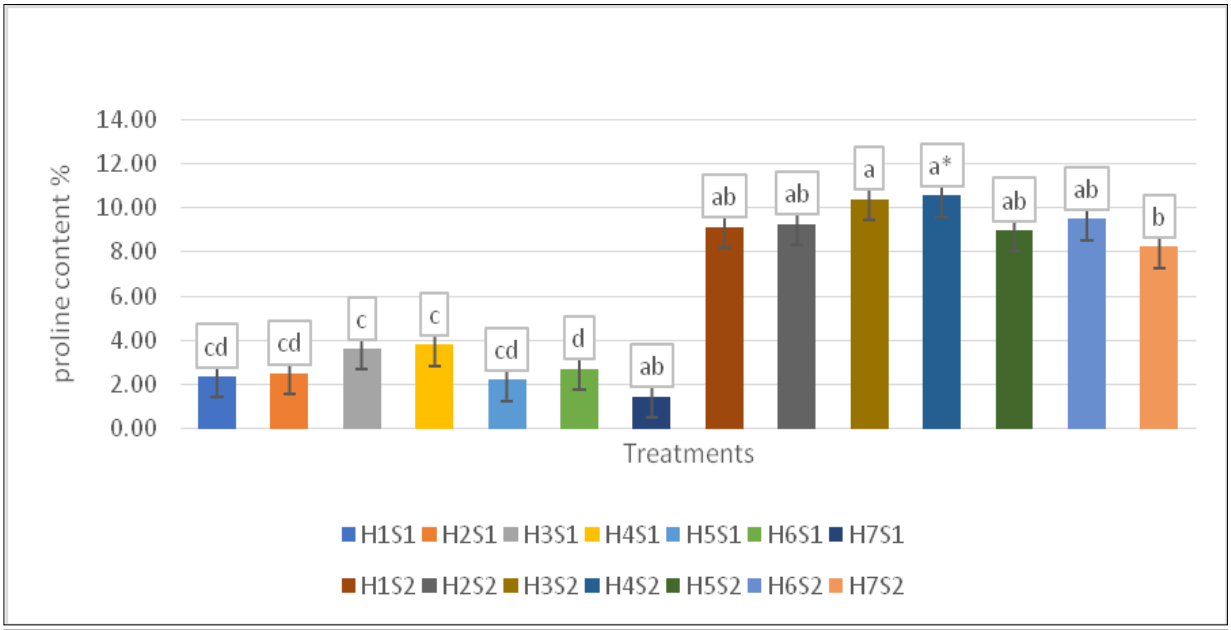


Fig. 2(b)

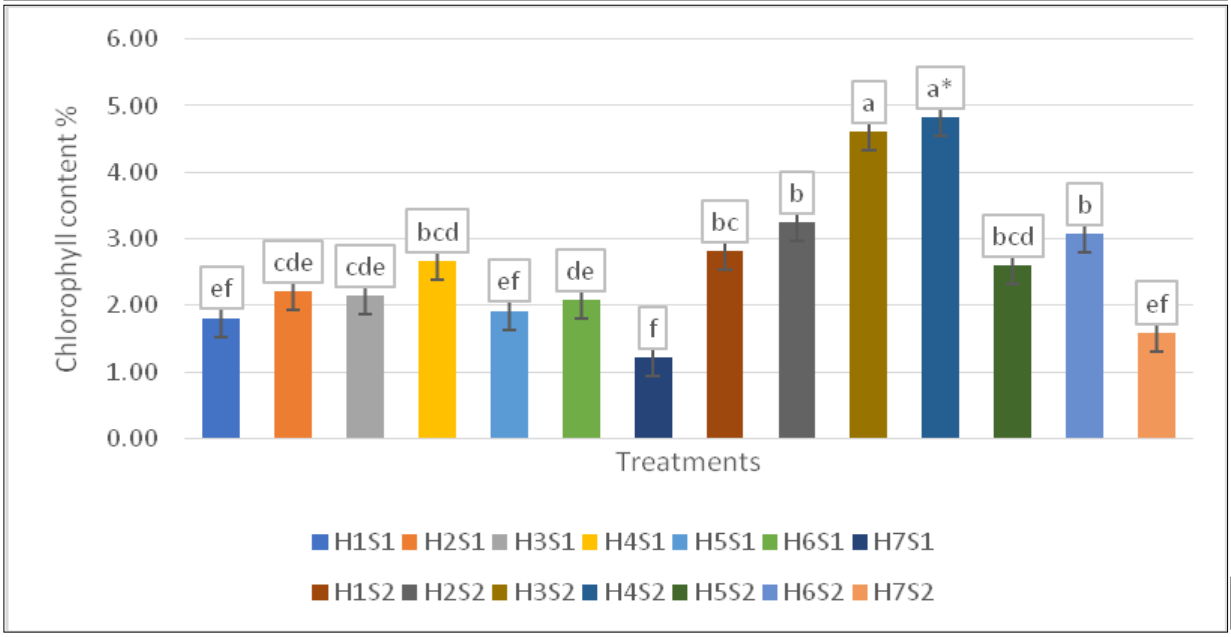


Fig. 2(c)

Fig. 2. Effect of plant hormones on plant physiological parameters of Kinnow mandarin.

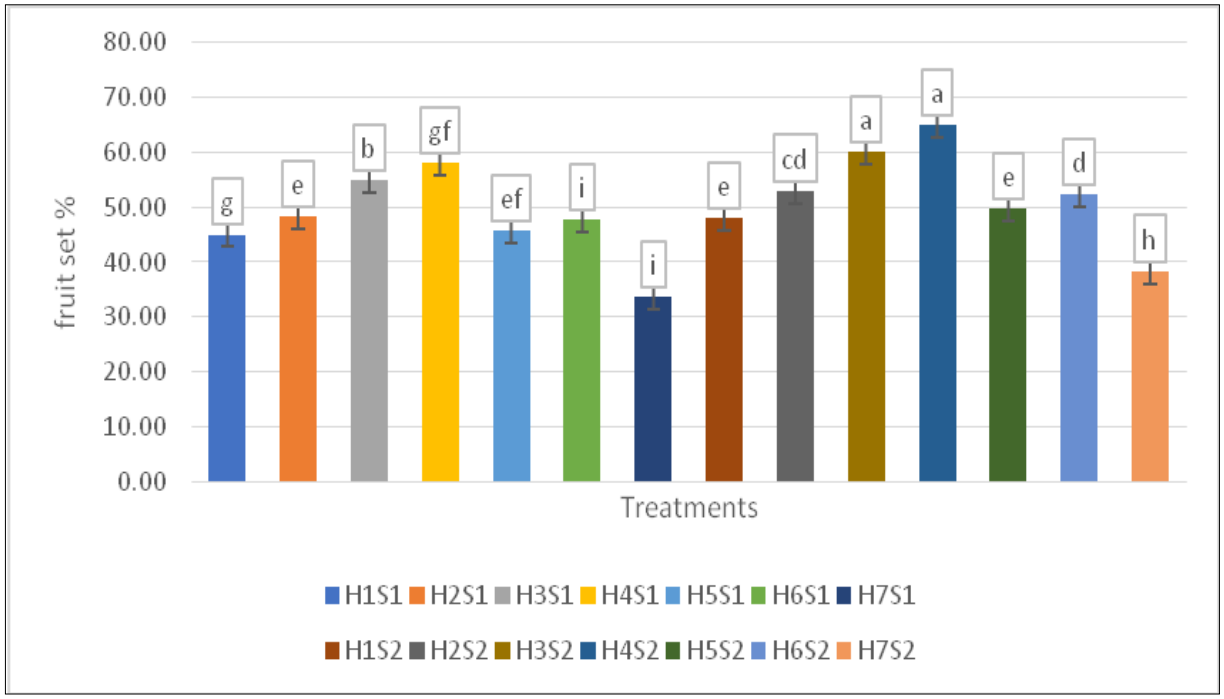


Fig. 3(a)

