Nitrogen enhanced sorghum morpho-physiological activity, and antioxidant capacity

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Abstract

Imbalanced mineral nutrition and scant information about nitrogen (N) in plants may result in reduction in sorghum morpho-physiological activities. However, farmers use higher or lower fertilizer doses regarding sorghum growth and yield. This study was undertaken to determine the response of sorghum morpho-physiological activities to different rates of nitrogen (N) during two growing seasons of 2017 and 2018. The treatments were consisted of a factorial combination of three N levels (N1 = 0 kg N ha^{-1}, N2 = 150 Kg N ha^{-1}, and N3 = 300 kg N ha^{-1}) and two varieties (V1 = CFSH30, and V2 = Siyong 3180). Treatments were arranged in a randomized complete block design with three replicates. Our results found that N application (N2 and N3) significantly increased plant growth and morpho-physiological activities; leaf length increased by 2.7–8.9%, leaf width by 0.4–4.5%, plant height by 27.8–20.5%, Specific leaf weight by 4.2–10.9%, leaf weight by 18.4–17.4%, and protein contents by 39.5–117.0% compared to N1. Interestingly, higher rates of N reduced number of plants m^{-2} by 42.4–24.8% but increased number of plants m^{-2} weight kg^{-1} by 11.6–62.8%. Moreover, compared with control, N enhanced CAT activity by 92.8-131.9%, SOD by 81.2–84.5%, and POD by 43.6–52.8% in 2017 and 2018. This study indicated that N3 significantly performed best among all treatments during the two growing seasons particularly in case of V1. Moreover, we also concluded that V1 performed better in terms of producing higher leaf width, plant height, specific leaf weight, stem weight, number of plants m^2 (weight/kg^{-1}), protein contents, and antioxidant enzymes activity as compared to V2.

Introduction

Sorghum (*Sorghum bicolor* L.) is an important crop grown for different end uses, including food, forage and industrial productions[1–2]. Worldwide, sorghum is cultivated in 42.12 million hectares with total and average production of 61.38 million tons, respectively[3]. However, its growth and yield is restricted by various biotic and abiotic factors including macronutrients deficiencies especially nitrogen[4].

Nitrogen (N) critically plays indispensable role in crop growth, development and yield formation. Adequat supply of N is crucial for maintaining the morpho-physiological and metabolic processes of the crops including nutrients uptake, antioxidant activities, photosynthesis, and respiration[5–6–7]. However, it's over supply is a serious problem in intensive agriculture production as it leads to soil acidification, enhancement of reactive N components in the environment as well as modification of soil N structure, with consequent deterioration of the ecosystem[8]. Therefore, N application in suitable ratio can candidly regulate crop growth and yield, and escape N pollution[9].

Non judicious use of N can encourage formation of reactive oxygen species (ROS) in crops, that leads to oxidative stress[10] and causes remarkable metabolic changes in plants that disrupt N metabolism[11]. Most of research findings depicts that excessive N supplement in crop production leads to environmental problems including eutrophication, emission of greenhouses gases, and acidic rain[12]. On the other hand, its deficiency causes early maturity and decreased yield along with poor quality[13]. In cereal crops, insufficient supply of N results in slender stalks, few tillers, smaller head, and grains with low protein contents.

Additionally, the highest effects of N deficiency decreased leaf length and width, light interception, and biomass and grains production[14]. Thus, monitoring of N in crops is vital to optimize its accurate rate for recommendation to the growers[15–6–7].

Substantial consideration has been received recently due to variation in nitrogen (N) availability caused by human activities[16]. Crop development and grain production vary broadly in response to N accessibility[17]. Recently, significant efforts have been made by researchers to develop N management tactics for increasing its use efficiency, by taking into consideration the 4R nutrient managing ideologies (accurate source, accurate rate, accurate time, and accurate placement[15].

Previous studies have been propounded that as compared to soybean and maize, sorghum is worthier pertinent for production on marginal land under suitable dose of N application[18,19]. The most considerable nutrient for enhancing yield and nutritional value of sorghum is N. In Brazil about 80 percent of sorghum production has reduced due to inappropriate rates of N, poor selection of sorghum species and inadequate soil management practices[20]. Deficiency of N in sorghum crops results in lower biomass production due to reduction in leaf area, chlorophyll contents, and photosynthetic rate[21]. Adams, Erickson, & Singh (2015)[22] reported, that 70 kg ha^{-1} N application was optimum to improve sorghum biomass yield (20.8 Mg ha^{-1}). Erickson, Woodard, & Sollenberger (2012)[23] conducted two studies and reported no significant effect of N on sorghum biomass yields.

