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The Influence of Nitrogen and Phosphorus on Watermelon Fruit Quality

Erone Vallantino Emongor[1*], Malikongwa Thatayaone[1] and Seoleseng Obonya Tshwenyane[1]

[1]Botswana University of Agriculture and Natural Resources, Crop Science and Production Department, Private Bag 0027, Gaborone, Botswana.

Authors' contributions

This work was carried out in collaboration between all authors. Author EVE designed the study, performed the statistical analysis, wrote the protocol and edited the final version of the manuscript. Author MT did data collection, managed the experimental processes and wrote the first draft of the manuscript. Author SOT managed the literature searches and editorial of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

This study was done to elucidate the effects of nitrogen (N) and phosphorus (P) on fruit quality of watermelon. The experimental design was a split-plot laid down in randomized complete blocks with three replications. The treatments were 0, 50, 100, 150 and 200 kg N/ha (main-plots) and 0, 25, 50 and 75 kg P/ha (sub-plots). There were no interactions between N and P treatments on all the dependent variables analyzed. Application of 50, 100, 150 and 200 kg N/ha to watermelon plants significantly ($P < .0001$) increased fruit quality (lycopene content, vitamin C, titratable acidity and fruit rind thickness) compared to fruit from control plants. However, P application had no significant ($P = .05$) effects on fruit lycopene content, vitamin C content, titratable acidity, soluble solids content and fruit rind thickness compared to fruit from control plants. In conclusion to optimize watermelon fruit quality, application of 150 kg N/ha and 50 kg P/ha to watermelon plants was recommended.

Keywords: Citrullus lanatus thunb; nitrogen; phosphorus; vitamin C; lycopene.

**Corresponding author: E-mail: vemongor@bca.bw, vemongor@gmail.com;*

1. INTRODUCTION

Watermelon fruit is nutritious containing 95% water, 0.5% ash, 0.01% oil, 0.5% fibre, 5% carbohydrates, 18-25 µg vitamin A, 0.04 mg thiamine, 0.03 mg riboflavin, 8.0 mg Ca, iron 0.0200 mg, niacin 0.6 mg, ascorbic acid 15.0 mg and potassium 6.0 mg per 100 g edible portion [1,2]. It may help reduce inflammation associated with conditions such as asthma, atherosclerosis, diabetes, colon cancer and arthritis [3]. It contains high levels of lycopene which is an antioxidant that reduces the risk of diseases related to oxidative stress, that arise as a result of imbalance in the human antioxidated status implicated in aging and a number of diseases such as cancer, atherosclerosis and rheumatoid arthritis [4]. Lycopene is a highly efficient oxygen radical scavenger and has been implicated in many epidemiological studies as providing protection against cancers especially in men [4]. Watermelon fruit also contains the amino acid citrulline. Citrulline is a precursor in the biosynthesis of arginine. Arginine boosts nitric oxide, which relaxes the blood vessels, thus having the same effect as viagra, which is used to treat erectile dysfunction in men [4]. Arginine has also been reported to significantly increase the production of sperms in humans [4,5]. Watermelon seeds may be roasted and eaten as snacks especially in Africa and Southern China [6, 7). Watermelon seeds contain 20-45% edible oil and 30-40% protein [7]. The husks of the seeds can be used as poultry feed. The leaves and young fruits can be utilized as vegetables. The peel of the fruit can be used for making jam [8]. In drought, the fresh watermelon and watermelon peels are used as livestock feed in southern Africa [8].

