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Foliar Supplementation of Clove Fruit Extract and Salicylic Acid Maintains the Performance and Antioxidant Defense System of *Solanum tuberosum* L. under Deficient Irrigation Regimes

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Abstract: A field trial was conducted twice (in 2020 and 2021) to evaluate the effect of clove fruit extract (CFE) and/or salicylic acid (SA), which were used as a foliar nourishment, on growth and yield traits, as well as physiological and biochemical indices utilizing potato (*Solanum tuberosum* L.) plants irrigated with deficient regimes in an arid environment. Three drip irrigation regimes [e.g., well watering (7400 m^3 ha^{-1}), moderate drought (6200 m^3 ha^{-1}), and severe drought (5000 m^3 ha^{-1})] were designed for this study. The tested growth, yield, and photosynthetic traits, along with the relative water content, were negatively affected, whereas markers of oxidative stress (hydrogen peroxide and superoxide), electrolyte leakage, and peroxidation of membrane lipids (assessed as malondialdehyde level) were augmented along with increased antioxidative defense activities under drought stress. These effects were gradually increased with the gradual reduction in the irrigation regime. However, under drought stress, CFE and/or SA significantly enhanced growth characteristics (fresh and dry weight of plant shoot and plant leaf area) and yield components (average tuber weight, number of plant tubers, and total tuber yield). In addition, photosynthetic attributes (chlorophylls and carotenoids contents, net photosynthetic and transpiration rates, and stomatal conductance) were also improved, and defensive antioxidant components (glutathione, free proline, ascorbate, soluble sugars, and α-tocopherol levels, and activities of glutathione reductase, peroxidase, superoxide dismutase, catalase, and ascorbate peroxidase) were further enhanced. The study findings advocate the idea of using a CFE+SA combined treatment, which was largely efficient in ameliorating potato plant growth and productivity by attenuating the limiting influences of drought stress in dry environments.

Keywords: potato; drought; antioxidant enzymes; salicylic acid; clove fruit extract; physio-chemical attributes

1. Introduction

As one of the most common *Solanaceae* crops, potato (*Solanum tuberosum* L.) is an important food crop, with a total global cultivation area of about 19,302,600.00 ha, ranking 4th after rice, wheat, and maize [1]. It is used for export and local consumption because it is a good source of vitamins, mineral nutrients, starch, organic acids, energy, etc., thus playing some crucial therapeutic roles for humans [2]. Like other crops, this crop is affected

by many factors related to the agricultural environment, including soil factors (e.g., biology, chemistry, and physics), in addition to the water regime.

Climate change is the major problem threatening agricultural production, and its threats tend to be magnified in the 21st century. Extreme changes in climatic factors, including precipitation, light, and temperature, lead to an excessive decrease in plant performance (e.g., growth and productivity) for many horticultural and field crops [3,4]. Increased temperature and reduced precipitation lead to heat stress and drought, respectively, especially in dry (water-deficient) environments [5,6].

The stresses (e.g., oxidative and osmotic) stimulated by drought adversely influence plant physio-chemical performance. Under severe drought, numerous plant species are dehydrated, causing the plant to die, or plant growth and resultant yield are greatly diminished in few species. In plants of some tolerant/adaptive crops, morphology and metabolic alterations occur in response to the stress of water deficiency, which are involved in the adaptation to these inevitable constraints in the agricultural environment [7].

Potato is among the most important crops that have the potential for study of their physiological and biochemical behaviors under stress conditions of water insufficiency due to naturally occurring genetic variations with drought resistance [8]. Water insufficiency diminishes cellular water content/turgor, which has undesirable influences on gas exchange traits resulting from stomata closure, which in turn diminishes transpiration and the rate of CO_2 assimilation, thus diminishing the net photosynthesis rate [9–11]. During water restriction, photosynthesis (an important process that makes cell organelles function well as a source of feeding) is suppressed. In addition, CO_2 availability and uptake, photochemical activity, and metabolism are adversely affected [12,13]. Free radical processes are activated during a condition of water stress, which has high potential for regression of photosynthesis, proteins, and other plant metabolites. In plant cells, oxidative stress is stimulated by drought by raising electron leakage to O_2 during respiration and photosynthesis, resulting in the excessive generation of ROS (species of reactive oxygen) [14,15], including $O_2^{\bullet -}$, OH^-, and H_2O_2. These ROS can directly attack lipids in cell membranes, deactivate enzymes involved in metabolism, and degrade nucleic acids resulting in death [16]. Plants, under non-stress conditions, are totally provided with various antioxidants forming a defensive system (e.g., enzymatic—superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, glutathione reductase, etc.—which link with non-enzymatic—carotenoids, ascorbate, proline, glutathione, α-tocopherol, etc.) to control ROS exhaustion in subcellular organelles and ensure that they are as balanced as possible when produced [17,18]. However, under stress, these defensive antioxidant components are used in plants to counteract oxidative stress as much as possible to avoid oxidative damage [19], and the extent of oxidative stress damage in plant cells depends on the antioxidant capacity. Among the components of effective defensive antioxidants, proline has a dual function in the two main mechanisms in stressed plants. It acts as an effective osmotic compound in favor of cellular osmotic modulation, membrane stabilization, and detoxification of harmful ions, and also acts as an effective antioxidant that contributes to the elimination of free radicals [20,21]. These defensive antioxidant components of the plant are in most cases not sufficient to sustain plant growth under severe stress, so plants must be provided with exogenous supports (e.g., plant extracts and/or antioxidants) to raise stress tolerance [22–26].

Under stress-free or undesirable conditions, plant growth and resultant yield can be ameliorated by foliar nourishment with a plant-based product (e.g., clove fruit extract; CFE) alone or with an antioxidant (e.g., salicylic acid; SA) due to a positive modulation of plant metabolism [22,27]. As noted earlier, an extract from a plant organ (e.g., grains, leaves, or roots) has been analyzed and diverse biostimulating substances have been detected, such as various antioxidant components, numerous nutrients, essential plant hormones, different osmotic molecules, and various vitamins. These biostimulating substances are absolutely necessary to strengthen the plant's defense system to effectively cope with various stressors. [8,13,22,25,26]. More recently, clove (*Syzygium aromaticum*) fruit extract

was found to provide anti-stress benefits to contribute to the sustainability of plant growth and production [22,28].

Salicylic acid (SA) is a phenolic antioxidant compound, messenger, or signaling molecule, and growth regulator to support plant growth and performance under different stressors. It helps plants to withstand various stressors by raising the plant's antioxidant capacity and suppressing ROS overproduction [29] by playing important roles in regulating some processes related to plant physiology and biochemistry. The uptake and transport of essential ions, in addition to the balanced permeability of cell membranes, are sustained by SA in favor of stressed plant growth [30]. The reinforcing effects of SA on stress adaptability and the evolution of plant damage are events dependent on the spraying method, time, and concentration of SA, in addition to the plant species [31]. In previous reports, exogenous application of SA to stressed plants has received particular attention of researchers because it potentiates the preservative influences on water-deficient plants, and the cytotoxic effects of water-deficient stress can be attenuated by exogenous supplementation of SA [30].

To the best of our knowledge, studies using SA alone to relieve the undesired influences of water-deficient stress in plants are numerous, and studies in which SA has been used with a plant extract are few. However, this is the first time that SA has been used in combination with clove fruit extract (CFE) as a bioactive stimulant to attenuate the undesired influences of water-deficient stress in potato plants. This contemporary study hypothesized that the application of CFE or SA alone will improve the growth and resultant yields of potato plants under water-deficient stress due to improvements in plant physio-chemical attributes (photosynthetic parameters, antioxidants, and osmoprotectants), but that application of both CFE and SA together (in combination) will outperform all single applications in improving drought-stressed plant growth and production. Therefore, this work was planned to assess the potential ameliorative influences of foliar nourishing with CFE and SA on growth, yield, and some physio-chemical traits of water-deficient-stressed *Solanum tuberosum* L. plants, and to create a relationship between the extent of tolerance (as the extent of improvement in plant growth and yield) and changes stimulated in the tested physio-chemical components.

2. Materials and Methods

2.1. Description of the Trial Site and Soil Analysis

A field trial was implemented twice in the 2020 and 2021 seasons on a private farm at the El-Salheya El-Gedida City (60.674625 N, 31.882901 E WGS), El-Sharkia Governorate, Egypt. The experiment area is located in a semi-arid region with average rainfall and temperature of 100−110 mm and 16.1−16.2 °C, respectively, for both seasons. Initial analysis of the trial soil site was carried out before planting in both seasons [32–34], and the data are presented in Table 1.

Table 1. Some major properties of the investigated soil.

Soil Characteristics	Unit	Values	
		2020 Season	2021 Season
Sand	%	90.50 ± 1.5	90.4 ± 1.3
Silt		4.78 ± 0.1	4.83 ± 0.13
Clay		3.72 ± 0.12	3.97 ± 0.16
Texture class		Sandy	
Organic matter	%	4.45 ± 0.05	4.57 ± 0.05
pH (in 1: 1 of soil: water suspension)		7.97 ± 0.04	7.99 ± 0.04

Table 1. *Cont.*

Soil Characteristics	Unit	Values	
		2020 Season	2021 Season
EC (in soil paste extract)	dS m^{-1}	1.69 ± 0.01	1.72 ±0.01
N-NH_4^+	mg kg^{-1} soil	14.8 ± 0.5	15.01 ± 0.4
N-NO_3^-		6.65 ± 0.1	6.71 ± 0.08
Total N		23.7 ± 0.05	27.5 ± 0.04
Total P		6.81 ± 0.1	6.37 ± 0.05
Total K		59.3 ± 0.3	56.6 ± 0.4

2.2. Plant Material and Irrigation Regimes

The area of the trials was plowed to depth of 0.4 m utilizing a moldboard plow and then divided into rows having a width of 0.8 m. Then, plots having a length of 4.0 m and width of 3.20 m (four rows for each plot) were established, with a row left between each of two plots as the cut-offs between treatments.

A drip irrigation net was constructed with a distance of 30 cm between each two drippers. The plants were subjected to three watering regimes: well watering (WW; 7400 m^3 ha^{-1}), moderate drought (MD; 6200 m^3 ha^{-1}), and severe drought (SD; 5000 m^3 ha^{-1}), and the irrigation intervals were identified according to the growth stage as recommended. The design applied to the experiments was a spilt-plot with three replications. The prime plots were randomly occupied by watering regimes. In the subplots, foliar treatments using clove fruit extract (CFE) and/or salicylic acid (SA) were randomly applied. By 8 January 2020, potato seed tubers (cv. Spunta; the most exported cultivar to European markets) were sown with a distance of 30 cm between each two plants. On 28 April 2020 (at maturity), the potato tubers were gathered manually. In the second season (2021) of experiments, all dates and times of sowing, treatments, and sampling applied in the 2020 season were similarly maintained. Before planting, all trial plots in the two seasons were fecundated with potassium fertilizer (K_2SO_4) in the amount of 100 kg K ha^{-1}. Nitrogenous fertilizer (NH_4NO_3) was utilized with an amount of 250 kg N ha^{-1}. The NH_4NO_3 fertilizer was applied in five equal portions with the first, third, fifth, seventh, and ninth irrigation.

