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Supplemental Sodium Nitroprusside and Spermidine Regulate Water Balance and Chlorophyll Pigments to Improve Sunflower Yield under Terminal Drought

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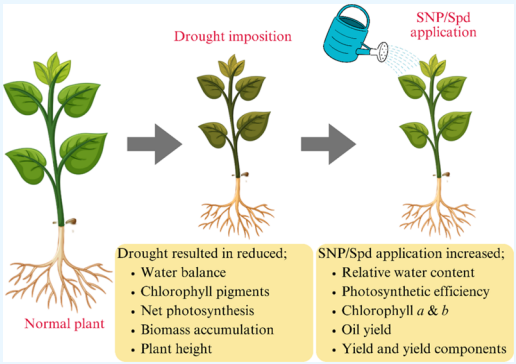
ABSTRACT: Drought is an inevitable environmental stress that drastically hampers the growth, productivity, and quality of food crops. Exogenous sodium nitroprusside and spermidine have decisive functions in the growth enhancement of plants; nevertheless, their specific role in mediating stress responses to improve drought tolerance in sunflowers at the reproductive stage (terminal drought) remains largely unknown. In the present study, we explored the positive effects of sodium nitroprusside and spermidine on physiological responses to increase in sunflower yield during periods of terminal drought. Initially, various doses (50, 100, 150, 200, 400 μM) for each sodium nitroprusside or spermidine were foliar sprayed to improve water content, chlorophylls, and biomass accumulation in sunflower seedlings under control (100% FC) and drought (60% FC) conditions. Optimized rates (100 μM for sodium nitroprusside) and (100 μM for spermidine) were further tested alone and in combination to assess drought tolerance potential and their ultimate impact on yield under drought stress. Drought exposure caused a marked reduction in relative water content (26%) and chlorophyll *a* (31%) and *b* (35%) contents; however, sodium nitroprusside and spermidine at 100 μM significantly improved the growth of sunflower (13%). Furthermore, combined use of sodium nitroprusside and spermidine at 100 + 100 μM markedly improved the achenes per head (16%), 1000-achene weight (14%), and ultimately grain (28%) and oil (21%) yields of sunflowers under drought stress. A strong association was found between the 1000-achene weight and the achene yield of sunflower. Hence, combined sodium nitroprusside and spermidine upregulate water balance and chlorophyll contents to increase sunflower yield under terminal drought.



1. INTRODUCTION

Drought as an extreme effect of climate variability has become a severe natural disaster to crop productivity, ecosystems, and humans.[1] Due to uneven climate patterns and extreme weather events, about 20% of the agricultural land area has been shifted to dry regions due to exposure to dry spells. Severity of drought is becoming more frequent and is expected to rise 1% by 2100. Currently, drought stress has become a major issue in agricultural production around the world.[69] As a more devastating stress factor, drought usually disrupts tissue water relations, nutrient absorption, and metabolic processes that makes the plants survival difficult to drought situations.[2,3] Drought also causes chlorosis and loss of cell turgidity, impairs net photosynthesis and root growth, assimilates translocation, imbalances membrane permeability, alters cell functions, and impairs enzyme activation and stomatal conductance, consequently reducing crop productivity.[4,5,70−72] As a main consequence of drought stress, overaccumulation of reactive oxygen species (ROS) causes oxidative damage to proteins, lipids, and membranes, thus minimizing normal functioning and even causing the death of plants.[6,7]

Use of mineral nutrients, phytohormones, osmoprotectants, and signaling molecules to plants has gained much attention to alleviate the losses induced by drought stress.[8,73] As an effective signal molecule, sodium nitroprusside application has proven effective in enhancing the flavonoids, phenolics, proline, soluble sugars, ascorbic acid, and glutathione metabolism in plants, which helps the plants to survive under adverse drought periods.[9,10] Recently, sodium nitroprusside has been suggested to contribute actively to maintaining membrane permeability for reducing ions leakage,

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preserving water balance, and improving chlorophyll content, photosynthesis, stomatal conductance, transpiration, and enzyme activation in plants under drought stress.[11,74,75] Exogenous sodium nitroprusside inhibits the gene expression of chlorophyllase, senescence hexokinase-1 gene, and senescence-associated gene 12 (SAG12) in further reducing chlorophyll degradation in drought-induced plants. Additionally, sodium nitroprusside upregulates iron homeostasis by restricting aconitase activity (Fe−S-containing enzyme) to adjust the iron concentration in plants for chlorophyll biosynthesis.[12,13] As a ubiquitous plant regulatory molecule, sodium nitroprusside has a key function in transcriptional modifications and lowering lipid peroxidation via direct scavenging of cellular free radicals, inducing drought resistance in plants.[14]

Additionally, spermidine, as a low-molecular-weight polyamine, also plays a crucial role in plant development under stressful environments. Exogenous spermidine application could effectively alleviate the drastic effects of drought via the accumulation of phytohormones and polyamines.[15,16] As previously reported, spermidine is involved in improving plant growth, health, and metabolism under drought stress by increasing the absolute water content, photosynthetic capacity, and antioxidative enzyme activities.[17] As a stress ameliorant, spermidine acts as a key scavenger for malondialdehyde and hydrogen peroxide via the upregulation of metabolic enzymes, which consequently improves drought tolerance in plants.[18] Moreover, spermidine induces cellular accumulation of proteins and organic solutes to retain a higher water balance in drought-stressed plants. Spermidine is also associated with maintaining cationic−anionic stability in plants, thus improving plant development under drought stress.[19,20]

Production of sunflowers in drought-prone areas is a huge challenge of drought stress, predominantly during drought at the reproductive (flowering) phase, which is the most sensitive growth stage.[21] Most of the studies have focused on the positive responses of sodium nitroprusside and spermidine on sunflower development under drought stress, while reports on increased drought tolerance in sunflower with sodium nitroprusside and spermidine protectants under terminal drought are less explained.

Here, we hypothesize that supplemental sodium nitroprusside and spermidine could potentially mediate the physiological processes in sunflowers for enhancing tolerance to terminal drought stress. Hence, the current studies were conducted with the objective of assessing the positive influence of sodium nitroprusside and spermidine on the relative water content, chlorophyll pigments, and growth of sunflowers and their further impact on the grain yield of sunflowers under terminal drought.

2. MATERIALS AND METHODS

2.1. Seed Source and Study Site. Mature seeds of sunflower (hybrid Hysun-33) used in the current experiments were obtained from ICI (Pvt.) Limited, Pakistan. The randomly selected and high-vigor seeds were surface sterilized with 10% NaOCl (sodium hypochlorite) solution for 5 min and then washed carefully with distilled water. The rinsed seeds were then air-dried to achieve the original moisture content.

The experiments were conducted at the Research Area, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan (latitude 30°−08′ N, longitude 71°−26′ E, and altitude 122 m).

2.2. Soil Analysis and Climatic Conditions. Prior to planting seeds, the experimental soil was analyzed for its physiochemical properties.[22] The textural class of the experimental soil was characterized by the hygrometer method.[23] The measured soil characteristics are listed in Table 1. The temperatures (minimum and maximum), rainfall, and relative humidity for both pot and field trials are presented in Table 2.

Table 1. Physical and Chemical Properties of the Experimental Soil[a]

soil properties	unit	pot soil	field soil
	Physical		
sand	%	56	60
silt	%	23	22
clay	%	21	18
textural class		clay loam	clay loam
	Chemical		
OM	%	0.53	0.55
pH		8.0	7.9
ECe	mS/cm	2.82	2.98
SP	%	35	34
N	%	0.041	0.065
P	mg/kg	9.23	11.8
K	mg/kg	197	224

[a]OM, organic matter; ECe, electrical conductivity of saturated soil extract; SP, saturation percentage; N, nitrogen; P, phosphorus; K, potassium.

