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Management of Ozone Stress Through Nutrient Amendments: Role of Biomass Allocation in Sustaining Yield in Selected Maize Cultivars

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Abstract: **In view of the present climate change scenario which significantly affects the agricultural sustainability, we need to develop certain strategies that help us to sustain agricultural productivity. Ground level ozone is an important component of climate change that has been proved to be the main culprit causing significant agricultural losses during the past few decades. The aim of present study is to evaluate the effectiveness of nutrient treatment in sustaining agricultural production under ambient ozone stress. Two varieties of maize (Zea mays L. var Malviya hybrid-2 and HHM-1) were taken as the experimental plants. Three doses of nutrients (NPK) recommended (N1), 1.5x recommended (N2) and 2x recommended (N3) were applied to the experimental plants grown under ambient ozone stress. Daytime eight hourly ozone concentrations varied from 49.2 to 59 ppb during the experimental period. Plants treated with nutrients responded better than the plants without nutrient, which served as control. Yield (test weight) increased significantly by 11, 46 and 44 % in Malviya hybrid-2 and by 11, 18 and 18.3 % in HHM-1 at N1, N2 and N3 treatments, respectively, as compared to control. Variations in the biomass allocation strategies during the vegetative and reproductive phases resulted in higher yield increments in Malviya hybrid-2 as compared to HHM-1. It was further observed that N2 treatment was sufficient to cause significant increments in yield as compared to control. The results of the present experiment clearly suggest that nutrient amendments can be effectively used in partially mitigating ambient ozone stress and sustaining agricultural productivity to some extent. However, more experimentation with different crop varieties is required to prove the expediency of nutrient amendments.**

Index Terms: **Ozone, agricultural production, sustainability, yield, mitigation.**

I. INTRODUCTION

Significant reductions in agricultural productions all over the globe is a well cited and evident fact (Peng et al., 2019; Emberson et al., 2018; Mills et al., 2018; Gao et al., 2017; Danh et al., 2016; Cotrozzi et al., 2016; Yi et al., 2016). O_3 concentrations are mounting rapidly in developing countries and are predicted to escalate in coming decades unless strict legislations to curtail the precursor emissions are implemented strictly (Cooper et al., 2014; Wild et al., 2012). The coincidence of occurrence of high O_3 concentration during the growth period of important agricultural crops has further intensified the negative effects of O_3 on crop production (Ainsworth, 2017). O_3 damages the sensitive crops like wheat, rice and soybean which stimulate the metabolically expensive defence mechanism at the cost of resource allocation away from growth and grain/seed production (Mills et al., 2018). Based on MOZRAT -2, Avnery et al. (2011a) estimated that global mean loss in the relative yield due to O_3 pollution for the year 2000 was 9.6, 3.9 and 11.2 % for wheat, maize and soybean, respectively. In a follow up study, it was predicted that by the year 2030, the global mean relative yield loss would be 10.6, 4.3 and 12.1 % for wheat, maize and soybean, respectively (Avnery et al., 2011b) under the optimistic emission scenario as defined by IPCC (Nakicenovic et al., 2000). On the basis of a meta-analysis study performed on 33 experiments, it was observed that the average yield loss associated with reducing mean O_3 concentration from 35.6-13.7 ppb was 8.4% (Pliejel et al., 2018). In India, O_3 pollution accounted for an annual loss of 4.2-15% and 0.3-6.3% in wheat and rice, respectively (Lal et al., 2017).

In spite of the peremptory consequences of O_3 on global crop production, developing strategies to mitigate O_3 stress in plants has attained little attention from the agronomists. In view of its multifarious nature of O_3, focussing on implementation of specific agronomic practices to minimise O_3 injury will be more favourable rather than investigating ways to increase crop yield (Mills et al., 2018). Supplemental nutrition can profoundly affect

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the sensitivity of plants towards O_3 stress through mechanisms that are still under debate (Harmens et al., 2017; Shang et al., 2018). Through planned experiments, many authors have observed that carbon allocation can be an important factor that can assist the efforts of O_3 stressed plants in sustaining yield upon nutrient amendments (Harmens et al., 2017; Watanabe et al., 2012). The present study was planned with the objectives of investigating the mechanistic approach adopted by maize, a C_4 crop, subjected to different doses of soil nutrient amendments, in sustaining O_3 induced yield losses under field conditions. The hypothesis that we propose are:

(i) Different cultivars of maize show differential behavior and adopt different mechanistic approach to manage O_3 stress upon similar soil nutrient amendments.

(ii) Biomass allocation and not the vegetative growth is the deciding factor in determining yield in both the cultivars under similar O_3 and soil nutrient amendment regimes.

II. MATERIALS AND METHODS

2.1 Study site

The study was conducted under natural field conditions at a rural site in Varanasi city located in the Eastern Indo Gangetic Plains of the Indian subcontinent at 25°14´ N latitude, 82°03´ longitude and 76.19 m above the sea level. The present experiment was carried out during the rainy season of the year 2017. Variations in climatological data during the study period are shown in Fig 1.

2.2 Plant Material

The experiment was performed on two maize (*Zea mays* L.) cultivars Malviya hybrid-2 and HHM-1. Malviya hybrid-2 was developed in 2007 by the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi by the cross with HUZ M185/HKI 1105. This cultivar matures in about 90-95 days and has an average yield of 54q ha^{-1}.

HHM-1 was developed in 2000 by CCS Haryana Agricultural University, Karnal by the cross with HKI 536/HKI 295. Maturity time for this cultivar is about 100-105 days with an average yield of 60 q ha^{-1}.

2.3 Experimental design

The design of the experiment was randomized split plot with cultivars as the whole plot and nutrient treatment as subplots. Each plot (1.5 ×1.5m) was separated by a trench (0.2 m wide and 0.5 m deep). The field was prepared by using standard agronomic practices. The soil of the experimental field was sandy loam in texture (sand 45%, silt 28% and clay 27%) having organic carbon 0.67%, pH 7.4, cation exchange capacity 17.8 meq%, nitrogen 0.12% and phosphorus 0.065%. The entire experimental area was ploughed to fine tilth and given a basal treatment of recommended doses of inorganic fertilisers (25 kg N, 50 kg P and 25 kg K per hectare of land) in form of urea, super-phosphate and muriate of potash, respectively). Seeds were hand sown in rows during the last week of June. After germination, plants were thinned to one plant per 15 cm. Distance between the rows was 20 cm. Three nutrient (NPK) treatments were given (i) recommended dose (N1), (ii) 1.5 times recommended dose (N2) and, (iii) 2 times recommended dose (N3). For N_1, 80, 40 and 40 kg ha^{-1}, for N2, 120, 60 and 60 kg ha^{-1}, and for N3, 160, 120 and 120 kg ha^{-1} of N, P and K were given, respectively. Control plots for each cultivar were also maintained in which no supplemental nutrients were added. Each treatment was replicated thrice. Watering was done using drip irrigation and a similar soil moisture regime was maintained in all plots. Nutrient treatment was given at 40 and 60 DAG, reflecting the vegetative and reproductive phase of the plants.

2.4 Ozone monitoring

Eight hourly ozone monitoring (0800- 1600 hours) was done at the experimental site from germination to harvest using O_3 Analyzer (model APOA 370, Horiba, Japan). The ambient O_3 concentration was measured by drawing air through a Teflon tube kept above the canopy.

Calibration of the monitoring instrument was conducted weekly by known concentration O_3.

