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Foliar salicylic acid application to enhance the morphophysiology of *Basella alba* and *Basella alba* var. *cordifolia* under water deficit stress

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Abstract. *Ayuningtias AW, Solichatun, Pangastuti A. 2024. Foliar salicylic acid application to enhance the morphophysiology of* Basella alba *and* Basella alba *var.* cordifolia *under water deficit stress. Nusantara Bioscience 16: 96-103.* Global climate change and increasing temperatures are becoming problems in the cultivation of medicinal plants such as *Basella alba* L. and *Basella alba* var. *cordifolia* (Lam.) M.R.Almeida. Foliar salicylic acid on leaves increases growth and productivity in medicinal cultivation. This study aims to determine the exogenous application of salicylic acid to increase the morphophysiology measurement in *B. alba* and *B. alba* var. *cordifolia* under water deficit stress. The study was carried out using a two-factor, completely randomized design consisting of salicylic acid concentration (0, 2, and 6 mM) and water deficit stress (100% (control), 75% field capacity (light stress), 50% wide field capacity (medium stress) and 25% field capacity (heavy stress). The observation results included morphophysiology. The best result on dry weight for *B. alba* was followed by SA 0mM+Medium stress with 5.45 g; in *B. alba* var. *cordifolia* was followed by SA 0mM+ Heavy stress with 3.09 g. Fresh weight for *B. alba* was followed by SA 4mM+ Heavy stress with 35.95 g, and *B. alba* var. *cordifolia* was followed by SA 0mM+ Heavy stress with 35.66 g. The shoot-to-root ratios in *B. alba* and *B. alba* var. *cordifolia* were followed by SA 2mM+Medium stress with 0.93 and 0.99, respectively. Quercetin in *B. alba* was followed by SA 4mM+ Heavy stress with 2.88% w/w, and *B. alba* var. *cordifolia* was followed by SA 6mM+Medium stress with 1.93% w/w. The gallic acid in *B. alba* and *B. alba* var. *cordifolia* was followed by SA 6mM+Medium stress with 10.21% w/w and 9.44% w/w. Prolin in *B. alba* and *B. alba* var. *cordifolia* was followed by SA 6mM+Medium stress with 8.74 and 9.73 µmol/gram wet weight. This study concluded that foliar salicylic acid application enhanced the morphophysiology, including growth, secondary metabolites, and proline accumulation of *B. alba* and *B. alba* var. *cordifolia* under water deficit stress.

Keywords: Basellaceae, climate change, medicinal, proline, secondary metabolites

INTRODUCTION

The environment controls medicinal plants' growth and output. Phytochemicals are also called anti-nutritional factors and are generally not essential for the body's normal functioning but have important therapeutic functions. Natural plant extracts can be used as a new source of antimicrobial agents with potential mechanisms of action. Plant extracts with antimicrobial properties can be used in therapeutic treatments (Ogbe et al. 2020).

Basellaceae is a family of plants used in the ancient Indian medical system known as Ayurvedic medicine. The plants *Basella alba* L. and *Basella alba* var. *cordifolia* (Lam.) M.R.Almeida are species from the Basellaceae family. The other names are Malabar spinach, white gondola (alba), red gondola (*B. alba* var. *cordifolia*), red Malabar spinach, and Ceylon spinach; only about 20 types of plants from this family are divided into five genera (Tjitrosoepomo 2007). *B. alba* has been used commercially in various products since ancient times. The phytochemical properties of *B. alba* are widely used for various purposes, especially in herbal medicine. This plant has been reported as androgenic, antiviral, antibacterial, antioxidant, antidiabetic, anti-inflammatory, antidepressant, antiulcer (because it heals canker sores), wound healing, nephron activity, and hepatoprotective (Deshmukh and Gaikwad 2014; Alakinde and Ojo 2018).

Global climate change and increasing temperatures are problems in the cultivation of medicinal plants. Water deficit stress is the most significant risk for global agricultural food security. If the temperature increases by about 2°C this century, one-fifth of the world's population will be severely affected by drought stress because of water deficit. Global warming is one of the biggest problems in agriculture worldwide and causes dryness. How plants deal with stress varies with different strategies, such as avoiding stress with a short life span, susceptibility to stress to death, and surviving by producing secondary metabolites. In recent decades, public interest in plant secondary metabolites has increased significantly due to their direct therapeutic effects or as precursors for drugs. Secondary metabolites in medicinal plants contain bioactive compounds but must be scaled up in large enough quantities for commercial needs because these compounds are not essential for plant growth (Salehi-Lisar and Bakhshayeshan-Agdam 2016; Ray et al. 2019; Sultan et al. 2019).