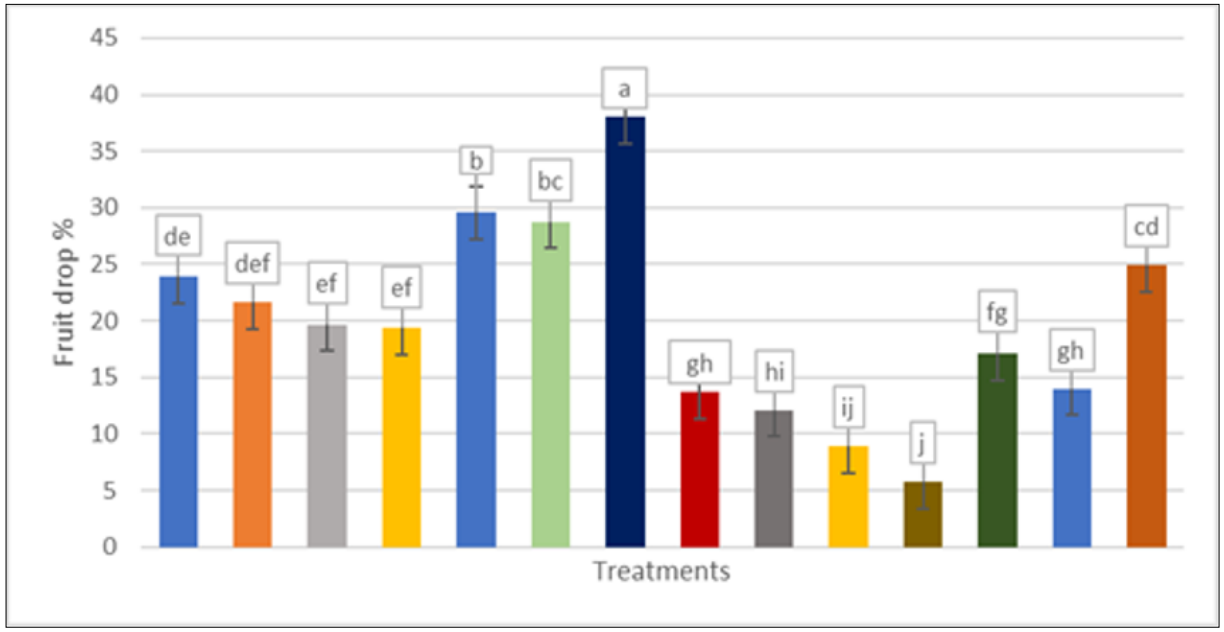


Fig. 3(b)

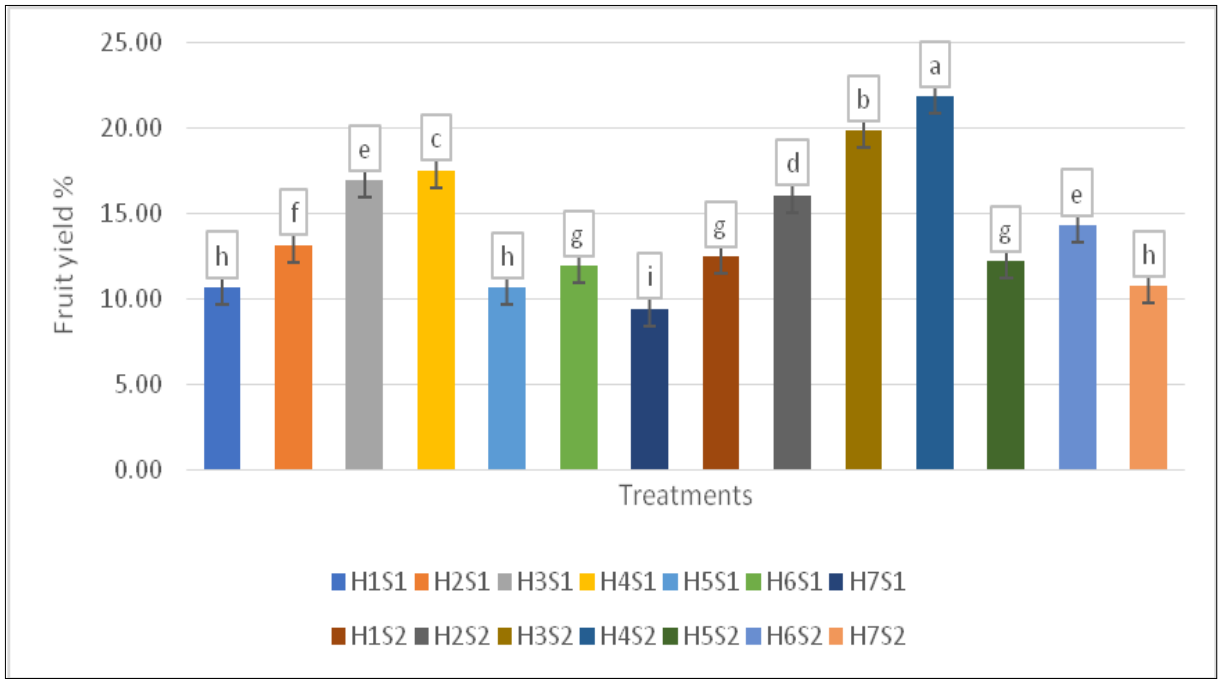


Fig. 3(c)

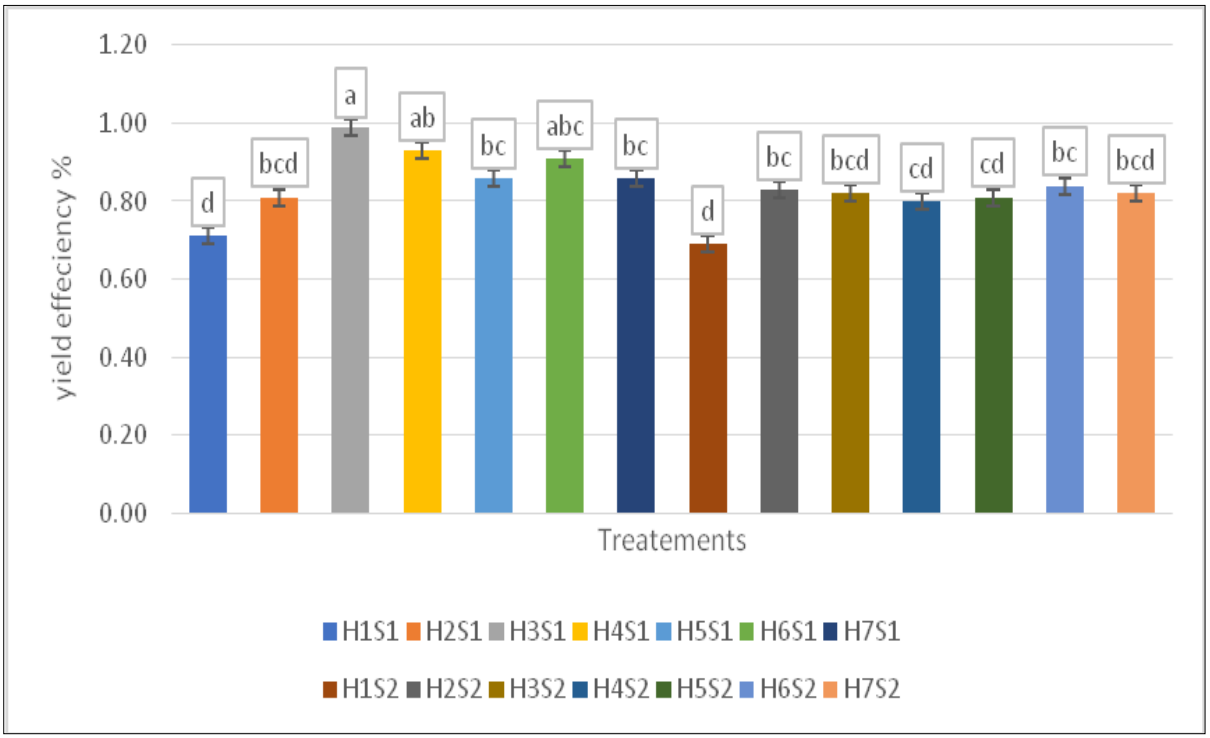


Fig. 3(d)