2.5 Plant Sampling and Analysis

Five plants from each replicate plot of treatment were selected randomly for both the cultivars at 50 days after germination (DAG) and 75 DAG for evaluating growth and biomass accumulation and allocation characteristics. Thus 15 plants per treatment were taken for each cultivar.

2.5.1 Plant growth characteristics

For growth characteristics, root and shoot lengths, number of leaves $plant^{-1}$ and leaf area $plant^{-1}$ were recorded. Leaf area was measured using portable leaf area meter (Model LI-3000, LI-COR, Inc., USA). For biomass determination, monoliths (10×10×20cm) containing intact roots were carefully dug out and were washed thoroughly by placing on a sieve of 1 mm diameter under running tap water to remove soil particles adhering to the roots. Component wise plant parts were separated, and oven dried at 80°C till a constant weight was achieved. The plant parts were then weighed separately and expressed in terms of g $plant^{-1}$. The biomass allocation pattern was studied by calculating different growth indices like specific leaf area (SLA), specific leaf weight (SLW), leaf area ratio (LAR), leaf weight ratio (LWR) by following the formulae modified by Hunt (1982). Root mass fraction (RMF), specific stem length (SSL) and crop growth rate (CGR) were estimated as per Poorter et al (2012). All the growth indices were calculated on the basis of dry weight data.

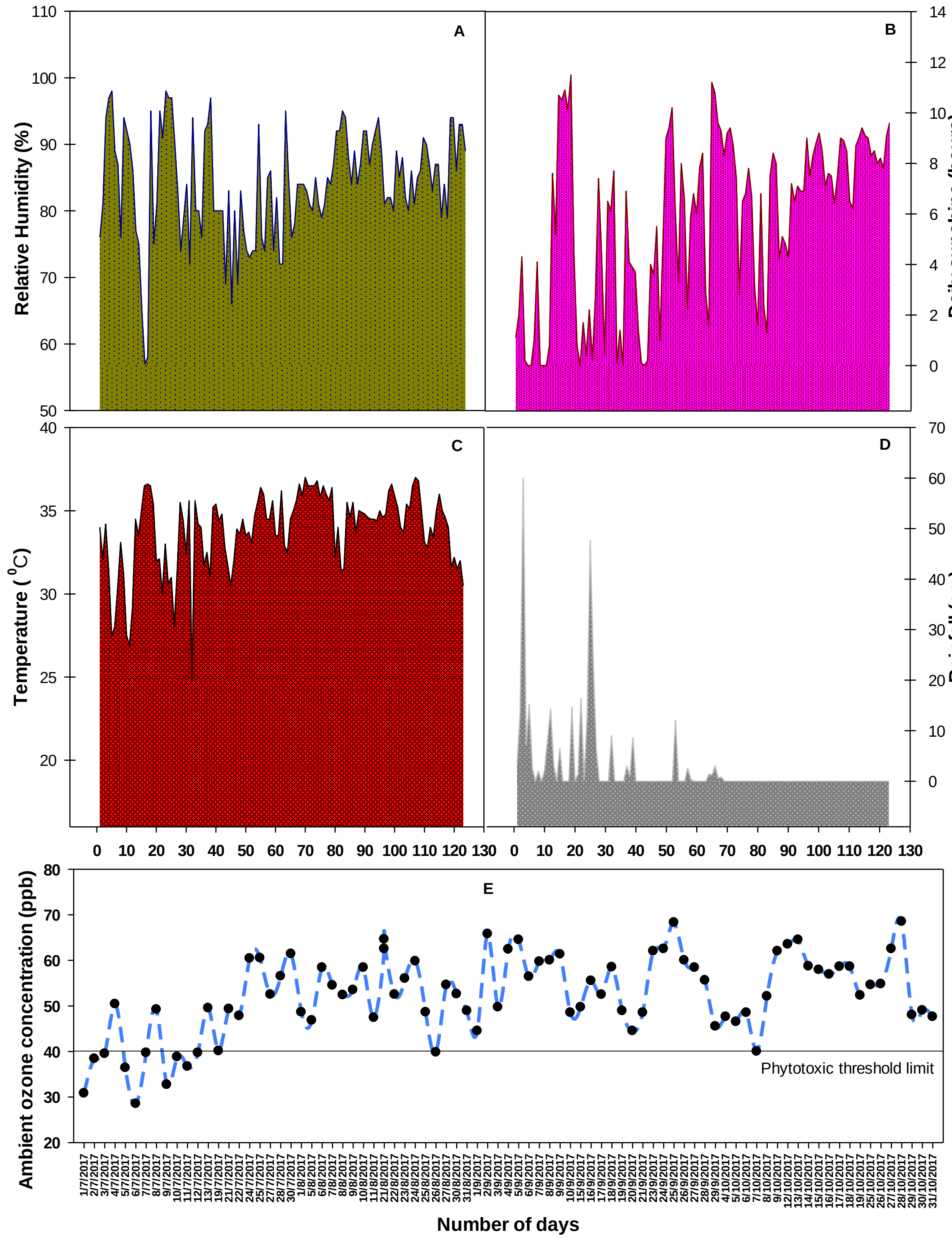

Fig 1. Variations in O_3 concentrations and meteorological parameters throughout the experimental period of maize. (a) Average daily relative humidity (%) (b) Average daily sunshine (hrs) (c) Average daily of ambient temperature (°C) (d) Average daily rainfall (cm) (e) Mean 8-hour day time O_3 concentration (ppb).

2.5.2 Carbohydrate contents

Reducing sugar was estimated by the colorimetric copper method of Somogyi-Nelson (Herbart et al., 1971). Total soluble sugars and starch contents were estimated by phenol-sulfuric acid method, with glucose as a standard (Rai et al., 2010). Wheat grains were grounded to make a powder for analysis of carbohydrate contents. 100 mg of samples were homogenized in 5 ml of 80% ethanol (v/v) and then centrifuged at 14,000 rpm for 20 min. The pellets were sequentially washed with 80% ethanol for five times and centrifuged at 14,000 rpm for 20 min after each washing. The supernatant collected was used for estimating total soluble and reducing sugars and pellets were used for starch content.

2.5.3 Nutrient contents

For estimating the nutrient quality, plant parts were separated, and oven dried at 80ºC, till a constant weight was obtained. Oven dried plant samples were grinded in a stainless-steel grinder and passed through a 0.5 mm sieve. Total nitrogen content was determined by Gerhardt Automatic N Analyzer (Germany). For P and K samples were digested following the protocol proposed by Allen et al. (1974) and the respective nutrients in the digested material were estimated with the help of Atomic Absorption Spectrophotometer (Model 2380, Perkin Elmer, USA).

2.5.4 Yield attributes

Yield attributes were assessed at the time of final harvest by harvesting 10 plants per treatment. Number of cobs plant^{-1}, Number of kernels cob^{-1}, weight of cobs plant^{-1}, weight of kernels cob^{-1} and weight of 1000 kernels (test weight) were recorded.

2.6 Statistical analysis

Significant differences between the treatments were evaluated for all the measured parameters using one-way analysis of variance (ANOVA). The data were tested for normality by Shapiro-Wilk test and found to be normal as p values were above 0.05. The individual and interactive effects of all three independent variables viz. age, treatment and mean O_3 concentration was analysed by performing 3x3factorial multivariate analysis ANOVA. Correlation coefficients were also performed between selected yield parameters and nutrient treatments. All the statistical tests were performed using SPSS software (SPSS Inc. Version 21.0, IBM Corp, Armonk, NY).

