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Physiological responses of arugula/nirá intercropping under different arrangements and growing seasons

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ABSTRACT

In intercropping between vegetable species, little is known about physiological responses. Thus, getting to know the physiological behavior of these crops is essential to define whether the species are efficient. The aim of this study was to evaluate the physiological responses of arugula/nirá intercropping under different arrangements and growing seasons. The experiment was conducted in a randomized block design, in a split-plot scheme, using the following cultivation arrangements: T_1 = arugula monocropping; T_2 = two rows of arugula with three rows of nirá (2R:3N); T_3 = two rows of arugula with two rows of nirá (2R:2N); T_4 = two rows of arugula with one row of nirá (2R:1N), in plots and growing seasons in the subplots (July 24[th] and September 3[rd]). We evaluated net photosynthesis rate, stomatal conductance, transpiration, internal carbon concentration, water use efficiency, leaf area and productivity. The highest rates of net photosynthesis were obtained in 2R:3N arrangement in the first growing season. Transpiration, stomatal conductance and internal carbon concentration were lower in the second growing season. The intrinsic and instantaneous water-use efficiencies were higher in the second growing season. Arugula monocropping and the 2R:1N arrangement reached similar productivity. The physiological responses of arugula were not influenced by the nirá cultivation arrangements, indicating that the crop can be arranged without harming the efficiency of the photosynthetic process. The 2R:1N intercropping allowed the best productive performance in the first growing season.

Keywords: *Eruca sativa*, *Allium tuberosum*, photosynthesis, physiological behavior.

RESUMO

Respostas fisiológicas da rúcula consorciada com nirá sob diferentes arranjos e épocas de cultivo

No consórcio entre hortaliças pouco se conhece sobre as respostas fisiológicas. Conhecer o comportamento fisiológico é fundamental para avaliar a eficiência dessas espécies. Sendo assim, objetivou-se avaliar as respostas fisiológicas da rúcula consorciada com nirá sob diferentes arranjos espaciais e épocas de cultivo. O experimento foi conduzido no delineamento em blocos casualizados, em esquema de parcela subdividida, com os arranjos de cultivo {T_1 = monocultivo de rúcula; T_2 = duas linhas de rúcula com três de nirá (2R:3N); T_3 = duas linhas de rúcula e duas de nirá (2R:2N); T_4 = duas linhas de rúcula e uma de nirá (2R:1N)}, nas parcelas e as épocas de cultivo nas subparcelas (24 de julho e 03 de setembro). Avaliou-se a taxa de fotossíntese líquida, condutância estomática, transpiração, concentração interna de CO_2, eficiência do uso da água, área foliar e produtividade. A maior taxa de fotossíntese líquida foi observada no arranjo 2R:3N para a primeira época de cultivo. A transpiração, condutância estomática e concentração de carbono interna foram menores na segunda época de cultivo. As eficiências intrínseca e instantânea do uso da água foram maiores na segunda época de cultivo. A rúcula em monocultivo e o cultivo consorciado 2R:1N alcançaram produtividade semelhante. As respostas fisiológicas da rúcula não foram influenciadas pelos arranjos de cultivo com nirá, indicando que a cultura pode ser arranjada sem prejuízo à eficiência do processo fotossintético. O consórcio 2R:1N possibilitaram o melhor desempenho produtivo na primeira época de cultivo.

Palavras-chave: *Eruca sativa*, *Allium tuberosum*, fotossíntese, comportamento fisiológico.

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Intercropping system is a very promising technique for vegetable producers. This system consists of growing two or more species simultaneously in the same planting area (Silva *et al.*, 2011; Hendges *et al.*, 2017, 2019; Viana *et al.*, 2021a,b). This technique provides an increase in profits per area, in productivity, sustainability of crops and more efficient use of the growing area and resources (Lira & Edilson, 2013; Damasceno *et al.*, 2016; Hendges *et al.*, 2017). However, in order to achieve these benefits, the crops must be adjusted in a way to maintain the greatest complementarity between each other, showing ability to establish a beneficial interspecific combination (Camili *et al.*, 2013). So that, before implementing intercropping, taking into consideration the traits and biological interactions of the chosen species is indispensable.

