Effect of Plant Spacing and Growth Regulator on Quality and Quantity of Mosambi Fruit Under Drip Irrigation

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Abstract

A field experiment was managed at the Water Management Research Farm, Renala Khurd, Pakistan to check the effect of the plant to plant spacing (3×3, 5×5, 7×7) and growth regulator (Isabion) on the quantity and quality of citrus fruit (Mosambi) under drip irrigation. The trial was arranged into a split-plot outline with six treatments under three blocks. Two foliar sprays were carried out at 40 days gap in September and November 2020. After 4 to 5 days of second foliar spray, data were collected. The results revealed that plant growth regulator isabion was more effective in improving plant traits like no. of fruits and leaf area in mosambi and plant to plant spacing only improved one plant trait, plant height. However, fruit weight was not affected by both plant to plant spacing and growth regulator treatments compared to control. The fruit quality analysis results showed that carbohydrate, vitamin, moisture and fat contents of mosambi fruits were increased by both plant to plant spacing and growth regulator treatments, while the increase in protein content was not found. In our study, plant growth regulator appeared to be more effective for improving plant traits while fruit traits were equally affected by both plant-plant spacing and growth regulator. In the future, both plant to plant spacing and growth regulator should be considered to improve citrus plant growth and fruit yield.
**Introduction**

Citrus fruits and their products are very common in both developing and developed countries due to their promoted taste, pleasant flavor, and budget-friendly economic reach. In the world, Pakistan is at the twelfth number in citrus yield [1]. For citrus production, drip irrigation is the worldwide best irrigation method because it permits water to run gradually to the roots of plants by saving water and nutrients either from above the soil surface or below the surface. One more vital point of drip irrigation as compared to flood irrigation is that less water is evaporated, so it has been found to be water-saving [2]. The productivity of citrus fruit depends on many factors and plant growth regulators (PGR) hold an important position among those. Many plant growth regulators have been determined for their commercial application in the citrus industry. In citrus fruit production, PGRs have been used for determining fruit set, flowering, and fruit drop [3]. In citrus orchards, PGRs are used to control reproductive and vegetative growth, improve fruit set, fruit growth and fruit quality [4]. Another essential factor that affects the efficiency of citrus is plant spacing. In citrus rootstock management, tree spacing has become an important factor due to the profit of higher tree density on initial production and economic returns [5]. However, fruit yield per unit land area becomes independent of tree spacing as the trees grow and compete and may decrease at closer spacing [6]. Fruits of citrus are not only high in macronutrients, such as simple sugar and dietary fiber but also contain important micronutrients such as potassium, calcium, magnesium, phosphorus, copper, folate, thiamin, niacin, vitamin B6, pantothenic acid and riboflavin, etc. For keeping normal growth and health, these macro and micronutrients are vital [4]. Mosambi juice is suggested for athletes as it decreases muscle cramps. It also has a vital role in skincare and is effective for curing scurvy. The yield (Kg per tree) of citrus comprises both fruit size and fruit number. Fruit number is the result of flower potency and fruit set. Fruit size is a function of cytokinesis and cell growth processes. For the marketability of citrus, fruit size is the main character. In the present study, we aimed to use a plant growth regulator (isabion) two times at three different plant-plant spacings in an orchard under drip irrigation and to evaluate their effects on qualitative and quantitative parameters of mosambi fruit.

**Materials and Methods**

**Experiment site and experiment design**

The study pertaining to see the effect of three different plant-plant spacings (3m×3m, 5m×5m, 7m×7m) and growth regulator (isabion) on citrus fruit (mosambi) under drip irrigation was conducted at the research area of Water Management Research Farm, Renala Khurd, Okara, Pakistan during the last week of September 2020. The area under citrus is 1.87 acres. Two foliar sprays of growth regulator (isabion) at 40-day intervals were carried out in September and November 2020. The first foliar spray was applied at the end of September and the second at the start of November. Each block was divided into two parts. One part was specified for the spray of isabion, and the other was taken without isabion as control. After 4 to 5 days of the second foliar spray of isabion, data were collected.