III. RESULTS

3.1 Ambient ozone concentration

The daytime eight hourly ozone concentration (M8) varied from 40.17 ppb in July 2017 to 55.4 ppb in September 2017. Mean value of M8 for the entire growth period of maize was recorded to be 50.50 ppb (Fig 1).

3.2 Morphological parameters:

The morphological parameters showed positive response under nutrient amendments as compared to control in both the cultivars during both sampling phases (vegetative and reproductive). In Malviya hybrid-2, root length increased significantly by 24.3, 30.7 and 67.3 % and shoot length showed significant increments of 31.8, 76.9 and 76.9%, respectively at N1, N2 and N3 treatments as compared to control during vegetative phase (Fig 2). During reproductive phase, root length improved significantly by 35, 42.4 and 48% and shoot length increased significantly by 11.5, 18.8 and 39%, respectively, at N1, N2 and N3 treatments as compared to control (Fig 2). Total plant length showed significant increments of 31, 73 and 79% during vegetative phase and by 12.8, 20 and 22.4%, respectively during reproductive phase at N1, N2 and N3 treatments, respectively, as compared to control. In HHM-1, significant increments of 30 and 67.5% were recorded during vegetative phase only in the shoot length of plants with N2 and N3 treatments as compared to control (Fig 2). During reproductive phase, root length increased significantly by 6.9, 15.9 and 38% at N1, N2 and N3 treatments, respectively, whereas shoot increased significantly by 12 and 28%, respectively, only at N2 and N3 treatments (Fig 2). Total plant length of HHM-1 cultivar increased significantly by 28.3 and 62 %, during vegetative phase and by 12.5 and 28.6% during reproductive phase, at N2 and N3 treatments, respectively, as compared to control. Total plant length varied significantly due to cultivar and amendment factors and their interactions except age × cultivar × amendment interaction (Table 1).

Number of leaves per plant increased significantly by 25, 45 and 45% during vegetative phase and by 15.9, 24 and 32% during reproductive phase at N1, N2 and N3 treatments, respectively, as compared to control in Malviya hybrid-2 (Fig 3). In HHM-1, the increment in the number of leaves per plant was significant only at N_3 treatment as compared to control during vegetative phase. During reproductive stage, however, this growth parameter increased significantly by 11.8, 23.5 and 41%, respectively, at N1, N2 and N3 treatments as compared to control (Fig 3). Number of leaves per plant varied significantly due to cultivar and amendment factors and due to age × cultivar interaction (Table 1). In Malviya hybrid-2, leaf area per plant showed significant increments of 31.8, 59.6 and 61.8% during vegetative phase and 37.5, 60.4 and 60.2% during reproductive phase, in N1, N2 and N3 treatments as compared to control (Fig 3). In HHM-1, significant increments of 40.5 and 61.3% during vegetative phase, and 21.8 and 45.3% during reproductive stage were recorded only at N1 and N2 treatments, respectively, as compared to control (Fig 3). Leaf area varied significantly due to all individual factors and their interactions (Table 1).

3.3 Total Biomass accumulation

In Malviya hydrid-2, root biomass increased significantly by 10.4, 19.8 and 22.9% and shoot biomass by 15.9, 46.9 and 49.2% during the vegetative phase in N1, N2 and N3 treatments, respectively, as compared to control (Fig 4). During the reproductive phase, significant increments of 46.3, 53.4 and 53.6% in root biomass and 38.5, 72.8 and 73% in shoot biomass were recorded (Fig 4). Total plant biomass showed significant increments of 14.6, 42.4 and 43.6%, during the vegetative phase and 38.8, 71.8 and 72%, during the reproductive phase, in N1, N2 and N3 treatments, respectively, as compared to control (Fig 4). In HHM-1, root biomass showed significant increase of 11.4, 35.4 and 57% during the vegetative phase and during the reproductive phase, it increased significantly by 30, 56 and 159 % at N1, N2 and N3 treatments, respectively, as compared to control (Fig 4).

Shoot biomass increased significantly by 7, 35 and 72 %, respectively during the vegetative phase and by 13, 53.5 and

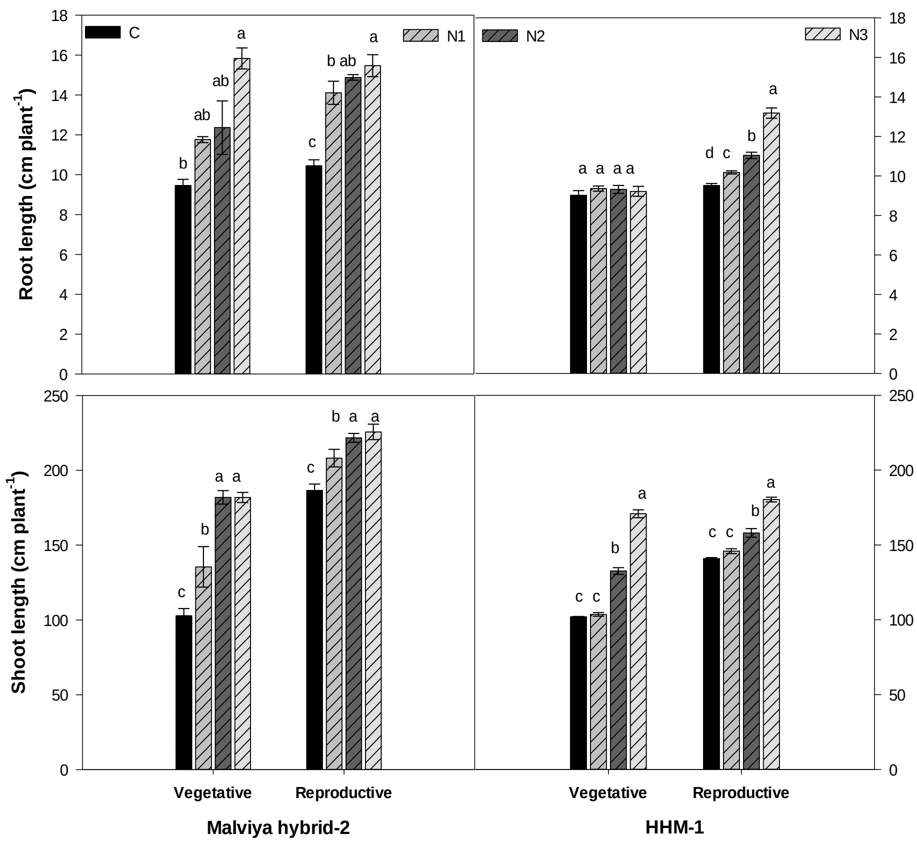

Fig 2. Root and shoot length of maize. cv. Malviya hybrid- 2 and HHM-1 grown at recommended (N1), 1.5 x recommended (N2) and 2 x recommended (N3) nutrient doses during vegetative and reproductive stages at ambient ozone concentration. Bar represents mean ± SE. Different letters indicate significant differences at $p < 0.05$ as per Duncan's test.