Salicylic Acid (SA) can increase growth measurements such as fresh weight, dry weight, root-to-shoot ratio, production of secondary metabolites, and proline accumulation for plants to defend themselves from stress by influencing physiological and biochemical aspects (Khalvandi et al. 2021). Serious water deficit stress conditions can change morphological, physiological, and biochemical. Morphological changes include dwarf plants, early maturity, and a high root-to-shoot ratio. Physiological changes are increased oxidative stress, high proline accumulation, and growth stops. Biochemical changes are ROS production, oxidative damage, antioxidant defense, and secondary metabolites (Seleiman et al. 2021). When exposed to stress, salicylic acid is involved in physiological processes such as photosynthesis, proline, nitrogen metabolism, glycine betaine production, and water-plant relations. It also protects plants. SA affects plant function in a dose-dependent (Khan et al. 2015). Spraying 140 mg l-1 SA, 4 g l-1 Zn, and 11.5 g l-1 GB increased photosynthesis and RWC, increased leaf gas exchange attributes, increased antioxidant enzyme activity, reduced MDA, H_20_2, and O_2 - in corn plants experiencing water deficit stress (Shemi et al. 2021). This study aims to determine the exogenous application of SA as a strong, cost-effective, environmentally friendly strategy to increase the growth measurements, synthesis, and accumulation of proline and secondary metabolites in *B. alba* and B. *alba* var. *cordifolia*.

MATERIALS AND METHODS

Experimental design

The *Basella* plants were grown in Assalaam Islamic Modern Boarding School greenhouse, Pabelan, Kartasura Sukoharjo Regency, Central Java, Indonesia (±114 meters above sea level). This study used a two-factor Completely Randomized Design (CRD) with 3 replications. The different concentrations of salicylic acid were 0 mM (control), 2 mM, 4 mM, and 6 mM; the water deficit stress treatment with 3 kg of planting medium was doused with water until the first drop came out. Pour 1 L of water as field capacity, then calculate soil water content with a 10 grams sample using the formula (Soil water content%) = (A-B)/A x 100%; A: Initial weight of soil sample before drying (g); B: Final soil weight after drying (g). Calculated watering concentrations of water Deficit Stress (DS) were 100% (control), 75% (light stress), 50% (medium stress), and 25% (heavy stress) based on field capacity. This treatment was carried out for 8 weeks after the seeds were planted in polybags at 8 weeks old.

Procedures

Plant materials

The *B. alba* and *B. alba* var. *cordifolia* seeds were obtained from traditional markets, "Ninu Farm," three seeds containing embryos were selected and planted in each polybag. The daily temperatures inside the greenhouse are about 23-31℃. The soil used is black with a sandy texture, has coarse grains, is not sticky, and has high porosity; the soil pH is 6.5. After the seeds were 8 weeks old, they were transferred to polybags with a diameter of 20 cm to be treated for 8 weeks. Salicylic acid with different concentrations, namely 2 mM, 4 mM, and 6 mM, was sprayed on the leaves every 2 days. Water deficit stress based on field capacity is provided every 3 days. Control plants were not given water deficit stress or salicylic acid treatment. Plant dry weight was calculated by weighing the total plant weight after drying for approximately 10 days by sun drying.

Growth measurements

Observational data are presented in grams (g) and measured using an analytical balance. The wet weight of the plants was weighed using an analytical balance at harvest time with the plants aged 4 months after planting. The wet weight of the plants was carried out by uprooting all the plants from the polybag, removing any soil that was still attached, and then weighing them using an analytical balance. The shoot-to-root ratio was obtained by comparing the root and shoot of dry weight.