**Quantitative data collection**

*Plant height and canopy measurement*

Height is the vertical distance from ground level to the highest point on the tree (a point referred to as the tip of the tree). Citrus plant height was measured by the direct height measuring tape method. The canopy is the covering of the plant. Citrus tree canopies were quite dense. The canopy was measured with the help of a measuring tape.

*Leaf area measurement*

To analyze individual leaf areas, an easy, cheap, and non-destructive millimeter graph paper method was used as described by Pandey and Sing [7].

*Quantity of fruits*

The quantity of fruits was estimated by the number of fruits. From each row, two plants were selected randomly, and fruits were counted manually.

*Fruit weight*

Fruits of different sizes (larger, medium, and small) were randomly selected, and their weights were measured using a weighing balance in grams.

**Biochemical analysis of mosambi fruits**

*Vitamin C (ascorbic acid) content analysis*

To determine vitamin C contents in fruits, the volumetric method was used. A total of 10 ul of the ascorbic acid solution in 0.1M phosphate-buffered
saline (pH 2.0) was pipetted into the electrochemical cell. As the voltammetry cell, different volumes of ascorbic acid solution (0.010 M) were added to the electrode surface. With cyclic voltammetry, the potential was scanned from -0.0 to +0.1 V and from 0.0 to +1.2 V. By measuring its voltammetric current, the quantity of ascorbic acid was obtained by differential pulse voltammetry. During the potential sweep, differential pulse voltammograms were recorded from -0.0 to -1.2 V [8]. The method was successfully implemented with 2 ul of the working solution dropped on the electrode surface.

Protein content analysis

To determine protein contents in fruits, a wet digestion method was used. In the digestion flask, 3g sample was placed and digested with digestion tablet (copper, potassium sulfate) and conc. H₂SO₄ at 370-400°C. For about an hour, the mixture was boiled until greenish color fumes appeared. To cool the digestion flask, 250 ml water was added. To neutralize the mixture, 10 ml of NaOH was used that released the NH₃ gas. For the titration of distillate, 0.1N H₂SO₄ was used to judge nitrogen content. Titration was done until orange color appeared. By using the formula below, protein content was calculated.

\[
\text{Nitrogen (\%)} = \text{vol. of 0.1} \times \text{NH}_2\text{SO}_4 \times \text{dilution vol. (250 ml)} / \text{sample weight} \times \text{distillate sample vol.} \times 100
\]

\[
\text{Crude protein (\%)} = \text{nitrogen (\%)} \times \text{factor (6.25)}
\]

Moisture content analysis

Moisture content was determined by using method no. 44-15 of AOAC [9]. In a pre-weighted crucible, 5g of sample was placed. To dry the sample, the crucible was placed in the hot air oven at 105°C temperature for 24 hours. Sample crucibles were removed from the oven when the samples were fully dried. Then crucibles were cooled down by placing them into a desiccator for 5-10 min. Noted the weight of the sample before and after the drying. To get the best results, three replicates were made. Moisture content was determined by the following formula:

\[
\text{Moisture (\%)} = (\text{initial weight of sample} - \text{final weight of sample}) / \text{initial weight of sample} \times 100
\]

Crude fat content analysis

By using the Soxhlet apparatus, method no. 30-10 of AOAC was used to estimate the crude fat content [9]. A total of 5g sample was placed in a thimble and then placed in an extraction tube. Apparatus was turned on and n-hexane drops started to fill down on the sample in the tube. when 6 cycles were completed, turned off the apparatus supply and residues were placed into pre weighted crucible. For 3-4 hours, the crucible was transferred into the oven to evaporate the n-hexane and then cool it in a desiccator and weighed again. The following formula was used to determine the percentage of crude fat content.

