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Effect of salicylic acid application in two basil cultivars submitted to water deficit

Efeito da aplicação do ácido salicílico em dois cultivares de manjericão submetidas ao déficit hídrico

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ABSTRACT

The water deficit is one of the factors that most limit the productivity of agricultural crops. Salicylic acid (SA) is a plant hormone that plays an important role in response to environmental stresses, including water deprivation stress. Basil (*Ocimum basilicum* L.) is a plant sensitive to water stress and its cultivation can be strongly affected by prolonged periods of drought. Thus, the objective of our study was to evaluate the possible attenuating role of AS in response to water stress in two basil cultivars. The experiment was conducted in a greenhouse of the Federal University of Agreste of Pernambuco (*Universidade Federal do Agreste de Pernambuco*). The experimental design was completely randomized, with eight treatments, being two varieties of basil (purple and green), two forms of application of SA (with and without application) and two forms of irrigation (irrigated and without irrigation). The concentration of salicylic acid used was 2mM and its application was exogenous. As for the results, it was observed a reduction of 15.7% of the dry mass of the aerial part of the basil plants when submitted to water deficit in relation to the irrigated plants. Significant differences were observed in the photosynthetic rate of the two varieties of basil when exposed to SA. These results suggest that the use of SA at the concentration of 2mM contributed positively to the growth of basil plants submitted to water stress as well as to irrigated plants.

RESUMO

O déficit hídrico é um dos fatores que mais limitam a produtividade das cultuas agrícolas. O ácido salicílico (AS) é um hormônio vegetal que possui um importante papel em resposta a estresses ambientais, incluindo estresse por privação hídrica. O manjericão (*Ocimum basilicum* L.) é uma planta sensível ao estresse hídrico e seu cultivo pode ser fortemente afetado por períodos prolongados de seca. Com isso, o objetivo do nosso trabalho foi avaliar o possível papel atenuador do AS em resposta ao estresse hídrico em duas cultivares de manjericão. O experimento foi conduzido em casa de vegetação da Universidade Federal do Agreste de Pernambuco. O delineamento experimental foi inteiramente casualizado, com oito tratamentos, sendo duas variedades de manjericão (roxo e verde), duas formas de aplicação do AS (com e sem aplicação) e duas formas de irrigação (irrigado e sem irrigação). A concentração de ácido salicílico usada foi de 2mM e sua aplicação foi exógena. Quanto aos resultados, observou-se uma redução de 15,7% da massa seca da parte aérea das plantas de manjericão quando submetidas a déficit hídrico em relação as plantas irrigadas. Foi observado diferenças significativas na taxa fotossintética das duas variedades de manjericão quando expostas ao AS. Esses resultados sugerem que o uso de AS na concentração de 2mM contribuiu positivamente para o crescimento das plantas de manjericão submetidas a estresse hídrico quanto para as plantas irrigadas.

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Introduction

Plants are sessile organisms inserted in highly heterogeneous environments. Therefore, they are exposed to constant environmental fluctuations throughout their life cycle. Water deficiency is one of the environmental factors that most limits plant productivity (Brodersen et al., 2019). In a condition of water deficit, various molecular, biochemical, physiological, morphological and ecological processes of plants are impaired. Damage caused by water deficiency, in scenarios of prolonged droughts, is potentially being aggravated by climate change, so mitigation has been a major challenge for the scientific community (EMBRAPA, 2020). Several strategies have been adopted to induce tolerance to water stress at different stages of plant growth, among the strategies are the application of exogenous regulators and synthetic hormones (Seleiman et al., 2021). Growth regulators, because they act in the processes of regulating plant developments and as signalers, play important roles in responses to plant tolerance to stresses (Khan et al., 2012).