Table 2. Meteorological Conditions at the Experimental Site during the Cropping Season of Sunflower[a]

month	temp. max. (°C)	temp. min. (°C)	RF (mm)	RH (%)
first quarter-Aug	36.7	29.8	0	57.5
second quarter-Aug	36.1	30.0	0	55.0
third quarter-Aug	37.5	28.8	0	50.8
fourth quarter-Aug	36.1	28.1	0	54.4
first quarter-Sep	36.4	37.4	0	53.9
second quarter-Sep	35.2	27.3	0	58.9
third quarter-Sep	37.4	28.3	0	52.1
fourth quarter-Sep	34.5	26.2	0	57.3
first quarter-Oct	35.6	26.4	0.1	54.2
second quarter-Oct	35.4	21.6	0	33.2
third quarter-Oct	34.1	17.3	0	29.6
fourth quarter-Oct	30.0	18.2	0	41.9
first quarter-Nov	28.6	16.5	0	39.9
second quarter-Nov	29.6	14.5	0	38.5
third quarter-Nov	26.4	11.3	0	37.0

[a]Temp. Max, temperature maximum; Temp. Min, temperature minimum; RF, rainfall; RH, relative humidity.

2.3. Experimental Details. *2.3.1. Pot Study: Dose Optimization for Sodium Nitroprusside and Spermidine Treatments under Drought Stress.* Uniform and randomly selected rinsed seeds (7/pot) were sown at 5 cm soil depth of 5 L capacity earthen pots. Each pot with 23.9 cm diameter and 25.4 cm height was filled with 4 kg of air-dry and ground soil mixture containing clay loam soil, sand, and peat material (3:1:0.5 w/w). All pots were arranged in a wire-house under

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natural light conditions during the cropping season of August 1 to 28, 2021 and sheltered from rainwater by a polyethylene plastic sheet. The optimum doses for urea (120 kg/ha), diammonium phosphate (84 kg/ha), and potassium sulfate (62 kg/ha), corresponding to 240, 168, and 124 mg/pot, respectively, were applied to fulfill the demands for NPK nutrients. All P, K, and 1/2 N was mixed with the top soil (0−15 cm) during pot filling, while the remaining 1/2 N was top-dressed at the four-leaves unfolded (BBCH code 14) stage. Weak seedlings were removed by thinning to keep three healthy plants in each pot.

At the five-leaves unfolded (BBCH code 15) stage, the plants were separated into experimental conditions, as one set of plants were retained at 100% FC (field capacity, control treatment), whereas another set was kept at drought level of 60% FC (drought treatment). The drought stress was first imposed by withholding the irrigation water until the plant leaves had shown wilting symptoms. The soil moisture content lost to fulfill the demands of evaporation and transpiration was added by weighing the pots two times a day until they gained 100 and 60% FC levels.

Foliar application of sodium nitroprusside (50, 100, 150, 200, 400 μM) (Na_2 $[Fe(CN)_5NO]\cdot 2H_2O$; purity ≥99.0%; Sigma-Aldrich) and spermidine (50, 100, 150, 200, 400 μM) ($H_2N(CH_2)_3NH(CH_2)_4NH_2$; purity ≥99%; Alfa Aesar, Germany), having Tween-20 (0.1%) as a surfactant, was done twice, i.e., at the time of drought stress initiation and 1 week later. A control treatment against sodium nitroprusside or spermidine solutions was also maintained by spraying plants with distilled water containing 0.1% Tween-20. Foliar application of sodium nitroprusside and spermidine elements under control and drought stress conditions (12 treatments comprising 2 drought levels × 6 sodium nitroprusside/spermidine levels) was repeated three times following the CRD factorial design. The plants were harvested at the 11-leave unfolded (BBCH code 21, 14 days of drought stress) stage for measuring water relations, chlorophyll contents, and biomass-related attributes.

2.3.2. Field Study: Inducing Drought Tolerance with Optimized Sodium Nitroprusside and Spermidine Treatments. Mature and healthy seeds were sown between August 5 and November 20, 2021, in well-prepared ridges using a seed rate of 5 kg/ha. The seeds were placed at proper depth using the hand-dibbling method in rows kept 75 cm apart by a plating distance of 25 cm. The recommended rates for nitrogen (120 kg/ha), phosphorus (84 kg/ha), and potassium (62 kg/ha) were applied through urea, diammonium phosphate, and sulfate of potash, respectively, to meet the nutrient demand of NPK fertilizers. All phosphorus and potassium and 1/3 nitrogen were mixed into the soil at the time of sowing. The remaining 2/3 nitrogen was broadcast into two splits, with each 1/3 at 6-leaves unfolded (BBCH code 16) and inflorescence emergence (BBCH code 51) stages. The less vigorous seedlings were uprooted at the 4-leaves unfolded (BBCH code 14) stage to maintain one plant for better crop establishment. To avoid the weed competition with crop plants, weeding was done manually.

Drought stress was enforced at the reproductive stage (flowering, BBCH code 61) by skipping the irrigation water. Five irrigations (375.10 mm irrigation water plus rainfall) were applied in the control plot and four irrigations (300.10 mm irrigation water plus rainfall) were applied in the drought treatment. An exogenous supply of optimized sodium nitroprusside (100 μM), spermidine (100 μM), and sodium nitroprusside (100 μM) plus spermidine (100 μM) including Tween-20 (0.1%) was done twice upon imposing a drought stress and repeated in 1-week intervals. The distilled water containing Tween-20 (0.1%) applied to the plants was considered the control treatment. This study had eight treatments and were replicated three times under an RCBD split-plot design. The crop was harvested 108 days after sowing.

2.4. Measurement of Relative Water Content. Fully expanded and young leaves was weighed to measure the fresh weight (W_1) and then soaked in distilled water (for 24 h at 4 °C) to record the turgid weight (W_2). The turgid leaf was then oven-dried (for 72 h at 70 °C) further to determine the dry weight (W_3).[24]

$$\text{relative water content (\%)} = (W_1 - W_3)/(W_2 - W_3) \times 100$$

2.5. Estimation of Chlorophyll Contents. Fresh leaf sample (0.5 g) was grounded and extracted overnight in 80% acetone. The filtrate was then centrifuged (25,000*g*) for 20 min at 4 °C. The filtered supernatant was then examined spectrophotometrically at 645 and 663 nm for calculating chlorophyll *a* and chlorophyll *b* contents.[25]

$$\text{chlorophyll } a \text{ (mg g}^{-1}) = [(12.7A663) - (2.69A645)] \times \text{acetone vol.}/1000 \times \text{leaf wt}$$

$$\text{chlorophyll } b \text{ (mg g}^{-1}) = [(22.9A645) - (4.68A663)] \times \text{acetone vol.}/1000 \times \text{leaf wt}$$

2.6. Biomass-Related Trait Measurement. After the plants were harvested from each pot, root and shoot lengths were measured from the base to the top. The harvested plants were separated into root and shoot portions, assessed for the fresh weight (only the shoot part), and then oven-dried for 72 h at 70 °C to record their dry weights (root and shoot parts). The fresh and dry weights were calculated on per plant basis.

2.7. Determination of Yield and Yield Attributes. At maturity, three plants from each treatment were selected randomly and measured for their average plant heights and head diameter. Average achenes/heads were counted by randomly selecting ten plants from each treatment. The 1000-achene weight was estimated by weighing five samples from the seed lot, each having 1000 achenes, and then averaging. The heads were separated, sun-dried, and threshed to calculate the achene yield. The plants (except achenes) from each plot were sun-dried, weighed, and then added to achene yield to determine the biological yield. The oil contents were measured using the Soxhlet Fat Extraction method[26] from each treatment and then achene yield was converted into oil yield. The ratio of the achene yield to biological yield was calculated as the harvest index.