Plant extraction

Plant extraction used all aerial parts: stems, leaves, and seeds. Extraction occurred after the plants were harvested or 88 days after planting. Next, dry all parts of the plant for 14 days at 50ºC, then finely grind the dried plant with a mortar. Extracts of *B. alba* and *B. alba* var. *cordifolia* were taken from each treatment as much as 0.3 grams sample and dissolved in absolute methanol sonicated at 50 Hz for 15 minutes and then centrifuged the extract at 5,000 rpm for 10 minutes. The centrifuged supernatant was transferred to the flacon bottle according to the treatment.

Secondary metabolites determination

Quercetin quantification by $AlCl_3$ method. 0.3 g of dry extracts of *B. alba* and *B. alba* var. *cordifolia* were dissolved in 10 mL of methanol each. The extract solution obtained was pipetted 1 mL plus 1 mL of 2% $AlCl_3$, and then 1 mL of 1M potassium acetate was added. The mixed solution was left for 1 hour at room temperature, and then a microplate reader (ELISA) was used to measure the absorption solution at a wave of 374 nm, repeated three times. Next, the wavelength was determined from the average absorption results. The quercetin standard series solutions preparation with 40 ppm, 60 ppm, 80 ppm, 100 ppm, and 120 ppm concentrations were obtained by diluting 1,000 ppm quercetin mother liquor. Then, 1 mL was added to 1 mL of 2% $AlCl_3$ and 1 mL of 1M potassium acetate. The standard solution that has been made is left for 1 hour at room temperature, and its absorbance is measured at a wavelength of 374 nm. Reading the concentration of flavonoids on the calibration line (μg/mL), the content of flavonoids in the extract is expressed in quercetin equivalents (mgQE/g extract). Quercetin levels are expressed in% w/w. The determination of gallic acid with the Folin ciocalteu method; a total of 0.3 g of dry extracts of *B. alba* and *B. alba* var. *cordifolia* were dissolved in 10 mL of methanol mixture each. The extract solution obtained was pipetted 1 mL plus the Folin-Ciocalteu

reagent, shaken, left for 3 minutes, added 1.2 mL of 7% Na_2CO_3 solution and left for 60 minutes at room temperature. The absorbance of the extract solution was measured with a Microplate reader (ELISA) at a wavelength of 725 nm and repeated 3 times. Then, the wavelength value was determined with the average absorbance value. Preparation of gallic acid standard series solutions at 40 ppm, 60 ppm, 80 ppm, 100 ppm, and 120 ppm concentrations were obtained by diluting 1,000 ppm gallic acid mother liquor. Then, 1 mL of Folin-Ciocalteu reagent was added, shaken, and left for three minutes. Added 1 mL of 20% Na_2CO_3 solution and then shake until homogeneous. Set aside one hour at room temperature and measure the absorbance at the maximum wavelength. A calibration curve was made for the relationship between gallic acid concentration (µg/mL) and absorbance (Supriningrum et al. 2020)

Calculation of proline accumulation

The accumulation of proline calculation begins by preparing a standard proline solution. Make a 2.5 µM stock solution diluted with 10 mL of 3% sulfosalicylic acid. Then, a proline standard was made with a concentration of 0.1 µM, 0.2 µM, 0.3 µM, and 0.4 µM. Next, each solution was mixed with ninhydrin acid and glacial acetic acid. Place the solution on a Microplate Reader (ELISA) to measure the absorption of the solution at a wave of 520 nm and repeat this three times, then determine the wavelength from the average absorption results. A standard proline curve was made by correlating the concentration of the standard solution with the absorbance results obtained from proline measurements of *B. alba* and *B. alba* var. *cordifolia* leaves. Y = ax+b, the absorbance value of the sample is entered as a Y value to obtain an X value (Bates 1973).

$$\text{proline} = \frac{\text{µmol proline/ml toluene}}{\text{Sample (g)}/5} = \frac{\text{µmol proline}}{\text{Fresh weight}}$$

Analysis of proline levels was carried out when the plants were 88 days after planting. The part used is the fresh leaves and the analysis of proline levels using the Bates method (1973). Samples of *B. alba* and *B. alba* var. *cordifolia* leaves were weighed as much as 2 g and then finely ground. Add 5 mL of 3% sulfosalicylic acid to the sample and filter using Whatman paper No. 1. Next, react 2 mL of filtrate with 2 mL of ninhydrin and 2 mL of glacial acetic acid. Next, it was heated in a water bath at 100°C for 1 hour, and the reaction was ended by placing the test tube in a glass beaker containing ice water. Next, 4 mL of the reaction mixture was extracted with 4 mL of toluene and shaken for 15-20 seconds. Toluene containing proline was pipetted using a micropipette and inserted into the microplate. The blank solution used is toluene. ELISA measured the absorbance of the solution on a Microplate reader (*Lab Geni*) at a wavelength of 520 nm.