Salicylic acid (SA) is a phenolic phytohormone that plays an important maintenance role against biotic and abiotic stresses, mainly by the production of osmolytes and secondary metabolite compounds (Khan et al., 2015). By influencing the regulation of the antioxidant defense system, stomatal movements, sweating and consequently the photosynthetic rate, SA shows itself as a promising hormone to relieve and trigger responses to environmental stresses (Nazar et al., 2015). Several studies show that the application of SA has a beneficial role against the damage caused by water stress, especially related to oxidative damage (Chen et al., 2014; Najafabadi and Ehsanzadeh, 2017; Wang et al., 2019; Sankari et al., 2019; Saheri et al., 2020; Zafar et al., 2021).

Basil (*Ocimum basilicum* L.) is an aromatic plant, belonging to the Lamiaceae family, rich in essential oils, with important medicinal and condimentary properties (Ribeiro et al., 2007; Pushpangadan and George, 2012). The Brazilian production of basil is practiced mainly by small producers and is focused on the commercialization of aromatic green leaves (May et al., 2008). However, in some regions of the Northeast there are productions on larger scales being destined for the production of the essential oil (Favorito et al., 2011). In the field, multiple factors can influence basil production, ranging from environmental conditions to the choice of cultivars or variety (Mulugeta and Radácsi, 2022). Basil is sensitive to water deficit, so measures that mitigate damage caused by stress and improve plant tolerance is very useful for basil production (Damalas, 2019). In this context, the present study aimed to analyze the role of salicylic acid in the growth of two basil cultivars under water deficit.

Material and methods

The experiment was conducted in a greenhouse of the Universidade Federal do Agreste de Pernambuco (*Universidade Federal do Agreste de Pernambuco* - UFAPE), during the months of September to November 2019. The place is characterized by 08°53'27"S of latitude, 36°29'48" W of longitude and altitude from 807 m, to 209 km of the state capital (Recife). The climate in the microregion of Garanhuns is tropical rainy, with dry summer; The rainy season begins in October and encompasses winter and early spring. The annual averages of temperature and relative humidity are, respectively, 21.1°C and 82.5%, with annual rainfall of 897 mm, being the wettest four-month period, represented by the months of May to August (Borges Júnior et al., 2012).

The experimental design was completely randomized (DIC), with two varieties of basil, green basil (MV) and purple basil (MR), two water conditions (irrigated and non-irrigated) and two forms of application of salicylic acid - SA (with application and without application). Each experimental unit was composed of two plants per pot. For the two green and purple varieties the treatments consisted of: Treatment 1 - Plants irrigated with salicylic acid (SA); Treatment 2 - Irrigated plants without SA; Treatment 3 - Non-irrigated plants without SA; Treatment 4 - Plants not irrigated with SA. The SA concentration used was 2mM (Carvalho et al., 2019).

The soil was collected in the sowing of Garanhuns, and presents the following characteristics: pH (water 1:25) = 6,5; H+ AI = 1,48 $cmol_c\ Kg^{-1}$; Al = 0,40 $cmol_c\ Kg^{-1}$; Na = 0,47 $cmol_c\ Kg^{-1}$; K = 0,77 $cmol_c\ Kg^{-1}$; Ca = 2,90 $cmol_c\ Kg^{-1}$; Mg = 2,60 $cmol_c\ Kg^{-1}$; P = 64,6 $mgKg^{-1}$; base saturation = 82%; field capacity = 0,110 g g^{-1} (Laboratory of physical chemistry of the IPA-Recife-PE, UFRPE-UAG, 2014). Each 5 kg plastic pot was filled with air-dried soil. The crop was being irrigated daily in the morning.