In intercropping cultivation, the species can compete with each other for water, light and nutrients, and this is the most difficult factor about managing this production system. The competition depends on, mainly, the population density and spatial arrangement used, as the number of plants per area increases, as well as they become closer, a reduction in resource availability may occur (Oliveira *et al.*, 2012, 2015). When crops are densely planted, shading between the companion species may occur, reducing light interception and photosynthetic rate and, consequently, the production of the species, due to the negative impact on the physiological traits of the plant (Costa *et al.*, 2014).

In this sense, the ideal spatial arrangement is one in which the species combine the use of the environmental resources, in order to maximize the physiological advantage of the intercropping system through synergistic or compensatory effects between crops, increasing the productive efficiency of vegetables (Oliveira *et al.*, 2015).

Some studies on vegetable intercropping system have shown very promising results, such as chicory and rocket (*Cichorium intybus*) (Cecílio Filho *et al.*, 2008); rocket and carrot (*Daucus carota*) (Lima *et al.*, 2013); collard and aromatic herbs (Hendges *et al.*, 2017); collard and spice species (Hendges *et al.*, 2019) and rocket and spice species (Viana *et al.*, 2021a,b). However, we highlight that little information was found about physiological behavior of the intercropped species during the period of coexistence in most of these studies.

Given the wide possibilities of establishing an intercropping between vegetables, no studies on arugula (*Eruca sativa*) and nirá (*Allium tuberosum*) intercropping had been carried out so far. Arugula is a short-cycle species, whose leaves are very appreciated in Brazilian cuisine. This crop is cultivated in all Brazilian regions (Oliveira *et al.*, 2015), mainly under monocropping system, despite having excellent potential for being intercropped with other crops (Nunes *et al.*, 2013).

Nirá is a spice species, widely used in Asian countries, still little cultivated in Brazil, though. This species belongs to the *Alliacea* family and also presents potential to be intercropped with vegetables, since in addition to increasing productivity in a complementary way, it contributes to reducing pest infestation (Souza & Macedo, 2007; Viana *et al.*, 2021a,b). In this context, the aim of this study was to evaluate physiological responses of arugula intercropped with nirá, under different spatial arrangements and growing seasons.

MATERIAL AND METHODS

The experiments were carried out from July to November, 2017, in the experimental area of Horta Didática at Universidade Federal do Ceará, Fortaleza-CE (3º44'17"S; 38º34'29"W and 21 m altitude). The local climate is 'As' (semi-humid tropical), with 26°C average temperature and 1,450 mm average annual rainfall (Alvares *et al.*, 2014). During the experiments, the maximum, minimum and average temperatures were 31.3°C, 21.3°C and 27.9°C.

The soil samples were taken out from the 0-20 cm layer. The chemical analysis showed the results: pH (H_2O)= 7.2; P= 344.3 mg dm^{-3}; K^+= 0.59 cmolc dm^{-3}; Ca^{2+}= 10.4 cmolc dm^{-3}; Mg^{2+}= 6.4 cmolc dm^{-3}; H+Al = 0.99 cmolc dm^{-3} and V= 95% extracted by Mehlich[1].

The experimental design used was randomized blocks, arranged in split-plot scheme in time (4x2) with four replicates. The plant spatial arrangements were put in the plots and, in the split-plots, the cycles of arugula production (first cycle, planting on July 24th and second cycle on September 3rd). The arrangements (treatments) consisted of single arugula crop and arugula/nirá intercropping, as follows: T_1 = arugula monocropping; T_2 = two rows of arugula with three rows of nirá (2R:3N); T_3 = two rows of arugula with two rows of nirá (2R:2N); T_4 = two rows of arugula with one row of nirá (2R:1N).

The experimental plot consisted of 2.0 m^2 area (1.0 x 2.0 m). The spacings were 0.2 x 0.2 m for arugula and 0.1 x 0.1 m for nirá. In intercroppings, nirá plants were spaced 0.20 m for arugula. The useful area of the plot consisted of four central rows of arugula and two central rows of nirá measuring 1.5 m^2. We evaluated 20 plants per plot.