Data analysis

Initial statistical analysis used normality and homogeneity tests, then continued with a two-way analysis of variance. After that, Duncan's multiple range test was continued at the 5% level to determine the real effect of each treatment. Data analysis using SPSS 26.0 software.

RESULT AND DISCUSSION

Analysis of growth characteristics of *B. alba* and *B. alba* var. *cordifolia* treated with salicylic acid under water deficit stress

Dry weight

The results are shown in Table 1; the highest value in dry weight was *B. alba* at treatment by SA 6mM+light stress with 5.45 g compared to control 1.42 g, while the lowest weight was treatment by SA 6mM+heavy stress 1.38 g. The highest value in *B. alba* var. *cordifolia* was in treatment SA 4mM+medium stress with a weight of 3.09 g compared to control 1.26 g, while the lowest weight was treated by SA 0mM+medium stress with 0.99 g. Dry weight indicates photosynthate assimilation results because the photosynthate is translocated from the roots to all parts of the plants, and there is an increase in protoplasmic addition due to the increased size and number of cells. So, dry weight also indicates the absorption of nutrients in a plant (Gardner et al. 1991).

Fresh weight

Table 1 shows the highest treatment in fresh weight study for *B. alba* was treatment by SA 4mM+heavy stress with 35.95 g compared to the control 25.81 gr while the lowest weight was treatment by SA 0mM+heavy stress with 18.68 g. The highest value in *B. alba* var. *cordifolia* was followed by SA 4mM+moderate with 35.66 g compared to the control 19.69 gr, while the lowest weight was treated by SA 0mM+medium stress with 18.51 g. Fresh plant weight shows the level of air absorption and nutrients plants absorb for metabolism (Noctor et al. 2018). In this study, the fresh weight of the initial plants had a uniform weight of ± 1.0-2.5 grams/plant. Treatment with water deficit stress (SA 0 mM) reduced the fresh weight of both *B. alba* and *B. alba* var. *cordifolia* plants, but salicylic acid increased the fresh weight.

Shoot-to-root ratio

The root ratio is a character that can be used to determine excess or lack of water. As shown in Table 1, the results showed that the highest shoot-to-root ratio in *B. alba* was in treatment SA 6mM+light stress with 0.93, while the lowest ratio in treatment SA 0mM+heavy stress with 0.21 or in the treatment with heavy stress. The best results for *B. alba* var. *cordifolia* were in treatment SA 6mM+light stress with 0.99, while the lowest ratio was followed by SA 0mM+heavy stress with 0.27 or in the treatment with severe water deficit stress.

Table 1. The growth characteristics of *B. alba* and *B. alba* var. *cordifolia* treated with salicylic acid under water deficit stress