2.3. Preparation and Analysis of Clove Fruit Extract (CFE)

Ten grams of clove fruits were dried, soaked in 1 L of distilled water (d-W), and incubated for 24 h at 50 °C. Then, the solution was filtered to a volume reaching 1 L with d-W. The resulting extract was analyzed [35–41] for the prime chemical ingredients, and the data are shown in Table 2.

Table 2. Some chemical constituents of clove fruit extract (CFE) (on dry weight basis).

Component	Unit	Value
Total phenolic compounds (TPC)	mg GAE g^{-1} CFE	323.79
Total flavonoids (TF)	mg QE g^{-1} CFE	34.65
Phenolic compounds		
3,4-Dihydroxybenzoic-acid	mg g^{-1} CFE	0.74
Ellagic-acid		0.62
Eugenol		104.7
Eugenyl-acetate		86.39
Gallic acid		18.33
Naphthalene		0.21
Tannic acid		0.78
Vanillin		1.49

Table 2. *Cont.*

Component	Unit	Value
Antioxidants and osmoprotectants		
Total free amino acid	g Kg^{-1} DW	70.2
Free Proline		0.19
Soluble sugars		0.56
Mineral nutrients		
Mg	g Kg^{-1} DW	3.2
Ca		12.3
Fe		1.3
P		11.8
K		16.5
N		16.9
Vitamins		
Vitamin A	mg Kg^{-1} DW	25.6
Vitamin E		55.2
Vitamin D		32.4
Vitamin C		36.9

The concentration of total phenolic compounds (TPC) in CFE was measured using a UV-spectrophotometer (Jenway-6705-UV/VIS), based on oxidation/reduction reaction, as reported in [35] using Folin–Ciocalteu reagent [36]. To 500 µL of diluted CFE (10 mg CFE in 10 mL D.W), 2.5 mL of Folin-Ciocalteu reagent (diluted 10 times with distilled water) and 2 mL of Na_2CO_3 (75 g L^{-1}) were added. The sample was incubated for 5 min at 50 °C, then cooled. For a control sample, 500 µL of distilled water was used. The absorbance was recorded at 763 nm. The TPC content was expressed as gallic acid equivalent (GAE) and calculated based on the calibration curve using the following linear equation:

$$y = 0.0189x + 0.0716 \quad (1)$$

$$R^2 = 0.9985$$

where y is the absorbance, x is the concentration (mg GAE g^{-1} extract), and R^2 is the correlation coefficient.

TF content was measured [37] with some modification. A 3 mL aliquot of 10 g/L AlCl3 ethanol solution was added to 0.5 mL of CFE (10 mg in 10 mL solvent). After 60 min, the absorbance at 420 nm was recorded. Total flavonoids content expressed as quercetin equivalent (QE) was calculated based on the calibration curve using the following equation:

$$y = 0.013x + 0.0166 \quad (2)$$

$$R^2 = 0.9968$$

where x is the absorbance, y is the concentration (µg QE), and R^2 is the correlation coefficient.

The antioxidant activity of CFE against DPPH radical emulsifier and β-Carotene/linoleic compared to gallic acid and TBHQ, and the absorbance of the ferric reducing power of CFE against gallic acid and TBHQ, are shown in Figure 1.

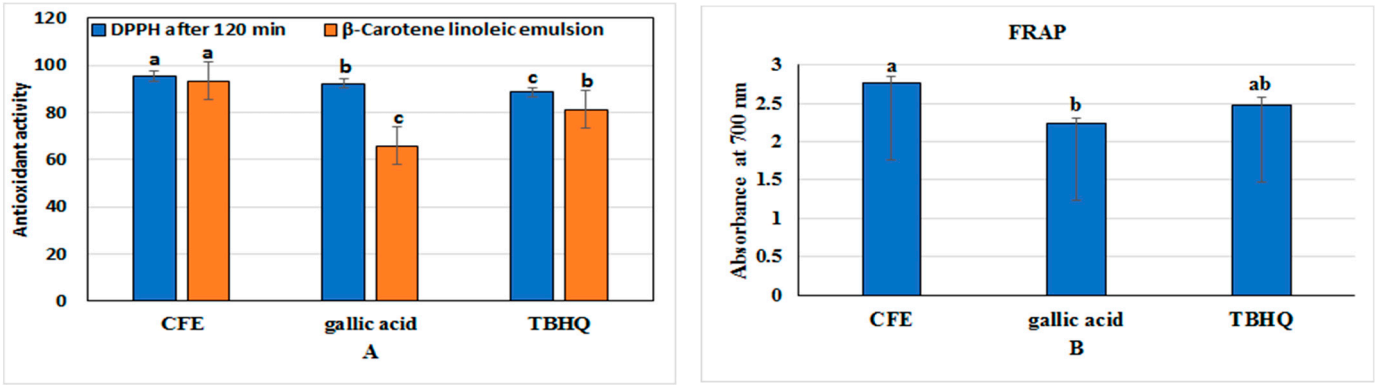

Figure 1. (**A**) Antioxidant activity of CFE against DPPH radical and β-Carotene/linoleic emulsion compared with gallic acid and TBHQ; (**B**) absorbance of ferric reducing power of CFE against gallic acid and TBHQ. Bars (represent treatment values) with different letters indicate significant difference between treatment values at $p \leq 0.05$.

The antioxidant activity was measured using three methods. All extracts were dissolved in DMSO except the aqueous extract that was dissolved in distilled water (10 mg in 10 mL solvent). Gallic acid and TBHQ were used as positive controls. Samples were analyzed in triplicate.

DPPH• free-radical scavenging activity: The electron donation ability of the obtained extracts was measured [38] by bleaching of the purple-colored solution of DPPH. A quantity of 100 µL of CFE (10 mg extract 10 mL^{-1} solvent) was added to 3.9 mL of 0.1 mm DPPH dissolved in ethanol. After 120 min, the absorbance was measured at 517 nm against the control. Percentage of antioxidant activity of free radical DPPH was determined as follows:

$$\text{Antioxidant activity (Inhibition) \%} = ((\text{A control} - \text{A sample})/\text{A control}) \times 100 \quad (3)$$

where A control is the absorbance of the control reaction and A sample is the absorbance in the presence of plant extract.

B-Carotene/linoleic acid bleaching: The ability of CFE, gallic acid, and TBHQ to prevent the bleaching of β-carotene was tested as reported in [39]. In summary, 0.2 mg β-carotene dissolved in 1 mL chloroform, 20 mg linoleic acid, and 200 mg Tween-20 were mixed in a round-bottom flask. After removal of chloroform, 50 mL of distilled water was added and the mixture was vigorously stirred. Aliquots (3 mL) of the emulsion were transferred to tubes containing clove extract or TBHQ. After mixing 0.5 mL of extract (10 mg extract in 10 mL solvent), an aliquot from each tube was transferred to a cuvette and the absorbance was measured (Abs0) at 470 nm. The remaining samples were placed in a 50 °C water bath for 120 min, then the absorbance was recorded at 470 nm (Abs120). A control without added CFE, gallic acid, or TBHQ was analyzed. Antioxidant potential was calculated as follows:

$$\text{Antioxidant activity (\%)} = (1 - (\text{Abs0sample} - \text{Abs120sample})/(\text{Abs0control} - \text{Abs120control})) \times 100 \quad (4)$$

where Abs0 sample is the absorbance of sample at zero time, Abs120 sample is the absorbance after 120 min, Abs0 control is the absorbance of control at zero time, and Abs120 control is the absorbance of control after 120 min.

Ferric reducing antioxidant power (FRAP): The reducing power of CFE was measured [40]. The reduction of Fe^{3+} to Fe^{2+} was tested by determining the absorbance of Perl's Prussian blue complex. A quantity of 100 µL of CFE (10 mg CFE in 10 mL D.W) was mixed with 1 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 1 mL of 1% $K_3Fe(CN)_6$. The mixture was incubated at 50 °C for 20 min. After 20 min of incubation, the mixture was acidified with 1 mL 10% of trichloroacetic acid, then 250 µL of $FeCl_3$ (0.1%) was added to the solution. Water was used as a blank and for control. Absorbance at 700 nm of this mixture was measured. Low absorbance indicates ferric reducing power

activity of the sample. Tert-butyl hydroquinone (TBHQ) and gallic acid were regarded as a positive control. Three replicates were analyzed for all samples.

HPLC analysis was applied according to [41] with a slight modification to determine phenolic and flavonoid compound contents using an Agilent Technologies 1100 series liquid chromatograph equipped with an autosampler. The analytical column was Agilent Eclipse XDB C18 (100 × 4.6 µm; 3.5 µm particle size). The diode array detector (DAD) was set to a scanning range of 180–420 nm. The mobile phase consisted of methanol (solvent A) and 0.1% formic acid (*v*/*v*) (solvent B). The flow rate was kept at 0.4 mL min^{-1} and the gradient program was as follows: 10% A—90% B (0–5 min); 20% A—80% B (5–10 min); 30% A—70% B (10–15 min); 50% A—50% B (15–20 min); 70% A—30% B (20–25 min); 90% A—10% B (25–30 min); 50% A—50% B (30–35 min) and 10% A—90% B (35–36 min). There was 5 min of post-run for reconditioning. The injection volume was 10 µL and peaks were monitored simultaneously at 280, 320, and 360 nm for the benzoic acid and cinnamic acid derivatives and flavonoids compound, respectively. All samples were filtered through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI) before injection. Peaks were identified by congruent retention times and UV spectrum and compared with those of the standards.

2.4. Applications of Clove Fruit Extract (CFE) and Salicylic Acid (SA)

At 20, 35, and 50 days after sowing, three foliar sprays each of the distilled water (control), CFE, SA, and CFE+SA were performed in the early morning utilizing a 20 L dorsal-sprayer to run-off. CFE and SA concentrations, in addition to spray application numbers and spray timings, were identified according to the findings of an initial pot experiment (data not shown). To optimize the penetration of the spray solution into the plant leaf tissues, the spray solutions received some drops of Tween-20 (0.1%, *v*/*v*) as a surfactant.

2.5. Assessment of Attributes Related to Growth and Yield

Fifty-five days after planting, 4 potato plants from the 4 rows of each experimental plot (a total of 12 plants from the three replicates) were randomly chosen to estimate shoot fresh and dry weights (g $plant^{-1}$) and leaf areas (cm^2 $plant^{-1}$). At harvest, fresh potato tubers samples were reaped from 40 plants from each plot (a total of 120 plants from the three replicates) to estimate average tuber weight (g), tuber number $plant^{-1}$, and total yield (ton fad^{-1}; fad = 4200 m^2 = 0.42 ha).

2.6. Assessment of Attributes Related to Plant Physio-Biochemistry

Four plants were gathered randomly for their leaves from each plot to evaluate the physio-chemical traits. The total contents of chlorophyll and carotenoids were measured after extraction utilizing fresh leaves and pure acetone following the procedures outlined in [42]. The rates of net photosynthesis and transpiration, and the conductance of stomata, were measured utilizing a photosynthetic system (portable, LF6400XTR, LI-COR, USA). Based on the procedure outlined in [43], the relative water content was measured.