Mineral nutrition of plants not only affects vegetative and reproductive organs, but also affects yield components of economic yield. In most crops, both quantity and quality are important yield components. For a productive fruit crop, N and P should be within a range referred to as sufficient, beyond this range the plant might be associated with excessive or deficiency of nutrients [9]. Yield production in the annual herbaceous crops of the Cucurbitaceae are affected by both factors that influence plant productivity and those that determine the partitioning of assimilates to reproductive tissues [10]. Fruit quality is an important criterion in the production of muskmelon, watermelon and winter squash. Production systems must provide conditions that allow fruit to develop acceptable sweetness and taste, and the size characteristics of the cultivar [10]. Watermelon fruits should have a minimum sugar content of 9-10% to be considered acceptable [11-13]. As yields of fruits increase, soluble solid content (SSC) and fruit size tends to decrease [14]. Factors that reduce the canopy photosynthetic rate, such as the extent of exposure of the leaf canopy to light or mineral deficiency of mineral nutrients such as N, P and potassium (K) may influence fruit quality and yield [10].

Few studies in literature have reported on the effects of N and P nutrition on watermelon fruit quality attributes such as fruit soluble solid content, titratable acidity, flesh firmness, rind thickness and fruit mineral content. Ferrante et al. [15] reported that application of N from 0 to 165 kg/ha to watermelons did not have an effect on fruit flesh firmness, skin and pulp content, carotenoid content, total phenols and vitamin C content. Shaoping et al. [16] reported that increasing N application rate above 200 kg/ha decreased fruit soluble solids content from 13.9% (200 kg N/ha) to 8.42% (300 kg N/ha). Olaniyi and Tella [17] reported that application of 60 kg N/ha and 25 kg K/ha significantly increased the seed protein, fat and mineral (P, K, Ca, Mg, Fe and Zn) contents of watermelon compared to control watermelon plants. Olaniyi [18] reported that application of 52.5 kg P_2O_5/ha was optimum for watermelon yield. While, Adeyemi [19] reported that in soil of low and medium fertility, application of 75 kg P_2O_5/ha resulted in optimum watermelon yield in Nigeria. In the Republic of South Africa, for high watermelon fruit yield and fruit of excellent quality, application of up to 180 kg N/ha and 73 kg P/ha in soils with lower than 20 ppm bicarbonate extractable P was recommended [20]. Botswana soils are deficient in P and low in N and organic matter [21-23]. In view of the conflicting information from different parts of the world and the lack of field studies in fertilizer requirements of watermelon in Botswana, this study was done with the objective to evaluate effects of N and P on fruit quality of watermelon.

2. MATERIALS AND METHODS

2.1 Experimental Site

The study was conducted in the Botswana University of Agriculture and Natural Resources (BUAN), Notwane Farm, located at 24º33'S; 25º54'E having an altitude of 994 m above sea level. The climate is semi-arid with average

annual rainfall of 538 mm. Most rains falls in summer, which generally starts in the late October and continues to March or April. During the rainy seasons, prolonged dry spells are common and rainfall trends are localized. The soils are deficient in phosphorus, have low levels of nitrogen and organic matter [24,25]. The soils are shallow, ferruginous tropical soils, mainly consisting of medium to coarse grains and sandy loams with low water holding capacity and subject to crusting after heavy rains [26].

2.2 Experimental Design

Two field experiments were carried out between September 2013 and May 2014. The experimental design was a split-plot laid down in randomized complete blocks with three replications. The treatments were N (main-plots) and P (sub-plots). Nitrogen in the form of limestone ammonium nitrate (LAN-28 % N) was applied at 0, 50, 100, 150 and 200 kg N/ha. Phosphorus was applied as single superphosphate (SSP-10.5% P) at 0, 25, 50 and 75 kg P/ha. The main-plot size was 12 m × 12 m while the sub-plots were 6 m × 6 m each. Before the start of the study, soil sampling was done at a depth of 0-30 cm for the determination of physico-chemical properties, and total N and P.