The seeds were planted in pots with a capacity of 5 kg and after 30 days of sowing the thinning was performed, leaving only two plants per pot. The application of AS was performed 48 days after sowing, being applied via foliar in the abaxial and adaxial part. Two applications were made with an interval of three days. After the second application of SA, water stress was initiated via suspension of irrigation. The suspension of watering was five days, where the experiment was completed and the morphological variables were performed: Plant height (cm): They were measured, with the aid of a tape, from the neck to the apex of the largest branch. The evaluation of gas exchange was performed from 9 to 11 a.m., using the IRGA (infrared gas analyzer IRGA (ADC-BioScientific Ltd. Modelo LCPro SD) in the irrigated plants, the non-irrigated plants were very withered and made the use of the IRGA impossible. At the time, the internal carbon concentration was measured (*Ci*) (μmol m^{-2} s^{-1}), stomatal conductance (*gs*) (mol of H_2O m^{-2} s^{-1}), transpiration (*E*) (mmol of H_2O m^{-2} s^{-1}), rate of net photosynthesis (*A*) (μmol of CO_2 m^{-2} s^{-1}), instant efficiency in water use (EiUA – A/E) calculated by relating it to liquid photosynthesis and perspiration [(μmol m^{-2} s^{-1}) / (mmol de H_2O m^{-2} s^{-1})] and the instantaneous efficiency of carboxylation (*EiC – A/Ci*) [(μmol m^{-2} s^{-1}) / (μmol m^{-2} s^{-1})] from the relationship between liquid photosynthesis and the internal concentration of carbon. The estimate of chlorophyll content was evaluated by means of SPAD index values, which were obtained by means of the portable chlorophyll meter, model (ClorofiLOG, Falker Agricultural Automation, Brazil). The dry mass of the plant was carried out in a forced air circulation oven at 70°C up to constant weight. Then, the material was weighed, obtaining the weight of the dry mass of the shoot and root.

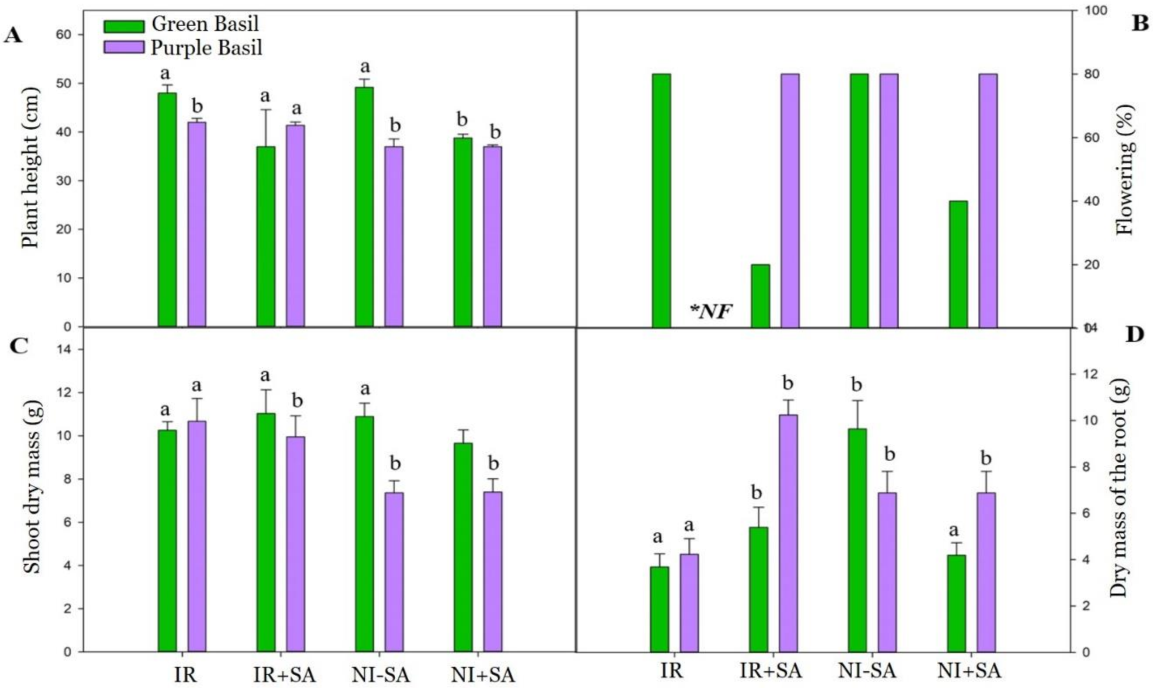
The data were statistically analyzed from the analysis of variance (ANOVA), by means of ASSISTAT and the comparison of means was made by the LSD test (Level Significative Difference) 5% probability. Person correlation analysis, principal component analysis (PCA) and cluster analysis were performed using the software R.