The stability index of cell membranes and the total leakage of inorganic ions from leaves were determined according to the procedures in [44]. A duplicate sample having a weight of 0.2 g was collected from the fully expanded leaf tissue and immersed in a test tube with 10 mL doubled distilled water. The samples were heated in a water both at 40 °C for 30 min and assessed for electrical conductivity (EC1; Starlac Industries, Ambala, Haryana, India). A similar set of leaf samples was heated at 100 °C for 10 min and assessed for EC2. The EC1 and EC2 ratio were used to determine the membrane stability index (MSI) through the following formula:

$$MSI\ (\%) = (1 - (EC_1/EC_2)) \times 100 \quad (5)$$

The total inorganic ions leaked out in the leaves was determined using 20 leaf discs 0.5 cm that were taken from the fully expanded leaf tissue and put into a boiling tube containing 10 mL of deionized water and electrical conductivity was measured (EC_a), and then the content was heated at 45 °C and 55 °C for 30 min each in a water bath and electrical

conductivity was measured (EC_b). Then, the content was boiled again at 100 °C for 10 min and electrical conductivity was recorded again (EC_c). The electrolyte leakage (EL) was calculated using the formula:

$$EL\ (\%) = (EC_b - EC_a)/EC_c) \times 100 \quad (6)$$

Malondialdehyde (MDA) was determined according to [45]. A weight of 0.1 g was taken from the fully expanded leaf tissue and homogenized with 5 mL 0.07% $NaH_2PO_4 \cdot 2H_2O$ and 1.6% $Na_2HPO_4 \cdot 12H_2O$ (50 mM) and centrifuged at 20,000× *g* for 25 min at 4 °C. The supernatant was used for the determination and the results of MDA were expressed as $A_{532–600}\ g^{-1}$ FW.

The superoxide ($O_2^{\bullet -}$) level in potato leaves was measured as $A_{580}\ g^{-1}$ leaf fresh weight following the procedure outlined in [46]. Potato leaf tissues (100 mg) were cut into 1 mm × 1 mm fragments and immersed for 1 h at room temperature in 10 mM K-phosphate buffer, pH 7.8, 0.05% NBT, and 10 mM NaN_3. Two milliliters of immersed solution was heated at 85 °C for 15 min and cooled rapidly. Optical density was measured colorimetrically at 580 nm and the $O_2^{\bullet -}$ content was expressed as $A_{580}\ g^{-1}$ FW.

The level of hydrogen peroxide (H_2O_2) was determined using the method outlined in [47]. H_2O_2 was determined in plant leaf tissue by the extraction in acetone, and then titanium reagent and ammonium were added to the extract and dissolved in sulfuric acid (1 M). Absorbance of the supernatant was measured at 415 nm. The results of H_2O_2 level were expressed as mole g^{-1} FW.

The methods outlined in [48,49] were utilized to assess the levels of both proline and total soluble sugars, respectively, in potato leaves. For proline, 0.1 g from the fully expanded leaf tissue was ground with 10 mL of 3% (*w*/*v*) aqueous sulphosalicylic acid, the homogenate was filtered through Whatman 2 filter paper, and 1 mL of filtrate was reacted with 1 mL acid ninhydrin reagent in addition to 1 mL glacial acetic acid in a test tube for 1 h at 100 °C. Then, the reaction was terminated in an ice bath. Toluene at 2 mL was added to the mixture and the upper toluene layer was measured at 520 nm using a UV spectrophotometer. For total soluble sugars, 0.2 g of leaves was washed with 5 mL 70% ethanol and homogenized with 5 mL 96% ethanol. The extract was centrifuged at 3500× *g* for 10 min. The supernatant was collected and stored at 4 °C. Freshly prepared anthrone (3 mL) was added to 0.1 mL of supernatant. This mixture was incubated in a hot water bath for 10 min. The absorbance was recorded at 625 nm with a Bausch and Lomb- 2000 Spectronic Spectrophotometer.

The α-tocopherol (α-ToCo) content (μmol g^{-1} leaf dry matter) was assessed by applying the procedures described in [50,51]. A stock solution was functioned using R-TOC to prepare standard solutions (20–200 μg mL^{-1}) and samples were saponified and homogenized. Saponification was implemented and the extraction was performed thrice. The filtrates were evaporated and the residues were dissolved with n-hexane (HPLC grade). With the HPLC system, α-TOC content was evaluated with a proper mobile phase (e.g., methanol: water, 94:6, respectively) with a 1.5 mL min^{-1} flow rate, and a UV detector set at 292 nm.

2.7. Determination of Enzymatic and Non-Enzymatic Antioxidant Activities

Fresh full expanded leaves of the same plants collected for physio-chemical attributes were used to evaluate the activities of different antioxidants. The procedure described in [52] was applied to extract the tested enzymes. Samples were washed with distilled water and surface moisture was wiped off. A weight of 0.5 g sample was homogenized in ice cold 0.1 M phosphate buffer (pH 7.5) containing 0.5 mM EDTA with a pre-chilled mortar and pestle. Then, the homogenate was transferred to centrifuge tubes and centrifuged at 4 °C in Beckman refrigerated centrifuge at 15,000× *g* for 15 min. The supernatant was transferred to 30 mL tubes and referred to enzyme extract. The activities of catalase (CAT, peroxidase (POD), ascorbate peroxidase (APX), superoxide dismutase (SOD), and

glutathione reductase (GR) enzymes were measured spectro-photochemically following the procedures depicted in [53–57], respectively.

CAT was assayed spectro-photo-chemically [53]. The enzyme extract (100 µL) was added to 100 µL of 100 mM H_2O_2 and the total volume was made up to 1 mL by 250 mM phosphate buffer pH 6.8. The decrease in optical density at 240 nm against a blank was recorded every 1 min. The activity POD was assayed using guaiacol as the substrate [54]. The reaction mixture consisted of 3 mL of phosphate buffer (0.1 M, pH 7.0), 30 mL of H_2O_2 (20 mM), 50 mL of enzyme extract, and 50 mL of guaiacol (20 mM). The reaction mixture was incubated in a cuvette for 10 min at room temperature. The optical density was measured at 436 nm and the enzyme activity was expressed as number of absorbance units g^{-1} leaf fresh weight. APX was assayed spectro-photo-chemically [55]. The assay was carried out at 25 °C in 1.0 cm light path cuvette and the reaction mixture consisted of 1500 < mu > l pH 7.0 phosphate buffer, 20 µL EDTA, 1000 µL sodium ascorbate, and enzyme extract (20 µL). After mixing, the reaction was initiated by adding 480 µL of H_2O_2 and the decrease in optical density at 290 nm against blank (without extract) was continuously recorded every minute (for two min). SOD activity was determined by recording the decrease in the absorbance of superoxide-nitro blue tetrazolium complex by the enzyme [56]. About 3 mL of a reaction mixture containing 0.2 mL of 200 mM methionine, 0.1 mL of 1.5 M sodium carbonate, 0.1 mL of 3 mM EDTA, 0.1 mL of 2.25 mM nitro-blue tetrazolium, 1.5 mL of 100 mM potassium phosphate buffer pH 7.0, 1 mL distilled water, and 0.05 mL of enzyme were taken in test tubes in duplicate from each enzyme sample. Two tubes without enzyme extract were taken as control. The reaction was started by adding 0.1 mL riboflavin (60 µM) and placing the tubes below a light source of two 15 W florescent lamps for 15 min. Reaction was stopped by switching off the light and covering the tubes with black cloth. Tubes without enzyme developed maximal color. A non-irradiated complete reaction mixture which did not develop color served as blank. Absorbance was recorded at 560 nm and one unit of enzyme activity was taken as the quantity of enzyme which reduced the absorbance reading of samples to 50% in comparison with tubes lacking enzymes. GR activity was measured after monitoring the oxidation of NADPH for three absorbance taken at 340 nm, and the activity was expressed as A_{564} min^{-1} mg^{-1} protein [57].

The procedures depicted in [58,59] were utilized to determine the levels (as µmol g^{-1} leaf fresh matter) of ascorbate and glutathione, respectively. To estimate the AsA content (µmol g^{-1} leaf fresh weight), the leaf extract was added to a mixture containing 30 mM of buffer (potassium phosphate, pH 7.4), TCA (2.5%), phosphoric acid (8.4%), bipyridyl (0.8%), and ferric chloride (0.3%). The reaction was conducted (40 °C, 30 min), and absorbance was read at 525 nm. To estimate GSH, leaf samples (0.5 g) were homogenized in 2 mL of 2% solution (v/v) of metaphosphoric acid and centrifuged for 10 min at 17,000× g. For neutralizing the supernatant, 0.6 mL of 10% solution (w/v) of sodium citrate was added. A 1.0 mL assay was prepared by adding 100 µL of each extract, distilled H_2O, 6 mM 5,5′-dithio-bis-2-nitrobenzoic acid, and 700 µL of 0.3 mM NADPH, and stabilized for 3–4 min at 25 °C. This whole assay was then added with 10 µL 5 mg mL glutathione (GSH) reductase to measure the absorbance at 412 nm. Standard curves were developed and readings were subjected for calculating the GSH concentrations expressed on fresh weight basis (µmol g^{-1} leaf fresh weight).

2.8. Statistical Analysis

Analyses of the resulting data were implemented applying IBM® SPSS® (SPSS Inc., IBM Corporation, New York, NY, USA) Statistics Version 25 (2017) for Windows. Two-way ANOVA was implemented to highlight the influence of three watering regimes (e.g., well watering, moderate drought, and severe drought) and four biostimulation treatments [e.g., control, clove fruit extract (CFE), salicylic acid (SA), and CFE+SA)], and their interactions. A p-value equal to or less than 0.05 was significant. A confidence interval was assessed at 95%.

3. Results

3.1. Growth, Yield, and Photosynthetic Attributes

All tested growth parameters [weight of fresh shoot (WFS), weight of dried shoot (WDS), and plant leaves area (LAP)], yield components [average plant tuber number (PTN), average tuber weight (TW), and total yield (TY)], and photosynthesis attributes [total chlorophyll content (TChC), total carotenoids content (TCarC), stomatal conductance (gs), net photosynthesis rate (Pn), and transpiration rate (Tr)] of potato plants were significantly reduced under the two drought levels [moderate drought (MD) and severe drought (SD)] compared to the control (well watering; WW) (Tables 3 and 4). The decreases in all growth and yield components were higher under SD than under MD. However, the application of SA and/or CFE significantly enhanced all of the above-mentioned growth and yield traits under WW and drought stress conditions compared to control plants. Improvements in all growth and yield traits were higher under drought stress than under WW. Among all foliar spray treatments, the combination of SA+CFE was the best treatment, increasing WFS, WDS, LAP, PTN, TW, TY, TChC, TCarC, gs, Pn, and Tr, on average, by 6.4, 6.1, 8.4, 5.8, 10, 6.8, 6.4, 13.6, 8.3, 4.9, and 13.3%, respectively, under WW compared to the SA+CFE-free control. Under MD, the SA+CFE treatment increased the above-mentioned growth and yield parameters by 20.1, 20.9, 10, 14.7, 5.5, 11.4, 25.7, 6.5, 17.1, 24.2, and 20.7%, respectively, compared to the corresponding control. Under SD, this best treatment increased the above parameters by 37.1, 36.9, 24.4, 14.5, 6.6, 21.5, 16.5, 8.5, 27.7, 13.6, and 23.9%, respectively, compared to the corresponding control.

Table 3. Influence of foliar nourishment with clove fruit extract (CFE) and salicylic acid (SA) on growth traits and yield component of potato plants grown under three irrigation regimes in two seasons.