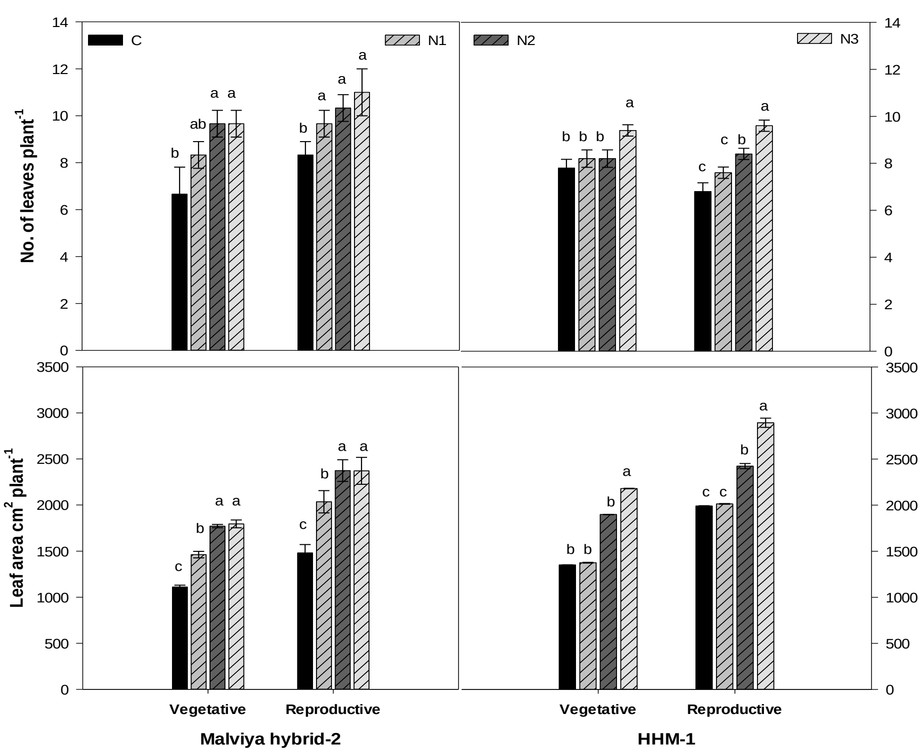

Fig 3. Leaf characteristics of maize cv. Malviya hybrid- 2 and HHM-1 grown at recommended (N1), 1.5 x recommended (N2) and 2 x recommended (N3) nutrient doses during vegetative and reproductive stages at ambient ozone concentration. Bar represents mean ± SE. Different letters indicate significant differences at $p < 0.05$ as per Duncan's test.

Table 1: Results of three-way ANOVA test of the morphological parameters of maize with their respective F values and level of significance.

	Root Length (cm plant^{-1})	Shoot Length (cm plant^{-1})	Total Length (cm plant^{-1})	Number of Leaves (plant^{-1})	Leaf Area (cm plant^{-1})	Root Biomass (g plant^{-1})	Shoot Biomass (g plant^{-1})	Total Biomass (g plant^{-1})
Age(A)	13.77***	736.77***	503.37	0.516	3.48***	72.5***	264.73***	196.67***
Cultivar(C)	48.84***	558.99***	428.4***	18.58***	56.93***	176.61***	1240***	1280***
Treatment(T)	14***	238.29***	177.89***	22.79***	280.49***	89.93***	166.37***	192.83***
A×C	0.207	89.34***	53.57***	18.58***	207.8***	221.94***	194.62***	115.01***
A×T	0.483	28.248***	18.6***	0.258	46.67***	26.14***	10.72***	15.52***
C×T	3.59*	22.77***	15.54***	1.11	49.34***	27.65***	78.6***	87.06***
A×C×T	2.5	3.76*	2.38	1.63	45.74***	50***	14.23***	22.4***

Table 2: Age wise changes in the growth indices of maize var Malviya hybrid-2 at different doses of nutrient treatments (Mean ± SE). Different letters indicate significant differences according to Duncan's test at $p < 0.05$.

	Crop Growth Rate (g g^{-1} day^{-1})		Specific Leaf Area (cm^{2}g^{-1})		Specific Leaf Weight (g cm^{-2})		Leaf Area Ratio (cm^2 cm^{-2})		Leaf Weight Ratio/Leaf Mass Fraction (g g^{-1})		Root Mass Fraction (g g^{-1})		Specific Stem Length (cm g^{-1})	
T	**V**	**R**	**V**	**R**	**V**	**R**	**V**	**R**	**V**	**R**	**V**	**R**	**V**	**R**
C	0.106^{c} ±0.006	0.875 ^{c}±0.009	333.13^{a} ±3.07	313.50^{a} ±8.18	0.003^{c} ±0.00003	0.0032^{c} ±0.00008	232.71^{a} ±49.55	66.78^{a} ±8.39	0.699^{a}± .155	0.212^{a}± 0.024	0.203^{a}± 0.038	0.045^{a}± 0.004	27.19^{a} ±4.11	8.79^{a} ±0.595
N1	0.122^{b} ±0.003	1.27^{b} ±0.004	318.42^{b} ±2.34	278.53^{b} ±7.43	0.0031^{b} ±0.00002	0.0036^{b} ±0.00001	266.65^{a} ±12.01	65.88^{a} ±5.08	0.837^{b}± .037	0.236^{b}± 0.017	0.198^{a}± 0.005	0.047^{a}± 0.006	30.60^{a} ±3.11	7.06^{b} ±0.223
N2	0.151^{a} ±0.003	1.57^{a} ±0.060	307.87^{c} ±1.98	260.48^{c} ±1.89	0.0032^{a} ±0.00002	0.0038^{a} ±0.00003	261.00^{a} ±23.31	62.21^{b} ±6.19	0.847^{b}± .076	0.239^{b}± 0.025	0.168^{a}± 0.001	0.040^{a}± 0.007	32.19^{a} ±2.25	6.04^{c} ±0.432
N3	0.152^{a} ±.0003	1.05^{a} ±0.020	311.58^{c} ±0.94	259.63^{c} ±3.57	0.0032^{a} ±0.00001	0.0039^{a} ±0.00005	261.54^{a} ±12.46	62.09^{b} ±7.92	0.839^{b}± .039	0.239^{b}± 0.031	0.171^{a}± 0.005	0.040^{a}± 0.011	32.04^{a} ±2.89	6.15^{c} ±0.570

T= Treatment; V= Vegetative; R= Reproductive; C= Control; N1= Recommended dose; N2= 1.5x Recommended dose; N3= 2x Recommended dose

Table 3: Age wise changes in the growth indices of maize var HHM-1 at different doses of nutrient treatments (Mean ± SE). Different letters indicate significant differences according to Duncan's test at $p < 0.05$.