2.8. Statistical Analysis. The linear model of Fisher's analysis of variance (ANOVA) technique was used to analyze the data for all parameters by STATISTIX 8.1 computer package. The treatments' differences were compared by the *posthoc* Tukey test ($P < 0.05$). A heat map for relative water contents, chlorophyll, and growth traits was constructed using

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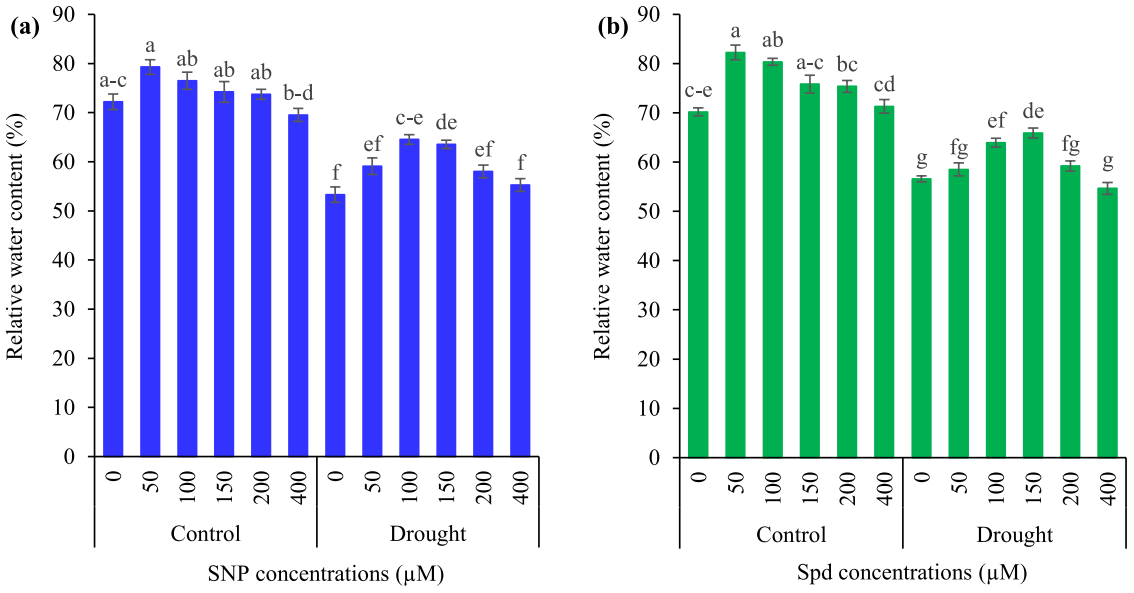

Figure 1. Relative water content (a, b) of 28-day-old sunflower plants as affected by sodium nitroprusside (SNP) and spermidine (Spd) grown under control and drought conditions. The values are means ± SE (n = 3), and sharing different letter(s) indicates the significant differences following Tukey's HSD test (P < 0.05). Drought stress: 100 and 60% FC; SNP: 50, 100, 150, 200, 400 μM; Spd: 50, 100, 150, 200, 400 μM.

the *R*-package. A correlation analysis was performed to assess the association among yield and yield-related traits.

The economic analysis was performed to assess the cost-effectiveness of sodium nitroprusside and spermidine treatments.[27] The cost of sodium nitroprusside and spermidine was 71,400 per 500 g and 18,700 per 1 g, respectively, in PKR (Pakistani rupees). The operational inputs such as land preparation, seed and its sowing, fertilization, irrigation, plant protection, intercultural practices, harvesting, threshing, and land rent were added to the fixed cost. The gross income was calculated using the average market price of sunflowers in Pakistan (PKR 95,000 per 1000 kg).

3. RESULTS

3.1. Pot Study: Sodium Nitroprusside and Spermidine Treatments Effects on Drought Stress. *3.1.1. Relative Water Content.* Drought prevalence remarkably (p < 0.05) reduced the leaf relative water contents by 26% in contrast to control (no drought) conditions (Supporting Table 1). Different doses of sodium nitroprusside significantly improved the relative water content, as a marked increase of 16% was observed with sodium nitroprusside at 100 μM supply in comparison to no sodium nitroprusside application under drought conditions. Higher rates for sodium nitroprusside (150−400 μM) caused a notable decline in relative water content, where a minimum increase of 3% was recorded with the highest rate of 400 μM to drought conditions (Figure 1a). Similarly, exogenous spermidine caused a significant increase in the leaf's relative water content under both control and drought conditions. A marked increase of 11% in the leaf's relative water content was exhibited with spermidine supply at 150 μM than with no spermidine application under drought stress. In contrast, plants supplemented with exogenous application of 100 μM spermidine showed the highest increase in leaf relative water content by 15% when exposed to control conditions (Figure 1b).

3.1.2. Chlorophyll Contents. Exposure to drought stress and exogenous sodium nitroprusside/spermidine significantly (p < 0.05) affected the chlorophyll *a* and *b* contents (Supporting Table 1). Plants showed a marked decline in the chlorophyll *a* content of 31% when subjected to drought treatment. Positive responses of foliar sodium nitroprusside for an increased chlorophyll *a* content are different for their diverse rates, as a maximum increase of 17% was exhibited with sodium nitroprusside application at 150 μM in parallel to no sodium nitroprusside application to drought stress conditions. Under control conditions of water application, plants treated with no sodium nitroprusside application revealed the highest increase in the chlorophyll *a* content of 15% (Figure 2a). Similarly, plants grown under drought stress conditions exhibited the highest decrease in the chlorophyll *a* content of 27% than that in control (no drought) plants, whereas exogenous spermidine supply at 150 μM increased the chlorophyll *a* content by 15% in comparison to no spermidine application. Under control conditions (no drought), the lowest rate for exogenous spermidine at 50 μM caused a maximum increase in the chlorophyll *a* content of 14% than no spermidine (Figure 2b).

Exposure to drought revealed a significant reduction in the chlorophyll *b* content of 35% as compared to the control water supply (no drought). Supplemental sodium nitroprusside at 100 μM markedly increased the chlorophyll *b* content by 19% than no sodium nitroprusside application to drought stress conditions. Contrarily, the highest increase in the chlorophyll *b* content by 15% was recorded with 150 μM sodium nitroprusside application under control (no drought stress) conditions (Figure 2c). Likewise, the chlorophyll *b* content was decreased by 33% in drought-prone plants, whereas spermidine application at 150 μM caused a maximum increase in the chlorophyll *b* content of 20% in drought stress conditions. Under control conditions of water supply, exogenous spermidine at 100 μM increased the maximum chlorophyll *b* content by 13% compared to no spermidine application. The highest rates for spermidine (200 and 400 μM) showed a minimum chlorophyll *b* content under both control and drought stress conditions (Figure 2d).

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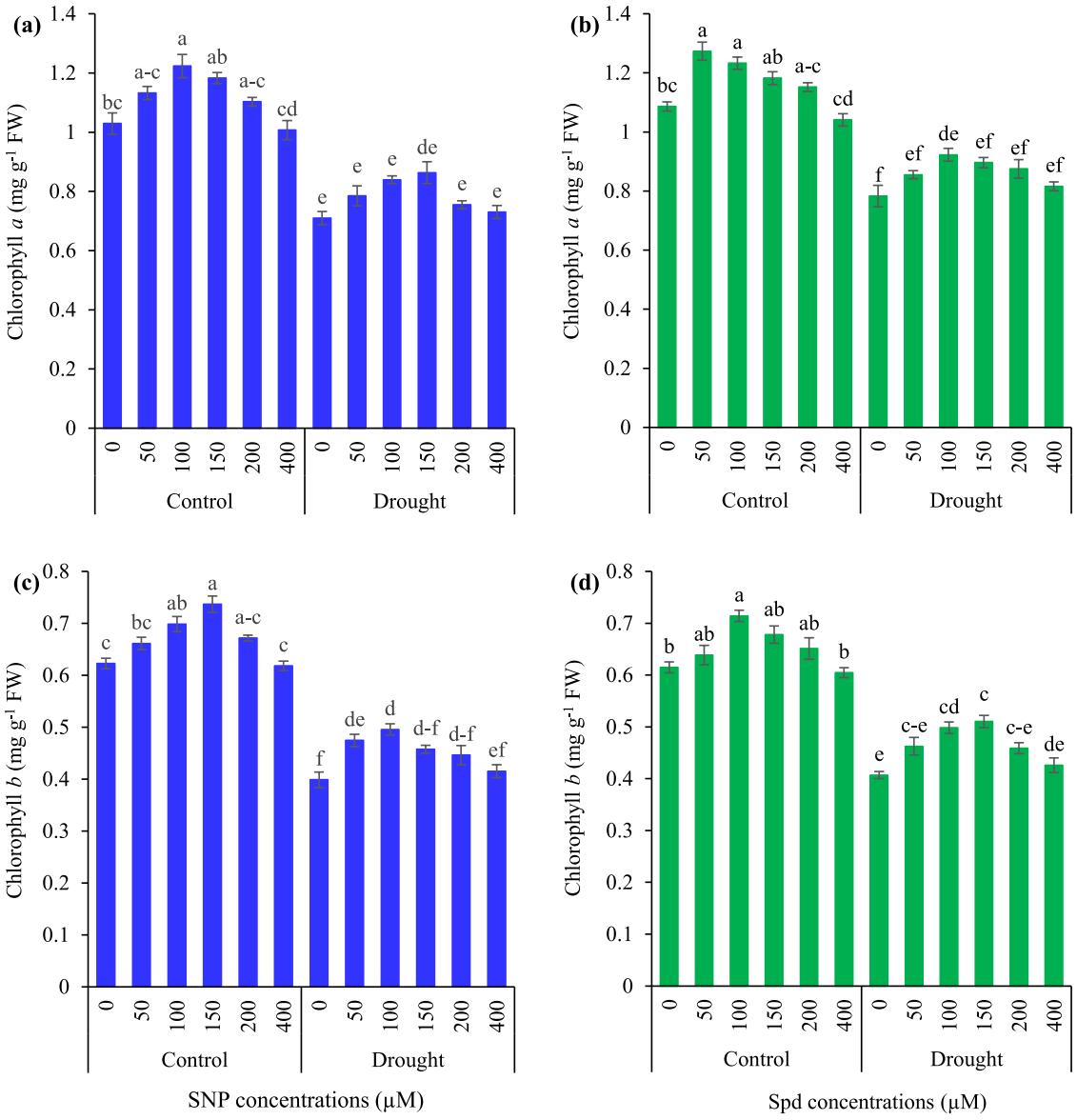