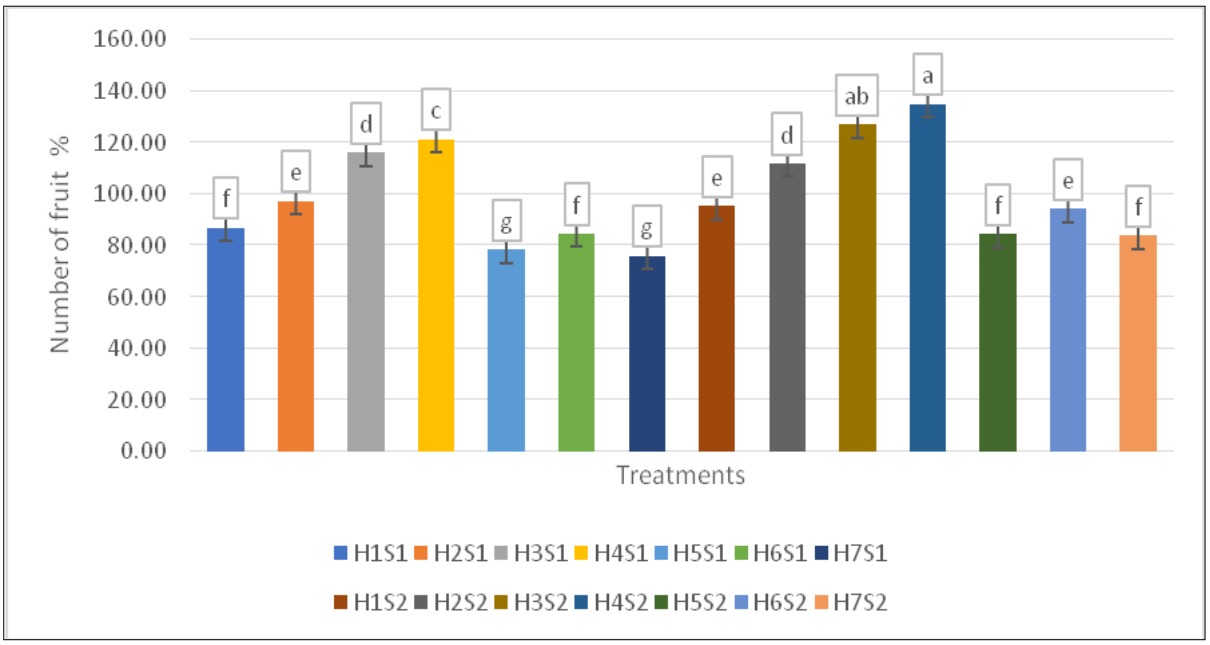


Fig. 3(e)

Fig. 3. Effect of plant hormones on plant flowering and yield parameters of Kinnow mandarin.

300 µM + FSFS and 60 FS DAFS) resulted in the highest FSFS (65.00 %) as compared with untreated plants. The foliar application of plant bioregulators significantly ($p \leq 0.05$) reduced the FD of Kinnow mandarin plants, with the highest FD (38.00 %) recorded under treatment H7 (control) and the lowest FD (8.88 %) recorded in treatment H4 (GA_3 at 150 µM), as shown in Fig. 3(b). However, the total number of fruits counted during each harvest across treatments and times consistently revealed a significant difference ($p \leq 0.05$), as shown in Fig. 3(e). The treatment combination H_4S_2 (GA_3 at 300 µM + at FSFS and 60 FS DAFS) found the highest number of fruits per plant (127 fruits plant^{-1}). The lowest number of fruits plants^{-1} (71.33) was recorded in H_7S_1 (control + at FSFS and 30 FS DAFS). Hence, the studies indicated that the maximum number of fruits per plant resulted from a higher FSFS. Plant hormones resulted in a highly significant increase in FY (21.86 kg plant^{-1}), with a p-value of 0.05 or less, recorded under treatment H_4S_2 (GA3 at 300 µM + at FSFS and 60 FSDAFS), as shown in Fig. 3(c). While the minimum increase in FY (9.45 kg plant^{-1}) was recorded in H_7S_1 (control + at FSFS and 30 FS DAFS).

The plant bio-regulator application at different stages recorded that the maximum increase in YE (0.99 kg cm^{-2}) was observed under the treatment combination H_4S_2 (GA_3 at 150 µM+At FS and 30 FS DAFS). The minimum increase in YE (0.69 kg cm^{-2}) was recorded in H_1S_2 (NAA at 100 µM + at FSFS and 30 FS DAFS).

Fruit quality parameters

The hormonal application at different stages showed a significant effect ($p \leq 0.05$) on fruit quality parameters (carotenoids, sugars, TSS and Ac) of Kinnow mandarin. The maximum increase in the carotenoid content (CRC) (5.50 µg g^{-1}) was found under the treatment combination H4S2 (GA3 at 300 µM + at FSFS and 60 FS DAFS), as shown in Fig. 4(a). The minimum increase in the CRC (1.53 µg g^{-1}) was recorded in H_7S_1 (control + at FSFS and 30 FS DAFS).

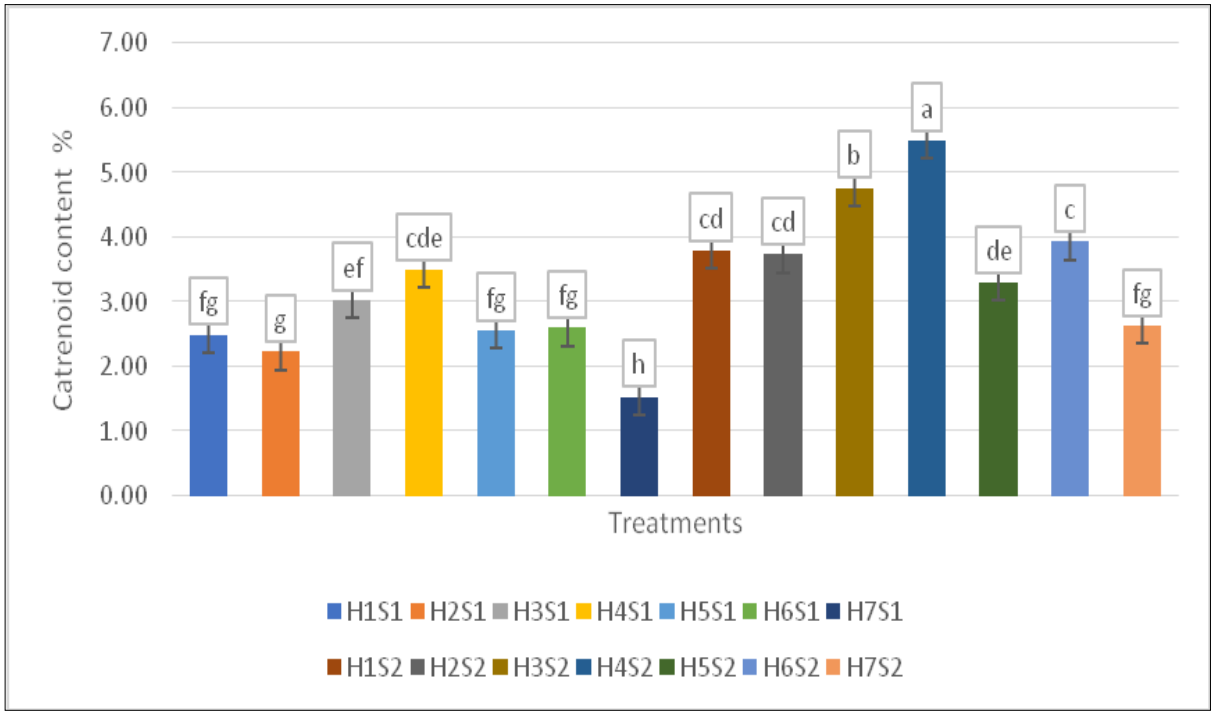


Fig. 4(a)