Parameters	Species	Treatment														
		Control	SA 2mM + DS control	SA 4mM + DS control	SA 6mM + DS control	SA 0mM + Light stress	SA 2mM + Light stress	SA 4mM + Light stress	SA 6mM + Light stress	SA 0 mM + medium stress	SA 2mM + medium stress	SA 4mM + medium stress	SA 6mM + medium stress	SA 0mM + heavy stress	SA 2mM + heavy stress	SA 4mM + heavy stress	SA 6mM + heavy stress
Dry Weight	*B. alba*	1.42± 0.01Ac	2.07± 0.27Ac	1.85± 0.14Ab	1.71± 1.23Aa	1.71± 0.12Bc	2.29± 0.06Bc	2.33± 0.33Bb	5.45± 0.48Ba	1.86± 0.04Cc	3.80± 0.58Cc	2.70± 0.73Cc	1.44± 0.06Ca	1.44± 0.31Cc	1.99± 0.24Cc	1.43± 0.05Cb	1.38± 0.12Ca
	B. alba var. *cordifolia*	1.26± 0.35Ac	1.06± 0.02Ac	1.55± 0.02Ab	1.77± 0.02Aa	1.37± 0.03Bc	1.08± 0.01Bc	2.46± 0.01Bb	1.63± 0.06Ba	0.99± 0.02Cc	1.91± 0.01Cc	3.09± 0.04Cb	1.72± 0.0.01Cca	1.48± 0.07Cc	1.44± 0.03Cc	1.06± 0.00Cb	1.32± 0.02Ca
Fresh Weight	*B. alba*	25.81± 4.31Ba	25.65± 4.65Ba	26.74± 5.80Ba	23.57± 3.44Ba	28.75± 1.30Ba	23.79± 4.87Ba	30.95± 1.76Ba	23.14± 3.52Ba	23.45± 2.38ABa	25.08± 2.22ABa	31.80± 4.43ABa	19.39± 2.93ABa	18.68± 2.41Aa	28.16± 3.86Aa	35.95± 4.13Aa	26.19± 3.76Aa
	B. alba var. *cordifolia*	19.69± 1.95Aa	23.89± 0.69Ab	22.33± 4.11Ab	24.04± 2.90Ab	22.06± 3.69Cb	25.95± 3.32Cb	30.56± 0.92Cb	21.00± 2.58Cb	18.51± 1.81BCb	23.47± 2.13BCb	35.66± 3.32BCb	23.74± 3.83BCb	24.74± 3.48ABb	32.97± 1.49Abb	21.56± 1.03ABb	25.14± 0.97ABb
Shoot-to-root ratio	*B. alba*	0.40± 0.003Aa	0.37± 0.001Ac	0.36± 0.002Ab	0.55± 0.002Ab	0.42± 0.001Ca	0.90± 0.001Cc	0.92± 0.003Cb	0.93± 0.003Cb	0.42± 0.003Ba	0.64± 0.001Bc	0.60± 0.001Bb	0.62± 0.001Bb	0.21± 0.003Ca	0.43± 0.002Cc	0.49± 0.003Cb	0.58± 0.001Cb
	B. alba var. *cordifolia*	0.42± 0.002Aa	0.38± 0.004Ad	0.64± 0.002Ac	0.64± 0.002Ab	0.35± 0.005Da	0.90± 0.004Dd	0.97± 0.002Dc	0.99± 0.002Db	0.38± 0.003Ba	0.66± 0.224Bd	0.69± 0.993Bc	0.75± 0.002Bb	0.29± 0.001Ca	0.77± 0.304Cd	0.58± 0.003Cc	0.27± 0.001Cb

Note: The averages marked with capital letters differ significantly between SA levels within each water availability level (FC), and the averages marked with lowercase letters differ significantly between water availability levels (FC) within each SA level based on the DMRT test on level 5%

Table 2. Secondary metabolites of *B. alba* and *B. alba* var. *cordifolia* treated with salicylic acid under water deficit stress

Parameters	Species	Treatment														
		Control	SA 2mM + DS control	SA 4mM + DS control	SA 6mM + DS control	SA 0mM + Light stress	SA 2mM + Light stress	SA 4mM + Light stress	SA 6mM + Light stress	SA 0 mM + medium stress	SA 2mM + medium stress	SA 4mM + medium stress	SA 6mM + medium stress	SA 0mM + heavy stress	SA 2mM + heavy stress	SA 4mM + heavy stress	SA 6mM + heavy stress
Quercetin (% w/w)	*B. alba*	1.2± 0.30Aa	0.99± 0.04Ba	1.15± 0.04Ca	1.34± 0.03Ba	0.33± 0.015Aa	1.41± 0.017Ba	1.52± 0.05Ca	1.69± 0.12Ba	0.75± 0.15Ab	1.8± 0.20Ab	1.87± 0.015Bb	2.04± 0.03Cb	0.27± 0.23Ac	2.33± 0.09Ab	2.88± 0.14Bb	1.80± 0.12Cb
	B. alba var. *cordifolia*	0.78± 0.14Ac	0.73± 0.25Bc	1.17± 0.04Dc	1.43± 0.04Cc	0.12± 0.03Aa	0.42± 0.03Ba	0.26± 0.03Da	0.15± 0.03Ca	0.04± 0.01Ad	1.16± 0.06Bd	1.59± 0.04Dd	1.93± 0.08Cd	0.02± 0.005Ab	1.37± 0.16Bb	1.41± 0.11Db	1.61± 0.04Cb
Gallic acid (%w/w)	*B. alba*	2.15± 0.39Aa	1.83± 0.31Ba	3.08± 0.14Ca	3.95± 0.11Da	0.27± 0.13Ab	3.74± 0.25Bb	5.05± 0.05Cb	5.25± 0.38Db	0.13± 0.05Ad	6.01± 0.12Bd	6.37± 0.13Cd	10.21± 0.14Dd	0.16± 0.005Ac	3.32± 0.12Bc	7.25± 0.09Cc	4.44± 0.19Dc
	B. alba var. *cordifolia*	3.18± 0.13Aa	4.57± 0.22Ba	4.73± 0.20Ca	5.21± 0.06Ca	2.53± 0.22Ac	6.40± 0.25Bc	6.9± 0.1Cc	7.16± 0.08Cc	0.86± 0.44Ad	7.38± 0.14Bd	9.31± 0.67Cd	9.44± 0.77Cd	0.19± 0.17Ab	6.31± 0.1Bb	8.08± 0.26Cb	7.34± 0.8Cb