Due to wide spread of N, modern agriculture is extensively polluting the natural environment[24]. Therefore, it is necessary to consume a suitable ratio of N to achieve higher yield of sorghum crop. N management at suitable rate is the main significant factor for improving soil fertility and obtaining higher yield[25]. In spite of the immense importance of N in crop production, impacts of higher levels of N application on morpho-physiological processes of sorghum growth and development traits are largely unknown. Maximum studies are addressing that sorghum requires low ratio of N as compared to maize and other crops[15]. However, much less attention has paid to examining the higher levels of N on sorghum morpho-physiological activities to achieve higher yield.

Based on these premises, a field trial was conducted to optimize a suitable level of N for healthier sorghum morpho-physiological activities and antioxidant capacity, and to evaluate appropriate sorghum varieties for higher biomass production.

Results

Leaf length (cm), leaf width (cm), plant height (cm) and specific leaf weight (g)

Varieties and varying levels of N significantly enhanced the sorghum growth parameters including leaf length, width, plant height, and specific leaf weight during the two consecutive growing seasons (Tables 1 and 3). It is clear that during the second year leaf length measured at the applied rates of N2 and N3 increased by 2.7 & 8.9% respectively as compared to N1. As compared with variety V1, V2 variety increased leaf length by 9.1%.

Table 1
Significance test of variation sources (mean square), and their effects on leaf length, width, plant height and specific leaf weight during 2017 and 2018.

Source	Leaf length (cm)	Leaf width (cm)	plant height (cm)	Specific leaf weight (g)
C (cultivar)	215.0**	10.4 **	221.0 ns	74.5**
R (ratio of N)	83.9 ns	0.5 ns	1823.5*	3.6*
Y (year)	517.4**	2.6 ns	28665.2**	381.9**
C×R	50.9 ns	0.7 ns	2194.0**	7.4**
C×Y	471.2**	9.0**	42.2 ns	13.0 **
R×Y	102.6*	0.2 ns	1741.2*	7.8**
C×R×Y	26.2 ns	1.3 ns	82.7 ns	9.1**
Error	35.2	0.7	483.6	1.1
Year				
2017	73.1 b	4.9 a	402 a	145.6 a
2018	68.7 a	4.6 a	50 b	113.0 b
N levels				
N1	69.7 b	4.7 a	121.6 b	32 b
N2	70.5 ab	4.6 a	135.6 a	38 a
N3	72.6 a	4.8 a	130.6 ab	36 a
Cultivars				
CFSH30	69.5 b	5.07 a	180.7 a	7.1 a
Siyong3180	72.3 a	4.4 b	127.8 a	5.4 b

C: cultivar; R: rates of nitrogen; Y: year. * and ** significant at 5 and 1% probability level, respectively.

Table 3
Effects of N rate on leaf length, width, plant height and specific leaf weight of two sorghum varieties during 2017 and 2018.

Variety	N rate (kg hm^{-2})	2017				2018			
		Leaf length (cm)	**Leaf width (cm)**	**plant height (cm)**	**Specific leaf weight (g)**	**Leaf length (cm)**	**Leaf width (cm)**	**plant height (cm)**	**Specific leaf weight (g)**
CFSH30	0	69.3 a	5.3 a	153.7 a	8.9 ab	67.7 a	5.0 a	103.5 a	6.5 a
	150	69.3 a	5.4 a	141.3 a	8.8 ab	66.4 a	4.2 a	115.0 a	3.8 b
	300	70.3 a	5.9 a	148.0 a	10.3 a	74.3 a	4.6 a	123.0 a	4.4 b
Siyong 3180	0	77.3 a	4.3 b	138.0 a	6.7 b	64.5 a	4.5 a	91.3 a	3.7 b
	150	77.0 a	4.4 b	152.3 a	7.4 b	69.5 a	4.6 a	134.0 a	3.7 b
	300	75.7 a	4.2 b	140.3 a	6.9 b	70.4 a	4.8 a	111.3 a	4.4 b

Sorghum plants were sampled on the 90th day after sowing in the year of 2017 and 2018. Different letters in the same column with in the same year in the table are statistically different at the 0.05 probability level by an ANOVA-protected test.