The soil was tilled, followed by pre-planting fertilization using 12 kg/m^2 organic compound (Guimarães *et al.*, 2016). The organic compound was prepared using cow manure and vegetable remains and showed the following chemical characteristics: N= 5935 mg dm^{-3}; P= 368.7 mg dm^{-3}; K^+= 2300 mg dm^{-3}; Ca^{2+}= 10.9 cmolc dm^{-3}; Mg^{2+}= 9.4 cmolc dm^{-3}; Zn= 98 mg dm^{-3}; Fe= 21.1 mg dm^{-3}; Mn= 67.7 mg dm^{-3}; Cu= 0.7 mg dm^{-3}; B= 1.6 mg dm^{-3}, extracted by Mehlich[1].

Seedlings of arugula cultivar 'Cultivada' (Topseed Garden®) were grown in 162-cell polypropylene trays, containing substrate based on organic compound and carnauba bagana (4:1 ratio) and kept in a protected environment. At 20 days after sowing (DAS), arugula seedlings were transplanted into seedbeds. Nirá was vegetatively propagated, using tillers of plants which were already being produced in the didactic vegetable garden of UFC. Before planting, tillers were separated and selected, roots were partially eliminated and shoots were cut (leaving approximately 3 cm of leaves). During the experiment, two cultivation cycles for arugula and one cultivation cycle of nirá were carried out. Transplanting of the first cycle of arugula was on July 24th and the second was carried out on September 3rd. For nirá, the transplanting was also on July 24th. The harvest of the first cycle of arugula was on August 27th, and the second cycle was harvested on October 8th; nirá was harvested on October 21th. We count a 35-day cycle after arugula transplanting and 80 days after nirá transplanting.

Weeds were controlled manually. The plants were irrigated daily, through micro-sprinkler irrigation system. The top-dressing fertilizations were performed by applying 5 kg/m^2 of organic compound, followed by scarification, at

7 and 21 days after the transplanting (DAT) of arugula seedlings. At 35 DAT, arugula was harvested.

Gas exchanges were analyzed for the arugula crop on the harvest day (35 DAT), for both cycles. The evaluated parameters were: net photosynthetic rate (P_N= μmol $CO_2/m^2/s$), stomatal conductance (g_s = mol $H_2O/m^2/s$), transpiration (E = mol $H_2O/m^2/s$), CO_2 concentration in the substomatic chamber (C_i = μmol CO_2/mol), ratio between CO_2 concentration inside the chamber and ambient concentration (C_i/C_a) leaf temperature (Tl) and instantaneous carboxylation efficiency (P_N/C_i). Using the data for gas exchanges, we determined the instantaneous water-use efficiency (P_N/E = WUE) and intrinsic water-use efficiency (P_N/g_s = WUE_i). We also evaluated the leaf area (LA = cm^2) and productivity (t/ha).

For gas exchanges, we used an infrared gas analyzer (IRGA), model LI6400XT, LI-COR, Biosciences In. Lincon, Nebraska (USA), to analyze fully-expanded leaves of the upper third of the plant, exposed to light, between 8:00 and 11:00 am, on a clear day, using artificial lighting of 1,200 μmol/m^2/s (flux density of photosynthetically active photons) in the evaluation chamber of the equipment, in order to maintain the most homogeneous environmental conditions during the evaluations. We measured the leaf area using an integrator, LI-COR® model LI 3100.

The data were submitted to Shapiro Wilk's test (normality test) and, Barlett's test (homogeneity of variance), then analysis of variance was carried out by F test and averages were compared using Tukey test at 5% significance, using Sisvar software (Ferreira, 2011).

RESULTS AND DISCUSSION

The variance analysis showed significant effect of growing seasons of arugula on net photosynthetic rate, transpiration, stomatal conductance, internal carbon concentration, ratio between internal and external CO_2 concentration, leaf temperature, intrinsic and instantaneous water-use efficiency. For the leaf area and productivity, an interaction effect between cultivation arrangements and production cycles was noticed.