Note: The averages marked with capital letters differ significantly between SA levels within each water availability level (FC), and the averages marked with lowercase letters differ significantly between water availability levels (FC) within each SA level based on the DMRT test on level 5%

Table 3. Proline accumulation of *B. alba* and *B. alba* var. *cordifolia* treated with salicylic acid under water deficit stress

Parameters	Species	Treatment														
		Control	SA 2mM + DS control	SA 4mM + DS control	SA 6mM + DS control	SA 0mM + Light stress	SA 2mM + Light stress	SA 4mM + Light stress	SA 6mM + Light stress	SA 0 mM + medium stress	SA 2mM + medium stress	SA 4mM + medium stress	SA 6mM + medium stress	SA 0mM + heavy stress	SA 2mM + heavy stress	SA 4mM + heavy stress	SA 6mM + heavy stress
Proline accumulation (µmol/gram wet weight)	*B. alba*	1.90± 0.3Aa	2.49± 0.35Ba	3.38± 0.12Ca	3.84± 0.34Da	2.12± 0.11Ac	4.95± 0.19Bc	5.57± 0.15Cc	5.87± 0.08Dc	3.29± 0.18Ad	4.47± 0.58Bd	6.57± 0.15Cd	8.74± 0.16Dd	3.58± 0.03Ab	2.31± 0.30Bb	3.62± 0.08Cb	4.97± 0.27Db
	B. alba var. *cordifolia*	1.28± 0.17Aa	3.12± 0.007Ba	3.51± 0.21Da	4.02± 0.16Ca	2.95± 0.12Ab	5.03± 0.04Bb	5.59± 0.09Db	6.08± 0.18Cb	2.29± 0.11Ad	6.14± 0.06Bd	9.35± 0.06Dd	9.83± 0.06Cd	2.87± 0.46Ac	6.22± 0.17Bc	8.05± 0.07Dc	5.20± 0.16Cc

Note: The averages marked with capital letters differ significantly between SA levels within each water availability level (FC), and the averages marked with lowercase letters differ significantly between water availability levels (FC) within each SA level based on the DMRT test on level 5%

Analysis of secondary metabolites of *B. alba* and *B. alba* var. *cordifolia* treated with salicylic acid under water deficit stress

Quercetin level

Table 2 shows the best treatment in quercetin for *B. alba* was in treatment by SA 4mM+heavy stress) with 2.88% w/w, the lowest ratio followed by SA 0mM+light stress with 0.27% w/w or in the treatment with heavy stress. The best ratio for *B. alba* var. *cordifolia* was in treatment by SA 6mM+medium stress with 1.93% w/w, while the lowest ratio was followed by SA 0mM+heavy stress with 0.02% w/w or in the treatment with severe water deficit stress.