Similar to leaf length, the leaf width was significantly affected by varieties and variety X year interaction. During the first year, application of N2 and N3 significantly increased leaf width by 0.4 & 4.5% respectively as compared to N1. V1 variety showed better performance and increased leaf width by 27.0 & 2.2% respectively during the two growing seasons as compared V2. During the second year, the higher rates of N2 and N3 significantly reduced leaf width by 6.4 & 0.21% respectively when compared with N1.

Furthermore, plant height was significantly affected by varying levels of N and varieties. As compared to N1, the application of N2 significantly increased plant height by 1.2 & 27.8% during two growing seasons, respectively. In 2017, the application of N3 reduced plant height by 1.2%. V1 performed better and enhanced plant height by 2.7 & 1.4% as compared with V2.

Moreover, specific leaf weight was influenced by all factors. The rates of N2 and N3 significantly increased specific leaf weight by 4.2 & 4.0% and increased by 10.5 & 10.9% respectively during the two growing seasons as compared to N1.V1 significantly enhanced specific leaf weight by 33.5 & 33.3% during 2017 and 2018 respectively as compared to V2.

Table 2
Significance test of variation sources (mean square), and their effects on soluble protein and antioxidant enzyme activity during two growing seasons.

Source	Soluble protein(mg g^{-1})	SOD (μg min^{-1})	CAT(μg min^{-1})	POD(μg min^{-1})
C (cultivar)	177.8**	3.2 ns	17252 ns	59714**
R (ratio of N)	257.6**	7343.9**	44508**	240062**
Y (year)	76.0 ns	43.7**	131252**	95926**
C×R	22.2 ns	12.9 ns	17558*	6612 ns
C×Y	0.8 ns	726.9**	3008 ns	10098*
R×Y	222.4**	177.4**	20008*	43825**
C×R×Y	46.6 ns	91.0**	14908 ns	3582 ns
Error	30.9	5.6	5357	2416
Year				
2017	14.0 a	23.0 a	150.9 a	271.7 b
2018	12.3 a	20.0 b	81.2 b	331.3 a
N levels				
N1	11.6 b	5.7 c	76.6 b	210.9 c
N2	11.6 b	25.3 b	127.5 a	324.4 b
N3	16.2 a	33.5 a	144.1 a	369.3 a
Cultivars				
CFSH30	14.4 a	21.7 a	128.7 a	278.0 b
Siyong3180	11.8 b	21.3 a	103.4 a	325.0 a

C: cultivar; R: rates of nitrogen; Y: year. * and ** significant at 5 and 1% probability level, respectively.

Stem weight (g) and leaf weight (g)

Various levels of N, and cultivar had significantly affected stem weight but no significant differences were observed for leaf weight (Table 4). As compared with V2, V1 showed higher stem weight and an increase by 36.0%. As compared with N1, the rate of N3 significantly increased stem weight by 0.5% and decreased by 17.0% with applied rate of N2.

Table 4

Effects of N rates on Stem weight, leaf weight, and sorghum twice cut (numbers of plant m^2 and weight kg^{-1}) of two sorghum varieties during 2017 and 2018.

Variety	N rate ($kg\ ha^{-1}$)	Stem weight (g)	Leaf weight (g)	2017		2018	
				Numbers of plant (m^2)	**Numbers of plant (m^2) weight kg^{-1}**	**Numbers of plant (m^2)**	**Numbers of plant (m^2) weight kg^{-1}**
CFSH30	0	83.6 ab	20.1 a	64.3 a	1.2 ab	38.0 a	1.2 b
	150	73.4 ab	16.0 a	38.7 a	0.8 ab	15.7 b	1.0 b
	300	105.9 a	18.0 a	36.0 a	1.5 a	23.7 ab	1.2 b
Siyong3180	0	77.3 ab	16.9 a	41.0 a	0.5 b	32.0 ab	1.0 b
	150	59.4 b	18.7 a	57.0 a	1.1 ab	24.7 ab	1.1 b
	300	55.7 b	18.8 a	48.3 a	1.3 ab	29.0 ab	1.8 a

Sorghum plants were sampled on the 90th day after sowing. Different letters in the same column in the table are statistically different at the 0.05 probability level by an ANOVA-protected test.