Results and discussion

Regarding the biometric parameters, it was observed that the height of the irrigated plants of the green cultivar is higher compared to the purple cultivar (Figure 1A). The application of SA resulted in an increase in relation to the height of the plants of the purple cultivar. After the application of the SA, no further statistical differences were observed between the cultivars (Figure 1A). Silva (2018), observed that basil plants of the green cultivar under water deficit showed a reduction of 14% of height when compared to irrigated plants. In the same work, it was observed that the application of 1mM of SA favored the growth in height of non-irrigated plants, indicating a potential attenuating effect of SA in relation to the height of basil plants of the green variety. Hussein et al. (2007) observed similar results, in which the exogenous application of 1mM of SA resulted in an increase in plant height, stem diameter and leaf area in corn plants. The irrigated basil plants of the purple cultivar present a late flowering compared to that of the green cultivar in the same condition (Figure 1B). However, when submitted to exogenous application of SA, the irrigated basil plants of the purple cultivar showed a flowering rate equal to the irrigated plants of the green cultivar. Interestingly, the plants of the green cultivar showed a 40% reduction in the flowering rate (Figure 1B).

Figure 1.

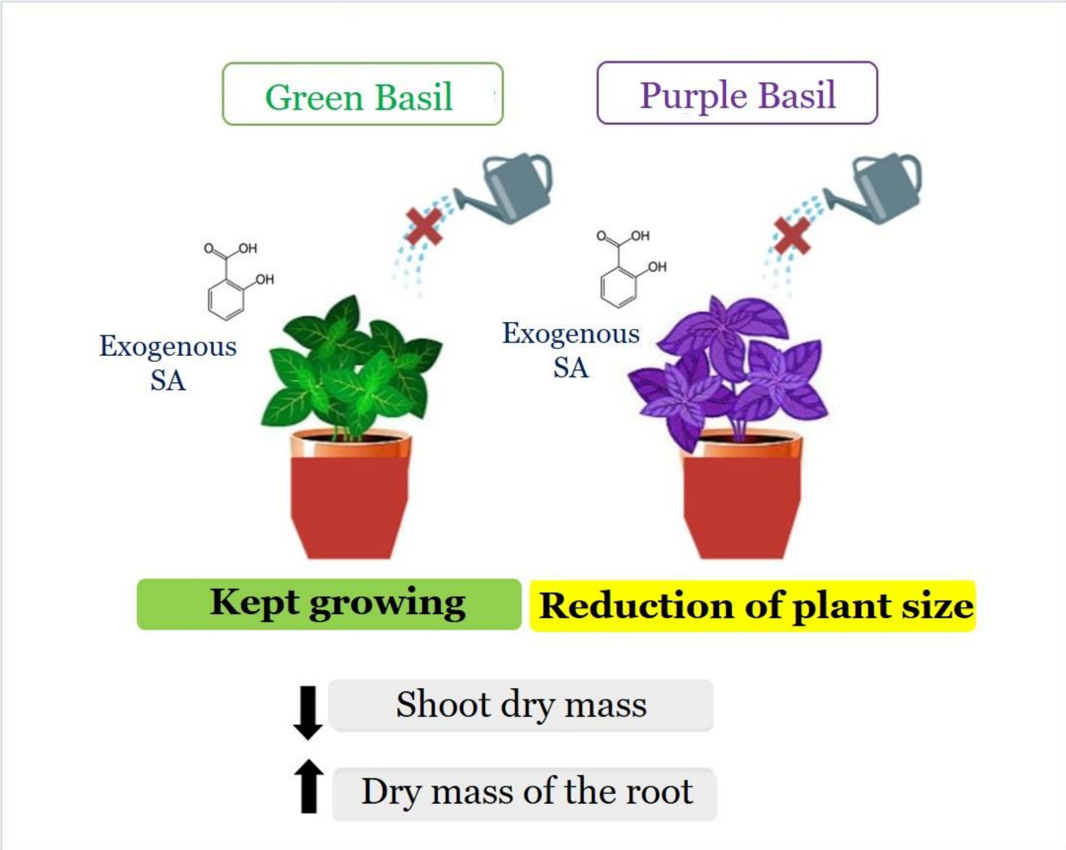
Evaluation of biometric parameters in green and purple basil cultivars under control conditions and under water deficit with and without exogenous application of SA. (A) Height of plants, (B) Percentage of flowering, (C) Dry mass of the area part, (D) Dry mass of the root.: Not irrigated and without application of SA; and NI+SA: Not irrigated and with application of SA. Averages followed by the same uppercase letter in the row and lowercase letter in the column do not differ from each other by the F test (p≤0,05). **NF*: It did not bloom; no plants of this treatment had bloomed until the day of the evaluation. Source: The authors (2023).