2.3 Cultural Practices

Citrullus lanatus Thunb variety 'Crimson Sweet' was used in the study. The seeds were directly planted at two seeds per hill at a spacing of 2.0 m (between rows) × 0.5 m (within rows). Seedling thinning was done two weeks after emergence leaving one seedling per hill. Fertilizer application was done by side banding about 5 cm away from the seedling. Nitrogen was applied in two splits. The first application was done two weeks after seedling emergence and the second application was done at the onset of flowering. Phosphorus was applied two weeks after emergence [9]. The soil was kept weed free by shallow cultivation between rows and around the watermelon plants with care to avoid root damage. The vines were trained to leave paths between the rows in order to give access for pest and disease control operations. Routine scouting of pests and diseases was done daily. Integrated pest management strategies such as sanitation, prevention, exclusion and elimination of pests and diseases were applied. To control fruit flies, aphids, thrips, leaf miners and cucurbit beetle, insecticides cypermethrin, chloropyrifos and methomyl were sprayed interchangeably on the late afternoons not to coincide with bee visitations. Irrigation with over-head sprinklers to a depth of 11 mm once a week was done in the absence of rainfall.

2.4 Dependent Variables Determined

The dependent variables that were determined included fruit rind thickness, titratable acidity, soluble solids content, water content, dry matter content, lycopene content, vitamin C content, and leaf and fruit N, P, K and Na.

2.4.1 Fruit maturity

The fruits were harvested manually about 85 days after planting. The maturity of the fruit harvested was indicated by the drying of the tendril, located at the insertion of the fruit peduncle into the stem and the colour of the side of the fruit which is in contact with the ground that changed from white to cream, in association with the hollow sound produced by tapping with a firm finger.

2.4.2 Fruit rind thickness

The rind thickness was determined on ten randomly selected fruit representative of the treatment at harvest. Each fruit was sectioned equatorially and the rind thickness measured using electronic veneer calipers.

2.4.3 Titratable acidity

Fruit titratable acidity was determined by taking ten randomly selected fruit/treatment/ replication. The fruit pulp was cut and mixed to form a composite sample. Then 100 g of the composite sample was weighed and 100 ml of distilled water added to the sample. The sample was then homogenized and filtered with five layers of cheese cloth. Then 20 ml of the filtrate was pipetted into a 50 ml conical flask and two drops of phenolphthalein indicator added. The sample was then titrated with 0.1 N NaOH to end point, and this was done in triplicates [27]. The results were expressed as total titratable acidity equivalents.

2.4.4 Soluble solids content

Ten randomly selected fruit per replication was used. The fruits were cut into sections at stem end, centre and blossom end. The fruit sections were blended separately. A drop of juice from these blended fruits sections was put on the hand refractometer (0-32%, AST, Japan).

2.4.5 Fruit mineral analysis

Ten randomly selected fruit per treatment per replication was cut (epidermis and mesocarp) and mixed to form a composite sample. A composite sample of 1 kg was weighed and oven dried at 66°C to constant weight (72 hours). The dried samples were ground using a sieve of size two and 1.25 g composite sample digested in 20 ml sulphuric acid (98%) and 4 ml hydrogen peroxide (30%) in a BD block at 330°C for 7 hours. Nitrogen was determined through distillation and titration using the micro-Kjeldahl method [27]. Phosphorus (P) was determined colorimetrically using sodium phenol and ammonium molybdate plus ascorbic acid method [27]. The absorbance was read on the UV Visible Spectrophotometer (UV-1602, IPC, Shimadzu, RSA). Potassium (K) and sodium (Na) were determined by atomic absorption spectrometry (Varian SpectrAA 300). Data was expressed as percentage or mg/g on dry weight basis.

2.4.6 Fruit vitamin C content

Vitamin C content was determined using titrimetric method according to AOAC [27]. The fruit pulp was cut and mixed to form a composite sample. Then 100 g of the composite sample was weighed. Then 100 g of the composite sample was homogenized with 100 ml of metaphosphoric acid-acetic acid solution. The sample was filtered with five layers of cheese cloth. Then 20 ml of the filtrate was pipetted into a 100 ml conical flask and two drops of thymol blue (0.04%) indicator added. The sample was titrated with 2,6-dichloroindophenol solution to end point. Also three sample aliquots containing the standard ascorbic acid solution (20 ml) with metaphosphoric acid-acetic acid solution (for correction or blank) was titrated with 2,6-dichloroindophenol. Calculation of vitamin C content was done according to AOAC [27] and expressed as mg/g.