Treatments		Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Leaf Area Plant^{-1} (cm^2)	Tuber Number Plant^{-1}	Average Tuber Weight (g)	Total Yield (Ton Fad^{-1})
Stress	**Foliar Spray**						
2020 Season							
Well-watered	Control	311.9 ± 3.2 d	33.5 ± 1.1 d	4158 ± 4.5 d	3.79 ± 0.09 c	191.3 ± 3.2 d	20.3 ± 1.1 d
	SA	322.2 ± 3.6 c	34.6 ± 1.3 c	4277 ± 4.6 c	3.89 ± 0.07 b	196.3 ± 2.5 c	21.1 ± 1.2 c
	CFE	327.1 ± 3.8 b	35.1 ± 1.2 b	4425 ± 4.8 b	3.97 ± 0.06 ab	203.1 ± 2.8 b	21.3 ± 1.3 b
	CFE+SA	331.7 ± 3.2 a	35.6 ± 1.5 a	4510 ± 4.4 a	4.02 ± 0.11 a	210.4 ± 2.7 a	21.7 ± 1.4 a
Moderate drought	Control	243.5 ± 3.6 h	26.1 ± 0.9 h	3577 ± 3.9 h	3.17 ± 0.07 g	171.6 ± 2.6 h	17.5 ± 1.2 h
	SA	262.6 ±3.8 g	28.2 ± 0.8 g	3709 ± 4.2 g	3.36 ± 0.08 f	174.2 ± 1.9 g	18.2 ± 1.4 g
	CFE	280.3 ± 4.1 f	30.1 ± 1.1 f	3713 ± 3.7 f	3.47 ± 0.08 e	177.5 ± 2.2 f	19.1 ± 1.0 f
	CFE+SA	292.2 ± 3.9 e	31.4 ± 1.3 e	3936 ± 3.8 e	3.64 ± 0.06 d	181.1 ± 1.8 e	19.5 ± 1.0 e
Severe drought	Control	152.6 ± 1.5 l	16.4 ± 1.4 l	2048 ± 3.5 l	2.60 ± 0.07 k	154.8 ± 1.6 l	13.3 ± 0.9 l
	SA	178.5 ± 1.5 k	19.1 ± 0.9 k	2185 ± 4.2 k	2.73 ± 0.08 j	159.9 ± 2.2 k	14.3 ± 0.8 k
	CFE	189.6 ± 1.8 j	20.3 ± 0.8 j	2371 ± 4.9 j	2.85 ± 0.06 i	162.1 ± 2.5 j	15.1 ± 0.9 j
	CFE+SA	208.9 ± 2.3 i	22.4 ± 1.2 i	2549 ± 3.8 i	2.97 ± 0.05 h	165.2 ± 2.3 i	16.1 ± 0.8 i
2021 season							
Well-watered	Control	309.3 ± 4.2 d	32.7 ± 1.4 c	4155 ± 5.3 d	3.75 ± 0.07 bc	190.4 ± 3.4 d	20.3 ± 1.0 c
	SA	319.4 ± 4.5 c	33.7 ± 1.7 b	4274 ± 5.6 c	3.84 ± 0.08 ab	192.3 ± 3.5 c	20.9 ± 1.0 b
	CFE	324.4 ± 3.8 b	34.4 ± 1.5 a	4423 ± 5.4 b	3.93 ± 0.09 a	202.7 ± 3.9 b	21.2 ± 0.9 b
	CFE+SA	329.1 ± 3.5 a	34.7 ± 1.3 a	4507 ± 4.8 a	3.96 ± 0.06 a	209.6 ± 2.8 a	21.7 ± 0.9 a
Moderate drought	Control	241.2 ± 3.8 h	25.4 ± 1.2 g	3574 ± 4.9 h	3.15 ± 0.07 e	171.0 ± 2.7 h	17.3 ± 0.9 g
	SA	260.3 ± 4.2 g	27.5 ± 1.3 f	3706 ± 5.6 g	3.43 ± 0.05 d	173.5 ± 3.5 g	18.1 ± 0.8 f
	CFE	278.0 ± 2.9 f	29.7 ± 1.4 e	3811 ± 4.7 f	3.45 ± 0.07 d	176.9 ± 1.6 f	18.9 ± 0.8 e
	CFE+SA	289.7 ± 2.3 e	30.9 ± 1.1 d	3935 ± 3.9 e	3.61 ± 0.08 c	180.4 ± 1.8 e	19.3 ± 0.9 d
Severe drought	Control	150.7 ± 2.7 l	16.1 ± 1.3 k	2045 ± 4.3 l	2.57 ± 0.09 h	154.3 ± 2.5 l	13.2 ± 0.8 k
	SA	176.6 ± 3.6 k	18.7 ± 0.9 j	2183 ± 3.6 k	2.70 ± 0.06g h	159.3 ± 2.7 k	14.2 ± 0.9 j
	CFE	187.6 ± 3.4 j	19.6 ± 0.9 i	2369 ± 5.2 j	2.82 ± 0.07 fg	161.5 ± 1.9 j	14.9 ± 0.7 i
	CFE+SA	207.0 ± 1.9 i	22.1 ± 1.0 h	2546 ± 5.3 i	2.95 ± 0.06 f	164.5 ± 1.8 i	16.1 ± 0.8 h

Mean values (n = 9) in each column for each year, ±SE. Means were compared at $p \leq 0.05$ by Duncan's Multiple Range Test. Mean pairs followed by different letters are significantly different. Control plants were sprayed with distilled water vs CFE extract or CFE+SA for the other treatments.

Table 4. Influence of foliar nourishment with clove fruit extract (CFE) and salicylic acid (SA) on total chlorophyll content (TChC), total carotenoid content (TCarC), net photosynthetic rate (Pn), stomatal conductance (gs), and transpiration rate (Tr) of potato plants grown under three irrigation regimes in two seasons.

Treatments		TChC (mg g^{-1} FW)	TCarC (mg g^{-1} FW)	Pn (μmol CO_2 m^{-2} s^{-1})	Tr (mmol H_2O m^{-2} s^{-1})	gs (mmol H_2O m^{-2} s^{-1})
Stress	**Foliar Spray**					
			2020 Season			
Well-watered	Control	2.09 ± 0.09 bc	0.74 ± 0.03 d	12.4 ± 1.1 d	6.34 ± 0.44 c	0.466 ± 0.02 d
	SA	2.12 ± 0.11 b	0.76 ± 0.04 c	12.6 ± 1.3 c	6.51 ± 0.42 bc	0.480 ± 0.03 c
	CFE	2.17 ± 0.12 ab	0.79 ± 0.04 b	12.8 ± 1.4 b	6.72 ± 0.45 b	0.493 ± 0.02 b
	CFE+SA	2.22 ± 0.10 a	0.84 ± 0.05 a	13.1 ± 1.5 a	7.17 ± 0.52 a	0.513 ± 0.04 a
Moderate drought	Control	1.65 ± 0.07 f	0.70 ± 0.03 e	8.33 ± 1.96 h	4.40 ± 0.41 fg	0.386 ± 0.01 h
	SA	1.77 ± 0.08 e	0.71 ± 0.04 e	8.84 ± 0.085 g	4.54 ± 0.46 f	0.413 ± 0.02 g
	CFE	1.93 ± 0.07 d	0.73 ± 0.03 d	9.78 ± 0.94 f	4.83 ± 0.38 e	0.430 ± 0.02 f
	CFE+SA	2.02 ± 0.10 cd	0.74 ± 0.03 d	10.3 ± 1.1 e	5.17 ± 0.37 d	0.453 ± 0.03 e
Severe drought	Control	1.33 ± 0.06 i	0.60 ± 0.011	6.18 ± 0.74 l	3.38 ± 0.33 j	0.260 ± 0.01 l
	SA	1.40 ± 0.08 h	0.63 ± 0.02 h	6.55 ± 0.73 k	3.67 ± 0.21 i	0.286 ± 0.01 k
	CFE	1.44 ± 0.07 h	0.65 ± 0.01 g	6.79 ± 0.62 j	4.07 ± 0.12 h	0.313 ± 0.02 j
	CFE+SA	1.54 ± 0.08 g	0.67 ± 0.02 f	7.10 ± 0.53 i	4.19 ± 0.32 gh	0.326 ± 0.02 i
			2021 season			
Well-watered	Control	2.07 ± 0.12 bc	0.73 ± 0.05 cd	11.9 ± 0.95 b	6.06 ± 0.39 c	0.463 ± 0.03 b
	SA	2.11 ± 0.10 b	0.75 ± 0.06 c	12.1 ± 1.3 ab	6.32 ± 0.38 bc	0.466 ± 0.03 b
	CFE	2.16 ± 0.11 ab	0.78 ± 0.07 b	12.2 ± 1.4 ab	6.50 ± 0.28 ab	0.476 ± 0.02 ab
	CFE+SA	2.21 ± 0.13 a	0.83 ± 0.06 a	12.4 ± 1.5 a	6.88 ± 0.15 a	0.4933 ± 0.03 a
Moderate drought	Control	1.55 ± 0.06 f	0.67 ± 0.03 f	7.92 ± 0.66 f	4.14 ± 0.34 f	0.373 ± 0.01 e
	SA	1.76 ± 0.07 e	0.70 ± 0.05 e	8.29 ± 0.74 e	4.28 ± 0.32 f	0.400 ± 0.01 d
	CFE	1.83 ± 0.08 d	0.70 ± 0.03 e	9.22 ± 0.85 d	4.67 ± 0.36 e	0.420 ± 0.02 cd
	CFE+SA	2.00 ± 0.13 cd	0.72 ± 0.04 de	9.89 ± 0.89 c	5.13 ± 0.37 d	0.436 ± 0.03 c
Severe drought	Control	1.22 ± 0.05 i	0.56 ± 0.01 h	6.14 ± 0.42 i	3.27 ± 0.19 g	0.243 ± 0.01 h
	SA	1.29 ± 0.03 h	0.60 ± 0.02 h	6.40 ± 0.43 hi	3.56 ± 0.12 g	0.276 ± 0.01 g
	CFE	1.33 ± 0.04 h	0.63 ± 0.03 g	6.53 ± 0.33 h	3.94 ± 0.13 f	0.300 ± 0.01 f
	CFE+SA	1.43 ± 0.06 g	0.59 ± 0.01 f	6.90 ± 0.36 g	4.05 ± 0.18 f	0.316 ± 0.02 f

Mean values (n = 9) in each column for each year, ±SE. Means were compared at $p \leq 0.05$ by Duncan's Multiple Range Test. Mean pairs followed by different letters are significantly different. Control plants were sprayed with distilled water vs CFE extract or CFE+SA for the other treatments.

3.2. Cell and Membrane Integrity and Oxidative Stress Markers

The relative water content (RWC) and membrane stability index (MSI) of leafy tissues were significantly diminished under MD and SD compared to the WW control (Table 5).

Reductions in RWC and MSI were more pronounced under SD (12.1 and 40.5%, respectively) than under MD (6.1 and 8.5%, respectively). However, the application of SA and/or CFE significantly improved RWC and MSI under WW, and under MD and SD compared to the control (without SA and/or CFE). Improvements in RWC and MSI were higher under MD and SD than under stress-free conditions. Among all foliar spray treatments, the SA+CFE combination was the best treatment. It increased RWC and MSI, on average, by 4.5 and 5.5%, respectively, under well watering (WW); by 5.1 and 5.3%, respectively, under MD; and by 5.0 and 3.5%, respectively, under SD, compared to the corresponding controls.