Figure 2. Chlorophyll *a* (a, b) and chlorophyll *b* (c, d) contents of 28-day-old sunflower plants as affected by sodium nitroprusside (SNP) (a, c) and spermidine (Spd) (b, d) grown under control and drought conditions. The values are means ± SE (n = 3), and sharing different letter(s) indicates the significant differences following Tukey's HSD test (P < 0.05). Drought stress: 100 and 60% FC; SNP: 50, 100, 150, 200, 400 μM; Spd: 50, 100, 150, 200, 400 μM.

3.1.3. Plant Biomass. A variability for growth traits of sunflower was considerably (p < 0.05) affected when subjected to drought stress and exogenous sodium nitroprusside/spermidine applications (Supporting Table 1). When exposed to drought stress, plants exhibited a maximum decrease in shoot and root lengths of 34 and 31%, respectively, than in control (no drought) conditions. The highest increase in shoot length of 9% was recorded in plants treated with sodium nitroprusside at 150 μM, whereas a higher rate of sodium nitroprusside (200 μM) increased the root at greater length by 17% in drought stress conditions (Figure 3a,c). Also, the highest increase in shoot and root lengths by 12 and 18% is depicted with exogenous spermidine application at 100 and 200 μM, respectively, under drought conditions. In contrast, application of spermidine at its higher rate of 400 μM in drought-stressed plants poorly increased the shoot and root lengths than under no spermidine supply (Figure 3b,d).

Plants grown under control conditions showed the highest increase in shoot fresh weight of 26% with exogenous sodium nitroprusside application at 150 μM, while 100 μM foliar supply caused a maximum increase of 13% under drought conditions (Figure 4a). Similarly, spermidine application at 100 μM enhanced the shoot fresh weight by 12 and 10% compared to other rates (50−400 μM) under both control and drought conditions, respectively (Figure 4b). The shoot dry weight was decreased by up to 33% in drought-stressed plants, whereas foliar sodium nitroprusside application at 100 μM caused a maximum increase in shoot dry weight of 31 and 15%, respectively, under both control and drought conditions (Figure 4c). For spermidine optimization, 100 μM foliar supply provided the highest shoot dry weight (14%) of plants than no spermidine when subjected to drought conditions. In contrast, a low dose of spermidine at 50 μM exhibited a maximum increase in shoot dry weight by 26% under control water conditions (Figure 4d). The root dry weight of sunflower

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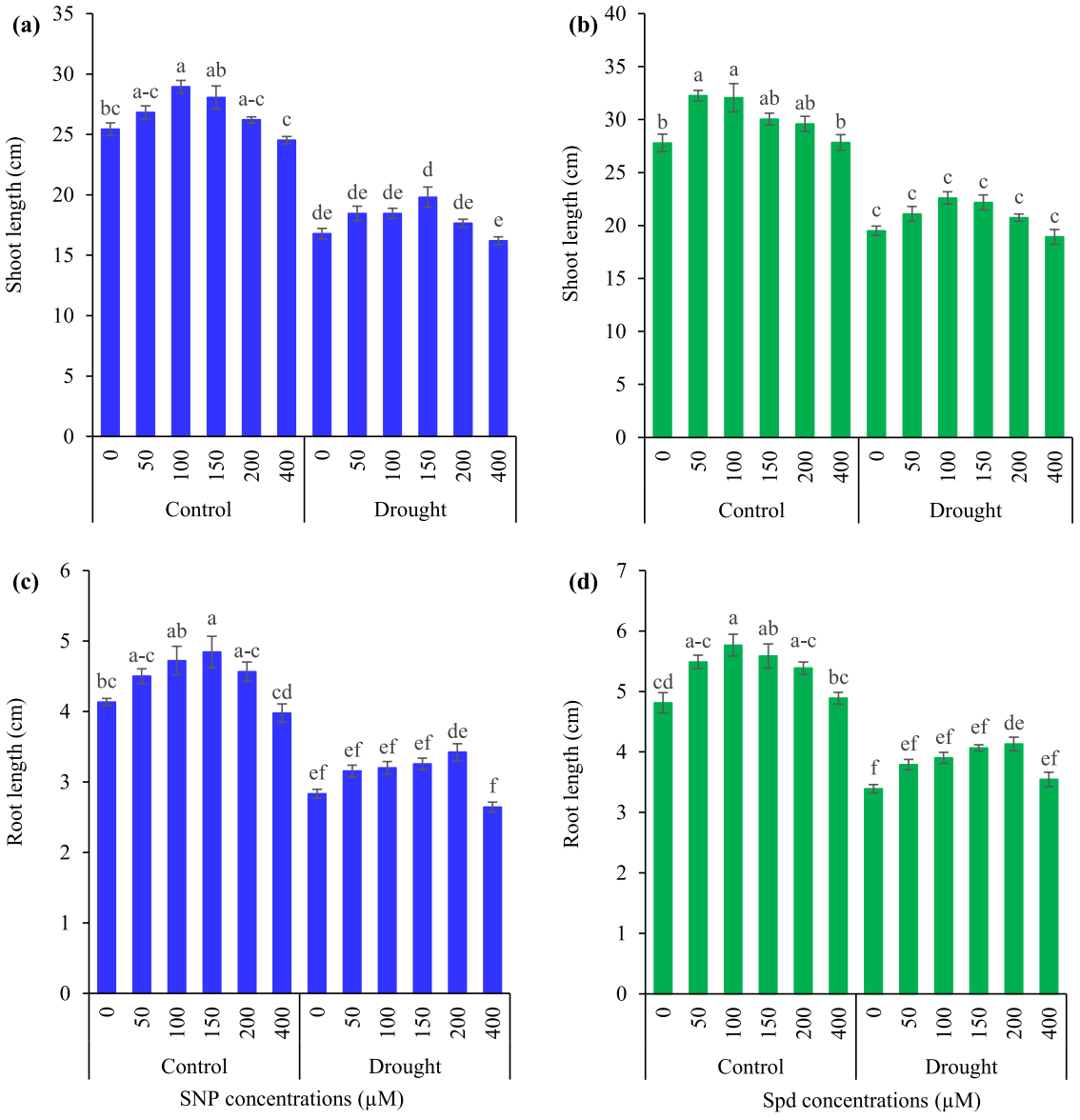

Figure 3. Shoot length (a, b) and root length (c, d) of 28-day-old sunflower plants as affected by sodium nitroprusside (SNP) and spermidine (Spd) grown under control and drought conditions. The values are means ± SE (n = 3), and sharing different letter(s) indicates the significant differences following Tukey's HSD test (P < 0.05). Drought stress: 100 and 60% FC; SNP: 50, 100, 150, 200, 400 μM; Spd: 50, 100, 150, 200, 400 μM.

plants was increased markedly by 17 and 10% with sodium nitroprusside and spermidine application at 150 μM, respectively, when grown under drought conditions (Figure 4e,f).

