ENHANCING PREHARVEST PHYSIOLOGY OF MULTI-PURPOSE PUMPKIN USING COMBINED NITROGEN, MULCH AND GIBBERELLIC ACID

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Abstract

In recent times, it has become apparent that multi-purpose pumpkin (*Cucurbita moschata* Duch.) is a very important crop since its leaves, fruits and seeds can be eaten as vegetables, snacks and blended dishes to boost household food and nutritional security. However, it is regarded as a low value crop, leading to poor cultivation, comprehension of preharvest physiological behaviour, and maximization of productivity in Kenya. Consequently, a study was conducted to assess the effects of combined nitrogen, mulch and gibberellic acid (GA_3) in enhancing its preharvest physiology of pumpkin. Four N rates of 0, 50, 100 and 150 kg N/ha supplied as CAN, three mulch types as none, unpainted, and black-painted rice straw, and three GA_3 rates of 0, 40 and 80 mg/L were assessed. The experiment was arranged in split-split plots in randomized complete block design, replicated three times in two seasons, and each plant occupied 2 m x 2 m space. Nitrogen was applied to main plots, mulch to sub-plots, and GA_3 to sub-sub plots. Pre-harvest physiological variables measured were stomatal conductance, chlorophyll, photosynthesis, and transpiration. Data values were subjected to analysis of variance using SAS Version 9.3. Separation of significant means was done using the least significant difference test at $\alpha = 0.05$. Results showed that nitrogen and mulch individually and interactively did not significantly ($P > 0.05$) affect most pre-harvest physiological parameters. The effect of GA_3 was not significant on stomatal conductance and leaf chlorophyll, but it was negative and significant on both photosynthesis and transpiration in season 1 which was drier than season 2. Based on the present results, the influence of combined nitrogen, mulch and GA_3 on multi-purpose pumpkin cannot be entirely depicted by analysing preharvest physiology. Adoption and application of treatments that promote desired preharvest physiology of multi-purpose pumpkin is recommended, including 50 kg N/ha to enhance stomatal conductance and leaf chlorophyll, and unpainted rice straws to enhance leaf chlorophyll content and photosynthesis.

Keywords: Chlorophyll content, Photosynthesis, Stomatal conductance, Transpiration

Introduction

Pumpkin is a fruit-vegetable belonging to the Cucurbitaceae family together with gourds, melons and squashes. The varieties of pumpkins grown widely in Kenya belong to *Cucurbita moschata* and *Cucurbita maxima*. Pumpkin is a native of Central America but is now domesticated in other tropical and subtropical countries (Fedha, 2008). Pumpkin leaves, fruits and seeds are rich sources of vitamins, minerals and antioxidants (Kiharason *et al.,* 2017). Pumpkin fruits are low in calorific content, making them weight-loss-friendly foods. Pumpkin nutrients and antioxidants are good in boosting the immune system, protecting eyesight, lowering risk of some cancers, and promoting heart and skin health (Ghanbari *et al*., 2007). However, it is regarded as a low value species, leading to poor cultivation and maximization of productivity in Kenya. Proper pumpkin growth, physiology, production, and quality require integration of inputs (Nakazibwe *et al.,* 2019). Growers concentrate on application of sole inputs at unverified rates, leading to poor preharvest physiology that does not support growth, development, productivity and quality well (Nakazibwe *et al.,* 2019). Ultimately, households and growers end up fetching low yields, income, and associated medicinal, food and nutritional benefits. This is despite the fact that the increased demand for pumpkins can be fulfilled by integrating practices that enhance pre-harvest physiology processes like photosynthesis, stomatal conductance and transpiration rate. Owing to the increasing need of pumpkin produce (leaves, fruits, seeds) in Kenya, determining of optimal inputs to enhance pre-harvest physiology is very imperative. The present study determined the effect of combined nitrogen fertiliser, mulch and GA_3 in enhancing preharvest physiology of pumpkin.

Nitrogen is an essential element in plant growth. Although ammonia ($NH_4^{+)}$, Nitrite (NO_2^-) and Nitrate (NO_3^-) account for less than 5% of the total N in the soil, Liu *et al.* (2014) indicated that N is a critical element that most plants absorb. Nitrogen is the most important element for proper plant growth and development, which substantially increases and enhances yields and quality, as it plays a critical role in biochemical and physiological processes (Ullah *et al.,* 2010). Nitrogen enhances total leaf biomass which is a determinant of pumpkin leaf vegetable yield (Nasim *et al.,* 2012). Mulch prevents fertilizer leaching since it slows down runoff, conserves soil moisture, increases soil temperature, suppresses weeds and prevents pathogen splash, thereby enhancing pumpkin shelf-life, yield and quality of pumpkin leaves, fruits and seeds (Cerniauskiene *et al.,* 2015). Gibberellic acid helps in transport of water and nutrients through the xylem and it influences many biochemical and physiological processes like photosynthesis, respiration, protein synthesis, cell extension, and wall thickness and stability (Abbas *et al.,* 2011), which are important in productivity and quality enhancement. It strengthens parthenocarpic flowers and fruits to prevent abortion, which is common in pumpkins particularly when pollination is inadequate (Mwaura *et al.,* 2014; Isutsa and Mwaura, 2017; Kiramana and Isutsa, 2019). Improving preharvest physiology of pumpkin through integrated inputs will ensure increment of yields, food, nutrition and income for producers, households and consumers (Nakazibwe *et al.,* 2019; Gomez *et al.,* 2020).