For net photosynthetic rate a difference between production cycles was noticed only for 2R:3N arrangement, being the highest value observed in the first production cycle, when the arugula was transplanted on the same day as nirá (Table 1). For Ci, the highest values were observed in the second cycle, in all spatial arrangements. These results can be explained by greater interference of nirá on arugula in the second cultivation cycle (35 DAT), since the higher nirá plants may have resulted in an interspecific competition, mainly for light. When a plant grows under the canopy of other plants, during the period of coexistence, the amount of light which reaches the leaves can become limiting (greater shading)

Table 1. Averages of net photosynthesis rate (P_N) and internal CO_2 concentration (C_i) of arugula under different cultivation arrangements with nirá and two arugula production cycles. Fortaleza, UFC, 2017.

Cultivation arrangements	Arugula production cycles			
	1st Cycle	2nd Cycle	1st Cycle	2nd Cycle
	P_N (μmol $CO_2/m^2/s$)		C_i (μmol CO_2/mol)	
Arugula	29.13 A	27.01 A	309.80 A	280.46 B
Arugula + nirá (2R:3N)	31.67 A	27.01 B	305.04 A	282.51 B
Arugula + nirá (2R:2N)	28.11 A	25.32 A	318.70 A	285.17 B
Arugula + nirá (2R:1N)	31.18 A	29.15 A	310.05 A	276.24 B
F Test				
Arrangements (A)	2.75		1.66	
Cycle (C)	18.68**		152.75**	
A x C	0.79		1.19	
Block	3.11**		5.00**	
CV (%) Arrangements	15.66		5.19	
CV (%) Cycles	11.41		3.99	

Means followed by same uppercase letters in the line did not differ significantly from each other by Tukey test ($p<0.05$). **Significant at 1% by F test.

Table 2. Averages of transpiration (E) and stomatal conductance (gs) of arugula under different cultivation arrangements with nirá and two arugula production cycles. Fortaleza, UFC, 2017.

Cultivation arrangements	Arugula production cycles			
	1st Cycle	2nd Cycle	1st Cycle	2nd Cycle
	E (mol $CO_2/m^2/s$)		g_s (mol $H_2O/m^2/s$)	
Arugula	12.57 A	10.23 B	0.886 A	0.539 B
Arugula + nirá (2R:3N)	12.62 A	10.40 B	0.916 A	0.525 B
Arugula + nirá (2R:2N)	13.03 A	9.81 B	0.968 A	0.480 B
Arugula + nirá (2R:1N)	12.84 A	10.39 B	0.978 A	0.536 B
F Test				
Arrangements (A)	0.287		0.99	
Cycle (C)	143.45**		465.76**	
A x C	1.65		2.53	
Block	14.10**		2.03	
CV (%) Arrangements	8.76		13.16	
CV (%) Cycles	9.85		12.99	

Means followed by same uppercase letters in the line did not differ significantly from each other by Tukey test ($p<0.05$). **Significant at 1% by F test.

compromising energy metabolism and reducing the rate of CO_2 assimilation (Su *et al.*, 2014), which favors the higher CO_2 concentration in the substomatic chamber (Van Loon *et al.*, 2014).

Transpiration and stomatal conductance were higher in the first production cycle, in all spatial arrangements (Table 2). These results reinforce the hypothesis that nirá caused greater shading on arugula plants, when arugula was transplanted later (35 DAT of nirá). Light has a proeminent role in stomatal regulation (Kerbauy, 2019). Under a greater light supply, plants tend to keep in a higher degree, and for longer time, the opening of the stomatal pore, since the hydration status of the leaf is adequate. In this condition, gas exchanges are favored: CO_2 influx into the leaf and water vapor efflux into the atmosphere (Taiz *et al.*, 2017). On the other hand, the reduction of stomatal opening and, consequently, transpiration as physiological responses of shaded plants are common.