2.4.7 Lycopene content

Fruit lycopene content was determined according to the method of Wayne et al. [28]. A composite sample of 2 g watermelon flesh was homogenized to a puree. 25 ml of hexane was then added to the watermelon puree and then placed on an orbital shaker to mix at 180 rpm for 15 minutes. The orbited watermelon puree containing hexane was left for 5 minutes to allow for phase separation. The absorbance of hexane (upper) layer was measured at 503 nm using a UV visible spectrophotometer (UV-160 IPC, Shimadzu).

2.5 Data Analyses

Due to the similarity of the data in the two trials, data was pooled during analysis [29]. The data collected was subjected to analysis of variance using the general linear model (Proc GLM) procedures of statistical analysis system (SAS) program package. Multiple comparisons among means was done using Protected Least Significant Difference (LSD) at $P = .05$. Proc univariate procedure was carried out on residuals to support assumptions of normality made.

3. RESULTS

3.1 Fruit Quality

Nitrogen fertilization significantly ($P < .0001$) influenced fruit lycopene, vitamin C, titratable acidity and N contents, and rind thickness of watermelon (Tables 1, 3). However, P and the interaction of N and P fertilization had no significant ($P = .05$) effect on fruit lycopene, vitamin C, titratable acidity and N contents, and rind thickness of watermelon. Application of N at 50, 100, 150 and 200 kg/ha significantly ($P < .0001$) increased fruit flesh lycopene (antioxidant) content compared to fruit from plants not applied with N (Table 1). However, there was no significant ($P = .05$) difference in the fruit flesh lycopene content of fruit applied either with 50, 100 or 200 kg N/ha (Table 1). The highest fruit flesh lycopene content was in fruit applied with 150 kg N/ha (Table 1). Nitrogen fertilizer application at 50, 100, 150 and 200 kg/ha significantly ($P < .0001$) increased fruit vitamin C compared to fruit from plants not applied with N (Table 1). However, there was no significant ($P = .05$) difference in the fruit vitamin C content of fruit applied either with 50, 100 or 200 kg N/ha (Table 1). The highest fruit vitamin C content was in fruit applied with 150 kg N/ha and it was significantly ($P = .05$) higher than the vitamin C content from watermelon plants applied with either 50, 100 or 200 kg N/ha (Table 1). Watermelon plants applied with 50, 100, 150 and 200 kg N/ha produced fruit with significantly ($P < .0001$) higher titratable acidity than fruit from plants not applied with N (Table 1). However, there was no significant difference in titratable acidity of fruit from plants applied with either 50, 100, 150, or 200 kg N/ha (Table 1). Nitrogen fertilizer application had no significant ($P = .05$) effect on the fruit soluble solids content (SSC) of watermelons (Table 1). Nitrogen fertilizer

application had a non-significant (*P* = .05) decrease in fruit SSC compared to fruit from plants not applied with N (Table 1). However, the fruit SSC significantly (*P* = .05) differed within the fruit (Table 1). The SSC from the middle section (portion) of the fruit was significantly (*P* = .05) higher than SSC from either the stem end or blossom end of the fruit (Table 3). The fruit SSC from the stem end or blossom end of the fruit did not significantly (*P* = .05) differ (Table 1). Nitrogen application significantly (P < .0001) increased the watermelon fruit rind thickness compared to fruit from control plants (Table 1). However, there was no significant (*P* = .05) difference in fruit rind thickness among the N rates (Table 1). Phosphorus fertilizer application had no significant (*P* = .05) effect on watermelon fruit lycopene, vitamin C, titratable acidity and SSC contents, and rind thickness (Table 2).

3.2 Fruit Mineral Content

The interaction of N and P fertilizer application had no significant (*P* = .05) effect on the fruit mineral content. Nitrogen fertilizer application significantly (*P* < .0001) increased the fruit N content compared to fruit from plants not applied with N (Table 3). Application of N at 50, 100, 150 and 200 kg/ha increased the fruit N content (Table 3). The highest fruit N content was in fruit applied with 200 kg/ha (Table 3). Increasing N application from 50 to 200 kg/ha significantly (*P* < .0001) increased N partitioning into the fruit (Table 3). However, N application had no significant (*P* = .05) effect on the partitioning of P, K and Na into the fruit (Table 3).