MATERIALS AND METHODS

Research Site

The field experiment was conducted in Chuka University Horticultural Research Farm between January 2019 and August 2020. The farm lies at 0^o 19' S, 37^o 38’ E and 1535 m above sea level. The annual temperature ranges from 12.2ºC to 23.2ºC. The site experiences two rainy seasons with the long rains occurring from March to June and short rains from October to December (Jaetzold *et al.,* 2006). The average annual rainfall is 1200 mm (http://en.climate-data.org). The soils are humic nitisols, deep, strongly weathered, well drained with clayey subsurface horizons, made of angular, blocky structural elements that easily crumble into polyhedricipeds with shiny faces, and high cation exchange capacity (Jaetzold *et al.,* 2006)).

Experimental Design and Treatments

A three-factor split-split experiment in a randomized complete block design (RCBD) with three replications was set up in two seasons from March 2019 to July, 2019 with 1,004 mm rainfall, and October 2019 to February 2020 with an average of 1,260 mm rainfall per year (https://www.worldweatheronline.com/chuka).

Each experimental plant occupied 2 m x 2 m space. The three factors tested were nitrogen, mulch and GA_3 in main-plots, sub-plots and split-plots, respectively. The nitrogen rates were 0, 50, 100 and 150 kg N/ha, applied in two equal doses at three weeks post-emergence and at the beginning of flowering. Amount of CAN fertilizer used per experimental unit was calculated as: a) 50 kg N/ha = 76.9 g CAN/4 m^2; b) 100 kg N/ha= 153.8 g CAN/4 m^2; c) 150 kg N/ha = 230.7 g CAN/4 m^2.

Mulch types used were none, unpainted and black-painted rice straws that were easily available near the experimental site in sufficient quantities. The black-painted and unpainted dry rice straws were placed on respective split plots after land preparation. Painting of the rice straws was done by dipping in a 200-L drum containing black paint solution and spreading out on the soil to air-dry. The rice straws were spread on plots to achieve 20 cm thickness. Planting holes were marked and opened in rice straw mulch before sowing pumpkin seeds.

The GA_3 rates used were 0, 40 and 80 mg/L. The GA_3 was dissolved in 50 ml alcohol and the volume made up to one litre stock solution by adding distilled water. The required rate of spray solution was prepared from the stock solution by diluting with distilled water. Commercial sticker drops were added to the solutions to facilitate uptake of GA_3. The GA_3 solution was applied to the plants using a 1-L hand-held sprayer during a calm morning. Low rate solution was applied first followed by high rate solution. Spraying was done once during the fourth week after emergence.

Pumpkin Establishment and Management

Three multipurpose pumpkin fruits of uniform size, free from diseases and pests and from one mother plant were used to extract seeds for the experiment. The fruits were sourced from farmers in the neighbourhood of the research site. Seeds were planted immediately after extraction (AOAC, 1995). The field was prepared to appropriate tilth required for pumpkin growth. All recommended phosphorus and potassium straight fertilizers were applied just before seed sowing. Two seeds were placed at the centre of each split-split plot and one was uprooted two weeks after emergence. All plots were kept weed-free through rogueing and manual weeding. Irrigation was done using drip tubes during drought. Insect pest and disease control was done when due using recommended pesticides and rates. Pumpkin vines were coiled when required while leaving them in contact with the soil. Data was taken from all the experimental plants, except the guard row ones.

Data Collection

The physiological parameters that were measured using non-destructive methods in this experiment were leaf chlorophyll content, stomatal conductance, photosynthesis status, and transpiration rate. Chlorophyll content was measured fortnightly using a chlorophyll meter, starting from the 6^{th} up to the 12^{th} week after emergence. A chlorophyll meter, SPAD 502 Plus (from Spectrum Technologies) with Data Loggers and RS-232 was used. Three fully expanded pumpkin leaves at the sixth node of three selected vines were used for measuring chlorophyll content using the non-destructive chlorophyll meter that gave rapid and accurate readings in ug/l.

A portable LI-6400 photosynthesis system was used to measure stomatal conductance fortnightly from the 6^{th} week up to the 12^{th} week after emergence. A leaf at the 6^{th} node of a selected vine was used and readings were obtained in mmol $m^{-2}s^{-1}$. Assessment of photosynthesis status as leaf gas exchange rate was done using the LI-6400 equipment starting from the 6^{th} week up to the 12^{th} week after emergence and readings were recorded in mg $m^{-2}s^{-1}$. The same LI-6400 portable photosynthesis system was used to measure transpiration rate fortnightly starting from the 6^{th} week up to the 12^{th} week after emergence and readings were recorded in mg’nr^s”l.

Data Analysis

The recorded data values on stomatal conductance, chlorophyll content, photosynthesis status and transpiration rate were subjected to analysis of variance, using the SAS software version 9.3. Subsequently, mean separation for significantly different treatments was done using the least significant difference test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Effect of Nitrogen on Preharvest Physiology

Nitrogen had no significant effect ($p > 0.05$) on the stomatal conductance in both seasons (Figure 1). Application of 50 kg N/ha produced the highest stomatal conductance of 0.49 mmol $m^{-2}s^{-1}$ and 0.80 mmol $m^{-2}s^{-1}$ in S1 and S2, respectively. The stomatal conductance was lowest in the control treatment during both seasons. Stomatal conductance was higher in S2 than in S1. Nitrogen had no significant ($p > 0.05$) effect on leaf chlorophyll in both seasons. However, the 50 kg N/ha produced the highest leaf chlorophyll content in both seasons (Figure 1). The control treatment produced the lowest leaf chlorophyll content during S1 and S2.