Electrolyte leakage (EL), malondialdehyde (MDA), superoxide ($O_2^{\bullet -}$), and hydrogen peroxide (H_2O_2) levels were gradually increased with a gradual decrease in the irrigation regime (from WW to MD and then to SD) (Table 5). The increases in El, MDA, $O_2^{\bullet -}$, and H_2O_2 were 63.5, 56.8, 57.2, and 85.3% under moderate drought and 109.7, 146.4, 90.6, and 193% under severe drought, respectively, compared to the normal control. However, foliar nourishing with SA and/or CFE for potato plants displayed a noticeable reduction in El, MDA, $O_2^{\bullet -}$, and H_2O_2 under normal and stressed conditions. The combination (SA+CFE) treatment was most functional in attenuating the harmful influences of drought and diminished El, MDA, $O_2^{\bullet -}$, and H_2O_2, on average, by 14.1, 6.3, 13.3, and 8.9%, respectively,

under well watering (WW); by 17.7, 26.7, 16.0, and 19.8%, respectively, under MD; and by 17.8, 17.7, 12.5, and 24,0%, respectively, under SD, compared to the corresponding controls.

Table 5. Influence of foliar nourishment with clove fruit extract (CFE) and salicylic acid (SA) on relative water content (RWC), membrane stability index (MSI), and electrolyte leakage (EL), as well as malondialdehyde (MDA), superoxide ($O_2^{\bullet -}$), and hydrogen peroxide (H_2O_2) levels of potato plants grown under three irrigation regimes in two seasons.

Treatments		RWC (%)	MSI (%)	EL (%)	MDA (μmol g^{-1} FW)	$O_2^{\bullet -}$ (A580 g^{-1} FW)	H_2O_2 (μmol g^{-1} FW)
Stress	**Foliar Spray**						
2020 season							
Well-watered	Control	85.7 ± 2.3 d	71.5 ± 2.2 c	7.07 ± 0.11 h	13.0 ± 0.69 i	0.37 ± 0.01 i	5.53 ± 0.21 i
	SA	87.3 ± 3.6 c	72.6 ± 2.5 b	6.86 ± 0.14 h	12.6 ± 0.88 i	0.36 ± 0.01 j	5.41 ± 0.26 i
	CFE	89.1 ± 3.5 b	74.5 ± 2.6 a	6.55 ± 0.19 j	12.5 ± 0.89 i	0.34 ± 0.02 k	5.21 ± 0.24 j
	CFE+SA	90.4 ± 2.8 a	75.4 ± 2.3 a	6.11 ± 0.18 j	12.4 ± 0.79 i	0.32 ± 0.01 l	5.05 ± 0.22 j
Moderate drought	Control	80.6 ± 3.6 h	65.7 ± 2.1 g	11.4 ± 0.32 d	20.5 ± 1.2 e	0.58 ± 0.03 e	10.3 ± 0.56 e
	SA	81.7 ± 3.9 g	67.0 ± 2.8 f	10.6 ± 0.35 e	18.6 ± 1.5 f	0.55 ± 0.04 f	9.54 ± 0.49 f
	CFE	82.8 ± 3.4 f	68.5 ± 2.9 e	9.92 ± 0.25 f	17.1 ± 1.4 g	0.52 ± 0.03 g	8.96 ± 0.39 g
	CFE+SA	84.8 ± 2.9 e	69.5 ± 3.1 d	9.42 ± 0.24 g	15.2 ± 1.1 h	0.49 ± 0.02 h	8.21 ± 0.48 h
Severe drought	Control	75.6 ± 2.5 l	42.2 ± 2.4 k	14.6 ± 0.29 a	36.6 ± 2.3 a	0.71 ± 0.04 a	16.1 ± 0.78 a
	SA	76.9 ± 2.6 k	48.8 ± 1.2 j	13.3 ± 0.28 b	33.5 ± 2.4 b	0.67 ± 0.03 b	13.9 ± 0.88 b
	CFE	77.9 ± 3.1 j	51.2 ± 1.7 i	13.1 ± 0.32 b	31.6 ± 2.4 c	0.64 ± 0.04 c	12.7 ± 0.79 c
	CFE+SA	79.3 ± 3.3 i	54.8 ± 1.6 h	12.1 ± 0.41 c	30.3 ± 1.5 d	0.62 ± 0.05 d	12.4 ± 0.95 d
2021 season							
Well-watered	Control	85.1 ± 4.1 b	70.8 ± 1.9 d	7.09 ± 0.32 h	13.7 ± 0.75 i	0.38 ± 0.02 i	5.68 ± 0.32 i
	SA	85.3 ± 4.5 b	71.9 ± 2.5 c	6.74 ± 0.36 hi	12.7 ± 0.74 i	0.37 ± 0.01 i	5.60 ± 0.33 ij
	CFE	86.6 ± 4.3 ab	73.5 ± 2.9 b	6.40 ± 0.25 ij	12.6 ± 0.86 i	0.35 ± 0.01 j	5.27 ± 0.34 jk
	CFE+SA	88.1 ± 4.8 a	74.8 ± 3.1 a	6.04 ± 0.29 j	12.6 ± 1.2 i	0.33 ± 0.01 k	5.16 ± 0.52 k
Moderate drought	Control	79.8 ± 3.5 d	65.2 ± 3.2 f	11.7 ± 0.45 d	21.4 ± 1.3 e	0.60 ± 0.03 e	10.5 ± 0.68 e
	SA	81.1 ± 3.2 cd	66.0 ± 1.9 f	10.8 ± 0.44 e	18.7 ± 1.5 f	0.55 ± 0.03 f	9.69 ± 0.78 f
	CFE	81.9 ± 3.9 c	67.3 ± 2.8 e	10.2 ± 0.52 f	17.4 ± 1.4 g	0.53 ± 0.04 g	9.25 ± 0.68 g
	CFE+SA	83.9 ± 3.3 b	68.4 ± 2.4 e	9.57 ± 0.56 g	15.5 ± 1.6 h	0.50 ± 0.03 h	8.47 ± 0.65 h
Severe drought	Control	74.4 ± 2.5 g	41.1 ± 1.3 j	15.1 ± 0.66 a	37.6 ± 2.3 a	0.72 ± 0.04 a	16.7 ± 0.79 a
	SA	75.9 ± 2.8 fg	47.6 ± 1.4 i	13.5 ± 0.69 b	34.2 ± 3.2 b	0.68 ± 0.03 b	14.2 ± 0.99 b
	CFE	76.7 ± 3.5 ef	50.5 ± 1.5 h	13.0 ± 0.66 c	32.1 ± 3.1 c	0.66 ± 0.02 c	13.1 ± 0.96 c
	CFE+SA	78.2 ± 2.8 de	53.9 ± 1.6 g	12.3 ± 0.68 d	30.7 ± 3.3 d	0.63 ± 0.01 d	12.5 ± 0.81 d

Mean values (n = 9) in each column for each year, ±SE. Means were compared at $p \leq 0.05$ by Duncan's Multiple Range Test. Mean pairs followed by different letters are significantly different. Control plants were sprayed with distilled water vs CFE extract or CFE+SA for the other treatments.

3.3. Osmoprotectants and Antioxidantive Status

Compared with the WW control, free proline (FPro), soluble sugars (SSug), α-tocopherol (α-ToCo), ascorbate (AsA), and glutathione (GSH) levels, in addition to superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) activities of the potato plant, were significantly increased under both MD and SD (Tables 6 and 7).

The increments in all of these osmoprotectants, and non-enzymatic and enzymatic antioxidants, were more pronounced under SD than under MD. However, the application of SA and/or CFE further increased all of the above-mentioned attributes under WW and under both MD and SD compared to the control (without SA and/or CFE). The increases in all of these osmoprotectants, and non-enzymatic and enzymatic antioxidants, were higher under drought conditions than under WW conditions. Among all foliar spray treatments, the combination of SA+CFE was the best treatment, increasing the levels of FPro, SSug, α-ToCo, AsA, and GSH, on average, by 3.3, 11.7, 12.6, 11.1, and 8.8%, respectively, under WW; by 17.0, 18.3, 20.0, 26.4, and 41.4%, respectively, under MD; and by 10.8, 9.6, 14.6, 15.9, and 6.5%, respectively, under SD, compared to the corresponding controls. Moreover, the SA+CFE treatment raised the activities of SOD, POX, CAT, APX, and GR, on average, by 8.7, 9.8, 6.8, 7.9, and 6.4%, respectively, under WW; by 13.0, 11.7, 5.2, 10.3, and 22.7%,

respectively, under MD; and by 8.5, 4.0, 10.6, 6.8, and 10.1%, respectively, under SD, compared to the corresponding controls.

Table 6. Influence of foliar nourishment with clove fruit extract (CFE) and salicylic acid (SA) on free proline, soluble sugars (S.sugar), α-tocopherol (α-TOC), ascorbic acid (AsA), and glutathione (GSH) contents of potato plants grown under three irrigation regimes in two seasons.

Treatments		Free Proline (μmol g−1 DW)	S.Sugar (mg g−1 DW)	α-TOC (μmol g−1 DW)	AsA (μmol g−1 FW)	GSH (μmol g−1 FW)
Stress	**Foliar Spray**					
			2020 Season			
Well-watered	Control	2.52 ± 0.11 j	10.7 ± 0.42 j	1.55 ± 0.07 k	1.20 ± 0.06 k	0.830 ± 0.02 i
	SA	2.58 ± 0.18 ij	11.4 ± 0.54 i	1.64 ± 0.09 j	1.25 ± 0.04 j	0.850 ± 0.04 i
	CFE	2.59 ± 0.15 ij	11.8 ± 0.49 h	1.71 ± 0.08 i	1.28 ± 0.06 ij	0.853 ± 0.06 i
	CFE+SA	2.61 ± 0.12 i	12.0 ± 0.63 h	1.75 ± 0.06 i	1.31 ± 0.03 i	0.906 ± 0.04 h
Moderate drought	Control	4.31 ± 0.19 h	16.4 ± 0.98 g	2.14 ± 0.11 h	1.54 ± 0.05 h	1.10 ± 0.07 g
	SA	4.55 ± 0.21 g	17.1 ± 0.87 f	2.25 ± 0.13 g	1.67 ± 0.06 g	1.66 ± 0.06 f
	CFE	4.79 ± 0.23 f	18.5 ± 1.1 e	2.41 ± 0.14 f	1.84 ± 0.07 f	1.39 ± 0.03 e
	CFE+SA	5.02 ± 0.24 e	19.6 ± 1.2 d	2.56 ± 0.13 e	1.96 ± 0.09 e	1.56 ± 0.05 d
Severe drought	Control	6.43 ± 0.29 d	23.3 ± 1.6 c	2.97 ± 0.15 d	2.36 ± 0.11 d	2.07 ± 0.13 c
	SA	7.72 ± 0.32 c	24.5 ± 1.4 b	3.18 ± 0.17 c	2.49 ± 0.14 c	2.15 ± 0.14 b
	CFE	6.88 ± 0.35 b	24.8 ± 1.5 b	3.28 ± 0.19 b	2.66 ± 0.16 b	2.19 ± 0.18 ab
	CFE+SA	7.08 ± 0.34 a	25.4 ± 1.5 a	3.39 ± 0.16 a	2.73 ± 0.18 a	2.23 ± 0.19 a
			2021 season			
Well-watered	Control	2.49 ± 0.11 g	10.5 ± 0.55 i	1.54 ± 0.05 l	1.14 ± 0.05 k	0.813 ± 0.03 h
	SA	2.53 ± 0.14 g	11.3 ± 0.64 h	1.62 ± 0.06 k	1.20 ± 0.08 j	0.823 ± 0.04 gh
	CFE	2.54 ± 0.13 g	11.6 ± 0.58 h	1.67 ± 0.08 j	1.24 ± 0.07 ij	0.833 ± 0.03 gh
	CFE+SA	2.57 ± 0.12 g	11.7 ± 0.72 h	1.73 ± 0.07 i	1.29 ± 0.07 i	0.883 ± 0.04 g
Moderate drought	Control	4.20 ± 0.22 f	16.3 ± 0.81 g	2.11 ± 0.12 h	1.52 ± 0.06 h	1.07 ± 0.08 f
	SA	4.51 ± 0.24 e	16.7 ± 1.2 g	2.20 ± 0.13 g	1.65 ± 0.07 g	1.21 ± 0.06 e
	CFE	4.76 ± 0.26 d	18.1 ± 1.4 f	2.38 ± 0.16 f	1.80 ± 0.08 f	1.33 ± 0.05 d
	CFE+SA	4.94 ± 0.33 d	19.1 ± 1.7 e	2.54 ± 0.11 e	1.91 ± 0.09 e	1.51 ± 0.08 c
Severe drought	Control	6.32 ± 0.36 c	22.8 ± 1.5 b	2.90 ± 0.13 d	2.33 ± 0.14 d	2.04 ± 0.16 b
	SA	6.50 ± 0.35 bc	24.1 ± 1.6 c	3.14 ± 0.14 c	2.46 ± 0.17 c	2.11 ± 0.18 a
	CFE	6.61 ± 0.41 b	24.5 ± 1.3 b	3.23 ± 0.16 b	2.64 ± 0.13 b	2.13 ± 0.14 a
	CFE+SA	7.05 ± 0.43 a	25.1 ± 1.6 a	3.34 ± 0.19 a	2.71 ± 0.18 a	2.15 ± 0.11 a