* and ** indicate statistical significance at the 0.05 and 0.01 probability level, respectively. NS indicates statistical insignificance.

Sorghum cutting (number of plants m^{-2} and weight kg^{-1})

Varieties and different rates of N had significantly affected the number of plants m^2 during the second cut but no significant differences were observed during the first cut (Table 4). The application of N significantly decreased number of plants m^2 by 9.1 & 42.4% at applied rates of N2 and decreased by 19.8 & 24.8% at the applied rates of N3 as compared to N1. V1 significantly reduced number of plants m^2 by 5.0 & 9.7% as compared with V2 during twice cuts.

Similarly, weight kg^{-1} was significantly affected by various rates of N and cultivars during twice cut. As compared to N1, the rate of N2 significantly increased weight kg^{-1} by 11.6% during first cut and reduced by 8.9% during the second cut. The higher rate of N3 had also significantly increased weight kg^{-1} by 62.8 & 30.4% during twice cut.

Protein contents, superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD)

Cultivars and various levels of nitrogen had significantly enhanced protein contents during the first year but no significant differences were observed during the second year (Table 5). Protein contents were significantly affected by N, and variety (Table 2). The application of N2 and N3 significantly increased soluble proteins by 39.5 & 117.0% during the second year and reduced by 21.9 & 1.7 during the first year as compared to N1. As compared to V2, V1 showed higher protein contents and increased by 21.7 & 13.5% respectively during two growing seasons.

Table 5
Effects of N rate on protein contents, super oxide dismutase (SOD), catalase (CAT), and peroxidase (POD) of two sorghum varieties during 2017 and 2018

Variety	N rate (kg ha^{-1})	2017				2018			
		Soluble protein (mg g-1)	**SOD (μg min-1)**	**CAT (μg min-1)**	**POD (μg min-1)**	**Soluble protein (mg g-1)**	**SOD (μg min-1)**	**CAT (μg min-1)**	**POD (μg min-1)**
CFSH30	0	15.8 a	6.8 d	71.7 b	204.6 c	10.3 a	4.5 d	87.5 a	183.3 c
	150	13.7 ab	24.6 b	185.0 ab	271.1 ab	10.4 a	25.4 c	64.2 a	299.5 bc
	300	16.6 a	36.8 a	174.2 ab	297.9 a	19.9 a	31.8 b	38.3 a	411.7 ab
Siyong 3180	0	14.6 ab	5.3 d	100.8 b	232.2 bc	5.9 a	6.1 d	46.7 a	233.4 c
	150	10.1 b	18.2 c	147.5 ab	307.0 a	12.3 a	33.1 ab	113.3 a	420.0 ab
	300	13.3 ab	28.2 b	226.7 a	317.5 a	15.3 a	37.1 a	137.5 a	450.2 a

Sorghum plants were sampled on the 90th day after sowing. Different letters in the same column in the table are statistically different at the 0.05 probability level by an ANOVA-protected test.

* and ** indicate statistical significance at the 0.05 and 0.01 probability level, respectively. NS indicates statistical insignificance.

Varieties and various levels of N had significantly affected CAT activity during the first year but no significant differences were observed during the second year (Table 5). CAT was significantly affected by cultivar, and the interaction between N and cultivar (Table 2). The CAT activity was increased with the applied rates of N2 and N3 by 92.8% and 131.9% respectively as compared to N1. V1 reduced CAT activity by 9.3% and 36.1% during the two growing seasons respectively as compared to V2.

Similarly, SOD was significantly affected during two growing seasons (Table 5). The activity increased by 74.4 & 81.9% at N2, and increased by 81.2 & 84.5% at N3 in 2017 and 2018 as compared with N1. As compared with V2, V1 increased SOD activity by 24.2% in 2017 and reduced by 23.0% in 2018.

Moreover, POD activity was significantly affected by cultivar and N during two years (Table 5). As compared with N1, at N2 POD activity increased by 24.6 & 43.4%, and at N3 increased by 28.8 & 52.8%, respectively during growing season. V1 showed lower performance and reduced POD by 10.7 & 22.0% during two years as compared with V2.