	Crop Growth Rate (g g^{-1} day^{-1})		Specific Leaf Area (cm^{2}g^{-1})		Specific Leaf Weight (g cm^{-2})		Leaf Area Ratio (cm^2 cm^{-2})		Leaf Weight Ratio/Leaf Mass Fraction (g g^{-1})		Root Mass Fraction (g g^{-1})		Specific Stem Length (cm g^{-1})	
T	**V**	**R**	**V**	**R**	**V**	**R**	**V**	**R**	**V**	**R**	**V**	**R**	**V**	**R**
C	0.140^{c} ±0.008	0.030^{c} ±.004	337.67^{a} ±2.77	438.40^{a} ±2.52	0.0030^{c} ±0.00002	0.0023^{c} ±0.00001	212.28^{a} ±5.94	286.03^{a} ±10.49	0.628^{bc} ±0.022	0.652^{ab}± 0.027	0.123^{a} ±0.006	0.137^{b} ±0.018	16.00^{a} ±0.92	23.43^{a} ±0.265
N1	0.152^{c} ±0.003	0.059^{b} ±.001	327.47^{ab} ±10.38	424.00^{a} ±15.86	0.0031^{bc} ±0.0001	0.0024^{b} ±0.00009	200.35^{a} ±4.92	250.46^{b} ±14.96	0.612^{c} ±0.013	0.590^{c} ±0.018	0.128^{a} ±0.003	0.154^{b} ±0.014	15.08^{a} ±0.20	21.42^{b} ±0.304
N2	0.191^{b} ±0.007	0.106^{a} ±.001	316.20^{bc} ±2.52	344.93^{b} ±4.33	0.0032^{ab} ±0.00003	0.0029^{a} ±0.00004	220.86^{b} ±5.77	226.10^{c} ±6.11	0.698^{a} ±0.023	0.655^{a} ±0.011	0.124^{a} ±0.013	0.139^{b} ±0.005	15.41^{a} ±0.16	17.12^{c} ±0.136
N3	0.241^{a} ±0.009	0.126^{a} ±.002	307.13^{c} ±5.2	351.77^{b} ±3.57	0.0033^{a} ±0.00005	0.0028^{a} ±0.00003	200.76^{b} ±2.09	216.38^{c} ±7.13	0.653^{b} ±0.004	0.615^{a} ±0.020	0.114^{a} ±0.008	0.179^{a} ±0.009	15.73^{a} ±0.12	16.42^{c} ±0.174

T= Treatment; V= Vegetative; R= Reproductive; C= Control; N1= Recommended dose; N2= 1.5x Recommended dose; N3= 2x Recommended dose

Table 4: Yield parameters of two maize varieties grown at different doses of nutrient treatments (Mean ± SE). Different letters indicate significant differences according to Duncan's test at $p< 0.05$.

Variety	Treatment	Number of kernel/cobs	Weight of kernel/cob	Weight of a cob
Malviyahybrid-2	C	172.00^{c} ±8.18	30.12^{c} ±3.64	41.46 ^{c}±6.30
	N1	204.00 ^{b}±6.55	41.74 ^{b}±6.02	53.91 ^{b}±2.46
	N2	241.66^{a} ±7.50	61.24 ^{a}±3.12	80.85 ^{a}±4.42
	N3	245.66 ^{a}±11.84	61.50 ^{a}±4.09	83.73 ^{a}±4.25
HHM-1	C	186.00^{c}±6.55	32.14^{c}±1.45	44.74^{c}±2.62
	N1	212.00^{b}±6.55	40.98^{b}±2.63	62.76^{b}±2.44
	N2	251.33^{a}±8.62	50.98^{a}±1.58	74.66^{a}±2.76
	N3	253.00^{a}±8.71	51.04^{a}±1.39	76.32^{a}±3.38
		Result of two-way ANOVA		
Cultivar(C)		8.40***	42.03***	45.68***
Treatment(T)		99.76***	136.24***	104.14***
C×T		0.198^{NS}	3.71*	6.53*

***= $p< 0.001$; * = $p< 0.05$; NS=Not Significant.

vegetative stage of HHM-1 cultivar, total plant biomass increased significantly by 7.8, 35 and 70.5 %, whereas it increased by 15.6, 54 and 92% during the reproductive stage at N1, N2 and N3 treatments, respectively, as compared to control (Fig 4). Total plant biomass showed significant variations due to all individual factors and their interactions (Table 1).

3.4 Growth indices and biomass allocation

Crop growth rate (CGR) increased significantly by 15, 42.5 and 43 % during the vegetative phase and by 46, 82 and 82 % during the reproductive phase in N1, N2 and N3 treatments, respectively, as compared to control in Malviya hybrid-2, whereas in HHM-1, it showed significant increments of 36 and 72 % in N1 and N2 treatments, respectively as compared to control during the vegetative phase and increased significantly by 85.7, 238 and 300 %, respectively, in N1, N2 and N3 treatments, respectively as compared to control during the reproductive phase (Table 2 and 3). Specific leaf area (SLA) in case of Malviya hybrid-2 declined significantly by 4, 7.5 and 6.5 % in N1, N2 and N3 treatments,

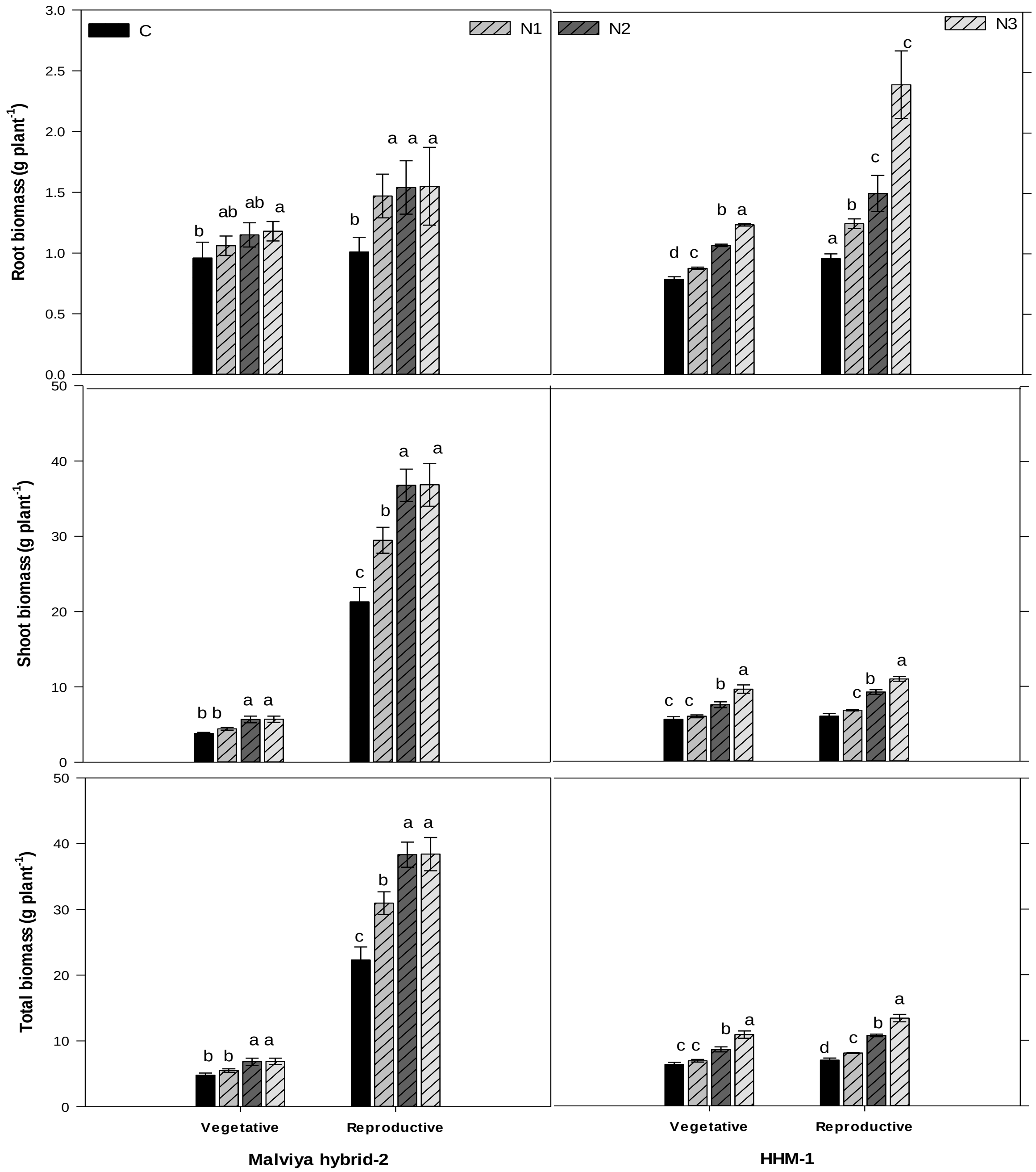

Fig 4. Root, shoot and total biomass of maize cv. Malviya hybrid- 2 and HHM-1 grown at recommended (N1), 1.5 x recommended (N2) and 2 x recommended (N3) nutrient doses during vegetative and reproductive stages at ambient ozone concentration. Bar represents mean ± SE. Different letters indicate significant differences at $p < 0.05$ as per Duncan's test.