The water deficit significantly reduced the dry mass of the shoot of the basil plants of the purple cultivar, regardless of the application of SA (Figure 1C). An increase in the dry mass of the roots was observed in both cultivars with the exogenous application of SA, both in the irrigated treatment and in the treatment of water deficit (Figure 1D). In general, the results indicate that basil plants under water deficit have a higher allocation of photo assimilates to the roots, favoring an increase in root growth of 36.62%. With water deficiency, the plant develops mechanisms to try to minimize these effects, one of them being the increase of the root system, favoring the capture of water in deeper layers of the soil (Pereira et al., 2009). Together, it was observed that in relation to growth, green basil variety was the least affected by the water limitation regime and maintained growth, while purple basil was the most affected by water deficit (Figure 2). These evidences indicate that SA was not as effective in attenuating the effect of water deficit in purple basil plants.

Figure 2.

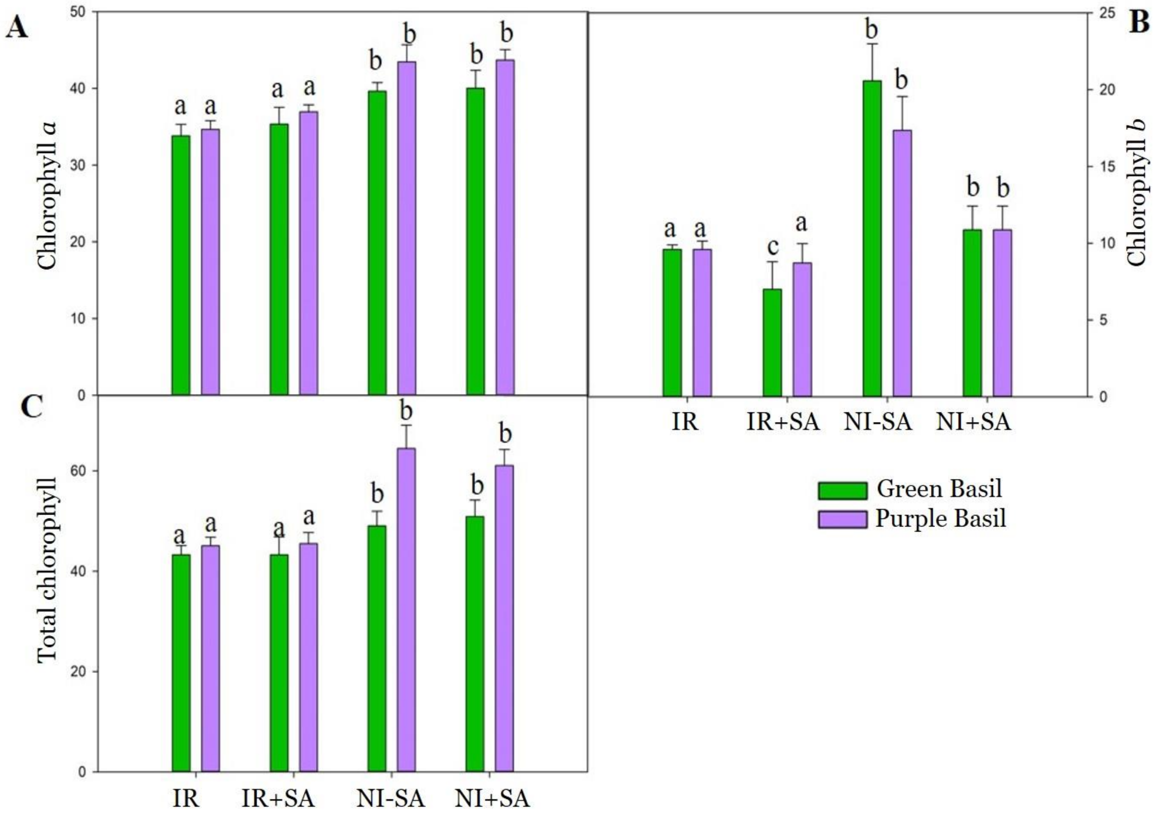
Illustration of the effect of SA application on plant growth of green and purple basil cultivars under water deficit. Source: The authors (2023).