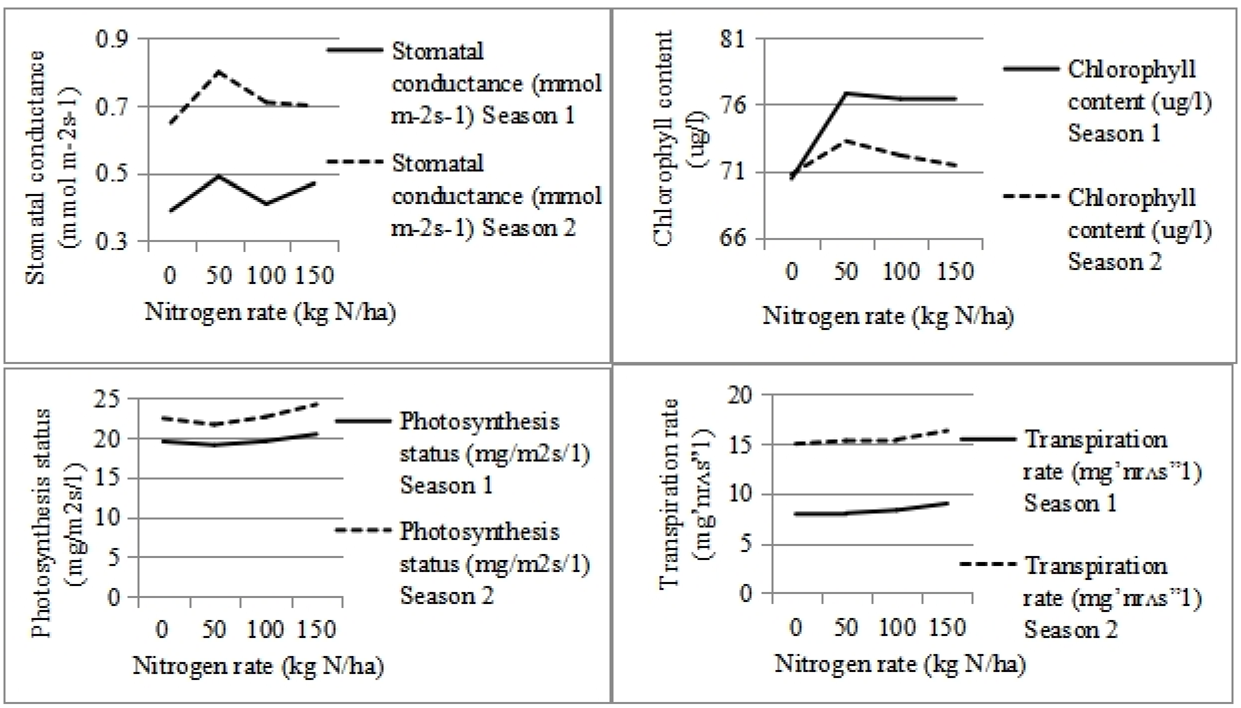

Figure 1: Effect of nitrogen on preharvest physiology

Nitrogen had no significant effect ($p > 0.05$) on the photosynthesis during S1 and S2, but application of 150 kg N/ha produced the highest photosynthesis of 20.44 mg $m^{-2}s^{-1}$ and 24.20 mg $m^{-2}s^{-1}$ during S1 and S2, respectively. The 50 kg N/ha treatment had the lowest photosynthesis of 19.07 mg $m^{-2}s^{-1}$ and 21.65 mg $m^{-2}s^{-1}$ during S1 and S2, respectively. Results showed that nitrogen had no significant ($p > 0.05$) effect on transpiration rate during S1 and S2, but application of 150 kg N/ha produced the highest transpiration rate of 8.96 mg’nr^s”1 and 16.30 mg’nr^s”1 during S1 and S2, respectively. Transpiration rate was lowest for the control treatment during both seasons.

Nitrogen has been found to play a critical role in chlorophyll synthesis and photosynthesis (Jasso-Chaverria *et al.,* 2005). Nitrogen insufficiencies have been reported to reduce individual and total leaf area, resulting in reduced surface light interception for plant photosynthesis and photosynthesis status (Cechin and Fumis, 2004). Reduced photosynthesis under nitrogen deficiency has been linked to low stomatal conductance and reduction of chlorophyll, carotenoid and protein contents (Correia *et al.,* 2005), which could explain why stomatal conductance and leaf chlorophyll content was lowest in the control treatments (Figure 1).

Effect of Mulch on Preharvest Physiology

Mulch had no significant ($p > 0.05$) effect on stomatal conductance during both seasons (Figure 2). Application of no mulch produced the highest stomatal conductance of 0.46 mmol $m^{-2}s^{-1}$ and 0.74 mmol $m^{-2}s^{-1}$ in S1 and S2, respectively. Results showed that stomatal conductance was lowest when painted rice straw mulch was applied. Stomatal conductance was higher in S2 than in S1. Mulch had no significant ($p > 0.05$) effect on leaf chlorophyll content during both seasons. Nonetheless, application of unpainted rice straw mulch produced the highest leaf chlorophyll content of 76.9 ug/l in S1 and 73.06 ug/l in S2.