Gallic acid level

The DMRT test at a 5% level showed significant results on the gallic acid content of the *Basella* plant. As shown in Table 2, the best treatment in gallic acid for *B. alba* was in treatment by SA 6mM+medium stress with 10.21% w/w while the lowest ratio followed by SA 0mM+heavy stress with 0.13% w/w or in the treatment with heavy stress. The best results for *B. alba* var. *cordifolia* were in treatment by SA 6mM+medium stress with 9.44% w/w, while the lowest ratio was in treatment by SA 0mM+heavy stress with 0.19% w/w or in the treatment with heavy stress.

Analysis of proline accumulation of *B. alba* and *B. alba* var. *cordifolia* treated with salicylic acid under water deficit stress

Proline accumulation

The DMRT test at a 5% level (Table 3) showed significant results on the proline accumulation of the *Basella* plant. The results indicate that the highest proline in *B. alba* was in treatment by SA 6mM+medium stress with 8.74 µmol/gram wet weight while the lowest ratio was treatment by SA 0mM+light stress with 2.12% µmol/gram wet weight. The best ratio for *B. alba* var. *cordifolia* was in treatment by SA 6mM+medium stress with 9.83 µmol/gram wet weight, while the lowest ratio was in treatment by SA 0mM+medium stress with 2.29 µmol/gram wet weight.

Discussion

The foliar salicylic acid application enhances the growth of B. alba *and* B. alba *var.* cordifolia

Basella plants easily adapt to various types of soil and climate and are considered one of the tropical spinach plants grown annually. *Basella,* known as Malabar spinach, is also planted because of its very important nutritional content (Adhikari et al. 2012; Sharma et al. 2022). The growth measurements observed were root shoot ratio, fresh weight, and final dry weight on *B. alba* and *B. alba B. alba* var. *cordifolia*. On the growth parameter, *B. Alba* and *B. alba* var. *cordifolia* have better results; this result aligns with Alakinde's and Ojo's (2015) research on *Basella* Linn's anatomy, which significantly differs in stress adaptation. *B. rubra* has anatomical characteristics that are more adaptive in dealing with stress with several features, including a multi-series epidermis, which prevents water loss; also, the long vascular bundles with a larger diameter allow plants to absorb water from the soil and distribute the water more effectively. The closed areoles and the absence of vein endings ensure water conservation; the final feature is the differentiation of the leaf mesophyll (Alakinde and Ojo 2015).

Plant dry weight is an indicator of the results of photosynthate assimilation, which is translocated from the roots to all parts of the plant and produced from an increase in protoplasm, so the size and number of cells also increase. SA activates the plant's defense system and helps adjust water needs when exposed to water deficit stress. In addition, SA increases the activity of SOD, CAT, and POD enzymes, reduces lipid peroxidation, and helps maintain PSII (Noctor et al. 2018). Applying salicylic acid increases fresh weight, which causes an active shift in photosynthesis processes. Salicylic acid sprayed on leaves modulates important enzymatic components such as monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; GR; GSH peroxidase, GPX) and non-enzymatic (including GSH) of the AsA-GSH pathway, as well as the glyoxalase system (Gly I and Gly II) and reduce oxidative stress in plants when exposed to drought stress (Alam et al. 2013; Sohag et al. 2020).

The ratio of shoots to roots is a characteristic that can determine excess or lack of water. Our research revealed that root growth is greater under water deficit stress than shoot growth. In water shortage conditions, plants allocate more photosynthate to the roots to absorb more nutrients. This causes root weight to increase while shoot weight decreases under water deficit stress conditions, making the ratio smaller (Table 1). Reduced shoot growth due to water deficit stress can be caused by loss of turgor, which causes limitations in cell enlargement and leaf expansion. Inhibition of leaf growth reduces photosynthesis due to stomata closing because it limits gas exchange. Disruption of photosynthesis also causes several enzymatic and hormonal changes (Tombesi et al. 2015; Xu et al. 2015; Furlan et al. 2017; Kou et al. 2022). Shoot-to-root ratio, fresh weight, and dry weight are closely related to photosynthesis. Salicylic acid sprayed on plants during drought accumulates more proline to maintain photosynthesis by stabilizing the Rubisco protein. SA also increases the activity of NR (nitrate reductase) and ATPS (ATP Sulfurylase) so that the metabolism of N (nitrogen) and S (sulfur) also increases. The increased N and S directly affect photosynthesis, so administering SA can control water deficit stress (Ashraf and Foolad 2007; Nazar et al. 2015).