3.1.4. Clustered Heat Map of Relative Water Content, Chlorophyll, and Biomass. A strong and positive association between chlorophyll *a* and *b*, shoot and root lengths, and shoot fresh and dry weights was revealed for sodium nitroprusside 100 and 150 μM (N), while the relative water content was positively clustered for sodium nitroprusside 50 μM (N). Contrarily, a positive relationship of chlorophyll *a* and *b*, relative water content, shoot and root lengths, shoot fresh weight, and root and shoot dry weights was depicted for spermidine 100 μM (N). A negative association of all traits was found for sodium nitroprusside and spermidine at 200 and 400 μM (D) (Figure 5).

3.2. Field Study: Drought Responses of Sunflower to Optimized Sodium Nitroprusside and Spermidine Treatments.

3.2.1. Yield and Yield Attributes. Drought stress and sodium nitroprusside/spermidine supply significantly (p < 0.05) affected the yield and yield-related traits (Supporting Table 2). Plants treated without sodium nitroprusside or spermidine showed a significant decline in plant height of 13% under drought stress compared with control water conditions. Combined application of sodium nitroprusside at 100 μM plus spermidine at 100 μM showed a maximum increase in plant height by 8% than no sodium nitroprusside or spermidine application to drought stress conditions (Figure 6a). Drought caused a significant decrease in the head diameter; however, a maximum decline of 27% was revealed in plants grown under drought conditions. Similarly, foliar sodium nitroprusside at 100 μM in combination with spermidine at 100 μM exhibited a significant increase in the head diameter by 11% under control water conditions. In contrast, the sole application of spermidine at 100 μM markedly increased the head diameter by 17% under drought

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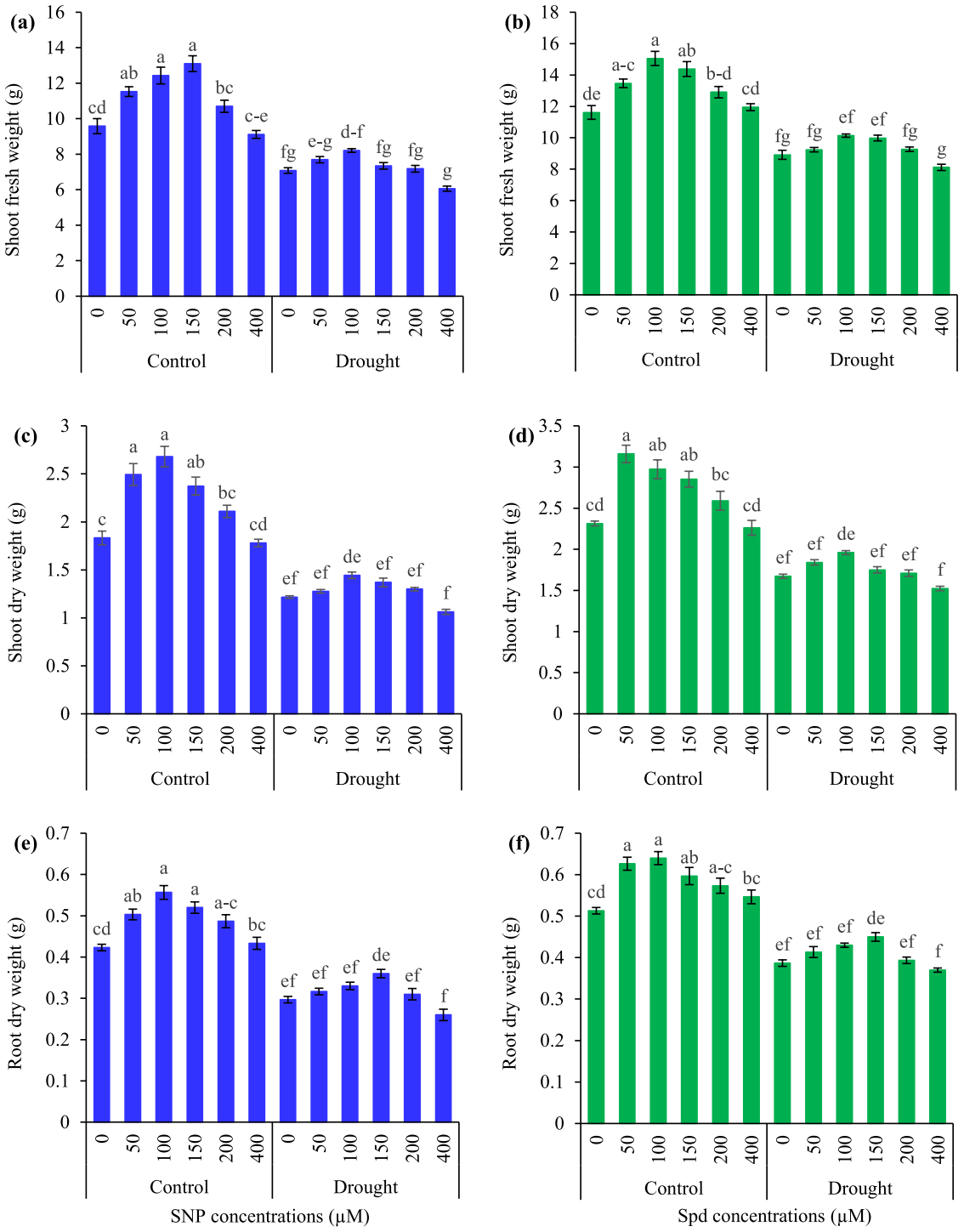

Figure 4. Shoot fresh weight (a, b), shoot dry weight (c, d), and root dry weights (e, f) of 28-day-old sunflower plants as affected by sodium nitroprusside (SNP) and spermidine (Spd) grown under control and drought conditions. The values are means ± SE (n = 3), and sharing different letter(s) indicates the significant differences following Tukey's HSD test (P < 0.05). Drought stress: 100 and 60% FC; SNP: 50, 100, 150, 200, 400 μM; Spd: 50, 100, 150, 200, 400 μM.

conditions (Figure 6b). Plants treated without sodium nitroprusside or spermidine exhibited a maximum decrease in achenes/head of 23% in drought stress compared to control conditions. Plants supplemented with sole application of spermidine at 100 μM showed a maximum increase in achenes/head of 16% when subjected to drought conditions (Figure 6c). The highest decline in 1000-achene weight by 24% was recorded in drought-stressed plants compared with to control plants. A foliar supply of combined sodium nitroprusside plus spermidine at 100 + 100 μM considerably increased the 1000-achene weight by 21 and 14% when grown under control and drought conditions, respectively (Figure 6d).