Discussions

Achievement of higher plant growth and yield mainly depends on knowing crop nutritional requirements throughout the cycle. Certainly, some visible symptoms and growth reduction in crops caused by biotic and biotic stress are diminished when the uses of nitrogen (N) application is tolerable and intensified by fertilizer deficiency[40]. N is a major required nutrient for the synthesis of some elements in plants such as amino acids, nucleic acid and organic acids, all of which are an important factors for plant growth and development[41]. Ahmad et al. (2019)[6] also reported that nutrients, mainly N application plays vital role in crop growth and productivity and/or is involved in various biological activities. N fertilization depends on plants species, environmental factors and soil physical properties including soil textures and soil fertility. Many countries in the world are still suffering from mismanagement of N application, so our study was to investigate whether varying levels of N1, N2, and N3 application could enhance morpho-physiological activities of the two sorghum genotypes.

In the current study, various levels of N and varieties significantly enhanced all the growth parameters of sorghum. Leaf width during the first year and specific leaf weight during two years were significantly enhanced at the applied rates of N2 and N3 respectively as compared with N1. This might be due to the variations in climatic conditions, longer growth period, or residual effects of N application. Our research findings are in accordance with the study of [21,42], who reported that year variances and climatic conditions had significant consequences on sorghum growth. Our findings further explored that V1 produced higher leaf width and specific leaf width as compared to V2. N treatments might had enhanced the growth characteristics of V1 variety via higher net photosynthesis activity and quick CO_2 assimilation[21–43].

Kropat et al. (2011)[44] also reported that application of N fertilizer resulted in enhanced leaf width, thickness and more resources being allocated to crops growth. In 'Hongyang' Kiwifruit (*Actinidia chinesis*) higher application of N consequenced in higher leaf width. N application led to increase in leaf width and mainly enhanced the size of an individual leaf [45]. Our results are also in agreement with the findings of [46], who reported that leaf width and specific leaf weight increased gradually with higher treatments of N as compared to untreated plants in Bellflower. Similar results are also reported by[47] in Aloe vera crops.

Additionally, in our study plant height and leaf length were significantly affected by year, and the interaction between N and year. This was consistent with results from[48] in sorghum, and[49] in marigold. Macronutrients promoted plant height and leaf length, particularly N enhanced length and numbers of internode that might be the possible reason of significance in plant height. Our research is supported by results of[32], who reported that plant height and leaf length were increased linearly from 4.65 to 4.95m as N fertilization increased from 0 to 268 kg ha^{-1}. Additionally, our results however, were not similar with the findings of[45] Kiwifruit and[50], who reported that leaf length was reduced under the higher ratio of N in cowpea. This reduction was observed due to some environmental factors such as light that directly influenced further leaf expansion under higher amount of N supply.

Furthermore, stem weight was significantly enhanced by varying levels of N, and cultivars. The applied rate of N3 increased stem weight as compared to N1. Actually, stem accumulates more N as compared to others crop growth parts might be the possible reason for higher stem weight. Our study is in unison with the results of[21], who reported that stem accumulates more ratio of N due to which crops produce higher biomass yield. Though, we did not measured N uptakes by sorghum, but higher plant growth and leaf length in the corresponding parameters signals to its relative higher uptakes[51].

Number of plants m^{-2} and weight kg^{-1} were significantly enhanced by different rates of N, and cultivars. Weight Kg^{-1} was increased at applied rates of N2 and N3 during twice cut as compared to the N1. The published literature pertains that N plays a vital role in plant biomass yield and plants population and plants ultimately produce higher weight with its increasing levels[45]. Barbanti, Di Girolamo, Grigatti, Bertin, & Ciavatta (2014)[52] reported higher numbers of plants with application of higher rate of N @ 250 kg ha^{-1} in maize crop. And less numbers of plants were observed at applied dose of N @100 kg ha^{-1} by[53].

Our findings further confirmed that soluble proteins were significantly affected by different levels of N, and cultivars. During the first year, protein contents were significantly increased by 39.3% and 117.0% at N2 and N3 application respectively as compared to N1. Comparing the two genotypes, V1 cultivar produced higher protein contents as compared to V2. In fact, the increase in soluble protein contents in V1 might be the possible result of the applied N fertilizer or of the genetic backgrounds of cultivar. Our research findings are however similar with the results of[54], who reported that protein contents significantly increased when the rate of N was increased.