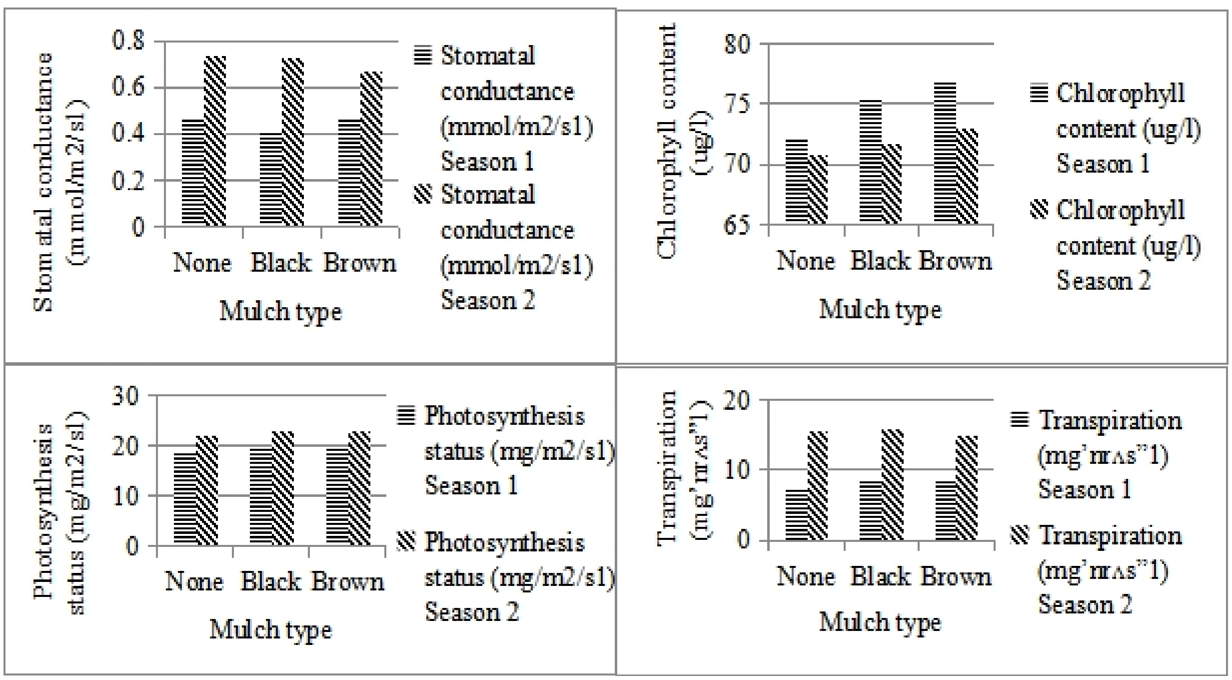

Figure 2: Effect of mulch on preharvest physiology

The effect of mulch was not significant ($p > 0.05$) on photosynthesis during S1 and S2. Application of unpainted rice straw mulch produced the highest photosynthesis of 19.90 mg $m^{-2}s^{-1}$ and 23.05 mg $m^{-2}s^{-1}$ during S1 and S2, respectively (Figure 2). Lowest photosynthesis status of 19.16 mg $m^{-2}s^{-1}$ and 22.32 mg $m^{-2}s^{-1}$ during S1 and S2, respectively, was produced when no mulch was applied. In addition, results showed that mulch had no significant ($p > 0.05$) effect on transpiration rate during S1 and S2. Application of black-painted rice straw mulch produced the highest transpiration rate of 8.79 mg'nr^s"1and 15.77 mg'nr^s"1 during S1 and S2, respectively. Transpiration rate increase was 105.8% during S2 compared to S1.

The present results could be explained by the fact that mulch improves leaf photosynthetic capacity leading to enhanced root growth and absorption of nutrients that in turn promote metabolic activities in plants. The increased metabolic activities result in increased leaf chlorophyll content, which induces more photosynthetic rates (Helaly *et al.,* 2017). Chlorophyll content plays a critical role in photosynthesis. Chlorophyll pigments absorb solar energy and transfer it to chemical energy used for synthesis of ATP and glucose.

A positive correlation between leaf nitrogen content and photosynthetic pigment content with mulch has previously been reported (Fritschi and Ray, 2007). This correlation was attributed to good environment modulation by mulch, stable moisture content and soil texture that result in unrestricted root growth, which subsequently increased nutrient absorption into plants. Also, mulch has been reported to be of significant importance in vegetable-fruit production through provision of conducive conditions for growth, prevention of soil nutrient leaching, erosion, and reduction of evapo-transpiration (Houles *et al.,* 2007). These moderative effects end up benefiting plant performance.

Effect of GA_3 on Preharvest Physiology

The applied GA_3 had no significant (P>0.05) effect on stomatal conductance during both seasons (Table 1). Control treatment produced the highest stomatal conductance of 0.46 mmol $m^{-2}s^{-1}$ during S1 and 0.73 mmol $m^{-2}s^{-1}$ during S2. Stomatal conductance of 0.40 mmol $m^{-2}s^{-1}$ and 0.70 mmol $m^{-2}s^{-1}$ in S1 and S2, respectively, was the lowest when 80 mg/L GA_3 was applied. The GA_3 had no significant (P>0.05) effect on leaf chlorophyll content in both seasons. The highest leaf chlorophyll of 75.2 ug/l and 72.41 ug/l was produced under the control treatment in both seasons. Leaf chlorophyll was relatively lower in S2 compared to S1 (Table 1).