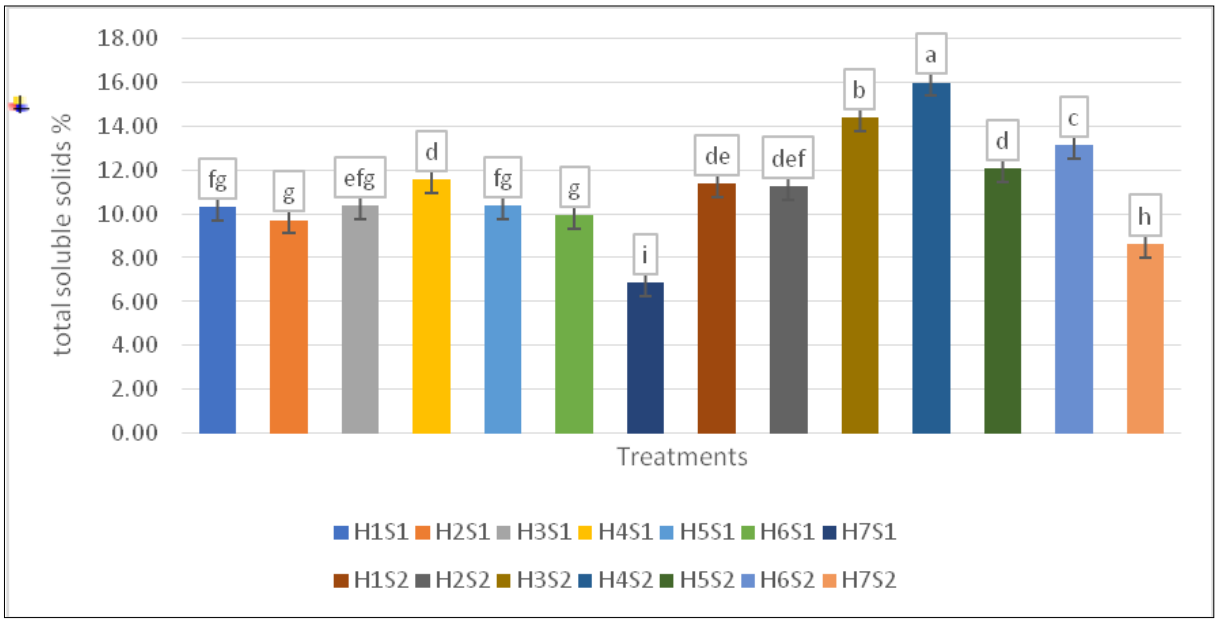


Fig. 4(b)

The maximum increase in TSS (16.01 °Brix) was recorded under the treatment combination H_4S_2 (GA_3 at 300 µM + at FSFS and 60 FS DAFS), as shown in Fig. 4(b). The minimum increase in TSS (6.85 °Brix) was recorded in H_7S_1 (control + at FSFS and 30 FS DAFS). The hormonal application and their stages showed a maximum AA (39.18 mg 100 g^{-1}) under the treatment combination H_4S_2 (GA_3 at 300 µM + at FSFS and 60 FS DAFS). While the minimum increase in AA (10.36 mg 100 g^{-1}) was recorded in H_7S_1 (control + at FSFS and 30 FSDAFS) as seen in Fig. 4(c). The minimum fruit Ac (0.48 %) was recorded with the treatment combination H_4S_2 (GA_3 at 300 µM + at FSFS and 60 FSDAFS). However, the maximum increase in Ac (0.84 %) was found in H_7S_1 (Control + at FSFS and 30 FSDAFS) as shown in Fig. 4(d). The plant hormonal application at critical stages showed a non-significant impact on the total sugar of Kinnow mandarin as recorded in Fig. 4(e). The maximum increase in total sugar (6.91%) was observed under treatment H4 (GA3 at 300 µM), whereas the minimum increase in total sugar (4.43 %) was recorded in treatment H_7 (control).

The combined effect of both stages with plant hormonal applications revealed that the highest increase in reducing sugar (4.76 %) was recorded under the treatment combination H_4S_2 (at FSFS and 60 FSDAFS). The lowest reducing sugar (2.14 %) was recorded in H_7S_1 (control + at FSFS and cell division) as seen in Fig. 4 (f). The application of plant bioregulators at different stages showed a significant effect ($p \leq 0.05$) on phenolic content (PhC), as shown in Fig. 4(g). The maximum increase in PhC (326.66 mg GAE g^{-1}) was found under the treatment combination H_4S_2 (GA3 at 300 µM + at FS and 60 FS DAFS). However, the minimum increase in the PhC (132.33 mg GAE g^{-1}) was recorded in H_7S_1 (control + at FS and 30 FS DAFS). The maximum increase in the antioxidant content (728.20 µmol L^{-1}) was observed under the treatment combination H_4S_2 (GA3 at 300 µM + at FS and 60 FS DAFS). The minimum antioxidant content (167.66 µmol L^{-1}) was observed with treatment combination H_7S_1 (control + at FS and 30 FS DAFS).

Principal component analysis

Principal component analysis (PCA) was performed to assess the pattern of variation and to identify the traits contributing most to genetic diversity among the studied treatment combinations (Fig. 5). The PCA reduced the dimensionality of the data and transformed

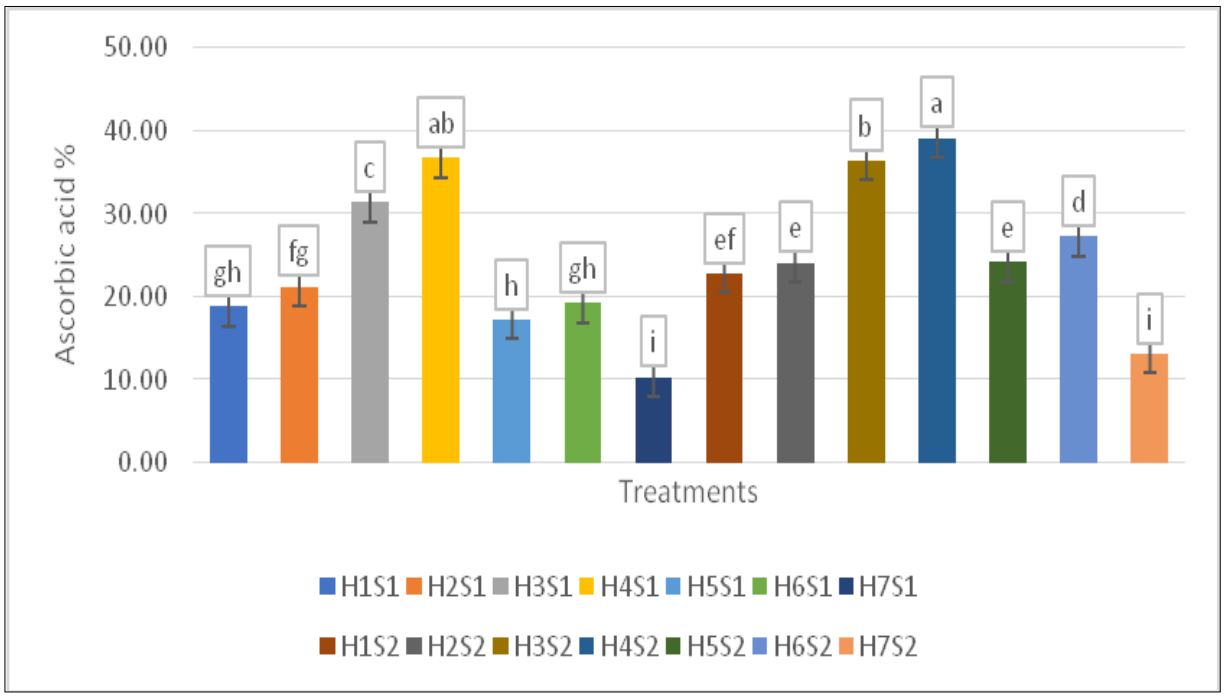


Fig. 4(c)