Exogenous foliar sodium nitroprusside at 100 μM plus spermidine at 100 μM application resulted in a maximum increase in achene yield by 25 and 28% than no sodium nitroprusside or spermidine supply under both control and

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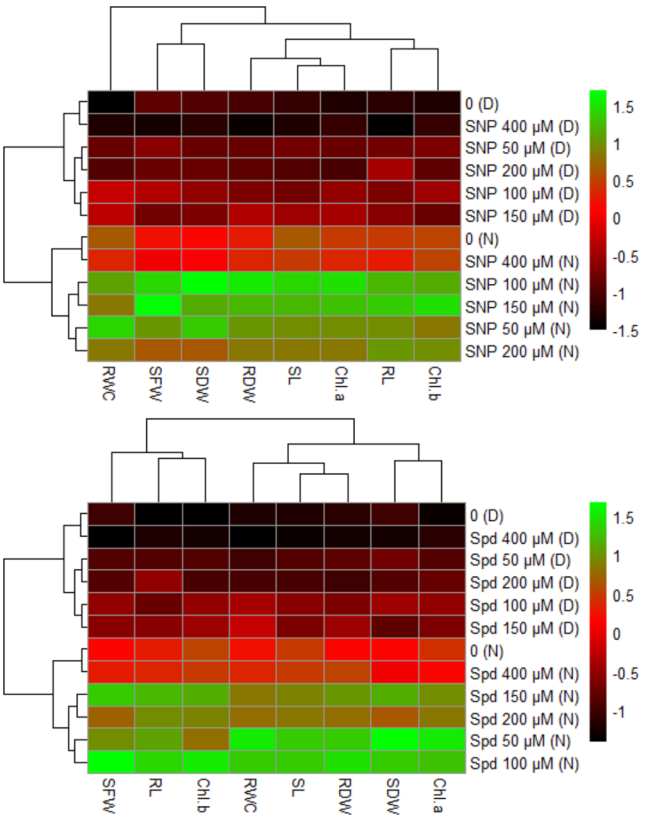

Figure 5. Clustered heat map of relative water content, chlorophyll pigments, and growth traits of sunflower following sodium nitroprusside (SNP) and spermidine (Spd) application. The change in colors indicates the significant differences at $P < 0.05$. RWC, relative water content; Chl. *a*, chlorophyll *a*; Chl. *b*, chlorophyll *b*; SL, shoot length; RL, root length; SFW, shoot fresh weight; SDW, shoot dry weight; RDW, root dry weight.

drought conditions, respectively (Figure 7a). Biological yield was considerably decreased by 11% in plants grown under drought stress, where supplemental sodium nitroprusside plus spermidine at 100 + 100 μM presented the highest increase in biological yield of 6% than no sodium nitroprusside or spermidine supply (Figure 7b). Drought drastically decreased the oil yield and harvest index of plants by 26 and 25%, respectively, than water control conditions. The maximum increase in oil yield and harvest index by 21 and 23%, respectively, was recorded in plants supplemented with combined sodium nitroprusside plus spermidine at 100 + 100 μM application in drought conditions (Figure 7c,d).

A strong and positive association of head diameter was found with achenes/head subjected to sodium nitroprusside or spermidine application under control and drought conditions. Similarly, the 1000-achene weight was positively correlated with achene and oil yields. A strong relationship of achene yield was determined with oil yield and harvest index (Figure 8).

Exogenous sodium nitroprusside significantly increased the net benefit (Rs. 101844.49 and 66219.79) and benefit−cost ratio (2.06 and 1.69) under both control and drought conditions. Sole spermidine supply or in combination with sodium nitroprusside negatively influenced the benefit−cost ratio due to its high market cost (Table 3).

4. DISCUSSION

Drought is a major environmental disaster that drastically affects crop growth and productivity. Drought prevalence in plants has been reported to reduce the metabolic processes, leaf turgor pressure, synthesis of photostabilizing pigments, water use efficiency, source−sink relationship, and nutrient absorption, thereby leading to reduced overall growth and development.[28−30] As a major yield-limiting factor, drought stress also causes severe damage to growth rates, physiological mechanisms, and seed development of crops,[32] thus reducing crop yield and quality. Under drought situations, plants tend to survive by the closing of stomata via accumulation of abscisic acid in the guard cells but resulting in reduced CO_2 uptake and assimilation for photosynthesis in plants.[31,76] Exogenous application of sodium nitroprusside and spermidine under drought conditions positively mediate the drought tolerance potential in sunflower plants by upregulating channels for ion movement, developing root system for higher water uptake, and reducing transpiration rate.[13,35] The present study is the first report that interprets the effects of various rates of sodium nitroprusside and spermidine on relative water content, chlorophyll pigments, and growth of sunflowers grown under drought conditions. The optimized rates of sodium nitroprusside and spermidine were further assessed for their positive responses to improving drought tolerance and yield of sunflowers under terminal drought.

Previously, it has been reported that drought is conducive to reducing stomatal opening, which contributes to a severe decline in leaf water content and turgidity in plants.[33,34] In the current findings, a prominent decrease in relative water content was found in drought-stressed plants, which might be related to imbalanced osmotic substances having key functions in maintaining the water balance of plants. Water deficiency stress also causes disruption in membranes' integrity, further aggravating a severe decline in leaf water contents.[36,37] It has been suggested that maintenance of water balance is the main challenge that plants face during drought periods.[38] Initially, plants regulate stomatal movement via osmotic adjustment, making their survival possible under stressful conditions. Plants accumulate various osmolytes (viz., ascorbic acid, sorbitol, proline, glycinebetaine, phenol, tocopherol, total free amino acids, total soluble carbohydrates, and inorganic ions) and try to sustain the turgor pressure of the cells.[10,39,40] Hence, maintenance of turgor pressure by regulating osmotic potential via cellular accumulation of organic solutes is an effective mechanism to improve the tolerance ability of plants but worked for short periods of drought.[41] In the current study, the addition of sodium nitroprusside and spermidine has been reported to recover the adverse effects of drought by improving the water status of plants. In fact, supplemental sodium nitroprusside application causes cellular accumulation of abscisic acid, which acts as osmoprotectants and protects the plants from oxidative damage via maintaining a higher leaf water potential.[42,43] In fact, sodium nitroprusside accumulates in plant roots and stimulates the auxin-dependent expression of certain genes. The expression of such genes further involves the development of root hairs and lateral roots, which uptake more water from the deep layers of plant rhizosphere, thus maintaining higher water balance in plants under drought situations.[44−46] In the present results, exogenous spermidine application retained higher relative water content in sunflower plants under drought stress conditions. The increase in water

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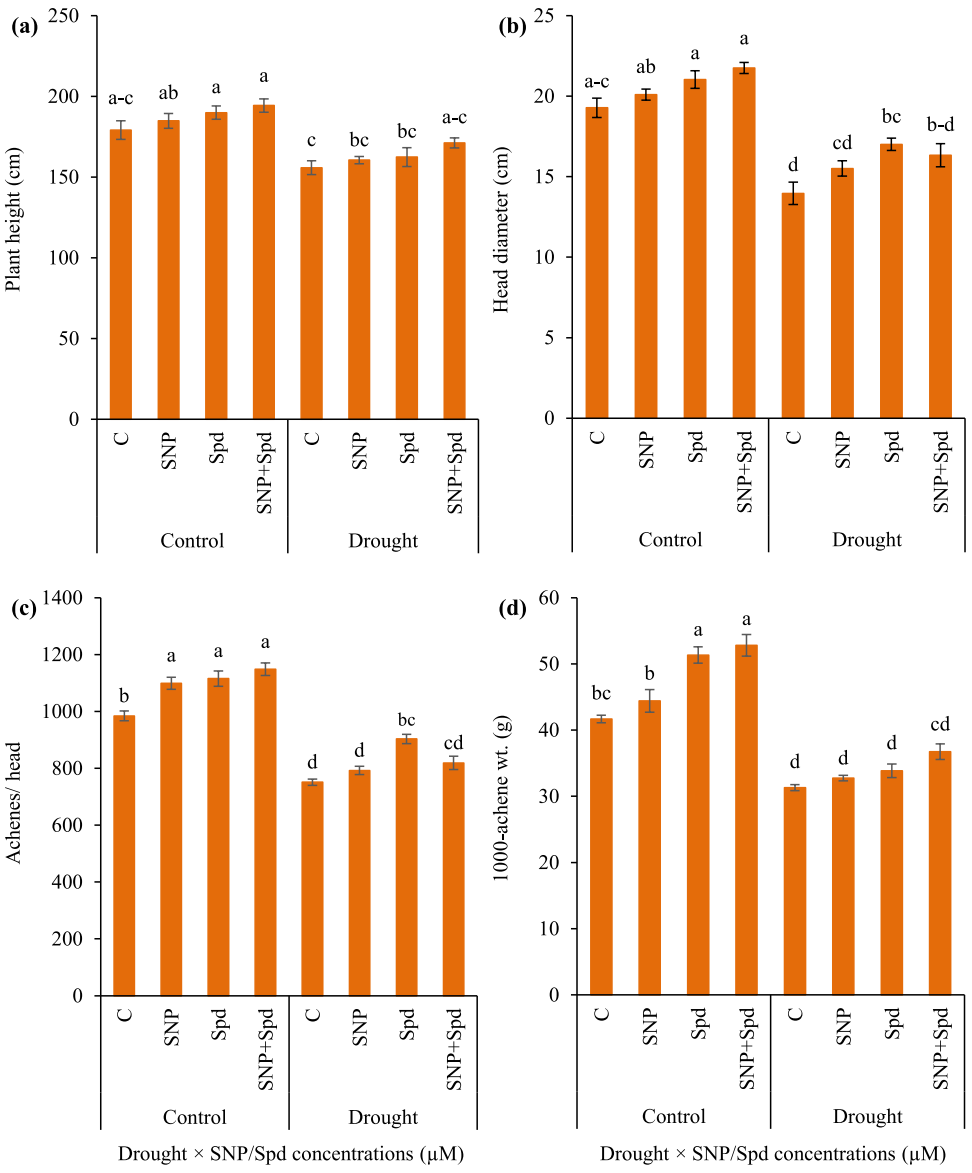