Mean values (n = 9) in each column for each year, ±SE. Means were compared at $p \leq 0.05$ by Duncan's Multiple Range Test. Mean pairs followed by different letters are significantly different. Control plants were sprayed with distilled water vs CFE extract or CFE+SA for the other treatments.

Table 7. Influence of foliar nourishment with clove fruit extract (CFE) and salicylic acid (SA) on peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), and glutathione reductase (GR) activities of potato plants grown under three irrigation regimes in two seasons.

Treatments		POX	CAT	APX	SOD	GR
Stress	**Foliar Spray**	**A_{564} min^{-1} mg^{-1} Protein**				
			2020 season			
Well-watered	Control	1.56 ± 0.06 k	39.8 ± 2.1 j	30.5 ± 1.1 k	4.14 ± 0.13 k	26.5 ± 1.2 k
	SA	1.63 ± 0.08 j	40.7 ± 1.9 i	32.0 ± 1.3 j	4.32 ± 0.21 j	27.3 ± 1.4 j
	CFE	1.67 ± 0.05 i	42.0 ± 1.6 h	32.9 ± 1.5 i	4.43 ± 0.15 i	27.7 ± 1.5 ij
	CFE+SA	1.70 ± 0.09 i	42.5 ± 1.7 h	33.3 ± 1.2 i	4.52 ± 0.23 i	28.4 ± 1.4 i
Moderate drought	Control	2.35 ± 0.11 h	48.1 ± 2.2 g	46.2 ± 1.6 h	6.63 ± 0.31 h	37.3 ± 2.1 h
	SA	2.46 ± 0.12 g	49.0 ± 2.5 f	47.9 ± 1.8 g	6.81 ± 0.35 g	39.1 ± 2.2 g
	CFE	2.57 ± 0.14 f	49.9 ± 2.8 e	49.8 ± 1.9 f	7.31 ± 0.36 f	43.0 ± 2.5 f
	CFE+SA	2.63 ± 0.12 e	50.5 ± 2.6 e	51.0 ± 1.8 e	7.51 ± 0.42 e	45.7 ± 2.3 e

Table 7. *Cont.*

Treatments		POX	CAT	APX	SOD	GR
Stress	**Foliar Spray**	**A_{564} min^{-1} mg^{-1} Protein**				
Severe drought	Control	3.15 ± 0.16 d	62.7 ± 3.2 d	63.9 ± 2.1 d	8.77 ± 0.35 d	57.7 ± 3.1 d
	SA	3.20 ± 0.17 c	65.3 ± 3.6 c	65.7 ± 2.2 c	9.18 ± 0.39 c	59.6 ± 3.6 c
	CFE	3.25 ± 0.19 b	68.2 ± 3.5 b	66.9 ± 2.5 b	9.40 ± 0.38 b	61.2 ± 3.5 b
	CFE+SA	3.30 ± 0.21 a	69.7 ± 3.8 a	67.8 ± 2.6 a	9.52 ± 0.46 a	63.0 ± 3.8 a
		2021 season				
Well-watered	Control	1.51 ± 0.07 j	38.9 ± 1.1 j	30.1 ± 1.4 h	4.11 ± 0.11 k	26.0 ± 1.5 j
	SA	1.59 ± 0.06 i	40.0 ± 1.5 i	31.1 ± 1.5 gh	4.23 ± 0.16 j	26.5 ± 1.6 j
	CFE	1.63 ± 0.05 hi	41.2 ± 1.4 h	31.9 ± 1.4 g	4.39 ± 0.18 i	26.7 ± 1.4 ij
	CFE+SA	1.67 ± 0.08 h	41.6 ± 1.9 h	32.1 ± 1.2 g	4.45 ± 0.15 i	27.5 ± 1.3 i
Moderate drought	Control	2.32 ± 0.13 g	47.2 ± 1.5 g	45.6 ± 1.5 f	6.60 ± 0.21 h	36.4 ± 2.1 h
	SA	2.44 ± 0.15 f	48.3 ± 1.9 f	47.0 ± 1.3 e	6.78 ± 0.32 g	38.4 ± 2.5 g
	CFE	2.54 ± 0.14 e	48.9 ± 2.1 ef	49.1 ± 1.4 d	7.20 ± 0.36 f	42.2 ± 2.6 f
	CFE+SA	2.59 ± 0.12 d	49.8 ± 2.3 e	50.3 ± 1.7 d	7.44 ± 0.35 e	44.8 ± 2.8 e
Severe drought	Control	3.11 ± 0.15 c	61.9 ± 2.6 d	63.0 ± 2.3 c	8.71 ± 0.39 d	56.5 ± 3.4 d
	SA	3.14 ± 0.14 bc	64.4 ± 3.1 c	64.8 ± 2.5 b	9.14 ± 0.32 c	58.3 ± 3.5 c
	CFE	3.16 ± 0.18 ab	67.2 ± 3.2 b	65.6 ± 3.1 b	9.31 ± 0.41 b	60.5 ± 3.9 b
	CFE+SA	3.21 ± 0.16 a	68.2 ± 35 a	67.8 ± 3.1 a	9.43 ± 0.42 a	62.8 ± 3.7 a

Mean values (n = 9) in each column for each year, ±SE. Means were compared at $p \leq 0.05$ by Duncan's Multiple Range Test. Mean pairs followed by different letters are significantly different. Control plants were sprayed with distilled water vs CFE extract or CFE+SA for the other treatments.

3.4. Traits Interrelationship

The association among evaluated morphological, yield, and physio-chemical traits of potato plants was estimated based on the analysis of principal components (Figure 2).

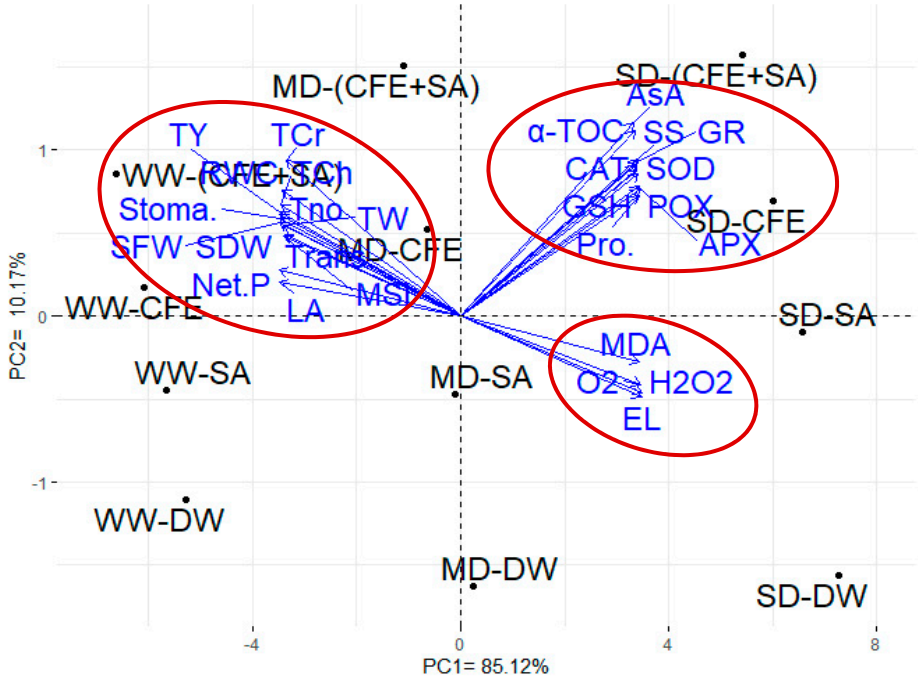

Figure 2. Biplot of the first two principal components for the morphological, yield, and physiochemical traits of potato plants. The morphological parameters comprised shoot fresh weight (SFW), shoot dry weight (SDW), and leaf area (LA). The yield parameters included tuber number (Tno), average

tuber weight (TW), and total yield (TY). The physiochemical parameters comprised total chlorophyll (TCh), total carotenoid (TCr), net photosynthetic rate (Net.P), transpiration rate (Trans.), stomatal conductance (Stoma.), relative water content (RWC), membrane stability index (MSI), and electrolyte leakage (EL), in addition to malondialdehyde (MDA), superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), proline (Pro.), soluble sugars (SS), α-tocopherol (α-TOC), ascorbic acid (AsA), glutathione (GSH), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), and glutathione reductase (GR). WW-DW, WW-SA, WW-CFE, and WW-CFE+SA were foliar applications using distilled water, salicylic acid, clove fruit extract, and salicylic acid + clove fruit extract under the well-watered treatments, respectively. MD-DW, MD-SA, MD-CFE, and MD-CFE+SA were foliar applications using distilled water, salicylic acid, clove fruit extract, and salicylic acid + clove fruit extract under the moderate-drought treatments, respectively. SD-DW, SD-SA, SD-CFE, and SD-CFE+SA were foliar applications using distilled water, salicylic acid, clove fruit extract, and salicylic acid + clove fruit extract under the severe-drought treatments, respectively.

4. Discussion

Drought is a serious environmental factor that threatens food security globally. Climate change increases temperatures and decreases rainfall, and thus increases the incidence of drought, especially in arid and semi-arid regions [60]. This diminishes the availability of water needed by plants, leading to severe weakness in growth and affecting the yields of plants, including potatoes [61–63]. Drought stress causes various harmful impacts on the performance of different crop plants by decreasing leaf uptake of photoactive radiation and decreasing radiation utilization efficiency [64]. It significantly depresses the membrane stability index (MSI), chlorophyll content, protein synthesis, and the rate of net photosynthesis (Pn) [7]. Moreover, it leads to toxic ion accumulations and disturbances in the attributes of gas exchange, all of which hinder photosynthesis, and thus the development, growth, and production of various crop plants [65–68]. Therefore, photosynthetic attributes have been utilized to assess the degree of tolerance to drought in crop plants [69].