respectively as compared to control during the vegetative phase and by 11, 16.9 and 17 % in N1, N2 and N3 treatments, respectively as compared to control during the reproductive phase of the plant (Table 2 and 3). In case of HHM-1, this growth index showed significant reduction of 3, 6 and 9 % during the vegetative phase and 3, 21 and 19 % during the reproductive phase in N1, N2 and N3 treatments, respectively as compared to control (Table 2 and 3). Specific leaf weight (SLW) showed a common trend of significant increments with increasing nutrient dose in both the cultivars at both sampling stages (Table 2 and 3). LAR showed significant decline at all the treatments during the reproductive stage in both the cultivars as compared to control (Table 2 and 3). RMF showed significant increase of 30.6 % at N3 treatment as compared to control only in HHM-1(Table 4 and 5). LMF increased significantly by 11.3, 12.7 and 12.7 % at N1, N2 and N3 treatment, respectively, as compared to control, only during the reproductive phase in Malviya hybrid-2 (Table 2 and 3). In HHM-1, LMF increased significantly at N2 and N3 treatments only, during the vegetative stage and at all the treatments during the reproductive stage, as compared to control (Table 2 and 3). SSL registered significant increments of 12.5, 18.4 and 17.8 % at N1, N2 and N3 treatments, respectively, as compared to control, during the vegetative stage and decreased significantly by 19.7, 31 and 30 % at N1, N2 and N3 treatments, respectively, as compared to control during the reproductive stage (Table 2 and 3). In HHM-1, SSL declined significantly by 8.5, 26.9 and 29.9 %, at N1, N2 and N3 treatments, respectively, as compared to control only during the reproductive phase (Table 2 and 3).

3.5 Carbohydrate content

Total soluble sugar (TSS) content in MH-2 cultivar increased significantly in all the treatments as compared to the control, however higher increments of 14.32 and 14.56 %, respectively, were observed in N2 and N3 treatments only (Fig 5). A very similar trend was observed in HHM-1 cultivar where N2 and N3 showed 19.47 and 21.05% increments. In case of reducing sugar, both the cultivars showed significant increments in all the treatments however greater increments were observed in N2 and N3 treatments of Malviya hybrid-2 cultivar which are 20.68 and 23.44 %, respectively, while HHM-1 showed 15.48 % increase in both the treatments (Fig 5). A similar trend of increase in starch content was also observed in Malviya hybrid -2 as well as HHM-1 cultivar where 49.08 and 54.52% increase were observed in former and in later, 46.03 and 47.44% was observed in N2 and N3 treatments respectively (Fig 5).

3.6 Nutrient contents

The total nitrogen (N) content in shoot, root and grains in N1 treatment of Malviya hybrid-2 cultivar were found to increase by 55.55, 12.5 and 21.42 %, respectively, while phosphorous (P) and potassium (K) content were found to increase by 15.89, 14.76, 12.20 % and 23.1, 35.67, 5.06 % respectively (Fig 6). A very similar trend in N, P and K increments was followed in N1 treatment of HHM-1 cultivar also. Considerable increments in N content were observed in N2 and N3 treatments as compared to the N1 viz. 99.02, 78.12, 50.84 % and 112.5, 65, 55.32 respectively in Malviya hybrid-2, however, lesser increments were seen in HHM-1 cultivar, given by 88.44, 73.35, 58% and 92.96, 71.52, 60.35% respectively (Fig 6). Similar trend was observed in P content of shoot and root of N2 and N3 treatments in both the cultivars, however, a substantial decrease in P and K was observed in grains of N2 as well as N3 treatments, given by 6.47, 9.03% in P and K respectively in N2 and 7.5, 3.31% in N3 in Malviya hybrid-2 (Fig 6). In case of HHM-1, the grain showed higher reductions in P and K values given by 8.01, 8.6 and 10.25, 7.76% in N2 and N3 respectively (Fig 6).

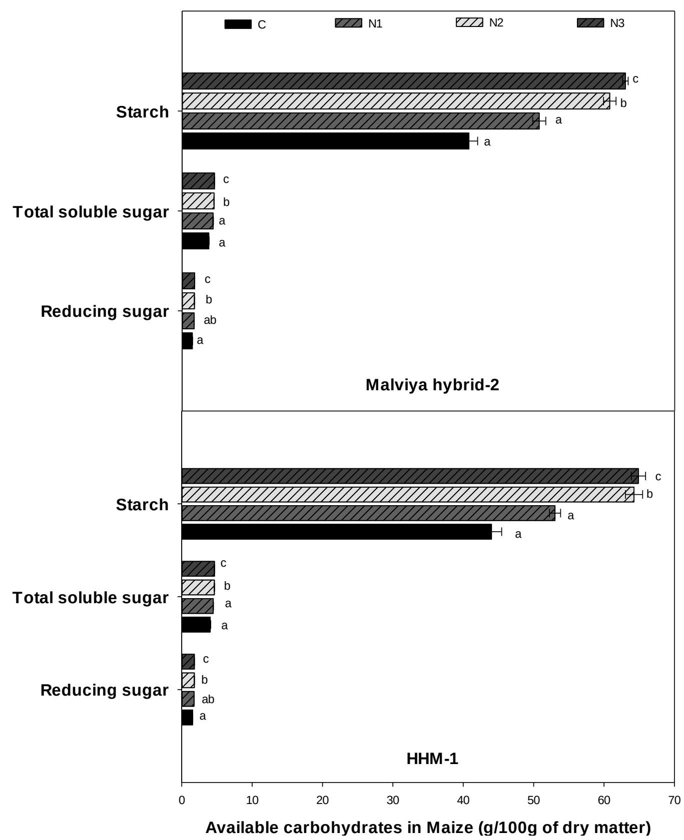

Fig 5. Contents of starch, total soluble sugars and reducing sugars in the kernels of maize cv. Malviya hybrid- 2 and HHM-1 grown at recommended (N1), 1.5 x recommended (N2) and 2 x recommended (N3) nutrient doses during vegetative and reproductive stages at ambient ozone concentration. Bar represents mean ± SE. Different letters indicate significant differences at $p< 0.05$ as per Duncan's test.