There was an increase in chlorophyll content in basil plants of both cultivars when submitted to water deficit and exogenous application of SA (Figure 3). The purple basil presented higher total chlorophyll content, differing statistically from the green variety (Figure 3C). Similar results were observed by Kordi et al. (2013), where they report that the application of SA in basil plants submitted to water deficit significantly increased photosynthetic pigments compared to the control. The results observed by Carvalho et al. (2020), show that there was an 18% reduction in the chlorophyll content of basil plants submitted to water deficit with SA, when compared to non-irrigated plants without SA. The chlorophyll content in the leaves depends primarily on the genetic load, as it varies according to the species, and genotypes within the species (Marenco et al., 2014)

Figure 3.

Impact of exogenous application of SA on the chlorophyll index in two basil cultivars under control conditions and under water deficit. (A) Chlorophyll a, (B) Chlorophyll B and (C) Total chlorophyll. IR: Irrigated; IR+SA: Irrigated and with exogenous application of SA; NI-SA: Not irrigated and without application of SA; and NI+SA: Not irrigated and with application of SA. Averages followed by the same uppercase letter in the row and lowercase letter in the column do not differ from each other by the F-test (p≤0,05). Source: The authors (2023).



Salicylic acid (SA) plays a very important role in the gas exchange of irrigated basil plants. The results showed significant differences between the genotypes, with no significant differences between the interactions (Table 1). There was a significant effect (0,01 =< p < 0,05) in the photosynthesis rate of the two varieties of basil when submitted to exposure to SA and compared with the control, where salicylic acid acted by increasing the photosynthetic activity of both (Table 1). The SA promoted a higher rate of photosynthesis for purple basil, also presenting a higher efficiency in water use. SA is used for regulation of several physiological mechanisms, among them is photosynthesis, growth and flowering (Hayat et al., 2010).

Table 1.

Stomatal conductance (gs), liquid photosynthesis (A), transpiration (E), internal carbon (Ci), instantaneous water use efficiency (USA) and intrinsic water use efficiency (EiUA) of two basil varieties (Purple and Green) submitted to salicylic acid application in irrigated plants.