There was a significant ($p < 0.05$) effect of GA_3 on photosynthesis in S1 (Table 1). The 0 g/L GA_3 had the highest photosynthesis of 20.69 mg $m^{-2}s^{-1}$ in S1, while application of 40 mg/L GA_3 had the highest photosynthesis of 23.31 mg $m^{-2}s^{-1}$ in S2. Lowest photosynthesis of 18.73 mg $m^{-2}s^{-1}$ and 21.86 mg $m^{-2}s^{-1}$ in S1 and S2, respectively, resulted when 40 mg/L GA_3 and no GA_3 were applied. There was a significant (P<0.05) effect of GA_3 on transpiration in S1 (Table 1). Highest transpiration rate of 9.045 mg’nr^s”1 and 15.92 mg’nr^s”1 during S1 and S2, respectively, was for no GA_3 and 40 mg/L GA_3. Transpiration rate was higher in S2 than in S1.

Photosynthesis and transpiration are associated with regulation of the opening and closing of stomata (Barbieri *et al.,* 2012). Transpiration is a key stimulant of weight-loss and quality deterioration in leafy vegetables (Hung *et al.,* 2011). The present results contradicted those of Miceli *et al.* (2019) on lettuce, where nutrient solution added with GA_3 enhanced physiology of leaves. Lettuce plants treated with GA_3 had significant increase in stomatal conductance. Application of GA_3 reduced photosynthesis status and leaf chlorophyll content, resulting in darker and less vivid greenish colour of lettuce (Miceli *et al.,* 2019). Transpiration rate reduced as GA_3 was applied, implying that that GA_3 promoted stomatal closure.

Table 1: Effect of GA_3 on preharvest physiology

GA_3 (mg/L)	Stomatal conductance ($mmol/m^2/s^1$)		Chlorophyll content (ug/l)		Photosynthesis status ($mg/m^2/s^1$)		Transpiration rate (mg’nr^s”1)	
	S1	S2	S1	S2	S1	S2	S1	S2
0	0.46	0.73	75.2	72.4	20.69a	21.86	9.045a	15.03
40	0.46	0.71	75.0	71.9	19.51ab	23.31	8.270a	15.92
80	0.40	0.70	74.7	71.3	18.73b	23.02	7.520b	15.55
P-value	*0.681*	*0.692*	*0.991*	*0.718*	*0.026**	*0.208*	*0.001**	*0.230*
LSD 5%	0.155	0.010	6.940	2.621	1.413	1.716	0.767	1.028

S1= Season 1 (March 2019-July 2019); S2= Season 2 (October 2019-February 2020)
*Means followed by the same letter or no letter within a column are not significantly different according to the LSD Test at $P = 0.05$

The reduction in chlorophyll content with increase in GA_3 rate could be attributed to dilution effect in enlarged and succulent leaves, as reported by Nagamani *et al.* (2015), who studied the effect of plant growth regulators in bitter gourd, using 50 ppm GA_3, 200 ppm NAA, 100 ppm maleic hydrazide, and 50 ppm Etherel. All the applied plant growth regulators had a positive effect on vegetative, flowering and fruit traits in both seasons, but the effects of the growth regulators were more evident in the rainy than in the spring-summer season. Application of 50 ppm GA_3, 200 ppm NAA, 50 ppm etherel was effective in enhancement of vegetative growth, fruit and seed yield, but spraying 50 ppm GA_3 twice at three leaf and tendril initiation stages was most effective in hybrid seed production of bitter gourd.

Effect of Nitrogen and Mulch on Preharvest Physiology

Interaction between nitrogen fertiliser and mulch had no significant effect on the preharvest physiology of multipurpose pumpkin as shown in Table 2. No nitrogen fertiliser with painted mulch and 50 kgN/ha with no mulch produced the highest chlorophyll in S1 and S2, respectively The highest stomatal conductance was achieved at 50 kgN/ha with no mulch and 150 kgN/ha with no mulch in S1 and S2, respectively. Photosynthesis status was highest at 100 kgN/ha with painted mulch and 150 kgN/ha with no mulch in S1 and S2, respectively. The 150 kgN/ha with painted mulch in S1 and 150 kgN/ha with no mulch in S2 gave the highest transpiration rate.

Effect of Nitrogen and GA_3 on Preharvest Physiology

Nitrogen and GA_3 had no significant effect on the preharvest physiology of multipurpose pumpkin as shown in Table 3. The 50 kgN/ha with 40 mg/L GA_3 and 50 kgN/ha with no GA_3 produced the highest chlorophyll content in S1 and S2, respectively. The highest stomatal conductance was achieved for 50 kgN/ha with 40 mg/LGA_3 and 150 kgN/ha with no GA_3 in S1 and S2, respectively. Photosynthesis status was highest for 100 kgN/ha and no GA_3 and 150 kgN/ha with 80 mg/L GA_3 in S1 and S2, respectively. The effect of the two factors on transpiration varied depending on the season (Table 3).