3.7 Yield attributes

Number of cobs per plant increased significantly by 50, 75 and 75% in both the cultivars Malviya hybrid-2 and HHM-1 at N1, N2 and N3 treatments, respectively, as compared to control (Table 4). Number of kernels per cob increased significantly by 18.6, 40.5 and 42.8%, respectively, in Malviya hybrid-2 and by 14, 35 and 36%, respectively, in HHM-1 at N1, N2 and N3 treatments as compared to control (Table 4). Significant increments of 94, 240 and 252.8%, respectively, were recorded for weight of cobs per plant in N1, N2 and N3 treated plants as compared to control in Malviya hybrid-2, whereas in HHM-1, the same parameter increased significantly by 110.7, 192.4 and 199 %, respectively, in N1, N2 and N3 treated plants as compared to control (Table 4). Weight of the kernels per cob increased significantly by 38.5, 103 and 104%, respectively in Malviya hybrid-2 and by 27.5, 58.6 and 58.8%, respectively, in HHM-1 in plants with N1, N2 and N3 treatments as compared to control (Table 4). Test weight (weight of 1000 kernels) increased significantly by 10.6, 45.8 and 43.8%, respectively, in N1, N2 and N3 treatments as compared to control in Malviya hybrid-2, where as in HHM-1, it increased significantly by 11, 18 and 18.3 %, respectively, N1, N2 and N3 treatments as compared to control (Table 4). Weight of kernels per cob, weight of cobs per plant and weight of 1000 seeds varied significantly due to all individual factors and their interactions (Table 4). Number of kernels per cob however showed significant variations due to cultivar and amendment factor only (Table 1).

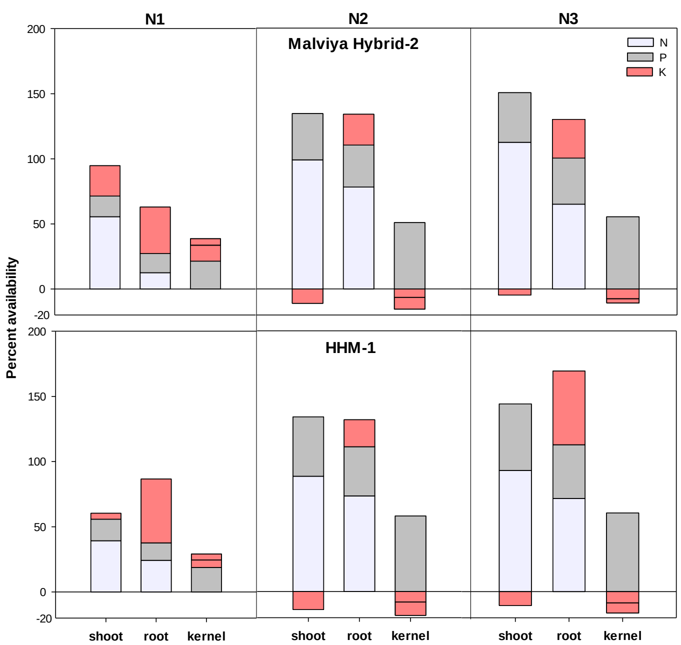

Fig 6. Nitrogen, phosphorous and potassium contents in root shoot and kernels of maize cv. Malviya hybrid- 2 and HHM-1 grown at recommended (N1), 1.5 x recommended (N2) and 2 x recommended (N3) nutrient doses during vegetative and reproductive stages at ambient ozone concentration. Bar represents mean ± SE. Different letters indicate significant differences at $p< 0.05$ as per Duncan's test.

IV. DISCUSSION

Mean O_3 concentration recorded during the experimental period was high enough to cause significant reductions in the agricultural productivity, as evident from the previous works done at the same experimental site (Ghosh et al., 2020; Yadav et al., 2019; Fatima et al., 2019; Singh et al., 2015; Tiwari et al., 2011). The average 8h (M8) O_3 concentration during the present study was observed to be 50.5 ppb, with mean monthly M8 O_3 concentration varying in the range of 40.17- 53.97 ppb. Yi et al. (2016) recorded average O_3 concentration (M7) during the maize growing season to be 40.7, 43.4 and 45.3 in 2013, 2014 and 2015, respectively. The observed O_3 concentrations at the experimental site were markedly higher than the global average O_3 concentration of 40.1 ppb as reported by Mills et al. (2018). Daytime 12 h mean O_3 concentration at the present experimental site increased from 24 to 43.85 ppb during the rainy season (July-October) from 2002- 2006 (Tiwari et al., 2006). Yadav et al. (2020) also observed an increasing trend of O_3 concentration at the present experimental site during the wheat growing season.

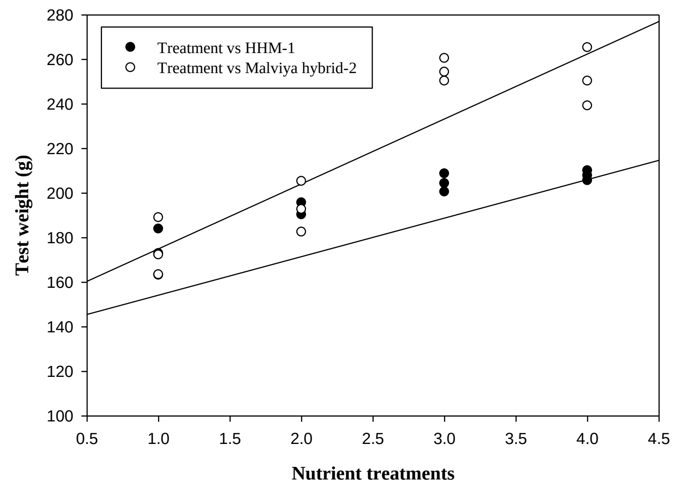

Fig 7. Correlation coefficient between test weight and nutrient doses of two maize cultivars.

Responses of growth parameters like root and shoot length, leaf number and leaf area clearly suggest that soil nutrient amendments were significantly effective in ameliorating O_3 injury in both the cultivars of maize, however, the magnitude of response was different in both the cultivars. Total plant length increased significantly in both the cultivars which was mainly attributed to the accession in shoot length. O_3 stress is known to reduce root growth which can be attributed to the damage to older leaves which serve as main source of photosynthate for root growth (Rai and Agrawal, 2010). In the present study, increased root length is attributed to the increased leaf area, particularly of the older leaves, which ensures a continuous supply of photosynthates for root growth. Singh et al. (2009) also reported significant increments in root and shoot length in mustard (*Brassica compestris* L. var Kranti) at 1.5 recommended NPK dose. Number of leaves plant^{-1} and leaf area plant^{-1} provide information regarding cell division and cell elongation which are two important aspects of plants growth. Studies have shown that O_3 stress has significant negative effect on both these parameters, cell division being more severely affected than cell elongation (Pandey and Agrawal, 1994; Tiwari et al, 2006; Tiwari and Agrawal, 2011). Soil nutrient amendments tend to minimize the effect of O_3 stress on number of leaves plant^{-1} and leaf area plant^{-}

[1] which is evident by the significant increments in these two parameters upon nutrient amendments, with more positive effects on leaf area. Similar response with respect to number of leaves plant^{-1} and leaf area was also reported from the two cultivars of wheat (HUW 510 and LOK-1) upon NPK treatment (Singh et al., 2015). Higher increments in leaf area in Malviya hybrid-2 reflect a better adaptation of this cultivar in ameliorating O_3 stress. Increments in number of leaves plant^{-1} and leaf area plant^{-1} upon nutrient amendments is a positive sign in O_3 stressed plants as these parameters determine the light interception capacity of the crop, enhance the photosynthetic ability, thus producing a positive effect on overall plant growth (Weraduwage et al., 2015). Feedback of growth indices like SLA, SLW, LAR and LWR also indicate towards the tendency to invigorate the response of leaf characteristics (number and area) in O_3 stressed plants upon nutrient amendments.