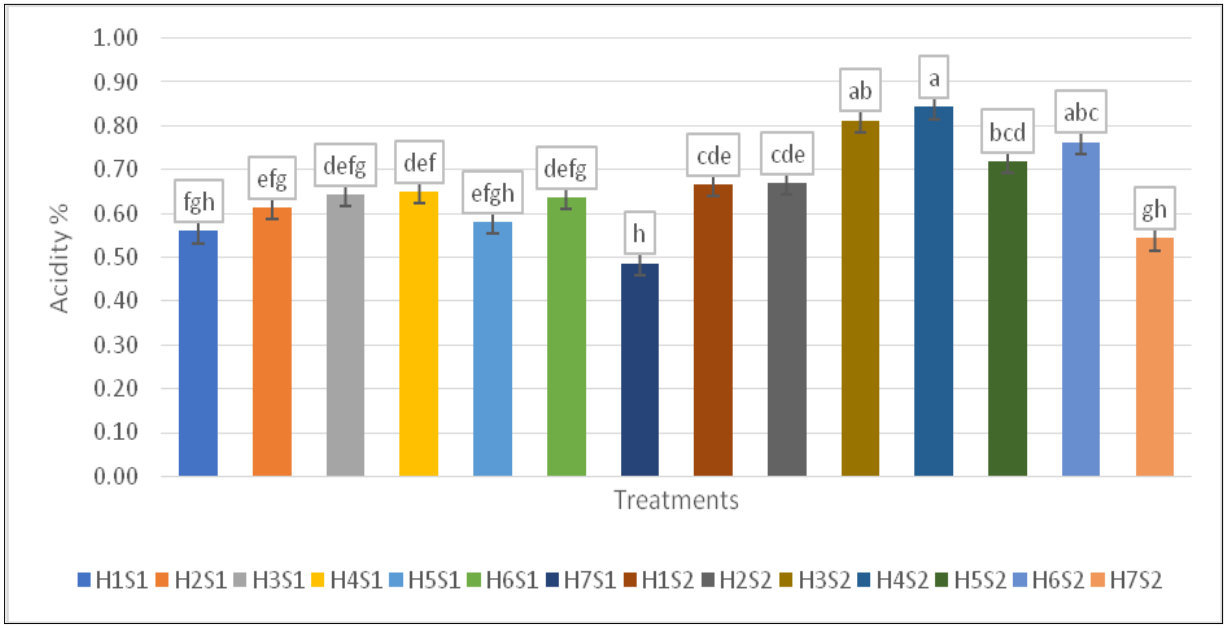


Fig. 4(d)

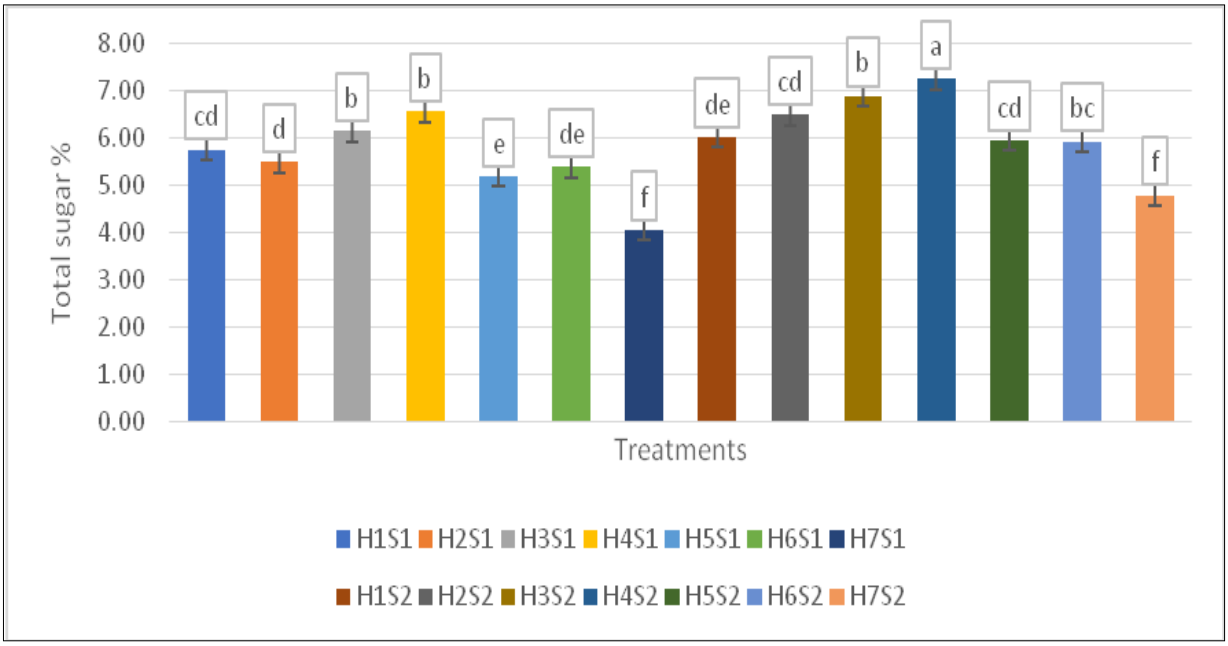


Fig. 4(e)

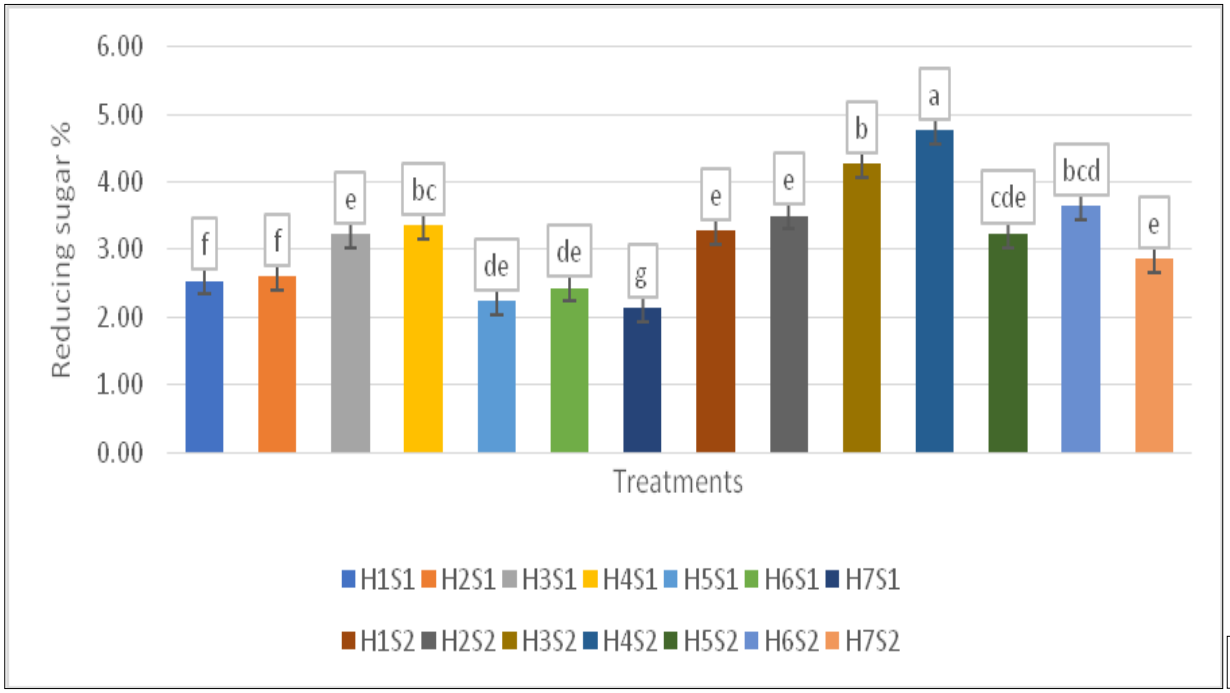


Fig. 4(f)