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Table 2: Effect of nitrogen and mulch on preharvest physiology

Nitrogen rate (kg/ha)	Mulch type	Chlorophyll (ug/l)		Stomatal conductance (mmol/m²/s¹)		Photosynthesis (mg/m²/s¹)		Transpiration (mg'nr^s"1)	
		S1	S2	S1	S2	S1	S2	S1	S2
0	None	69.7	69.47	0.313	0.673	18.56	21.24	7.41	14.47
	Black	84.7	69.49	0.352	0.703	20.13	22.66	8.07	15.54
	Brown	74.7	73.13	0.513	0.726	19.82	23.43	8.20	15.9
50	None	74.4	73.39	0.648	0.675	18.66	22.68	7.10	15.84
	Black	75.0	73.25	0.435	0.674	18.80	22.25	8.77	15.14
	Brown	80.9	73.02	0.345	0.592	19.74	20.02	8.09	13.96
100	None	74.4	72.09	0.358	0.757	19.49	22.94	7.79	15.72
	Black	76.5	71.14	0.426	0.741	20.28	22.40	8.99	15.80
	Brown	78.2	73.10	0.421	0.618	18.85	22.52	8.08	14.71
150	None	70.8	71.94	0.445	0.848	19.93	25.34	8.19	16.77
	Black	68.0	69.37	0.409	0.816	20.23	23.93	9.34	16.59
	Brown	68.8	72.90	0.561	0.740	21.16	23.31	9.33	15.53
p-value		0.162	0.785	0.26	0.076	0.952	0.170	0.978	0.361
LSD 5%		13.6	5.629	0.277	0.265	3.729	4.486	2.908	3.844
CV%		7.3	5.0	40.1	16.0	11.5	7.4	15.0	7.5

S1= Season 1 (March 2019-July 2019), S2= Season 2 (October 2019-February 2020)
*Means followed by the same letter or no letter within a column are not significantly different according to the LSD Test at *P*=0.05

Table 3: Effect of nitrogen and GA_3 on preharvest physiology

Nitrogen rate (kg/ha)	GA_3 (mg/L)	Chlorophyll (ug/l)		Stomatal conductance (mmol/m²/s¹)		Photosynthesis (mg/m²/s¹)		Transpiration (mg'nr^s"1)	
		S1	S2	S1	S2	S1	S2	S1	S2
0	0	75.1	70.38	0.405	0.693	20.58	21.88	8.46	14.96
	40	76.7	70.85	0.339	0.747	18.78	23.03	7.76	15.57
	80	77.5	70.96	0.386	0.661	19.17	22.42	7.47	15.39
50	0	77.1	75.70	0.386	0.593	19.88	20.46	8.57	14.29
	40	80.3	72.42	0.693	0.659	19.24	22.23	8.11	15.66
	80	72.9	71.54	0.386	0.689	18.08	22.26	7.28	14.99
100	0	76.6	71.07	0.445	0.655	20.78	22.28	9.02	14.66
	40	74.0	74.26	0.409	0.778	19.61	24.13	8.46	16.53
	80	78.5	71.09	0.378	0.682	18.22	21.46	7.38	15.03
150	0	70.2	72.48	0.557	0.841	21.25	22.81	10.10	16.20
	40	68.9	69.95	0.401	0.746	20.39	23.85	8.70	15.91
	80	72.0	71.78	0.457	0.817	19.44	25.93	7.96	16.78
P-value		0.927	0.486	0.400	0.546	0.979	0.975	0.927	0.726
LSD 5%		15.47	5.02	0.282	0.267	3.098	4.600	2.731	3.866
CV%		7.3	5.0	40.1	16.0	11.5	7.4	15.0	7.5

S1= Season 1 (March 2019-July 2019), S2= Season 2 (October 2019-February 2020)
*Means followed by the same letter or no letter within a column are not significantly different according to the LSD Test at *P*=0.05

Effect of Mulch and GA_3 on Preharvest Physiology

Mulch and GA_3 had no significant effect on the preharvest physiology of multipurpose pumpkin (Table 4). Black painted mulch with 40 mg/L GA_3 in S1 and no mulch with no GA_3 produced the highest chlorophyll in S2. No mulch with 40mg/L GA_3 produced the highest stomatal conductance in both seasons. Photosynthesis status was highest where unpainted mulch with no GA_3 and no mulch with 40 mg/L GA_3 in S1 and S2, respectively were used. Transpiration was highest where black with 0 mg/L GA_3 and no mulch with 40 mg/L GA_3 in S1 and S2, respectively, were used.

Table 4: Effect of mulch and GA_3 on preharvest physiology

Mulch type	GA_3 rate (mg/L)	Chlorophyll content (ug/l)		Stomatal conductance (mmol/m^2/s^1)		Photosynthesis (mg/m^2/s^1)		Transpiration (mg'nr^s"1)	
		S1	S2	S1	S2	S1	S2	S1	S2
None	0	75.9	72.32	0.397	0.698	19.48	22.31	8.14	14.0
	40	69.5	72.1	0.597	0.834	19.41	24.55	7.77	16.86
	80	71.5	70.75	0.376	0.683	18.62	22.3	6.97	15.27
Black	0	71.9	69.45	0.442	0.748	21.29	21.75	9.89	15.35
	40	78.6	71.47	0.375	0.715	19.39	22.96	8.71	16.02
	80	76.4	71.6	0.400	0.738	18.91	23.71	7.78	15.92
Brown	0	76.4	75.46	0.542	0.642	21.32	21.50	9.13	14.77
	40	76.8	72.03	0.409	0.648	19.67	22.42	8.33	14.86
	80	77.6	71.68	0.430	0.717	18.65	23.04	7.81	15.45
P-value		0.162	0.647	0.416	0.444	0.763	0.695	0.841	0.47
LSD 5%		10.73	4.708	0.261	0.168	2.721	2.779	1.488	1.727
CV%		7.3	5.0	40.1	16.0	11.5	7.4	15.0	7.5