Phosphorus fertilizer application had no significant (*P* = .05) effect on fruit mineral partitioning of N, K and Na, but significantly (*P* < .0001) increased the fruit P content (Table 4). Application of P at 25, 50 and 75 kg/ha, significantly (*P* < .0001) increased fruit P partitioning compared to fruit from control plants (Table 4). There were significant (*P* = .05) fruit P content differences among the P fertilizer application rates (Table 4). Application of P above 50 kg/ha resulted in a significant (*P* = .05) decrease in fruit P content (Table 4).

Table 1. Effect of nitrogen fertilization on fruit lycopene, vitamin C, soluble solids content and rind thickness

Nitrogen kg/ha	Lycopene (mg/kg)	Vitamin C (mg/g)	Titratable acidity (g/100 ml juice)	Fruit soluble solids content (%)			Rind thickness (mm)
				Stem end	Middle	Blossom end	
0	11.85d	7.70c	0.12b	6.73a	8.94a	6.75a	9.12b
50	18.49bc	12.27b	0.15a	6.78a	8.25a	6.70a	12.32a
100	19.19b	13.24b	0.14a	6.74a	8.70a	6.60a	12.12a
150	23.35a	15.17a	0.14a	6.71a	8.83a	6.71a	12.80a
200	15.98c	12.96b	0.14a	6.69a	8.25a	6.61b	12.40a
Significance	****	****	****	NS	NS	NS	****
LSD	2.9	0.99	0.01	0.93	0.89	1.04	1.60

*****, NS. Significant at P < .0001 or non-significant, respectively. Means separated using the Least Significant Difference (LSD) at P = .05; means with the same letter(s) are not significantly different*

Table 2. Effect of phosphorus fertilization on fruit lycopene content, titratable acidity, soluble solids content and rind thickness

P kg/ha	Lycopene (mg/kg)	Vitamin C (mg/g)	Titratable acidity (g/100 ml juice)	Soluble solids content (%)			Rind thickness (mm)
				Stem end	Middle	Blossom end	
0	17.65a	12.01a	0.12a	5.82a	8.40a	6.09a	10.98a
25	17.73a	12.39a	0.13a	6.37a	8.66a	6.38a	11.63a
50	18.82a	12.34a	0.13a	6.47a	8.74a	6.24a	12.01a
75	18.88a	12.33a	0.12a	5.98a	8.58a	6.18a	10.73a
Significance	NS	NS	NS	NS	NS	NS	NS
LSD	2.66	0.89	0.01	0.83	0.80	0.93	1.42

NS, non-significant at P = .05. Means separated using the Least Significant Difference (LSD) at P = 0.05; means with the same letter (s) are not significantly different

Table 3. Effect of nitrogen fertilization on fruit N, P, K and Na content

kg N/ha	N (%)	P (%)	K (%)	Na (mg/g)
0	1.02e	0.32a	2.35a	1.15a
50	2.07d	0.34a	2.34a	1.29a
100	2.60c	0.32a	2.23a	1.34a
150	2.93b	0.31a	2.44a	1.14a
200	3.34a	0.29a	2.19a	1.10a
Significance	****	NS	NS	NS
LSD	0.17	0.17	0.26	0.30

*****, NS significant at P < .0001 or non-significant, respectively. Means separated using the Least Significant Difference (LSD) at P = .05. Means within the column(s) followed by the same letter (s) are not significantly different*

Table 4. Effect of phosphorus fertilization on fruit N, P, K and Na content

kg P/ha	N (%)	P (%)	K (%)	Na (mg/g)
0	2.28a	0.11d	2.20a	1.13a
25	2.41a	0.33c	2.32a	1.22a
50	2.43	0.44a	2.34a	1.23a
75	2.45	0.39b	2.35a	1.24a
Significance	NS	****	NS	NS
LSD	0.17	0.05	0.12	0.26