\[
\text{Crude fat (\%)} = \text{n-hexane residue weight} / \text{sample weight (g)} \times 100
\]

Carbohydrates content analysis

A total of 0.1 g fruit sample was added in 5 ml of 2.5N HCl and heated in a water bath for 3 hours. It was neutralized by adding sodium carbonate and volume was increased to 100 ml. Each experimental sample was mixed with 1 ml of 5% phenol solution and 5 ml of 96% sulfuric acid solution. It was then kept at 30°C for 20 min. The color absorption was later determined at 490 nm using a spectrophotometer. Glucose was used as a standard and using the standard curve, we calculated the contents of total carbohydrates present in the sample solution [9]. The final calculations were made using the following formulas:

Absorbance corresponds to 0.1 ml of the test = ‘x’ mg of glucose

\[
100 \text{ mL of the sample solution contains} = (x' / 0.1) \times 100 \text{ mg of glucose} = \% \text{ of total carbohydrates present}
\]

Statistical analysis

Standard procedures were followed to collect the data on quantity and quality parameters. The data collected were statistically analyzed using Fisher’s analysis of variance technique with computer package M. STATEC for statistical analysis and the treatment mean was compared by the least significant test [10].

Results and Discussion

Quantitative data results

Number of fruits

Data regarding the number of fruits affected by spacing and growth regulator (isabion) is given in Table 1. It is clear from the results that overall, 7x7 m plant to plant spacing showed significantly lower
number of fruits compared to 3×3 m and 5×5 m spacings and the use of growth regulator (isabion) significantly increased the number of fruits of mousambi. These results are in accordance with the results of Dogar et al., who reported the maximum number of fruits in mousambi that was planted at 11×22 ft spacing distance [11].

**Leaf area**

Data regarding leaf area affected by spacing and growth regulator (isabion) is given in Table 1. It is clear from the results that the use of growth regulator (isabion) treatment significantly increased the leaf area of mousambi. While all three plant-plant spacing treatments did not show a significant effect on the leaf area of mousambi. These results are in accordance with the observations of Qureshi et al., who reported that growth regulators are more effective in increasing the leaf area of mousambi [12].

**Plant height**

Data regarding plant height affected by spacing and growth regulator (isabion) is given in Table 1. It is clear from the results that plant to plant spacing significantly influenced the plant height of mousambi and maximum plant height was obtained at 5×5 m spacing. While the use of growth regulator (isabion) did not show a significant effect plant height of mousambi. These results are not in line with the observations of Qureshi et al., who reported that growth regulators are more effective in increasing the plant height of citrus [12].

**Plant canopy**

Data regarding plant canopy affected by spacing and growth regulator (isabion) is given in Table 1. It is clear from the results that plant to plant spacing, the use of growth regulator (isabion) treatment both did not show a significant effect on the canopy of mousambi plant. These results are not in line with the observations of Ladanija et al., who reported that under wide spacing plantation, plant canopy gradually increases over the years [13].

**Fruit weight**

Data regarding fruit weight affected by spacing and growth regulator (isabion) is given in Table 1. It is clear from the results that the use of growth regulator (isabion) treatment did not show overall a significant effect on the fruit weight of mousambi. However, plant to plant spacing showed a significant effect overall and 3×3 m and 5×5 m spacing showed higher fruit weight compared to 7×7 m plant-plant spacing. These results are not in