S1= Season 1 (March 2019-July 2019), S2= Season 2 (October 2019-February 2020)
*Means followed by the same letter or no letter within a column are not significantly different according to the LSD Test at *P*=0.05

Effect of Nitrogen, Mulch and GA_3 on Preharvest Physiology

Highest conductance of 1.33 mmol m^{-2}s^{-1} was for $N_1M_0GA_1$ in S1. Lowest stomatal conductance of 0.27 mmol m^{-2}s^{-1} was for $N_0M_0GA_1$ (Table 5). In S2, highest stomatal conductance of 0.92 mmol m^{-2}s^{-1} was for $N_2M_0GA_1$, while the lowest of 0.42 mmol m^{-2}s^{-1} was for $N_1M_2GA_0$. The $N_1M_0GA_1$ (50kg N/ha, no mulch and 40 mg/L GA_3) and $N_2M_0GA_1$ (100 kg N/ha, no mulch and 40 mg/L GA_3) had the highest combined effect on stomatal conductance in S1 and S2, respectively. No significant effect was observed due to interactive effect of nitrogen, mulch and GA_3 on stomatal conductance in both seasons.

Leaf chlorophyll content of 90.2 ug/l was highest for $N_0M_1GA_2$, while the lowest of 63.8 ug/l was recorded for $N_3M_2GA_1$ in S1. In S2, highest leaf chlorophyll content of 80.45 ug/l was obtained for $N_1M_2GA_0$, while the lowest of 64.32 ug/l resulted for $N_0M_1GA_0$. The $N_0M_1GA_2$ (0 kg N/ha, black-painted rice straw mulch and 80 mg/L GA_3) and $N_1M_2GA_0$ (50 kg N/ha, unpainted rice straw mulch and 0 mg/L gibberellic acid) had the highest combined effect on leaf chlorophyll content in S1 and S2, respectively. No significant effect was observed due to interactive effect of nitrogen, mulch and GA_3 on leaf chlorophyll content in S1 and S2. The highest photosynthesis status of 22.33 mg m^{-2}s^{-1} was for $N_2M_1GA_0$, while the lowest photosynthesis status of 16.94 mg m^{-2}s^{-1} was obtained for

$N_2M_2GA_2$ during S1 (Table 5). On the other hand, 27.13 mg $m^{-2}s^{-1}$ was the highest photosynthesis status obtained for $N_3M_0GA_2$ in S2, while $N_1M_2GA_0$ had the lowest photosynthesis status of 17.67 mg $m^{-2}s^{-1}$. The effect of $N_2M_1GA_0$ (100 kg N/ha, black-painted rice straw mulch and 0 mg/L GA_3) and $N_3M_0GA_2$ (150 kg N/ha, no mulch and 80 mg/L GA_3) produced the highest interaction of nitrogen, mulch and GA_3 on photosynthesis status in S1 and S2, respectively. No significant effect was observed due to interaction effect of nitrogen, mulch and GA_3 on photosynthesis status in S1 and S2.