In this study, the decline in plant growth and production under water deficit stress (Table 3) is likely due to the detrimental osmotic influence of drought stress causing a decrease in photosynthetic pigments, disturbances of gas exchange attributes, cell integrity and water balance, and elevations in oxidative stress markers ($O_2^{\bullet -}$ and H_2O_2) of stressed plants (Tables 4 and 5). These drought-inhibitory effects stimulate closure of stomata, disorder of ionic balance, and decrease in photosynthesis, thus inhibiting plant growth and production [7,11,70]. Conversely, osmoprotective compounds [free proline (FPro) and soluble sugars (SSug)], and non-enzymatic [FPro, α-tocopherol (α-ToCo), ascorbate (AsA), and glutathione (GSH)] and enzymatic [superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR)] antioxidants were noticeably elevated in potato plants to enable them to tolerate drought stress. However, the potato plants failed to perform well under the droughts tested (Tables 3–5). Therefore, it was necessary to treat the potato plants externally with stimulating adjuvants such as clove fruit extract (CFE) and/or salicylic acid (SA) to enable them to overcome the water deficit stress. Hence, foliar spraying with CFE and/or SA noticeably enhanced growth parameters, yield traits, and physio-biochemical attributes of water deficit-stressed potato plants.

Analysis of CFE revealed the presence of flavonoids, phenolic compounds (i.e., 3,4 dihydroxybenzoic-acid, ellagic-acid, eugenol, eugenyl-acetate, gallic-acid, naphthalene, and tannic acid), antioxidants and osmoprotective compounds (i.e., FPro, GSH, and SSug), base nutrients (i.e., Ca, Mg, Fe, N, K, P), and vitamins (i.e., vit. A, vit. E, vit. D, and vit. C; AsA) (Table 2). These diverse components in CFE indicate that it can be utilized as an effectual plant biostimulant.

Under drought stress, the application of CFE significantly improved potato plant growth characteristics (e.g., weight of fresh and dried shoots, and plant leaf area), which may be attributed to the reinforced mobilization of growth-related metabolites/inorganic solutes such as ascorbate (vit. C), Ca^{2+}, and K^+ in CFE (Table 2) to the growing plumule and/or increased activity of amylase and reducing sugars, which contribute to early

(seedling) vigor and thus promoted plant growth [71,72]. In addition, the importance of CFE as a plant growth biostimulator is because it contains many phenolic compounds and flavonoids along with osmoprotectants, vitamins, and many essential nutrients (Table 2). The phenolic compounds (PhCs) of CFE are essential to relieve the harmful influences of water limitation stress in the plant [73]. They have been considered antioxidants and bioremediators to affect different processes related to plant physiology, development, and growth promotion, including seed germinability and processes related to cell division and chlorophyll biosynthesis [74,75]. Under multiple stressors, PhCs accumulate in stressed plants and represent distinct defense mechanisms to boost tolerability and adaptability of stressed plants by playing essential roles in multiple physiological processes [76,77]. In association with PhCs, flavonoids, as an essential component of CFE, have been confirmed for biosynthesis and accumulation in water-deficient plants to enhance plant resistance against the harmful influences of water-deficiency stress [78–80]. Accumulation of flavonoids in the cytoplasm can lead to detoxification of harmful H_2O_2 molecules, resulting in stress stimulated by water limitation [81]. Gallic acid (GLA), as one of the PhCs present in CFE, boosts plant growth under some stresses, including osmotic stress conditions due to its primary role in enhancing the level of indole acetic acid (IAA) in favor of stimulating cell division or elongation [82,83]. In addition, phenolic acids (PhAs) found in CFE (Table 3), restrict the production of ROS harmful products. They have a strong antioxidant capacity (due to the many hydroxyl groups placed in their structure) to restrict the undue (excessive) excitation energy of chlorophyll under stress. Moreover, PhAs are further synthesized due to stress as a stress defense mechanism and can serve as substrates for peroxidases [84,85].

As a non-enzymatic phenolic antioxidant, salicylic acid (SA) is a plant growth regulator, and a messenger or signaling molecule for modulating plants' responses to different stressors. When applied to potato plant leaves, it significantly improved photosynthetic pigments and this improvement was increased with the combined use of SA and CFE under drought stress (Table 4). This affirmative finding may be due to the influence of SA on increasing the endogenous content of cytokinins, which enhance differentiation of chloroplasts and biosynthesis of chlorophylls, and prevent degradation of chlorophylls [86,87], in addition to CFE benefits. Under the harmful influences of water deficit stress, SA application increased stomatal conductance in drought-stressed plants (Table 4) in favor of maintaining photosynthetic activity and reducing stress damage [88]. In this study, a high chlorophyll content along with stomatal conductivity combined with high photosynthetic capacity were the pillars of SA application responsible for the improvement in growth and yield components, and the total yield of tubers under water restriction. These positive influences were more pronounced when SA was applied in combination with CFE (Tables 3 and 4).

As important components of CFE, macronutrients, including K^+ and N, moderate the harmful influences of stress on plant performance in the form of growth and production [89–91]. Micronutrients also boost drought stress tolerance by improving plant root and overall plant growth, resulting in enhanced nutrient uptake and content [92]. Based on the foregoing, foliar nourishment with CFE may provide an active onset of earlier emergence and more timely termination of other phenological features [28].

Exogenous SA treatment attenuated the harmful influences of drought stress on characteristics of plant growth due to SA-induced increases in mineral uptake, CO_2 assimilation, and photosynthetic rate [93,94], along with the sustained role of SA on cell membranes and antioxidant function in favor of metabolic activity that may increase plants' stress tolerance [88,95]. Moreover, in our study, when the integrated SA+CFE was applied, the best results were obtained under drought stress conditions, which greatly relieved the harmful influences of drought stress by limiting the decline in potato plant growth and productivity (Table 3). In this study, integrated application of SA+CFE magnified the leaf area (Table 3), which collected the maximum content of chlorophylls (Table 4), whereby maintaining green leaf area maximized leaf photosynthesis (Table 4), and increased the capacity of the sink being met by providing photoassimilates of green leaves [96].

Drought stress, in this study, caused a decrease in chlorophyll content related to photosynthesis and gas exchange (Pn, Tr, and gs) relative to normal conditions. The reduction in these attributes was neatly connected to the degree (moderate or severe) of water restriction stress (Table 4). All these attributes have the same trend as that of tissue health status (RWC and MSI, Table 5). Reducing gs under drought stress can protect the plant because it allows for water conservation and improved WUE. Zhou [97] concluded that the reduction in photosynthesis is commonly because of the limitation of stomata under drought adverse conditions due to diminished gs and intercellular CO_2 levels. In the current study, the reduction in RWC under drought stress was accompanied by a reduction in gs and Tr, demonstrating that the limitation of stomata mainly led to the decrease in Pn. Because CO_2 fixation in the "Calvin cycle" is stress sensitive [98], it likely limits photosynthesis and potato plant leaves absorb more light energy than photosynthetic CO_2 fixation can take up. Reducing photosynthetic CO_2 fixation decreases the utilization of NADPH, which lowers the $NADP^+$ level [99]. Because $NADP^+$ is the primary acceptor of electrons in PSI, depletion of $NADP^+$ quickens the electron transfer from PSI to O_2, which leads to H_2O_2 generation from $O_2^{\bullet -}$ [14]. Plants have some protective mechanisms (e.g., Mehler reaction [14], photorespiration [100], and non-photochemical quenching [101]) that can dissipate excess energy but the energy dissipated by these mechanisms remains negligible.

Data of our study showed that applying CFE+SA kept chlorophyll at the highest content along with enhancing gas exchange parameters (Table 4). These affirmative findings are likely due to that CFE contains the essential nutrients related to photosynthesis and chlorophyll molecules, such as N, Mg, and Fe. Both Mg and N are central components of the chlorophyll molecule. Mg acts as a Rubisco activator to influence the CO_2 assimilation in leaves and N is a pivotal component of cellular proteins [102]. In addition, Fe plays a functional role in activating enzymes related to the biosynthesis pathway of chlorophylls and some antioxidative enzymes such as APX and GSH that eliminate ROS and restrict the degradation of chlorophylls [103]. Moreover, K as a CFE nutrient plays a stomata regulatory role and controls the rate of photosynthesis in a plant growing under saline conditions [104] due to its role as a major osmotic substance to maintain high water content in tissues under stress [105]. Regulation of stomata depends on the supply of K in the guard cell and leaf apoplast [106], and the role of K in stomata regulation is a key controlling factor in photosynthesis [107].

Vitamin C (Ascorbate, AsA) is one of the stimulants found in CFE and is an important antioxidant in the defense system of the plant. It is implicated in many biological activities (e.g., antioxidant, enzymatic co-factor, and donor/receptor in transport of electrons either at plasma membranes or in chloroplasts) in the plant, all of which are related to the plant's ability to tolerate the influences of oxidative stress [108]. In chloroplasts, the "Halliwell-Asada pathway" shows that APX utilizes AsA to oxidize monodehydro-ascorbate (MDHA) to elevate dehydro-ascorbate (DHA). This step is followed by a reduction in both DHA and MDHA to replenish the AsA pool. This scavenging type can be observed near PSI to diminish the hazards of ROS escaping, and to reduce ROS reactions with each other [109].

Drought stress, in our study, noticeably raised electrolyte leakage (EL) in leaf tissues and malondialdehyde (MDA) (Table 5). Under drought stress conditions, leafy stomata mostly reduces the fixation of CO_2, whereas transfer of electrons and light reaction proceeds normally. In addition, NADP acceptance of electrons is restricted and therefore O_2 can serve as an electron acceptor resulting in more production of ROS ($O_2^{\bullet -}$, H_2O_2, and OH^-), which leads to cell membrane lipid peroxidation and an increase in EL [110,111]. However, the application of CFE or SA significantly decreased EL and MDA, and the combined use of CFE+SA was most functional in tolerating drought stress in potato plants (Table 5). EL enables the assessment of cell membrane injury by subjecting plants to drought stress. Sustaining cell membrane integrity under drought stress is an intrinsic portion of the drought tolerance mechanism [112]. In addition, bypassing the use of CFE or SA alone, the combined application of CFE+SA significantly improved cell integrity by increasing the relative water content (RWC) and membrane stability index (MSI) under water limitation stress (Table 5). Both RWC and MSI are useful measures of the plant physiological water

status [113]. Under stress, plant water status is very sensitive and thus predominant in estimating plant stress response [114]. Drought stress diminishes the root hydraulic conductivity, leading to diminished water flow from the absorbing roots to the shoot system, even in osmotically modified plants [115]. Photosynthesis is associated with the plant transpiration rate, and the inhibition of transpiration is an authoritative and rapid measure of the toxic influences of stress [116]. Therefore, a decrease in leaf RWC occurred in drought-stressed potato plants as a result of decreased water flow from absorbing roots to the shoot system, which may be due to the closure of stomata due to a diminished rate of transpiration (Table 4), causing inhibitory influences on plant growth and production. SA increases RWC and boosts tolerance to water deficit stress in plants [117], which is likely due to the increased level of ABA by SA, ultimately helping to maintain the desired plant water balance [118].