For ratio between CO_2 concentration inside the chamber and ambient CO_2 concentration (C_i/C_a) the authors noticed higher values in the first growing cycle, whereas for leaf temperature (Tl), higher values were reported in the second cycle (Table 3). These results are related to g_s. The higher the stomatal conductance, the lower the resistance to CO_2 diffusion inside the leaf, which increases C_i/C_a ratio. On the other hand, the smaller the stomatal opening, the lower the transpiration rate. Thus, evaporative cooling due to water loss tends to be lower, promoting leaf heating (Taiz *et al.*, 2017).

In relation to instantaneous carboxylation efficiency (P_N/C_i) no difference was noticed. For intrinsic and instantaneous water-use efficiency, higher values were obtained in the second growing season (Table 4), showing that higher quantity of CO_2 was absorbed, to detriment of less water loss (lower g_s and E).

In the intercropping of arugula and spice species (coriander, green onion and parsley), Viana *et al.* (2021a) observed no influence of this intercropping system on physiological responses of arugula, only for growing seasons, corroborating the results in this study. Hendges *et al.* (2017) evaluated the intercropping of collard with spice species (green onion, coriander, basel and parsley) and observed that the coriander was prejudicial to the photosynthetic performance of collard. This fact points out that the physiological responses of intercropped crops vary according to the chosen species and growing arrangement.

For leaf area (LA) and productivity, we verified an interaction effect ($p≤0.01$) between growing arrangements and production cycles. In the first cycle, all the intercropped treatments showed higher averages for LA in relation to monocropping, whereas in the second cycle, monocropping and intercropping,

Table 3. Averages of the ratio between internal and external CO_2 concentration (C_i/C_a) and leaf temperature (Tl) of arugula under different cultivation arrangements with nirá and two arugula production cycles. Fortaleza, UFC, 2017.

Cultivation arrangements	Arugula production cycles			
	1st Cycle	2nd Cycle	1st Cycle	2nd Cycle
	C_i/C_a		Tl (°C)	
Arugula	0.829 A	0.733 B	31.38 B	33.47 A
Arugula + nirá (2R:3N)	0.816 A	0.739 B	31.37 B	34.48 A
Arugula + nirá (2R:2N)	0.820 A	0.741 B	31.50 B	33.39 A
Arugula + nirá (2R:1N)	0.825 A	0.706 B	31.24 B	33.96 A
F Test				
Arrangements (A)	0.45		0.39	
Cycle (C)	127.35**		62.34**	
A x C	1.40		0.83	
Block	5.54**		20.54**	
CV (%) Arrangements	6.81		5.59	
CV (%) Cycles	5.19		4.67	

Means followed by same uppercase letters in the line do not differ statistically from each other, by Tukey Test, $p≤0.05$. **Significant at 1% by F test.

Table 4. Averages of intrinsic water use efficiency (P_N/g_s = WUEi, μmol/m/s H_2O) and instantaneous water-use efficiency (P_N/E = WUE, μmol CO_2/mol H_2O) of arugula under different cultivation arrangements with nirá and two arugula production cycles. Fortaleza, UFC, 2017.

Cultivation arrangements	Arugula production cycles			
	1st Cycle	2nd Cycle	1st Cycle	2nd Cycle
	WUEi		WUE	
Arugula	32.20 B	54.05 A	2472.38 A	2652.84 A
Arugula + nirá (2R:3N)	34.80 B	51.29 A	2633.32 A	2630.71 A
Arugula + nirá (2R:2N)	28.63 B	53.18 A	2238.97 B	2625.31 A
Arugula + nirá (2R:1N)	32.41 B	55.75 A	2538.82 A	2808.53 A
F Test				
Arrangements (A)	0.76		1.13	
Cycle (C)	273.03**		7.77**	
A x C	1.85		1.20	
Block	6.18**		7.34**	
CV (%) Arrangements	17.64		18.93	
CV (%) Cycles	14.94		14.23	

Means followed by same uppercase letter in the line do not differ statistically from each other, by Tukey Test, $p≤0.05$. **Significant at 1% by F test.

Table 5. Averages of leaf area (LA) and arugula productivity under different cultivation arrangements with nirá and two arugula production cycles. Fortaleza, UFC, 2017.