Table 5: Effect of nitrogen, mulch and GA_3 on preharvest physiology

Treatment	Stomatal conductance ($mmol/m^2/s^1$)		Chlorophyll content (ug/l)		Photosynthesis status ($mg/m^2/s^1$)		Transpiration rate (mg’nr^s”1)	
	S1	S2	S1	S2	S1	S2	S1	S2
$N_0M_0GA_0$	0.35	0.78	71.9	72.5	19.48	23.85	7.62	15.10
$N_0M_1GA_0$	0.36	0.61	80.5	(64.3)	21.80	20.12	8.73	14.53
$N_0M_2GA_0$	0.65	0.69	72.8	74.3	20.46	21.67	9.03	15.23
$N_0M_0GA_1$	(0.27)	0.81	69.3	70.2	17.53	21.95	7.70	15.87
$N_0M_1GA_1$	0.35	0.72	83.3	68.8	19.13	22.48	7.75	15.22
$N_0M_2GA_1$	0.40	0.71	77.3	73.6	19.67	22.67	7.82	15.62
$N_0M_0GA_2$	0.32	0.73	67.9	65.7	18.67	17.93	6.92	12.45
$N_0M_1GA_2$	0.35	0.78	**90.2**	75.7	19.47	25.37	7.73	16.87
$N_0M_2GA_2$	0.49	0.77	74.0	71.5	19.38	23.95	7.75	16.85
$N_1M_0GA_0$	0.34	0.68	81.6	76.1	18.03	23.87	6.85	16.00
$N_1M_1GA_0$	0.47	0.69	77.8	70.6	19.69	19.85	10.43	14.82
$N_1M_2GA_0$	0.35	(0.42)	71.9	**80.5**	21.93	(17.67)	8.43	(12.07)
$N_1M_0GA_1$	**1.33**	0.74	71.0	71.3	20.12	22.62	8.28	16.65
$N_1M_1GA_1$	0.38	0.72	79.8	75.5	18.74	23.33	8.32	16.27
$N_1M_2GA_1$	0.37	0.52	90.0	70.5	18.87	20.73	7.73	14.05
$N_1M_0GA_2$	0.39	0.61	70.4	72.8	17.82	21.55	(6.17)	14.87
$N_1M_1GA_2$	0.46	0.62	67.5	73.7	17.98	23.57	7.55	14.33
$N_1M_2GA_2$	(0.31)	0.84	80.8	68.1	18.44	21.65	8.12	15.77
$N_2M_0GA_0$	0.40	0.54	79.5	69.8	19.42	20.18	9.15	13.45
$N_2M_1GA_0$	0.46	0.83	81.5	70.6	**22.33**	22.23	9.58	15.33
$N_2M_2GA_0$	0.48	0.60	68.8	72.9	20.60	24.42	8.33	15.20
$N_2M_0GA_1$	0.39	**0.92**	70.7	75.5	19.58	26.08	7.35	17.42
$N_2M_1GA_1$	0.42	0.70	68.3	73.3	20.26	24.15	8.82	16.90
$N_2M_2GA_1$	0.41	0.72	83.1	73.9	19.01	22.17	9.20	15.25
$N_2M_0GA_2$	0.36	0.82	73.2	71.0	19.47	22.57	6.88	16.3
$N_2M_1GA_2$	0.4	0.69	79.8	69.5	18.26	20.82	8.57	15.17
$N_2M_2GA_2$	0.37	0.54	82.5	72.8	(16.94)	20.98	6.70	13.63
$N_3M_0GA_0$	0.50	0.80	70.6	70.9	20.87	21.33	8.92	15.30
$N_3M_1GA_0$	0.48	0.86	65.8	72.3	21.32	24.82	**10.8**	16.72
$N_3M_2GA_0$	0.69	0.87	74.1	74.2	20.30	22.27	10.73	16.58
$N_3M_0GA_1$	0.40	0.87	67.1	71.4	20.39	**27.57**	7.75	**17.52**
$N_3M_1GA_1$	0.35	0.73	75.8	68.3	19.43	21.88	9.95	15.72
$N_3M_2GA_1$	0.45	0.65	(63.8)	70.2	21.34	22.10	8.58	14.48

Treatment	Stomatal conductance (mmol/m^2/s^1)		Chlorophyll content (ug/l)		Photosynthesis status (mg/m^2/s^1)		Transpiration rate (mg'nr^s"1)	
	S1	S2	S1	S2	S1	S2	S1	S2
$N_3M_0GA_2$	0.43	0.88	74.6	73.5	18.54	27.13	7.92	17.48
$N_3M_1GA_2$	0.40	0.87	73.0	67.5	19.92	25.10	7.28	17.32
$N_3M_2GA_2$	0.54	0.71	68.4	74.4	19.84	25.57	8.67	15.53
P-value	0.700	0.225	0.850	0.276	0.990	0.163	0.563	0.271
LSD 5%	0.51	0.38	23.12	9.13	5.35	6.38	3.52	4.61

S1= Season 1 (March 2019-July 2019); S2= Season 2 (October 2019-February 2020).
Bolded values = Highest; Bracketed values = Lowest

The highest transpiration rate of 10.80 mg'nr^s"1 was for $N_3M_1GA_0$, while the lowest 6.85 mg'nr^s"1 was for $N_1M_0GA_0$ in S1. The 17.52 mg'nr^s"1 was the highest transpiration rate in S2 for $N_3M_0GA_1$, while $N_1M_2GA_0$ had the lowest 12.07 mg'nr^s"1 (Table 2). The $N_3M_0GA_1$ (150 kg N/ha, no mulch and 40 mg/L GA_3) had the highest interactive effect in S2. No significant effect was observed due to interactive effect on transpiration rate in both seasons. No significant interactive effect of nitrogen fertiliser, mulch and GA_3 on physiological parameters (stomatal conductance, chlorophyll content, photosynthesis status and transpiration rate) resulted in the present study. This result was supported by Siddiqui *et al.* (2008), who found net photosynthesis, stomatal conductance, and chlorophyll content were positive, but not significant in *Brassica juncea*. Leilah and Khan (2021) reported that timely spraying of GA_3 at the early vegetative stages leads to rapid leaf growth and photosynthesis achieved surpassed the basic needs of sugar beet.

Conclusion and Recommendations

Nitrogen and mulch individually, their two-way interactions as well as combined nitrogen, mulch and GA_3 do not significantly differ in influencing stomatal conductance, leaf chlorophyll content, photosynthesis and transpiration. Gibberellic acid effect does not significantly affect stomatal conductance and leaf chlorophyll, but it negatively and significantly affect both photosynthesis and transpiration, depending on the season. The present non-significant results imply that the effect of combined nitrogen, mulch and GA_3 on multi-purpose pumpkin cannot be entirely depicted by analysing preharvest physiology, but requires analysing other responses.

Nevertheless, the use of 50 kg N/ha to enhance stomatal conductance and leaf chlorophyll and unpainted rice straws to enhance leaf chlorophyll and photosynthesis, as opposed to black-painted rice straws that had the highest transpiration rate, is recommended. Furthermore, the use of 50 kg N/ha in combination with 40 mg/L GA_3 and with or with no mulch to enhance stomatal conductance and leaf chlorophyll content is also recommended. Adoption and application of combined rates of nitrogen, mulch and GA_3 that promote the desired preharvest physiology of multi-purpose pumpkin is also recommended.

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