LWR/LMF is a good index for studying the strategy adopted by plants with respect to photosynthetic allocation in order to minimize O_3 stress. In Malviya hybrid-2, LWR/LMF significantly increased upon soil nutrient amendments, however the increments were less during the reproductive stage as compared to the vegetative stage, suggesting that during the reproductive stage, more biomass was translocated away from the leaves. Since RMF did not show any significant variation in the above case, the probability of biomass translocation towards the developing pods is clearly evident. In HHM-1, LWR/LMF did not follow any definite trend to be correlated with biomass allocation towards reproductive organs. Data suggest that HHM-1 focussed more on biomass accumulation in vegetative plant parts rather than allocation towards the reproductive parts. This is further evident through the luxurious vegetative growth of HHM-1 as compared to Malviya hybrid-1. An overview of these responses leads to the conclusion that the two selected cultivars of maize showed mechanistic alternation in translocation of the photo assimilates from leaves towards the developing cobs. The differential response of the two cultivars towards biomass allocation and accumulation is reflected in two-way ANOVA studies which showed significant effects of age, cultivar and treatment interactions on plant biomass.

Yield reduction is a well-known feature of plants growing under O_3 stress (Yadav et al., 2020, Ghosh et al., 2020; Peng et al., 2019, Fatima et al., 2019). McGarth et al. (2015) calculated the O_3 induced yield losses of about 10 %, for maize in United States between the period 1980-2011. In the present study, an increment in the yield characteristics in both the cultivars suggest a positive effect of soil nutrient amendments on O_3 stressed plants. Malviya hybrid-2 was more responsive in ameliorating O_3 stress as compared to HHM-1, under similar nutrient amendments regimes. This is evident from higher increments in the weight of kernels per cob and test weight (weight of 1000 seeds) in Malviya hybrid-2 as compared to HHM-1. The yield response of the two maize cultivars was directly correlated to the distinctive biomass allocation strategy of the two cultivars. There have been quite a few studies which have shown that the proportion of above-ground biomass allocated to the reproductive parts to be negatively affected by O_3 stress (Betzelberger et al., 2010; Pleijel et al., 2014). The positive effects of soil nutrient amendments were further confirmed by the significant positive correlation between test weight of the plants and nutrient doses (Fig 7). The value of correlation coefficient (r^2) between test weight and nutrient doses was higher for Malviya hybrid-2 (r^2= 0.804) as compared to HHM-1 (r^2= 0.146) which suggest that Malviya hybrid-2 responded more positively to nutrient amendments. The weight of the cobs per plant was however, higher in HHM-1 as compared to Malviya hybrid-2 at all the nutrient treatments. These results can be explained by the fact that a more proportion of the photosynthate allocated towards the developing cobs was utilized in formation of husk leaves in HHM-1. Since HHM-1 is a slow growing cultivar, the period of cob development overlapped with high O_3 concentration. The lower value of CGR in HHM-1 as compared to Malviya hybrid-2, during the reproductive phase confirmed the slow growing nature of HHM-1. Formation of more husk leaves in HHM-1 can be considered as an adaptive mechanism to shield the developing cobs from the direct effect of O_3 stress. Increments in husk leaves of cobs in two maize cultivars (HQPM1 and DHM117) at two elevated doses of O_3 (ambient +15 ppb and ambient + 30 ppb) were also reported (Singh et al., 2014). Increase in the test weight of the kernels did not increase significantly after N2 treatment in both the cultivars which suggest that N2 treatment was sufficient in ameliorating O_3 stress in maize plants. Therefore, in spite of the fact that vegetative characteristics responded more positively at N3 treatment in HHM-1, yield increments were significantly high only at N2 treatment as compared to control. Studies have shown that application of nutrients above a certain dose results in increased O_3 uptake by the plants and the repair mechanisms are no longer able to counteract O_3 induced damage (Harmens et al., 2017; Marzuoli et al., 2018).

In our study, starch contents in grains increased significantly in nutrient treated plants as compared to control along with concurrent increase in soluble and reducing sugars in nutrient treated plants. O_3 stress is known to reduce the starch contents in grain with an increase in soluble and reducing sugars (Yadav et al., 2020). The results of our study suggest that soil nutrient amendments enhanced the allocation of sucrose towards developing kernels. It has been proved that O_3 stressed plants have a tendency to divert more of their photo-assimilates towards strengthening the defense system of plants. In the present study, soil nutrient amendments were capable of reducing O_3 injury due to which allocation of photosynthates towards the developing cobs was boosted. In the present study, increments in soluble sugars and reducing sugars were more in Malviya hybrid-2 as compared to HHM-1. In case of HHM-1, perhaps the photosynthates were diverted towards strengthening of its defense mechanism, rather than their accumulation in kernels. Nitrogen remobilization to the reproductive parts is a sensitive parameter which increases in O_3 stressed plants due to O_3 induced senescence. In our case, although the amount of nitrogen in kernels increased upon soil nitrogen treatment, percentage of nitrogen allocated towards the developing kernels declines significantly with increasing nutrient doses, reflecting a reduction of O_3 stress in nutrient treated plants. The yield quantity and quality characteristics showed no significant difference between N2 and N3 nitrogen treatment doses, indicating that N2 treatment is sufficient in partial amelioration of O_3 injury.

CONCLUSION

The present study clearly demonstrated that soil nutrient amendments can be used as an effective measure to manage O_3 stress in plants. Both the cultivars responded positively towards soil nutrient amendments; however, the magnitude of response varied. The variations in the response of the plants towards nutrient treatments can be attributed to the difference in the biomass allocation strategies adopted by the two cultivars. Positive effects of nutrient amendments were more prominent in Malviya hybrid-2 as compared to HHM-1 cultivar of maize. The variability of the two cultivars in their biomass allocation strategy was clearly evident as the fast-growing cultivar Malviya hybrid-2 was able to allocate more biomass towards the developing cobs, thus sustaining higher yield as compared to HHM-1, which was slow growing. The carbohydrate contents of the kernel also suggest that HHM-1 was utilizing its photo-assimilates more towards defence rather than enhancing yield. Development of more husk leaves in HHM-1 is actually a protective measure adopted by the plants to protect the developing cobs from direct O_3 stress. The mechanistic approach of HHM-1 was more towards protection against O_3 stress which is evident from the development of more husk leaves. Malviya hybrid-2, on the other hand focussed on its biomass allocation strategy, diverting more photosynthates towards the developing cobs. Soil nutrient amendments not only improved the kernel quality characteristics but also partially annulled O_3 induced retention of carbohydrates in the vegetative parts, thus translocating them towards the developing cobs. Dose response studies suggest that 1.5 times recommended dose of soil nutrient amendments was sufficient in partial amelioration of O_3 stress. Higher nutrient dose (2 times recommended) although positively affected the vegetative growth but was not effective in sustaining yield in both the cultivars as compared to lower nitrogen treatment. Based on the results of this experiment, both the cultivars can be recommended to be used for agricultural practices in areas experiencing higher O_3 concentration with a proper and planned management of nutrient application. Malviya hybrid-2, however, is a more favored cultivar owing to the more positive response of its yield attributes towards nutrient treatment upon exposure to ambient O_3.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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