The antioxidant enzymes catalase (CAT) was significantly affected by different rates of N, and cultivars during the first year. But no significant differences were observed during the second year Moreover, SOD and POD was significantly affected by various rates of N and cultivar in 2017 and 2018. The antioxidant activities were significantly increased at the applied rate of N2 and N3 as compared with N1. Therefore, applying N can improves the antioxidant defense mechanism of sorghum crop, which is efficient enough to remove ROS. According to[37], fertilizer application increased the antioxidant enzymes activities and avoided ROS increment in *Alnus cremastogyne* under stress condition. Other researches also indicated that increase in antioxidant enzymes activity might be due to the composition of chemical fertilizers and their effect on soil biota and plant metabolism[6–55]. ROS ($O_2^{\bullet-}$ and H_2O_2) works as a signaling molecule in plant cells; but their excessive production in plant cells can activate fragmentation of DNA, proteins degradation, lipid peroxidation, and may even lead to cell demise[56]. Our results are in accordance with the findings of[6,57], who reported that combined application of NPK at increasing levels significantly increased antioxidant activities in cotton crop.

Materials And Methods

Site Description and Soil preparation

A two-year field experiment was conducted in the same field in 2017 and 2018 at the Experimental Farm of Yangzhou University, Jiangsu Province, China (32.30∘ N, 1198 25∘ E). The previous crops grown on field were cotton (*Gossypium hirsutum* L.) and castor (*Ricinus communis* L). The soil was sandy loam of a Typic fluvaquents Entisols. Soil organic carbon was determined by using the Walkley-Black chromic acid wet oxidation approach[26]. Soil N content was measured by following the Kjeldahl approach[27]. The available phosphorus (P) was determined by following the Micro-Vanadate-Molybdate approach[28] The available potassium (K) was formulated by following the neutral ammonium acetate extract approach using a flame photometry[29]. The soil contained 1.22% organic matter, 1.0 g kg^{-1} total N, 14.1 mg kg^{-1} P, and 77.3 mg kg^{-1} K (pH 7.1).

Experimental design and Plant materials

The study was designed as a two factors factorial experiment following randomized complete block design with the split-plot arrangement. The first factor included two varieties CFSH30 and Siyong 3180 abbreviated as V1 and V2 and the second factor was N rates of 0, 150, and 300 kg N ha^{-1}, abbreviated as N1, N2, and N3 respectively.

Ethical Statement

Forage Sorghum variety CFSH30 from Canada was used in this study. The variety was improved by Dr OM Dangi in 2012 and provided by Canada Environmental Renew Co. Ltd. Siyong 3180 variety from China was collected in 2018 by Mengma Agriculture Company plz. The plant materials were provided by Chinese Academy of Agricultural Sciences.

The seed was stored in brown bags in fridge (4 ^{O}C) to maintain good germination ability. Before sowing, seeds of each variety were selected for uniform color and size. Germination percentage was recorded 90.5% and 90.7% for CFSH30 and Siyong 3180. On May 26, both in 2017 and 2018, the seed was hand broadcast at the rate of 4.5 kg ha^{-1} in 18 plots of 10.5 m^2 (3.5 m × 3. 0 m) sizes with plot to plot distance of 40 cm.

Phosphorus (P) was broadcasted twice at equal rate of 60 kg ha^{-1} per time at seeding and plant elongation stages[30]. The N was applied as urea to all treatments in solid form. The N and P fertilizer were applied during sowing at the same time to each plot by hand-broadcasting. Others field practices, like, mowing between lines, weeding, and pest and disease control were carried out in accordance with local recommendations.

Observation and measurement

Plant growth parameters

On the 90th day of seeding (DAS) ten uniform plants were randomly collected in each plot and leaf length (cm) and width (cm) were measured[31].

Plant height (cm) was measured using a survey rod from the bottom of soil to the top of the panicles[32].

To determine specific leaf weight, the second top leaves were selected and weighted through electronic balance.

To determine biomass production, whole plants were harvested at 90th day after sowing from the center of plots and hand-separated into leaf and stem. The fresh weight of leaves and stem were examined[33].

Sorghum was cut repeatedly: into two cut-systems. Raw materials were accumulated and determination were made on the numbers of plant m^2 and weight kg^{-1} through electrical scale[34].