Cultivation arrangements	Arugula production cycles			
	1st Cycle	2nd Cycle	1st Cycle	2nd Cycle
	LA (cm^2)		Productivity (t/ha)	
Arugula	480.08 bB	836.08 aA	10.09 bB	20.23 aA
Arugula + nirá (2R:3N)	1087.35 aA	549.21 bB	9.24 bA	6.10 cB
Arugula + nirá (2R:2N)	1097.69 aA	545.13 bB	9.27 bA	6.97 cA
Arugula + nirá (2R:1N)	1043.07 aA	843.71 aB	14.73 aA	14.97 bA
	F Test			
Arrangements (A)	9.22**		40.51**	
Cycle (C)	23.23**		6.41*	
A x C	19.29**		38.99**	
Block	0.60		0.87	
CV (%) Arrangements	13.45		15.95	
CV (%) Cycles	16.91		12.08	
			Productivity (t/ha)	
nirá			49.70 a	
Arugula + nirá (2R:3N)			16.92 b	
Arugula + nirá (2R:2N)			13.08 b	
Arugula + nirá (2R:1N)			8.95 b	
			F Test	
Arrangements			31.40**	
Block			1.15	
CV (%)			30.00	

Means followed by same uppercase letters in the line and lowercase in the column do not differ statistically from each other, by Tukey Test, $p \leq 0.05$. *,**Significant at 5 and 1%, respectively, by F test.

2R:1N arrangement, were superior (Table 5). Comparing the growing cycles, except for monocropping, all treatments showed higher LAs when arugula and nirá were transplanted on the same day (July 24th).

For productivity, we observed superior values for intercropping, 2R:1N arrangement, in the first cycle and monocropping in the second cycle. Evaluating these results, we could verify a direct relationship of leaf area and plant production. Thus, plants with larger leaf areas are able to intercept a greater amount of light, which is converted into photoassimilates, increasing the production of plant biomass (Taiz *et al.*, 2017; Viana *et al.*, 2021a,b).

Evaluating intercropping of arugula with spice species (coriander, green onion and parsley), Viana *et al.* (2021b) observed that arugula intercropped with coriander showed the best agronomic performance, productivity and biological efficiency. In another intercropping of collard with spice species (green onion, coriander, basel and parsley), Hendges *et al.* (2019) verified that intercropping collard with parsley showed the best agronomic performance, land-use efficiency and productivity, due to higher productivity of the main crop and absence of damage to production. These results corroborate the results in this study, showing that the 2R:1N arrangement was the most productive, pointing out that the efficiency and productivity of the intercropping depend on the complementarity between the species, as well as the spatial arrangements between them.

In intercropping with higher population density (2R:3N and 2R:2N), the authors verified lower productivity of arugula due to higher competition between crops. In the intercropping with lower population density (2R:1N), better productive performance was verified, resulting in an optimization in the use of available resources in the production area, allowing a relationship of spatial complementarity between crops (Hendges *et al.*, 2017, 2019). In relation to growing seasons, the late association of arugula with nirá, especially under higher population density, interfered negatively in productivity. That was because in the second production cycle, nirá plants were high, so that more shading was observed. In addition, these nirá plants competed intensively for production factors (water, light, nutrients and CO_2) with arugula, resulting in less temporal complementarity between crops.

Nirá monocropping showed higher productivity, considering the highest population density per unit area. In average, monocropping showed a yield 298% higher than intercropping cultivation system (Table 5). In intercropping treatments, the highest population densities of nirá could have increased the competition for water, light, and nutrients of arugula crop, resulting in a lower productivity. This competition results in a decrease of light intensity, causing a reduction in photosynthetic activity, with a concomitant decrease in the production of photoassimilates by the plant, which reduces the accumulation of mass in plants (Taiz *et al.*, 2017).

The spatial arrangements did not show any change considering physiological responses of arugula. The growing seasons influenced all variables related to gas exchange and production. The intercropping system of two rows of arugula cultivation alternated with one row of nirá (2R:1N) was the arrangement which showed the best productive performance in the first growing cycle, whereas in the second cycle, monocropped arugula was superior.

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