Figure 6. Plant height (a), head diameter (b), achenes/head (c), and 1000-achene weight (d) of sunflower plants as affected by sodium nitroprusside (SNP) and spermidine (Spd) grown under control and drought conditions. The values are means ± SE (n = 3), and sharing different letter(s) indicates the significant differences following Tukey's HSD test (P < 0.05). Drought stress: control and drought at reproductive stage; SNP: 100 μM; Spd: 100 μM.

content in plants with spermidine application might be due to developing an extensive root system for more soil water uptake. As suggested in previous reports, spermidine is also involved in controlling hydraulic or hormonal signals of stomatal regulation to conserve higher water contents in drought-prone plants.[47,48]

In the present results, chlorophyll pigments (chlorophyll a and b) were markedly decreased in sunflower plants by means of drought stress. Drought adversities in terms of reduced chlorophyll pigments (a and b) might be attributed to cellular accumulation of ROS. Overgeneration of ROS such as hydrogen peroxide (H_2O_2), superoxide anions (O_2), and hydroxyl radicals (OH^{-1}) in drought-prone plants is closely related to increased ROS-induced chlorophyllase activity, which is liable for the degradation of chlorophyll pigments.[3,49] In our results, sodium nitroprusside- and spermidine-treated plants showed a marked increase in chlorophyll pigments (a and b) under drought conditions. A notable increase in chlorophyll contents (a and b) might be attributed to the key role of sodium nitroprusside in alleviating ROS damages by enhancing activities of antioxidative enzymes, scavenging of free radicals, and making them less reactive through the induction of H_2O_2-suppressing enzymes.[50,51] Additionally, exogenous spermidine supplementation enhanced the chlorophyll pigments in drought-stressed sunflower plants, which might be due to the fact that spermidine enters the chloroplast rapidly and reduces the drought-induced degradation of chlorophylls, thus improving the tolerance of plants to drought stress.[52,53]

Drought-induced reduction in the growth of sunflower plants was exhibited in the current results. A remarkable decline in shoot and root lengths and fresh and dry weights could be related to reduced stomatal conductance, membrane permeability, and carbohydrate partitioning in plants due to drought stress exposure. Moreover, drought stress also causes a loss of leaf water relations and overproduction of ROS,

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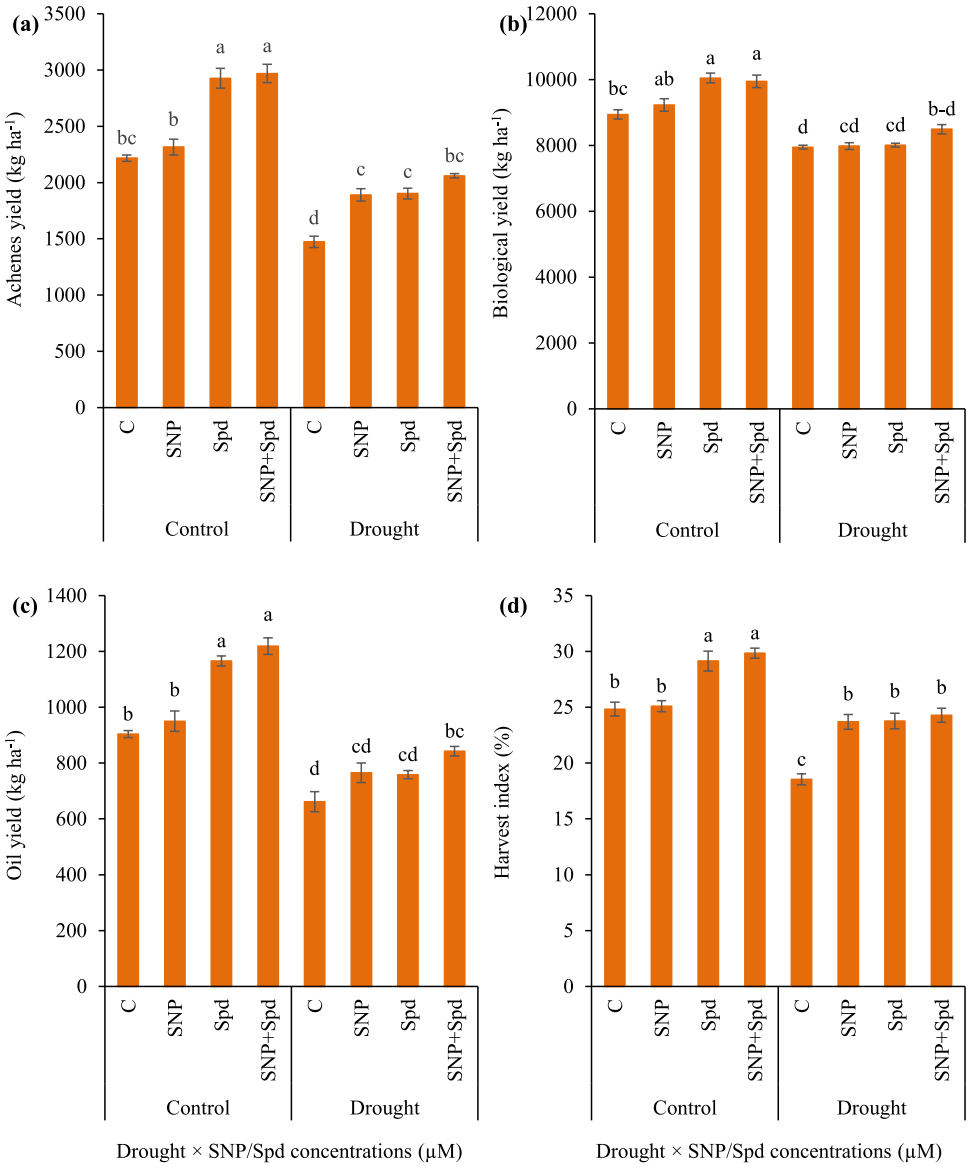

Figure 7. Achene yield (a), biological yield (b), oil yield (c), and harvest index (d) of sunflower plants as affected by sodium nitroprusside (SNP) and spermidine (Spd) grown under control and drought conditions. The values are means ± SE (n = 3), and sharing different letter(s) indicates the significant differences following Tukey's HSD test ($P < 0.05$). Drought stress: control and drought at reproductive stage; SNP: 100 μM; Spd: 100 μM.