*****, NS significant at P < .0001 or non-significant. Means separated using the Least Significant Difference (LSD) at P = .05. Means within the columns followed by the same letter (s) are not significantly different*

4. DISCUSSION

4.1 Effects of N and P on Watermelon Fruit Quality

Nitrogen fertilizer application significantly increased the watermelon fruit lycopene content, vitamin C and rind thickness compared to control plants. Lycopene is a carotenoid under the group of compounds known as isoprenoids. Isoprenoids are biosynthesized from acetyl-CoA via mevalonic acid and isopentenyl pyrophosphate [30,31]. Plastid isoprenoids (gibberellins, abscisic acid, photosynthesis-related pigments such as carotenoids and the phytol moiety of chlorophylls, and the side chain of electron carriers such as plastoquinone K1, α-tocoquinone, and α-tocopherol) are derived from isopentenyl pyrophosphate [32,33]. The increase in watermelon fruit lycopene content due to N fertilizer application was attributed to the role of N in many compounds and enzymes involved in the mevalonate and isopentenyl pyrophosphate pathway. Phosphorus application in the current study had a non-significant increase in the fruit lycopene content because P is a component of the isopentenyl pyrophosphate which is an important macromolecular in the synthesis of carotenoids. Ferrante et al. [15] reported that application of N from 0 to 165 kg/ha to watermelons did not have an effect on fruit flesh lycopene content and total phenols. Kobryn and Hallmann [34] observed that application of 140 and 210 mg N/dm of rockwool increased the lycopene content of tomato.

Rind thickness is genetically controlled (quantitative trait with a larger contribution to its inheritance from additive than dominant genetic components), but can also be influenced by cultural practices such as plant nutrition [9, 35]. Nitrogen increased the watermelon rind thickness because of the role of N in fruit growth and development. Maluki et al. [36] reported that N, P and the interaction of N and P at different rates had no significant effect on watermelon fruit rind thickness.

Nitrogen application increased the watermelon fruit vitamin C and titratable acidity contents, but it had no significant effect on fruit soluble solids content. The increase in fruit quality was attributed to the role of N and P in photosynthates biosynthesis which are converted into organic acids and starch during fruit growth and development. Subsequently during fruit ripening the starch is converted to soluble solids content. The interaction of sugars and organic acids are important in the flavor of fruits. In general, the concentration of acids declines during fruit ripening, but the total number of acids increase [37]. Maluki et al. [36] reported that N

and P interaction increased watermelon fruit quality by increasing fruit soluble solids content, low acidity and firmness. While Aguyo et al. [38] reported that increased nutrient application enhanced fruit quality of watermelons. Uwah et al. [39] reported that application of 120 and 180 kg N/ha increased the watermelon fruit soluble solids content (sugars) by 80 and 108%, respectively, compared to fruits from plants where N was not applied. Similar results have been reported [40-42].

4.2 Effect of N and P on Fruit Mineral Content

Nitrogen and P fertilizer application independently increased fruit N and P contents. Application of N and P fertilizers increased the uptake and partitioning of N and P to the watermelon fruit hence explaining the increase in fruit N and P content in fruits from plants applied with fertilizers. Inorganic and organic fertilizers are sources of essential mineral nutrients such as N and P [9,43,44]. Nitrogen and P fertilizer application improves the uptake and partitioning of these nutrients within plants [9,25,43].

5. CONCLUSION

In conclusion under sandy loam soils, the optimal N and P fertilizer application rate for high watermelon fruit quality was 150 kg N/ha and 50 kg P/ha. Application of N and P lower than 150 and 50 kg/ha, respectively, resulted in decrease in fruit lycopene, vitamin C, titratable acidity and N contents, and rind thickness. The authors recommend that further research in N and P fertilizer application rates should be done under different soils and agronomic practices of watermelon in Botswana.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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