### Table 1 Effect of plant spacing and isabion on the quantitative parameters of mousambi fruit.

| Parameters        | Spacing | Isabion | Control | Mean (spacing) |
|-------------------|---------|---------|---------|----------------|
| No. of fruits     |         |         |         |                |
| 3×3               |         | 164.13\(^a\) | 24.17\(^b\) | 94.14\(^a\) |
| 5×5               |         | 71.67\(^b\)  | 91.33\(^a\) | 81.50\(^b\) |
| 7×7               |         | 39.00\(^c\)  | 36.67\(^b\) | 37.83\(^c\) |
| Mean (treatment)  |         | 91.60\(^a\)  | 50.72\(^b\) |                |
| Leaf area (\(\text{cm}^2\)) |         |         |         |                |
| 3×3               |         | 25.18\(^a\)  | 16.33\(^b\) | 20.76 |
| 5×5               |         | 29.58\(^a\)  | 20.08\(^ab\) | 24.83 |
| 7×7               |         | 19.92\(^ab\) | 15.33\(^b\) | 17.62 |
| Mean (treatment)  |         | 24.89\(^a\)  | 17.25\(^b\) |                |
| Plant height (\(\text{ft}\)) |         |         |         |                |
| 3×3               |         | 11.25\(^a\)  | 11.67\(^b\) | 11.46\(^b\) |
| 5×5               |         | 14.75\(^a\)  | 14.75\(^a\) | 14.75\(^a\) |
| 7×7               |         | 10.00\(^b\)  | 11.50\(^ab\) | 10.75\(^b\) |
| Mean (treatment)  |         | 12.00    | 12.64   |                |
| Plant canopy (\(\text{ft}\)) |         |         |         |                |
| 3×3               |         | 22.75    | 23.50   | 23.12 |
| 5×5               |         | 21.25    | 23.00   | 22.12 |
| 7×7               |         | 22.00    | 21.25   | 21.62 |
| Mean (treatment)  |         | 22.58    | 22.00   |                |
| Fruit weight (\(\text{g}\)) |         |         |         |                |
| 3×3               |         | 141.33   | 151.67  | 146.50\(^a\) |
| 5×5               |         | 137.00   | 143.61  | 140.33\(^a\) |
| 7×7               |         | 125.33   | 125.67  | 125.50\(^b\) |
| Mean (treatment)  |         | 134.55   | 140.37  |                |

Different small English letters show significant differences in each treatment at different spacings.
Different capital English letters show significant differences in the overall treatment effect.
Table 2 Effect of plant spacing and growth regulators on quality parameters of mosambi fruit.

| Parameters     | Spacing | Isabion | Control | Mean (spacing) |
|----------------|---------|---------|---------|----------------|
| Carbohydrate contents | 3×3     | 16.64   | 15.92   | 16.28          |
|                 | 5×5     | 16.09   | 15.16   | 15.62          |
|                 | 7×7     | 16.64   | 15.67   | 16.15          |
| Mean (treatment)|         | 16.45<sup>A</sup> | 15.58<sup>B</sup> |               |
| Fat contents    | 3×3     | 0.59<sup>bc</sup> | 0.51<sup>c</sup> | 0.55          |
|                 | 5×5     | 0.63<sup>b</sup> | 0.69<sup>ab</sup> | 0.66          |
|                 | 7×7     | 0.72<sup>a</sup> | 0.72<sup>a</sup> | 0.72          |
| Mean (treatment)|         | 0.64 |        | 0.64          |
| Moisture contents| 3×3     | 5.01<sup>a</sup> | 4.71<sup>bc</sup> | 4.86          |
|                 | 5×5     | 4.91<sup>b</sup> | 4.37<sup>cd</sup> | 4.64          |
|                 | 7×7     | 4.28<sup>cd</sup> | 4.20<sup>d</sup> | 4.24          |
| Mean (treatment)|         | 4.73 |        | 4.42          |
| Protein contents| 3×3     | 1.45 | 1.30 | 1.37          |
|                 | 5×5     | 1.46 | 1.36 | 1.41          |
|                 | 7×7     | 1.40 | 1.38 | 1.39          |
| Mean (treatment)|         | 1.43 |        | 1.34          |
| Vitamin contents| 5×5     | 24.53<sup>b</sup> | 23.71<sup>c</sup> | 23.53          |
|                 | 7×7     | 25.72<sup>a</sup> | 22.54<sup>c</sup> | 25.54          |
|                 | 3×3     | 25.36<sup>a</sup> | 24.27<sup>a</sup> | 24.53          |
| Mean (treatment)|         | 25.20<sup>a</sup> | 23.87<sup>B</sup> |               |

Different small English letters show significant differences in each treatment at different spacings. Different capital English letters show significant differences in the overall treatment effect.

line with the observations of Gosh and Pal, who showed maximum fruit weight in an inter-cropping trail of three-year mosambi at 5×5 m spacing [14].