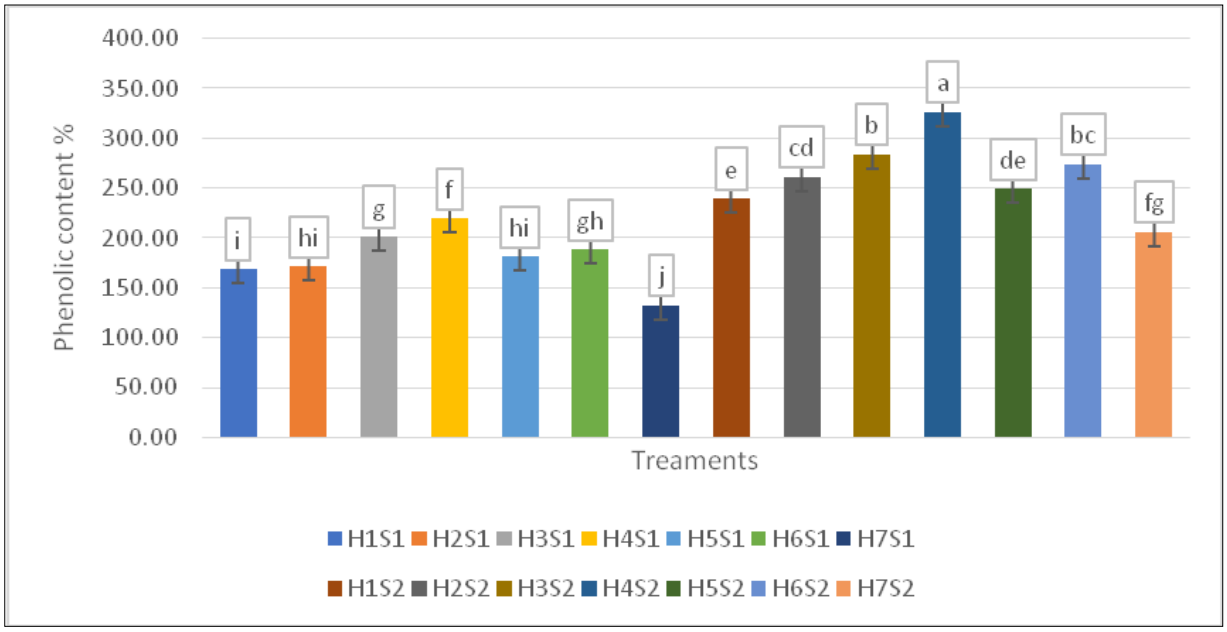


Fig. 4(g)

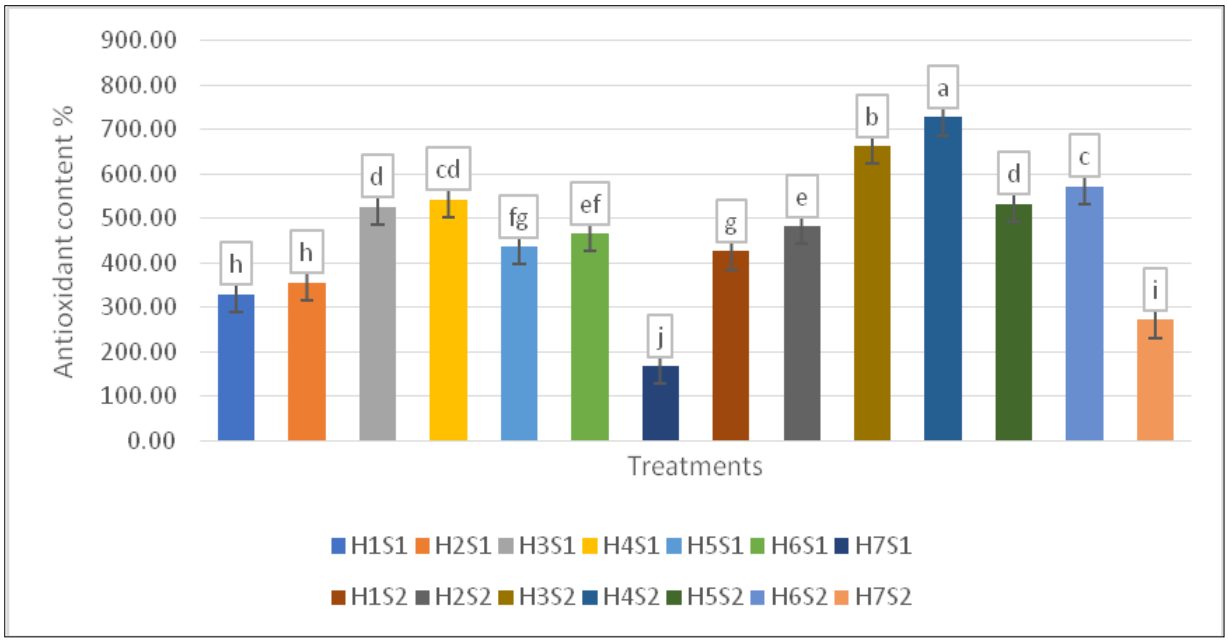


Fig. 4(h)

Fig. 4. Effect of plant hormones on plant physico-chemical parameters of Kinnow mandarin.

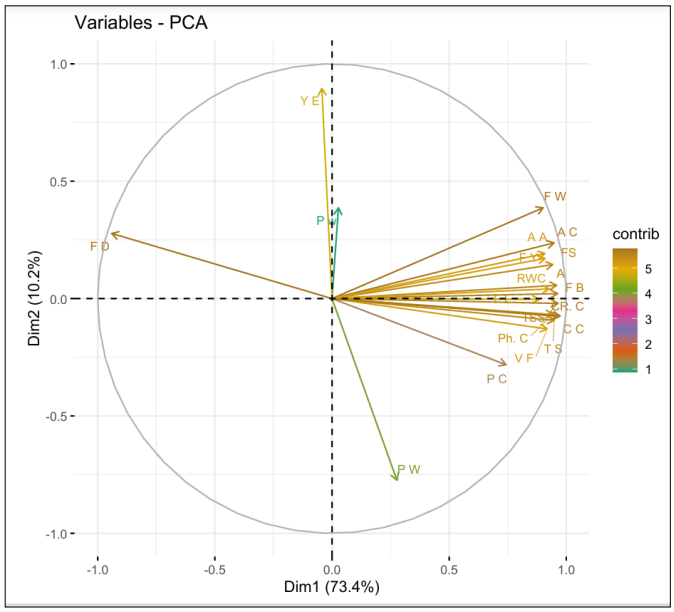


Fig. 5(a)

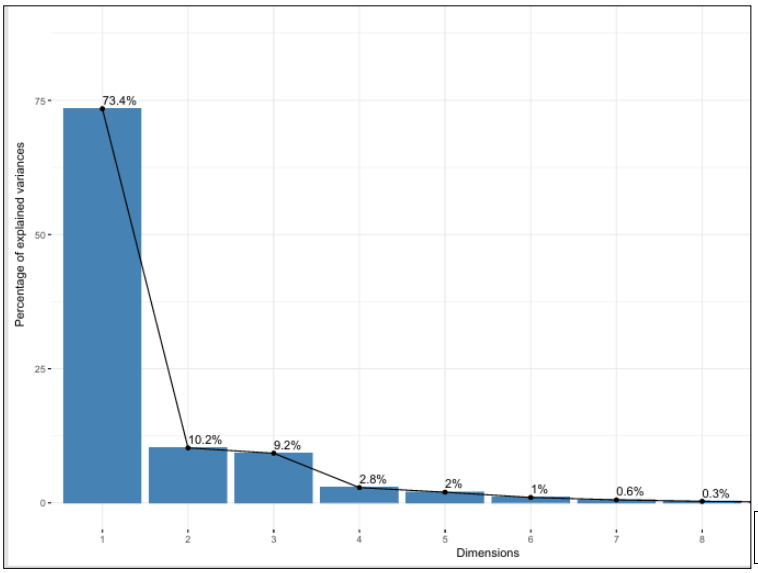


Fig. 5(b)