Determination of physiological parameter

0.5 g leaves sample were used to determine protein soluble activity. The sample was homogenized at 4 ^oC in 5 mL Na-phosphate buffer (pH 7.2) and directly centrifuged at 4^oC at 10,000 rpm for 15 minutes. The supernatants were kept on ice for further examination. Soluble protein content was measured by using the Coomassie blue dye-binding assay following the method of[35]. Absorbance readings were converted into protein contents using bovine serum albumin (BSA) as the standard curve[36]. Supernatants and dye were pipetted in spectrophotometer cuvettes and absorbance was recorded using a spectrophotometer (Model 721, Shanghai Mapada Instruments Co. Ltd, Shanghai, China) at 595 nm.

To determine antioxidant activities, 0.5 g fresh leaves were crushed in a mortar containing 5 ml extraction buffer (50 mM Tris-HCl [pH 7.0], 0.1 mM EDTA, 1 mM AsA, 1 mM dithiothreitol and 5 mM $MgCl_2$). The resultant homogenates were centrifuged at 10,000 g for 15 minutes at 4^0C, and the obtained supernatants were used to determine the activity of catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD)[6–7–37]. SOD activity was measured by computing the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT)[38]. The structure of the reaction combination (3 ml) was 50 mM of Tris-HCl (pH 7.8), 13.37 of mM methionine, 0.1 mM of NBT, 0.1 mM of riboflavin, 0.1 mM of EDTA and 0.1 ml of the enzyme extract. Peroxidase (POD) and catalase (CAT) activities were assessed following the methods of Tariq et al. (2018)[37] with minor changes. Due to guaiacol oxidation, POD activity was evaluated by calculating the increase in absorbance at 470 nm. The reaction combination was composed of 50 mM Tris-HCl (pH 7.0), 10 mM of guaiacol, 5 mM of H_2O_2 and 0.1 ml of the enzyme extract. One unit of POD activity was determined as an absorbance variation of 0.1 units per min. In addition, CAT activity was measured by determining the decrease in the absorbance of H_2O_2 at 240 nm, respectively. The structure of the reaction combination (3 ml) was 50 mM of Tris-HCl (pH 7.0), 0.1 mM of EDTA, 12.5 mM of H_2O_2 and 0.1 ml of the enzyme extract. One unit of enzyme activity was distinct as a 0.01-change in the absorbance at 240 nm per min.

Statistical analysis

Compound data analysis of two years (2017 and 2018) was conducted based on randomized complete block design with split-plot arrangement (two cultivars and three N rates). Analysis of variance was carried out using Mstate-C sofware[39]. Least significant difference test (LSD) was used when the F values were significant ($P \leq 0.05$).

Conclusions

The study examined the effects of various levels of N on morpho-physiological activities of two sorghum varieties. We concluded that sorghum growth and physiology responded more to higher rates of N as compared with N1. The application of N2 and N3 enhanced all the morpho-physiological activities such as leaf length, leaf width, plant height, Specific leaf weight, stem weight, leaf weight, weight kg^{-1}, protein contents, and antioxidant enzymes activities. Moreover, higher application of N reduced the number of plants m^2 during two cuts as compared to N1. Both sorghum varieties exhibited potential increase but V1 showed higher morpho-physiological activity as compared with V2. All these indices sorted out the best practices for achieving higher sorghum biomass yield but N3 was much appropriated during the two growing seasons particularly for V1 variety. Therefore, it can be concluded from the results that higher rate of N3 and V1 cultivar is promising alternative for good economic revenues at Yangtze River China. However, additional investigation is required to examine the impact of nitrogen from different sources in more sorghum varieties. Consequently, nitrogen fertilizer management is needed to provide growth and yield of crops.

Declarations

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Author contributions statement

The field and design were arranged by Zhou Guisheng and experiment were conducted by Irshad Ahmad. Data was collected by Irshad Ahmad and analyzed by Guanglong Zhu and Xudong Song. Muhi Eldeen Hussein Ibrahim help in the collection of data. The article has been written by Irshad and reviewed by Yousaf Jamal, the paper grammatically checked by Shahid Hussain, Ebtehal Gabralla and Wangjian jun.

Additional Information

Competing of interests

The authors declare no competing of interests.

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