Peroxidation of membrane lipids and EL in plants are usually signaled by free radicals that induce membrane degradation or damage in stressed plants [119]. In this study, peroxidation of membrane lipids was evaluated as the level of malondialdehyde (MDA). This is considered a stress biochemical indicator, because it suppresses biomass production and diminishes the plant's adaptability to stress [120]. MDA concentration and damage to membranes increase in water-deficient plants due to increased ROS generation [121]. In stressed plants, MDA stimulates the adverse ROS action, whereas carotenoids induce protection from ROS [122]. Application of CFE and/or SA, in which the CFE+SA combination was preferred, attenuated the adverse influences of water deficiency on potato plant cell membranes by reducing water stress-induced MDA while increasing the antioxidant enzyme activities such as APX and SOD (Tables 5 and 7). The elevation in MDA was associated with elevated levels of $O_2^{\bullet -}$ and H_2O_2 as ROS, which were suppressed by CFE+SA treatment (Table 5).

During stress, the closure of the stomata is stimulated by ABA to avert the plant water imbalance so that CO_2 fixation is reduced. This in turn reduces the oxidation of NADP, the first acceptor of electrons during photosynthesis. Therefore, when ferredoxin is decreased in the photosystem, free oxygen radicals are produced by the Mehler reaction [123]. This transfer generates one, two, and three electrons forming $O_2^{\bullet -}$, H_2O_2, and $OH^{\bullet}$, respectively, with grave consequences for proteins, lipids, and DNA [16]. Therefore, the cell membrane integrity, in addition to enzyme activities and the function of the photosynthetic machinery, are affected [124]. Table 5 shows that levels of $O_2^{\bullet -}$ and H_2O_2 were raised under drought but were suppressed by the application of CFE and/or SA treatments. This may be because CFE is rich in some antioxidants such as proline, amino acids, and phenolic components (Table 2), which are absorbed by plant leaves and reinforce the endogenous antioxidant system to overcome drought stress by reducing ROS damage. PhCs have a powerful antioxidant capacity capable of attenuating oxidative stress and scavenging toxic H_2O_2 and the phenol/ascorbate/POX system in stressed plants [125]. In this respect, [82] reported that treating the stress-treated roots with GLA decreases H_2O_2 content.

A common consequence of plant exposure to drought and osmotic stress is the accumulation of toxic ROS, which cause damage to proteins, lipids, carbohydrates, and DNA [126]. To avert the accumulation of toxic ROS, the activity of SOD increases, as the first defense line, to protect from excessive formation of $O_2^{\bullet -}$ (from reduced oxygen) by converting it to H_2O_2 in plants [16], so SOD activity is boosted by application of GLA to stressed rice roots [82]. Among all SOD isozymes, Mn–SOD1 and Mn–SOD9 expression is the most strongly stimulated by GLA application, indicating that it is essential for protection against stress in comparison with other enzymes [82]. Our study indicated that PhCs, as a component of CFE, directly contributed to the potato plant antioxidant capacity against water deficit stress. In this regard, [127] confirmed a correlation between the plant content of PhCs and endogenous antioxidant activity/capacity. This status is reflected in the overall level of GAL present in the CFE, possibly to overcome the accumulation of $O_2^{\bullet -}$ and H_2O_2, along with the positive action of SA that can directly scavenge the $O_2^{\bullet -}$ and H_2O_2 in a non-enzymatic manner [128].

Proline is a non-enzymatic antioxidant and a main component of CFE (Table 2). It plays an essential role in the osmotic modulation of the cell when the plant accumulates it under stress conditions [129]. In this study, it was positively altered in the potato plants, because it significantly increased under drought stress and was further accumulated by application of CFE and/or SA, favoring the combined CFE+SA treatment (Table 6). Accumulation of proline limits ROS damage to plants and improves plant drought tolerance, and it also physically quenches $^1O_2$ radical or reacts directly with $OH^\bullet$ [130]. The elevated level of proline and some other ingredients of CFE that most likely transferred to plants by foliar nourishment improved the antioxidant system of potato plants to counteract the degradation caused by drought stress [7]. PhCs such as GAL present in CFE may play an essential role in increasing proline accumulation under stress conditions, as indicated by [82], who reported that GLA application increases proline content under stress conditions. They added that the accumulation of proline by applying GLA under stress may be associated with the restoration of cell volume and turgor, and the protection and stabilization of membrane structures and enzymes. In addition, the use of SA increases the resistance to drought through the accumulation of various osmotic compounds including soluble sugars and proline, which are essential for the osmotic regulation mechanism [88].

The drought stress reaction in plants typically incorporates a decline in cellular water potential by optimizing osmotic adjustments or net groupings of solutes, and significant usage of both the persistence of cell water contents and turgor [131]. Under drought stress, like proline, soluble sugars accumulate, which ensures the cell by keeping the harmony between osmotic cytosol quality, vacuole quality, and external condition [56]. CFE components and/or SA likely play a crucial role in osmotic modification and may help modulate gene expression, playing a role in storage functions, metabolic processes, and tolerance [22,25]. Taha [132] noted that stress causes ROS production, causing oxidative damage and a modification in antioxidant activity. Non-enzymatic lipophilic antioxidants such as tocopherols (as one of the CFE components) can scavenge free radicals and ROS under stress conditions [133]. Our results indicated that the α-ToCo content was significantly elevated by CFE and/or SA treatment under drought stress conditions (Table 6).

Antioxidants with small molecular mass (non-enzymes) play a pivotal role against oxidative stress, and are a major intrinsic portion of the plant's defense system along with the enzymatic antioxidants, which are the other portion. Regularly, APX activity must be strictly modulated with the balance of the ASA and GSH pool, resulting in improved antioxidant capacity of plant cells and suppressed oxidative damage [134]. Ascorbate (AsA) can donate electrons in various enzymatic and non-enzymatic reactions so that it is a high strict ROS scavenger. It can protect cell membranes by directly eliminating $O_2^{\bullet-}$ and OH^- [135]. Glutathione (GSH) and AsA are the prime antioxidants of the AsA-GSH cycle, which help diminish H_2O_2 in plant cells. Mostly, glutathione reductase (GR), MDHAR, and DHAR are accountable for providing APX with substrates via the formation of GSH and AsA [136]. Drought stress caused the level of H_2O_2 to increase so that the ASA content was indicated as being responsible for the stress [137]. In this study, drought stress boosted the levels of GSH and AsA in comparison with the non-stressed control, and the combined CFE+SA application further elevated the GSH and AsA levels under the tested stress (Table 6). In this regard, [138] demonstrated that application of a *Carthamus tinctorius* extract containing a phenolic component under stress minimizes $O_2^{\bullet-}$ and H_2O_2 accumulations because the phenolic component contributes to an increase in non-enzymatic compounds such as AsA, which directly eliminates $O_2^{\bullet-}$ and H_2O_2. Moreover, [82] indicated that applying GAL (a component of CFE) boosted the AsA and GSH levels of rice plants under stress conditions. Collectively with SA, they cause increases in GSH and AsA levels to protect plants from ROS overproduction and membrane injury, or they can lead to the biosynthesis of other substances that have protective influences on stressed plants [139].

As an essential part of the plant's antioxidant defense system, the enzymes SOD, CAT, POX, APX, and GR, possessing the first line of defense of SOD, all noticeably increased

under the adverse conditions of irrigation water restriction and further increased with applying the combined CFE+SA treatment to avoid cellular damage by scavenging ROS such as $O_2^{\bullet -}$ and H_2O_2 (Tables 5 and 7). As reported in many studies, by eliminating H_2O_2, CAT is the prime ROS scavenger in plants. It is exclusively present in peroxisomes and glyoxysomes, and may block the formation of OH^- radicals accountable for peroxidation of cell membrane lipids and many influences on plant growth [140,141]. In the current study, drought-stressed potato plants treated with CFE and/or SA, in which the CFE+SA combined treatment was preferred, showed strong activation of CAT (Table 7), thus blocking oxidative damage in plant tissues, as indicated by the diminished levels of H_2O_2 and MDA (Table 5). Moreover, the activity of SOD (mutates $O_2^{\bullet -}$ to H_2O_2) under water shortage stress was elevated and further increased by the application of CFE+SA (Table 7). Like CAT, APX removes H_2O_2, and its activity is increased in several plant species under stress [7,11]. Application of CFE containing some phenolic components and other antioxidants increased the activity of plant antioxidants under stress-free and adverse conditions. In this respect, [127] confirmed a correlation between the plant content of PhCs and endogenous antioxidant activity/capacity. Our results showed that, like CFE, foliar nourishment with SA increased enzymatic activity in potato plants. Increased enzymatic binding activity by SA application in extracts of soybean leaves has also been confirmed by [142]. SA causes a balanced modification in the antioxidant enzyme activities based on ROS levels in plant tissues to enhance plants' tolerance to water deficiency. A similar mechanism of tolerance to SA-induced multiple drought stresses has been reported in lemongrass [143].

Our findings indicated that applying CFE and SA in integration resulted in potato plant growth and productivity, in addition to physiological and biochemical attributes including the components of the plant's antioxidant system, that were more effective and exceeded the corresponding results obtained with application of CFE or SA alone (Tables 3–7). This may be attributed to the positive complementarity of beneficial SA mechanisms in favor of increasing potato plant stress tolerance along with beneficial mechanisms of CFE due to the diversity of its bioactive components in favor of improving potato plant tolerance to the drought stress under study.

The interrelationship among the evaluated parameters (Figure 2) indicates that the yield parameters are positively associated with total chlorophyll, Pn, Tr, gs, MSI, and RWC (physiological parameters). We speculate that the high values of these physiological parameters are associated with the greater total yield and its contributing traits, especially under the conditions of drought stress. In addition, proline and soluble sugars showed a highly positive association with antioxidant activity and carotenoids content. Furthermore, the yield traits exhibited a highly negative association with H_2O_2 and $O_2^{\bullet -}$. In accordance with these results, it is interesting to note that the specific physiological and biochemical parameters are closely associated with yield-related traits under water-deficient conditions.

5. Conclusions

The present study confirmed that the harmful influences of drought stress on potato plant growth and production can be attenuated by applying clove fruit extract (CFE) and/or SA, in which the combined CFE+SA treatment is favored. These applications can protect potato plants against damage caused by drought stress. Foliar nourishment with CFE in combination with SA was the most functional application in providing potato plants with the greatest drought tolerance when grown under moderate drought and severe drought conditions. Decreased oxidative stress ($O_2^{\bullet -}$ and H_2O_2) following the reduction in EL and MDA, along with an increase in the antioxidant defense system components [e.g., carotenoids, osmoprotectants (free proline and soluble sugars), non-enzymatic antioxidants (proline, ascorbate, α-tocopherol, and glutathione), and enzymes (superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, and glutathione reductase)] under drought stress was obtained by the most effective combined CFE+SA treatment, thus supporting potato plants to sustain cell membrane integrity and tissue water balance, and improv-

ing plant growth and yield. Therefore, the study findings advocate the idea of using a CFE+SA combined treatment as foliar nourishment as an important practical approach that effectively and economically achieves food security. This approach was largely efficient in ameliorating potato plant growth and productivity by attenuating the limiting influences of drought stress in dry environments.

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