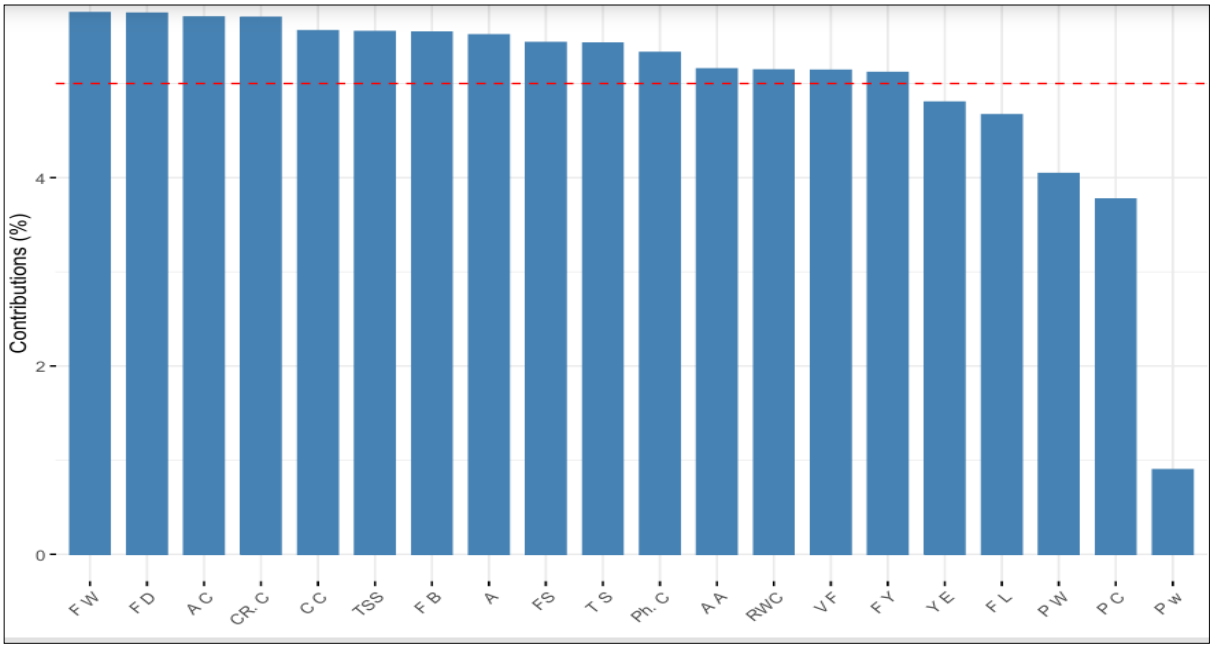


Fig. 5(c)

Fig. 5. Principle component analysis: (a) PCA biplot, (b)scree plot and (c) bar graph presenting contribution of each trait.

AA: Ascorbic acid; Ac: acidity; CC: chlorophyll content; CRC: carotenoid content; FD: fruit drop; FB: fruit breadth; FL: fruit length; FS: Fruit set; FW: fruit weight; FY: fruit yield; PhC: phenolic content; PC: proline content; PW: peel weight; RWC: relative water content; TSS: total soluble solid; TS: total sugar; VF: volume of fruit; YE: yield efficiency.

correlated variables into a smaller number of independent principal components (PCs). The analysis revealed that the first five principal components accounted for 97.73 % of the total variability present among the genotypes. Among them, PC1 explained the maximum variation (73.43 %) with an eigenvalue of 14.7, followed by PC2, which contributed 10.20 % of the total variation with an eigenvalue of 2.05. Together, PC1 and PC2 explained 83.67 % of the cumulative variation, indicating that these two components captured most of the variability and were therefore sufficient for graphical interpretation through the PCA biplot. Principal component 3 contributed 9.24 % variation, while PC4 and PC5 explained comparatively lower variation of 2.84 % and 1.98 %, respectively.

The PCA biplot illustrated the relationship among traits. Traits located close to each other in the biplot indicated positive association, whereas traits positioned in opposite directions showed negative correlation. Traits situated far from the origin contributed more strongly toward overall variability. Similarly, genotypes scattered farther apart exhibited greater genetic divergence, suggesting substantial variability among the studied material.

The contribution plot of individual traits indicated that FS, FD, antioxidant content, CRC, CC, TSS, fruit weight (FW) and related biochemical traits contributed more prominently toward total variation. In contrast, traits with lower contribution percentages had comparatively smaller influence on total variability.

Overall, the PCA results demonstrated considerable variability among treatment combinations and highlighted important morphological, yield and biochemical traits responsible for differentiation, which can be effectively utilised for selection.

Correlation analysis

Pearson's correlation coefficient indicates relationships among flowering, yield, fruit physiology and biochemical parameters of Kinnow mandarin. Fig. 6 provides a visual representation of the relationships among various variables. Correlation analysis revealed significant associations among morphological, yield and biochemical traits. Fruit yield showed strong positive correlations with fruit length (FL) (r = 0.95), fruit breadth (FB) (r = 0.94), FW (r = 0.96), volume of fruit (VF) (r = 0.97), CRC (r = 0.98), CC (r = 0.97), TSS (r = 0.96), antioxidant content (r = 0.90) and RWC (r = 0.90), indicating that improvement in these traits may directly enhance yield performance. Yield efficiency also exhibited strong positive correlations with FB (r = 0.94), antioxidant content (r = 0.90), RWC (r = 0.90), FW (r = 0.88) and CC (r = 0.87), suggesting their collective contribution toward productivity. Fruit weight demonstrated strong positive correlations with FB (r = 0.98), VF (r = 0.96), TSS (r = 0.94), CC (r = 0.93) and CRC (r = 0.92), indicating that larger fruits contributed substantially to overall FW and yield. Similarly, TSS showed positive correlations with antioxidant content (r = 0.94), RWC (r = 0.85), CRC (r = 0.87) and CC (r = 0.86), suggesting simultaneous improvement in fruit quality parameters. Biochemical traits such as antioxidant content, PhC, CRC, CC and RWC were positively and significantly associated with each other. Antioxidant content showed strong positive correlations with RWC (r = 0.95), FB (r = 0.94) and FW (r = 0.88). Chlorophyll content also exhibited a high positive correlation with CRC (r = 0.97). In contrast, FD exhibited strong negative correlations with FS (r = -0.83), AA (r = -0.89), FY (r = -0.91), CRC (r = -0.93), CC (r = -0.89) and antioxidant content (r = -0.91), indicating that increased FD adversely affected yield and quality

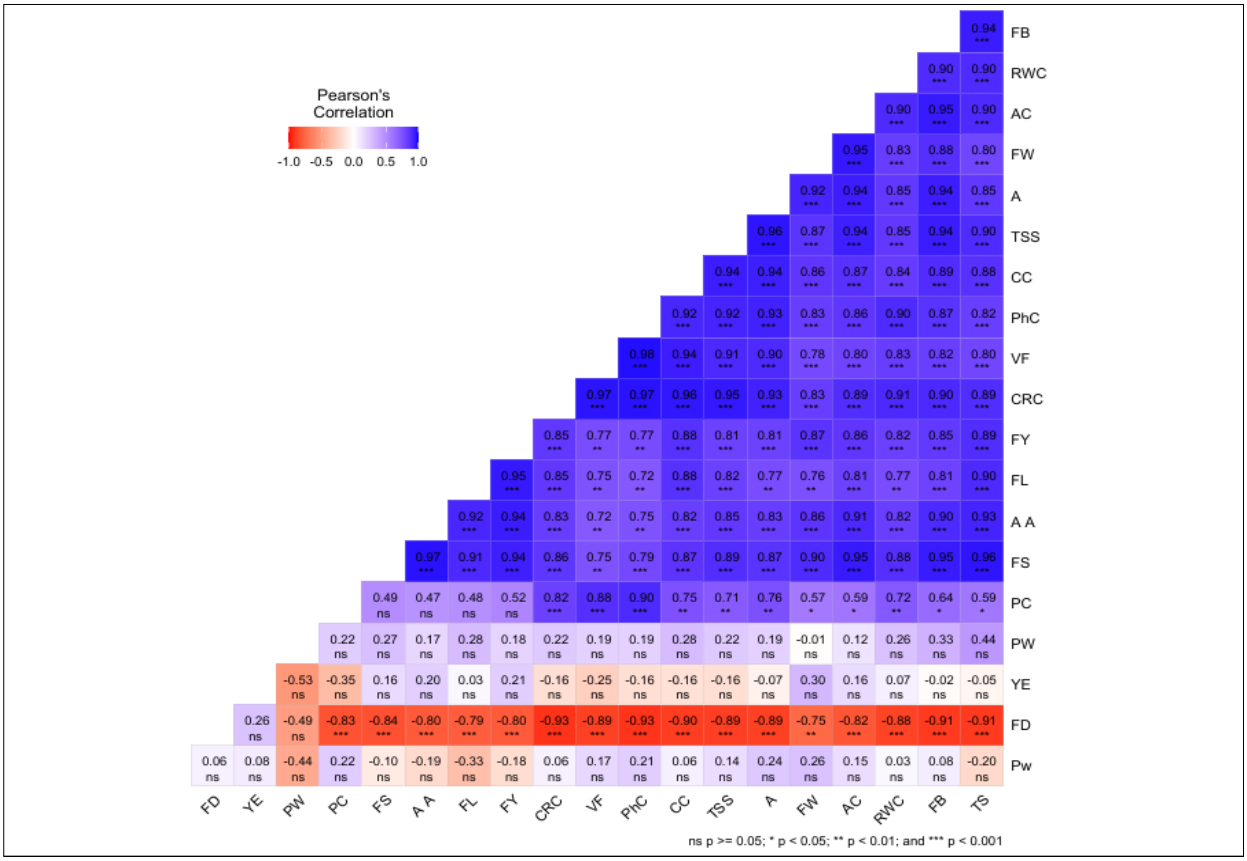


Fig. 6. Correlation among the various traits.