**Biochemical analysis results**

*Carbohydrate contents*

Data regarding the carbohydrates contents in mosambi fruit as affected by plant growth regulator (isabion) and plant-plant spacing are given in Table 2. It is clear from the results that overall, the use of plant growth regulator isabion significantly increased the carbohydrate contents in mosambi fruit from 15.16% to 16.64% compared to control, while three plant-plant spacing treatments did not show significant differences among each other. These results matched with the observations of Bharti et al., who observed the effect of plant growth regulators on mosambi biochemical parameters and reported increase in total sugar, ascorbic acid and other antioxidant compounds [15].

*Fat contents*

Data regarding the fat contents in mosambi fruit as affected by plant growth regulator (isabion) and plant-plant spacing are given in Table 2. It is clear from the results that the use of plant-plant spacings significantly affected the fat contents; however, those were not different statistically between corresponding plant growth regulator isabion and control treatments. The overall effect of both plant growth regulator and plant-plant spacing was also not significant. These results matched with the observations of Maity et al., who observed the effect of foliar spray on pomegranate cultivars, which resulted in increased seed oil content [16].

*Moisture contents*

Data regarding the moisture contents in mosambi fruit as affected by plant growth regulator (isabion) and plant-plant spacing are given in Table 2. It is clear from the results that the use of plant-plant spacings significantly affected the fruit moisture contents and higher moisture contents were found at 3×3 plant-plant spacing. The overall effect of both plant growth regulator and plant-plant spacing was also not significant. These results are in accordance with that of Rodrigues and Subramanyam, who sprayed mandarin orange plants with CLPA, 2, 4, S-T and NOA8 and found increase in the juice and peel moisture contents [17].

*Protein contents*

Data regarding the protein contents in mosambi fruit as affected by plant growth regulator (isabion)
and plant-plant spacing are given in Table 2. It is clear from the results that both the use of plant growth regulator isabion and plant-plant spacing did not affect the protein content in mosambi. These results are not in accordance with the that of Khandaker et al., who studied the effects of growth regulators on the phytochemical properties of the wax apple fruit and reported that growth regulators greatly increased phenol and flavonoid content, vitamin C content and produced higher phenylalanine ammonia lyase (PAL) and antioxidant activity [18].

Vitamin contents

Data regarding the vitamin contents in mousambi fruit as affected by plant growth regulator (isabion) and plant-plant spacing are given in Table 2. It is clear from the results that three plant-plant spacings showed significant differences among each other for plant growth regulator and control treatments. However, overall effect of plant-plant spacings was not significant while plant growth regulator isabion showed significant effect. These results are in accordance with the observations of Mir and Itoo, who observed that the effect of exogenous applications of growth regulators leading to an increase in ascorbic acid or vitamin C in mosambi fruit [19].

Conclusions

For mosambi plant, plant growth regulator significantly increased the number of fruits and leaf area, while the overall effect of plant to plant spacing was significant on plant height and number of fruits compared to control treatment. Similarly, plant growth regulator significantly increased the fruit carbohydrate and vitamin contents, while the overall effect of plant to plant spacing was non-significant on all tested qualitative parameters compared to control. These results revealed that both plant growth regulator and plant to plant spacing are important parameters; however, plant growth regulator appeared to more important parameter to consider in future studies to improve the yield and quality of mosambi fruits.

Conflict of Interest

The authors have no conflict of interest to declare.

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