resulting in reduced growth of plants.[7,54] As explained in previous reports, drought-induced ROS synthesis causes oxidative injury to proteins, nucleic acids, lipids, chlorophyll pigments, membrane permeability, and photosynthetic electron transport chain in plants. This oxidative damage to various physiological processes ultimately leads to reduced growth performance of plants. Furthermore, the destructive effects of drought stress in terms of reduced normal functioning of photosynthetic systems (PS-I and PS-II) involved in the efficient growth of plants are likely the main consequence of ROS.[3,49] In the present results, supplemental sodium nitroprusside and spermidine caused a prominent increase in shoot height and fresh and dry weights of sunflower plants. The increase in the growth of plants with sodium nitroprusside supplementation might be ascribed to its easy absorption to intracellular spaces of membranes. This cellular penetration of sodium nitroprusside provides strength to biological system of cells and ultimately enhancing growth efficiency of plants under drought stress.[55] Furthermore, exogenous sodium nitroprusside in combination with phytohormones during early growth periods has also been reported to be involved in improving the stages of plant development. Recently, exogenous sodium nitroprusside supply under drought situations has been reported to upregulate the photosynthetic efficiency, water balance, and stomatal movement and reduce membrane leakage of plants, further improving growth performance of plants.[11,46,56] In the present study, exogenously applied spermidine is also advantageous for growth enhancement and increasing tolerance to drought stress in sunflower plants.[15] It is evident that spermidine has been documented to regulate the antioxidative defense system, starch metabolism, and associated gene expression, suggesting its essential role in enhancing germination and development of plants under drought stress environments.[53,57] Previous studies have described that spermidine is actively involved in maintaining ionic channels, ion homeostasis, cellular structures, membrane

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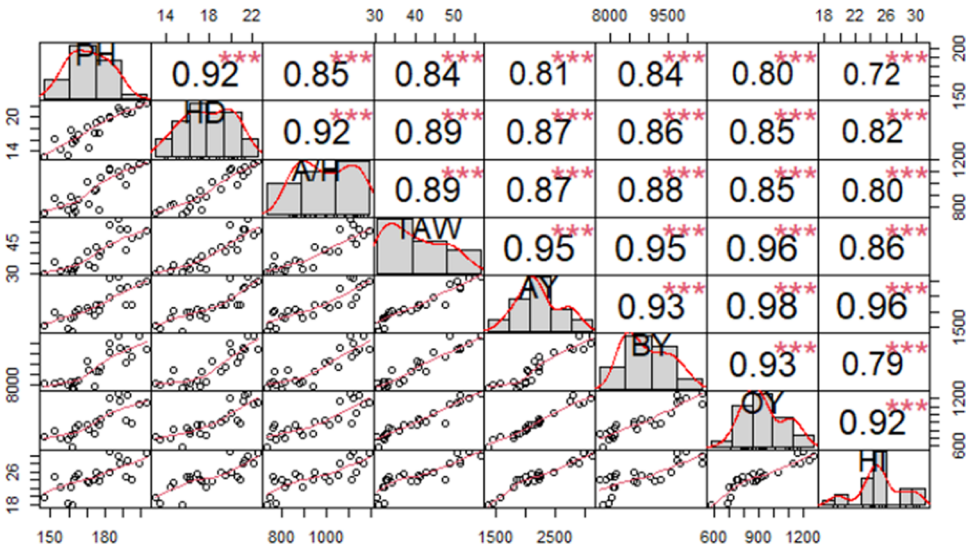

Figure 8. Pearson correlation coefficient (r) with density and frequency distribution, and values comprising significance (*) of correlation coefficients (r) for yield and yield traits of sunflower following sodium nitroprusside (SNP) and spermidine (Spd) application. PH, plant height; HD, head diameter; A/H, achenes/head; TAW, 1000-achene weight; AY, achene yield; BY, biological yield; OY, oil yield; HI, harvest index.

Table 3. Net Return and Benefit−Cost Ratio of Sunflower Plants[a]

drought stress/SNP/Spd treatments	variable cost (Rs.)	fixed cost (Rs.)	total cost (Rs.)	gross income (Rs.)	net benefit (Rs./ha)	benefit−cost ratio
			Control			
C	4948	90,486	95,434	189,611	94,177	1.99
SNP	5688	90,486	96,174	198,018	101,844	2.06
Spd	58,617	90,486	149,103	250,259	101,156	1.68
SNP + Spd	59,357	90,486	149,843	253,907	104,064	1.69
			Drought			
C	4207	90,486	94,693	125,999	31,306	1.33
SNP	4946	90,486	95,432	161,652	66,220	1.69
Spd	57,876	90,486	148,362	162,650	14,288	1.10
SNP + Spd	58,615	90,486	149,101	176,130	27,029	1.18

[a]C, no SNP/Spd; SNP, sodium nitroprusside; Spd, spermidine.

permeability, ATP synthesis, and scavenging of free radicals (ROS), thus improving plant growth under drought conditions.[58]

In the present study, a marked reduction in yield and yield-related attributes of sunflower under drought stress was ascribed to inhibited water balance, leaf turgidity, cell division and expansion, RuBISCO enzyme activity, regeneration of RuBP, ATP synthesis, and nutrient uptake.[59] The drought consequences in plants involve regulation at physiological, molecular, biochemical and morphological levels, primarily impeding the photosynthetic efficiency, compatible osmolytes accumulation, and ROS equilibration, which results in remarkable change in leaf morphology and yield reduction.[60,77] It is recently documented that limited water availability to plants instigated a decrease in leaf surface area, which reduced the translocation of photosynthates to sinks and sunlight efficiency to perform optimal photosynthetic activities, hence leading to decreased grain weight and senescence of leaves during periods of full blooming.[61] In our results, supplemental sodium nitroprusside and spermidine remarkably increased the grain weight, achene, and biological yields, which might be related to the upregulation of transcript expression, osmoregulation, ion uptake, physiological processes, root development, seed germination, and increased antioxidant activities under drought stress.[62,63] Previous reports also suggest that spermidine supply promotes cellular processes such as programmed cell death, cell division, differentiation and proliferation, protein translation, and DNA synthesis, aggravating growth developmental processes in plants under drought situations.[64−66] Previously, it has been well documented that sodium nitroprusside in combination with spermidine could effectively promote the accumulation of secondary metabolites and activity of RuBisCO enzyme for carboxylation and strongly bind to thylakoid chlorophyll protein complexes for increasing photosynthetic efficiency,[67,68] thereby improving the yield performance of plants under drought stress.

5. CONCLUSIONS

This study increased our understanding of how exogenous sodium nitroprusside and spermidine application modulate the physiological responses for inducing drought tolerance in sunflower, particularly under terminal drought stress. Exogenous sodium nitroprusside at 100 μM and spermidine at 100 μM were identified as the most effective rates to improve leaf relative water content, chlorophyll pigments, and growth traits in drought-induced sunflower plants. During periods of terminal drought, combined foliar application of sodium nitroprusside and spermidine markedly alleviated the adversities of drought and contributed better to inducing drought resistance in sunflower plants through improved water

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relations and chlorophyll contents. Supplemental sodium nitroprusside and spermidine also improved the achenes per head, 1000-achene weight, and achene and oil yields under drought stress conditions. Hence, foliar sodium nitroprusside and spermidine appear important for drought tolerance of sunflowers during terminal drought periods. However, further research is needed to assess the key effects of sodium nitroprusside and spermidine for improving the adaptability and productivity of other food crops against abiotic stresses, particularly drought stress.

■ ASSOCIATED CONTENT

Data Availability Statement

All data sets analyzed in the present study are included in the manuscript.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c02061.

Summary of two-way ANOVA for the effects of drought, sodium nitroprusside/spermidine, and their interaction treatments on relative water content, chlorophyll, and biomass of sunflower (Supporting Table 1); summary of ANOVA for the effects of drought, sodium nitroprusside/spermidine, and their interaction treatments on yield and yield attributes of sunflower (Supporting Table 2) (PDF)

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Author Contributions

M.A.S. was responsible for conceptualization and supervision. I.H. performed the investigation and methodology. S.A.F. and H.N.F. performed the formal analysis. K.M. and G.A. were responsible for laboratory analysis. K.S.A., W.H., S.Y., and M.A. wrote, reviewed, and edited the manuscript. M.A.E., H.O.E., and F.U. were responsible for funding acquisition. G.A.A. performed the methodology. The final version of the manuscript was approved by all of the authors.

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Notes

The authors declare no competing financial interest.

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