AA: ascorbic acid; Ac: acidity; CC: chlorophyll content; CRC: carotenoid content; FD: fruit drop; FB: fruit breadth; FL: fruit length; FS: FS; FW: fruit weight; FY: fruit yield; PhC: phenolic content; PC: proline content; PW: peel weight; RWC: relative water content; TSS: total soluble solid; TS: total sugar; VF: volume of fruit; YE: yield efficiency.

traits. Peel weight (PW) showed weak or non-significant associations with several desirable traits, suggesting comparatively lower contribution toward yield improvement. Proline content showed moderate positive correlations with FS (r = 0.48), AA (r = 0.50), FL (r = 0.75), FY (r = 0.78) and CC (r = 0.72), suggesting its role in physiological regulation and maintenance of fruit quality. Overall, the correlation matrix indicated that fruit size, biochemical quality and physiological traits were positively interrelated and contributed significantly toward FY and quality improvement.

Discussion

Plant physiological responses under rainfed stress conditions

The present study demonstrated that exogenous application of PGRs significantly improved the physiological performance of Kinnow mandarin under rainfed conditions, where intermittent water deficit is a major constraint to productivity. Among the treatments, GA_3 applied at the cell elongation stage was the most effective in enhancing RWC, chlorophyll concentration and proline accumulation. The higher RWC observed in GA_3-treated plants indicates improved plant water status and cellular hydration, which is particularly important under moisture-limited environments. Gibberellins are known to promote cell elongation, membrane stability and stomatal regulation, thereby improving water retention capacity and maintaining turgor during stress conditions (31). The increased proline accumulation further supports the enhanced stress tolerance in treated plants, as proline functions as an osmoprotectant involved in osmotic adjustment, stabilisation of cellular membranes and scavenging of reactive oxygen species under drought stress (32).

Similarly, CC was significantly higher in GA_3-treated plants, suggesting improved photosynthetic efficiency and delayed leaf senescence. Under rainfed conditions, maintenance of chlorophyll is essential for sustaining carbon assimilation and assimilate supply to developing fruits. The present findings agree with reports describing the stimulatory role of GA_3 in chloroplast development and chlorophyll biosynthesis (33). However, some earlier studies reported a reduction in CC following GA_3 application. Such discrepancies may be attributed to differences in citrus cultivars, concentration and timing of GA_3 application and environmental conditions. In the present investigation, the application of GA_3 during the active cell elongation phase under moderate water stress may have favoured chlorophyll retention by delaying senescence and maintaining leaf metabolic activity. Thus, the enhanced physiological status observed in the present study likely contributed to improved fruit retention and yield under rainfed conditions.

FS, fruit retention and yield

The foliar application of PGRs, particularly GA_3, significantly improved FS, fruit retention, yield and YE in Kinnow mandarin. The superior performance of GA_3 applied at the cell elongation stage may be attributed to its direct involvement in cell elongation, assimilate partitioning and hormonal regulation during early fruit development. Gibberellic acid promotes active sink strength in developing fruits, thereby enhancing the translocation of carbohydrates and nutrients toward reproductive organs. Improved assimilate mobilisation ensures adequate nourishment of young fruits, resulting in better fruit growth and reduced premature abscission (34).

The reduced FD observed under GA_3 treatment may also be associated with its antagonistic interaction with ethylene and abscisic acid, both of which are involved in abscission processes. Gibberellic acid likely suppressed the formation of the abscission layer by maintaining auxin activity in the fruit pedicel, thereby delaying senescence and improving fruit retention (35). In addition, GA_3 is known to stimulate pollen tube growth, ovary development and fertilisation efficiency, which collectively contribute to higher FS (2, 33). The present findings are in agreement with previous reports in citrus where GA_3 application improved fruit retention and yield through enhanced hormonal balance and carbohydrate availability. Variations reported among studies may be due to differences in environmental conditions, tree age, orchard management practices and timing of growth regulator application. Under the rainfed conditions of central Punjab, the beneficial effects of GA_3 were likely more pronounced because improved water relations and assimilate partitioning helped plants overcome stress-induced FD.

The increase in FW and overall yield observed in the present study further confirms the role of GA_3 in promoting cell elongation and expansion during fruit growth. Since the treatment was applied at the cell elongation stage, the hormonal effect coincided with the critical phase of rapid fruit enlargement, which explains the substantial improvement in fruit size and productivity.

Fruit quality attributes

GA_3 treatment also significantly influenced fruit quality parameters of Kinnow mandarin. The increase in TSS may be attributed to enhanced photosynthetic activity and efficient translocation of assimilates from source leaves to developing fruits. Gibberellic acid is known to stimulate enzymes such as amylase and invertase, which facilitate the conversion of stored carbohydrates into soluble sugars, thereby improving fruit sweetness and maturity characteristics (36). The reduction in fruit Ac observed under GA_3 treatment may be associated with accelerated metabolic conversion of organic acids during fruit ripening, resulting in improved flavour quality. These findings are in agreement with earlier reports on citrus fruits where GA_3 enhanced sugar accumulation and reduced Ac through improved assimilate mobilisation.

Ascorbic acid content was also significantly increased by GA_3 application, indicating enhanced nutritional quality of fruits. This increase may be linked to GA_3's stimulatory effect on enzymatic pathways involved in vitamin C biosynthesis, as well as delayed senescence and improved metabolic activity within fruit tissues (37). Although total sugar content did not differ significantly among treatments, numerically higher values in GA_3-treated fruits suggest that enhanced sugar synthesis may have been balanced by increased utilisation during rapid fruit growth and respiration (38).

The improvement in PhC and antioxidant activity further indicates the positive influence of GA_3 on fruit biochemical quality. Phenolic compounds are important secondary metabolites associated with antioxidant defence and stress adaptation. The higher phenolic accumulation observed in the present study may result from GA_3-induced activation of the phenylpropanoid pathway (39). Consequently, increased antioxidant activity in GA_3-treated fruits may be attributed to enhanced synthesis of phenolics and flavonoids, which play a major role in scavenging free radicals and improving fruit nutritional value (40). Under rainfed conditions, enhanced antioxidant metabolism may also help mitigate oxidative stress caused by water deficit, thereby contributing to better fruit

quality and shelf life.

Overall, the present findings indicate that GA_3 application at the cell elongation stage effectively improved physiological resilience, fruit retention, yield and fruit quality of Kinnow mandarin under rainfed conditions through coordinated regulation of water relations, assimilate partitioning, hormonal balance and antioxidant metabolism.

Conclusion

Kinnow mandarin is an important fruit grown in tropical and subtropical areas. The productivity and quality are usually impaired in the central region of Punjab due to the coincidence of short spells of water deficits at critical stages, i.e., at the fruit and cell division stage and at the FS and cell elongation stage. In this study, we found that applying plant hormones at critical stages significantly affects fruit quality, productivity and the physiological status of the plant. The best treatment combination, H4S2 (GA3 at 300 µM + at the FS and cell elongation stage) application at the FS and cell division stage and at the cell elongation stage, significantly increases plant morphology, flowering, yield, plant physiology and fruit physico-chemical growth of Kinnow mandarin. Overall, the study found that hormonal application at critical stages improves overall productivity and quality of Kinnow mandarin.

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Authors' contributions

RK and RS were responsible for drafting and writing the manuscript. RS, NC, JS, HS and GS contributed to the study's conceptualisation and provided essential project supervision. RK and HS conducted the data analysis, while RK and S were instrumental in preparing the research framework. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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