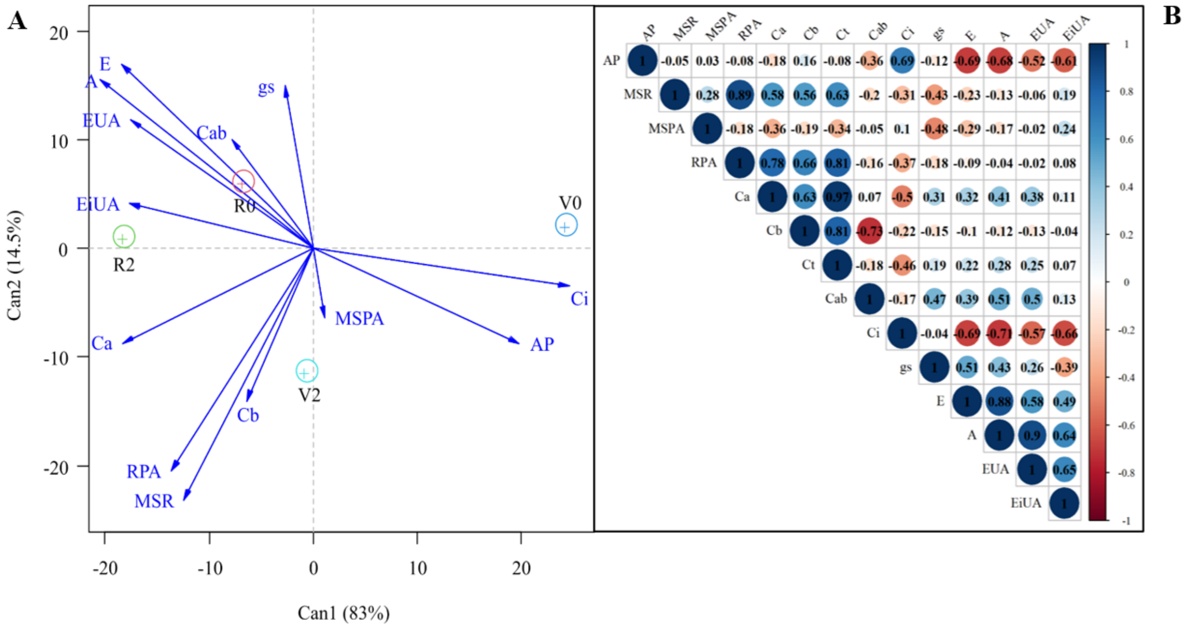
Variety	*gs*			*A*		
	Salicylic acid			Salicylic acid		
	0	2	Average	0	2	Average
Purple	0,044 aA	0,050 aA	0,047	6,34	6,97	6,66 a
Green	0,046 aA	0,034 bB	0,04	3,84	3,72	3,78 b
Average	0,045	0,042		5,09 A	5,34 A	
Variety	*E*			*Ci*		
	Salicylic acid			Salicylic acid		
	0	2	Average	0	2	Average
Purple	1,66 aB	1,80 aA	1,73	228 bA	230 bA	229
Green	1,36 bA	1,26 bA	1,31	299 aA	255 aB	277
Average	1,51	1,53		263	243	
Variety	EUA			EiUA		
	Salicylic acid			Salicylic acid		
	0	2	Average	0	2	Average
Purple	3,82	3,88	3,85 a	145	142	143 a
Green	2,86	2,96	2,91 b	86	118	102 b
Average	3,34 A	3,42 A		115 A	130 A	

Averages followed by the same uppercase letter in the row and lowercase letter in the column do not differ from each other by the F test ($p \leq 0{,}05$). Source: The authors (2023).

Figures 4 A and B illustrates the correlations of growth variables, chlorophyll indices and gas exchange. It was observed that dimension 1 was responsible for explaining most of the results, where, through cluster analysis, it was observed that only water stress was responsible for the grouping of treatments.

Figure 4.

Analysis of canonical variables and confidence ellipses of growth, chlorophyll indices and gas exchange of two varieties of basil (Purple and Green) submitted to the application of salicylic acid (A). Pearson's correlation for growth variables, chlorophyll indices and gas exchange of two varieties of basil (Purple and Green) submitted to the application of salicylic acid (B) in irrigated plants and under water deficit. Source: The authors (2023).



Conclusions

In irrigated plants, salicylic acid contributed to the growth in height of green basil and significantly increased the photosynthetic rate of green and purple basil varieties. Regarding the plants submitted to water deficit, the SA favored the growth of the roots of the green and purple basil plants and contributed to the increase of the total chlorophyll content for the green and purple basil plants. Therefore, SA provides a beneficial effect in relation to increases in photosynthesis and plant height, as well as the increase in dry mass of roots is an indication of its mitigating role to water stress.

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