The foliar salicylic acid application enhances the morphophysiology of B. alba *and* B. alba *var.* cordifolia

Proline production during long periods of water deficit stress increases because proline is synthesized in the leaves and transported to the roots to overcome water shortages. This study proved that salicylic acid in both *Basella* plants increased proline accumulation (Table 3). Several studies report that exogenously administered salicylic acid can reduce the adverse effects, increasing growth, photosynthesis, and proline accumulation in water deficit stress. Wang et al. (2022) stated that proline in the roots,

stems, and leaves of the germplasm of watermelon M08 strain Y34 was very significant when exposed to water deficit stress. Profiles and enzyme activity measurements revealed that the ClP5CS1 gene contributes primarily to leaf proline synthesis via the Glu pathway. Nazar et al. (2015) stated that proline production increased in *Brassica juncea* (L.) Czern. given 0.5 mM SA by increasing γ-glutamyl kinase (GK) and decreasing proline oxidase (PROX) activity. Salicylic acid significantly inhibits the activity of 1-aminocyclopropane carboxylic acid synthase (ACS) in *B. juncea* plants during water deficit stress. The increase in proline during water deficit stress regulates water balance as an osmoprotectant so that plants are protected from oxidative stress; thus, the photosynthesis process runs well. Exogenous application of SA increases proline content due to increased pyrroline-5-carboxylate reductase activity by converting pyrroline-5-carboxylate into proline so that proline synthesis increases. The study proves that SA can improve the antioxidant defense system of plants and increase the levels of osmotic regulatory substances to remove ROS. These results follow our observations that the levels of ROS and MDA were significantly reduced in plants after SA treatment (Sharma et al. 2023).

Currently, drought is the most critical abiotic stress and affects agriculture throughout the world. The main negative impact that occurs due to drought is oxidative damage. Flavonoids are a non-enzymatic antioxidant that increases plant defense against drought stress. During abiotic stress conditions, modifications to plant flavonoid biosynthesis occur for the defense system. Plants with higher concentrations of flavonoids cope better with oxidative stress. Flavonoids are synthesized in plants via the shikimate and phenylpropanoid pathways. Exogenously administered salicylic acid in appropriate doses activates several genes associated with cellular processes and responses to environmental stimuli or stress conditions. The expressed genes were directly and indirectly related to phenylpropanoid metabolism. Salicylic acid works on signaling pathways to increase plant tolerance to stress so that yields remain good. Apart from that, salicylic acid plays an important role in increasing physiological processes, stomatal conductance, photosynthesis rate, and chlorophyll content under stress conditions (Arbona et al. 2013; Li et al. 2013; Nazar et al. 2015; Shomali et al. 2022).

Quercetin can protect chloroplasts from singlet oxygen produced by visible light. An increase followed a rapid increase in PAL activity in quercetin levels. This is in line with research (Khalil et al. 2022), which states that salicylic acid has a positive impact on increasing secondary metabolites in *Eriocephalus africanus* L. Adegoke and Ojo (2017) stated that phenolic and flavonoid content in *B. alba* greater than in *B. alba* var. *cordifolia*. Salicylic acid influences receptor transcription to produce defense gene expression, increasing plant immunity. Prolonged drought stress increases excitation energy and ROS levels, but salicylic acid works the opposite, namely increasing the antioxidant system in plants experiencing stress (Badri et al. 2008; Gondor et al. 2016; Kumar et al. 2021; Peng et al. 2021).

Phenol content is a good indicator for assessing environmental stress and plant metabolism tolerance. It can scavenge free radicals such as ROS, reduce singlet oxygen, break down peroxides, and inhibit autooxidation of lipids and plant antioxidant compounds necessary to protect plants against proliferation and oxidative stress. The increase in phenol content is due to the induction of special defense mechanisms that protect the cytoplasm and chloroplasts from oxidative damage. Moreover, Salicylic acid has been shown to help the synthesis of phenolic compounds such as the PAL enzyme, which catalyzes the phenylpropanoid pathway to form trans-cinnamic acid, the main mediator of phenolic biosynthesis (Ksouri et al. 2008; Oh et al. 2009; Sharma et al. 2019; Moradbeygi et al. 2020; Shohani et al. 2023).

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